Physiology, Blooms and Prediction of Planktonic Cyanobacteria

6

Roderick L. Oliver, David P. Hamilton, Justin D. Brookes and George G. Ganf

Contents

Summary 155

6.1 **Introduction** 156
6.1.1 Cyanobacterial Blooms 156
6.1.2 Modelling 156

6.2 **Light Capture and Photosynthesis** 158
6.2.1 Spectral Influence 160
6.2.2 Photoacclimation, Photoadaptation and Photoinhibition 162

6.3 **Photosynthesis and Cellular Metabolism** 163
6.3.1 Modelling of Photosynthesis 164

6.4 **Nutrients** 166
6.4.1 Phosphorus 166
6.4.2 Nitrogen 166

6.5 **Nitrogen Fixation** 169

6.6 **Nitrogen Storage** 172
6.6.1 Nitrogen Starvation 172

6.7 **Cellular Elemental Stoichiometry** 172

6.8 **Whole Lake Nutrient Influences** 173

6.9 **Modelling Nutrient-Dependent Growth Rates** 174

6.10 **Modelling Metabolic Interactions and Cellular Growth** 174

6.11 **Buoyancy and Its Regulation** 176
6.11.1 Gas Vacuole Structure 176
6.11.2 Pressures Acting on Gas Vesicles 176
6.11.3 Buoyancy Regulation 177
6.11.4 Sinking or Floating 178
6.11.5 Colony Formation and the Effect on Buoyancy 180
6.11.6 Within-Population Variability 180
6.11.7 Effects of Turbulence 181

6.12 **Population Losses** 181
6.12.1 Chytrid Infections 182
6.12.2 Programmed Cell Death 182
6.12.3 Cyanophages 183

6.13 **Water Movement and Blooms** 183

6.14 **Ecosystem Models That Include Water Movement** 185

6.15 **Conclusions** 186

References 187

R.L. Oliver (✉)
CSIRO Land and Water, CSIRO, PMB2, Glen Osmond, SA 5064, Australia
e-mail: Rod.Oliver@csiro.au

D.P. Hamilton
Environmental Research Institute, The University of Waikato, Hamilton, New Zealand
e-mail: d.hamilton@waikato.ac.nz

J.D. Brookes • G.G. Ganf
School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, SA 5005, Australia
e-mail: Justin.brookes@adelaide.edu.au; George.ganf@adelaide.edu.au

Summary

This chapter addresses some of the challenges associated with trying to model population fluctuations, bloom formation and collapse of planktonic cyanobacteria. It is argued that improved modelling and prediction rely on a better understanding of the physiological responses of cyanobacteria to the physical and chemical characteristics of their environment. In addition there is a need to better understand the complex trophic interactions that influence population dynamics. The high variability of cyanobacterial populations represents a major challenge for models attempting to make predictions at the whole lake scale. Many of the physiological attributes described within specific models do not capture the dynamics of cyanobacteria, because of the extensive parameterisations required by the array of descriptive algorithms. The physiological attributes to be modelled include the ability to fix nitrogen, storages of both nitrogen and phosphorus, capture light across a range of wavelengths with

B.A. Whitton (ed.), *Ecology of Cyanobacteria II: Their Diversity in Space and Time*,
DOI 10.1007/978-94-007-3855-3_6,

specific accessory pigments, form colonies or filaments, and regulate buoyancy through the balance between gas vacuoles and cellular constituents. Recruitment of populations from sediments may also be important in bloom formation, but is not considered in this chapter. Although there is a commonality in models of cyanobacteria and micro-algae with their descriptions of photosynthesis, nutrient uptake, movement and grazing, there is a need to differentiate the cyanobacteria based on their key attributes, if their occurrence and succession are to be predicted separately from the micro-algae. The challenge is to develop models that incorporate complex physiological processes, responsive to changes at a range of ecosystem scales, but without excessive calibration of the key underlying algorithms. One suggestion is to turn from the single limiting-factor modelling approach that creates a plethora of disjointed algorithms and develop bio-mechanistic representations of integrated cellular function that incorporate dynamic responses to multiple effectors.

6.1 Introduction

6.1.1 Cyanobacterial Blooms

Cyanobacteria are ancient organisms (Schopf and Packer 1987; Chap. 2) whose blooms have been recorded through historical times (Francis 1878; Codd et al. 1994). Cyanobacterial blooms have detrimental effects on the quality of water supplies, partially due to the large biomass that can develop, because of the production of compounds that affect water flavour (Izaguirre et al. 1982) and also because of the risk of the toxins that are produced by some of the bloom forming species (Falconer et al. 1983; Carmichael 1992; Codd 1995; Azevedo et al. 2002).

The physiological adaptations that cyanobacteria have evolved to scavenge for limiting resources have proved highly successful in allowing them to occupy a niche in the pelagic zone of lakes (Vincent 2009). Increased occurrences of cyanobacterial blooms are frequently related to human influences in modifying the physical and biogeochemical conditions of aquatic systems (Reynolds 1987; Oliver and Ganf 2000). A shift in the phytoplankton community composition to more frequent dominance by cyanobacteria also alters the structure and trophic functionality of aquatic ecosystems by changing the flow paths of energy and nutrients.

An ability to predict the occurrence and extent of cyanobacterial blooms relies on developing models that can describe the growth, losses and distribution of planktonic cyanobacteria in response to different or changing environmental conditions. Benthic stages, including overwintering of vegetative cells (Chap. 7) or production of akinetes and their subsequent germination (Faithful and Burns 2006), may also be an important part of the life cycle of cyanobacteria, but knowledge of the environmental attributes that influence these stages is rudimentary and application of mechanistic models of these processes would be premature and is therefore not included in this chapter. Suitable models could provide the early warnings required by water resource managers to activate alleviation strategies including enhanced treatment processes and alternative water supplies (Ferguson 1997). There is also the expectation that improved model predictions might help identify new means of reducing bloom occurrences. Understanding how altered environmental conditions enhance the development of cyanobacterial blooms is also important for efforts aimed at restoring the community structure and trophic functionality of modified aquatic ecosystems.

The bloom-forming cyanobacteria are part of the diverse group of phototrophs that comprise the phytoplankton and are influenced by the same array of environmental conditions as their more taxonomically diverse counterparts, the eukaryotic micro-algae. Like the micro-algae, they are oxygenic phototrophs and capture most of their energy from sunlight, using this energy source to drive nutrient uptake and cellular metabolism that results in cell maintenance and growth. They are also impacted by losses similarly to the eukaryotic micro-algae, through depletion of nutrients and energy, grazing, sedimentation and microbial attack. It is not surprising that the approaches used in modelling the growth, physiology and ecology of cyanobacteria parallel those developed for planktonic micro-algae (e.g. Robson and Hamilton 2004). However, the prokaryotic cyanobacteria differ significantly from their eukaryotic counterparts in many aspects and these differences need to be incorporated into models that not only simulate cyanobacteria populations separately from eukaryotic populations but may also provide differentiation of individual populations of cyanobacteria. In this chapter cyanobacterial physiology is reviewed to elucidate the important processes, from the level of genes to populations, which need to be modelled to advance understanding of the complex interactions between hydrodynamics, biogeochemistry and cyanobacterial physiology.

6.1.2 Modelling

A complete understanding of the causal factors leading to cyanobacterial blooms is lacking. Hence a number of different modelling techniques have been adopted to predict the timing, spatial distribution and magnitude of cyanobacterial blooms, and to derive knowledge about their dynamics. None of these models is perfect and, so long as there is argument about how blooms come about, there will not be a definitive model. There has been considerable debate about the approaches used in modelling phytoplankton dynamics (e.g. Flynn 2003a, 2005). The current debate has been stimulated by acknowledgement that many of the current ecosystem models are based around outdated paradigms of phytoplankton physiology. Progress has not corresponded with what might

be expected from the exponential improvement in computing power, and new information on cellular functioning that has come from molecular approaches (Bhaya et al. 2000) and significantly enhanced measuring equipment (Flynn 2005). Such new developments, often described by explicit models of specific processes, have not been captured in comprehensive models of phytoplankton dynamics at larger (e.g. lake) scales or within the trophic structure of the aquatic system.

Much of the debate has also centred on empirical versus mechanistic models, a discussion that has been ongoing for decades. Modelling approaches have lagged behind the progress in allied fields (Zhao et al. 2008). Part of the debate revolves around the need to balance simplicity and realism, often resulting in empirical models (Flynn 2005), but in some cases the call for more detailed modelling has led to highly complex formulations for which the parameters may be difficult to assess and not easily extended to the system scale. Functional representations of key processes may provide the tools with which to link small-scale and large-scale representations (Zhao et al. 2008). The need for process focussed models becomes particularly evident when considering the varying responses to environmental conditions of different taxonomic and functional groups of phytoplankton. The aim of the models is to capture the competitive potential of different phytoplankton species or groups and therefore the seasonal sequences and shifts in community composition, including responses to major environmental alterations such as eutrophication and climate change (Brookes and Carey 2011; Kosten et al. 2012). Good process based models of phytoplankton will differentiate the characteristics of cyanobacteria from those of other phytoplankton, particularly characteristics that may contribute to blooms of cyanobacteria (Robson and Hamilton 2004). Such characteristics include buoyancy regulation, nutrient storage capacity, the capability to absorb light of different wavelengths, and the cellular balances between energy capture, nutrient uptake, nutrient assimilation, cellular composition and cellular metabolism. Different cyanobacterial taxa also vary in their relative capacity and efficiency in relation to these processes. For instance, it is known qualitatively how variations in nitrogen and phosphorus species and supply, stratification and light availability allow different cyanobacteria taxa to dominate (Carey et al. 2012), however the current suite of mechanistic models is severely challenged when presented with predicting the dominance of particular cyanobacterial taxa, due to limitations in the level of model process description and differentiation amongst the different taxa.

There are two main categories of models that have been used for simulating cyanobacteria: deterministic mathematical models and artificial neural network models (Guven and Howard 2006). The latter group includes a variety of modelling techniques such as genetic algorithms, Bayesian belief networks (Hamilton et al. 2007), fuzzy logic (Laanemets et al. 2006), and machine learning techniques that are designed to progressively adapt knowledge and statistical tools to an observed data set, and therefore to gradually reduce the error in predictions. In the case of Bayesian belief networks there is a judgment call developed through shared experience, in order to allocate a probability for the formation of blooms. Arhonditsis et al. (2007) provided an example of a coupled deterministic-Bayesian model in using a Bayesian calibration process to derive parameter values for a deterministic model. In some cases artificial neural networks have also been combined with deterministic models of lake hydrodynamics to capture the way in which cyanobacteria are affected by water mixing and transport processes (Ibelings et al. 2003). This chapter does not present details of artificial neural network models, but instead focuses on mathematical models with equations based around empirical or process representations of present knowledge of the ecology and physiology of cyanobacteria.

As part of our description of mathematical models of cyanobacteria we deal only briefly with hydrodynamic models. The reader is best advised to consult literature on physical limnology (Imberger and Patterson 1990; Imboden 2004), modelling of hydrodynamics (Hodges et al. 2000) and applications of coupled hydrodynamic-ecological models for cyanobacteria biomass prediction (Ibelings et al. 2003; Robson and Hamilton 2004; Hense and Burchard 2009) to understand the way these models represent hydrodynamics and are coupled with ecological models.

A major challenge for any model to simulate successfully the formation and magnitude of a bloom is to have a spatial scale suitable to encompass the variations in cyanobacterial concentrations within the waterbody. The difficulties in taking accurate measurements of cyanobacteria concentrations make this a particular challenge. Surface blooms are often most pronounced in calm conditions or on leeward shores under low wind speeds. Because wide variations can occur on space scales of a few millimetres (Fig. 6.1) to hundreds of metres (Fig. 6.2) and across whole lakes (Fig. 6.3), different sampling techniques can bias observations (Ahn et al. 2008). These depend, for instance, on the position and volume of sample collected or, in the case of *in vivo* fluorometry, the particular water mass sampled by the fluorometer. Furthermore, the usual quantitative measure of biomass is to collect a volume of water, either at a specific point in a waterbody or integrated with respect to depth, and then to make cell counts and possibly dimensional analyses of different species via light microscopy, to provide information on cell concentrations and biovolumes. Such a tedious and time-consuming process offers no opportunity to match the gridded nature of outputs from 3-D models and provides only limited capacity to validate 3-D coupled hydrodynamic-ecological model used to simulate cyanobacteria biomass. However, there are techniques that can markedly increase

sampling ability in a waterbody. For instance, flow cytometry can provide high throughput of water samples. Some fluorometers exploit the differences in spectral fluorescence associated with specific cyanobacterial pigments (phycocyanin or phycoerythrin), providing greater flexibility in interpreting the results than solvent-extracted chlorophyll *a* or chlorophyll fluorescence alone. Confidence in the results requires comprehensive calibration of the sensors.

Descriptions of mechanistically based phytoplankton models have been produced and a call has been made to create more unified modelling frameworks (Baumert and Petzoldt 2008; Mooji et al. 2010). This would help integrate efforts to develop comprehensive models. This approach has the added advantage of identifying the experimental and field studies needed to provide measurements to support the modelling, and perhaps lead to an international cooperative programme to provide the information (Mooji et al. 2010). It could also help to develop confidence in model predictions outside the dataset and ranges used for the initial model application and calibration.

6.2 Light Capture and Photosynthesis

Cyanobacteria carry out oxygenic photosynthesis in a manner similar to that found in the chloroplasts (Tandeau de Marsac and Houmard 1993). Although the chlorophyll *a* containing reaction centres of Photosystem I (PSI) and Photosystem II (PSII) are similar in cyanobacteria and microalgae, the major antennae, or light harvesting complexes

Fig. 6.1 Variability of cyanobacteria blooms in space: the small-scale: (all in New Zealand except for **c**.) (**a**) Lake Ohinewai, Waikato (A. Daniel); (**b**) Lake Rotorua (C. Zhang); (**c**) Lake Mendota, Wisconsin, USA (C. Spillman); (**d**) Lake Rotoehu, Rotorua (M. Landman); (**e**) Lake Ngaroto, Waikato (W. Powrie); (**f**) Lake Rotoroa, Waikato (W. Paul); (**g**) Lake Rotoiti, Rotorua (N. Miller); (**h**) Lake Ngaroto, Waikato (All photos with permission)

Fig 6.1 (continued)

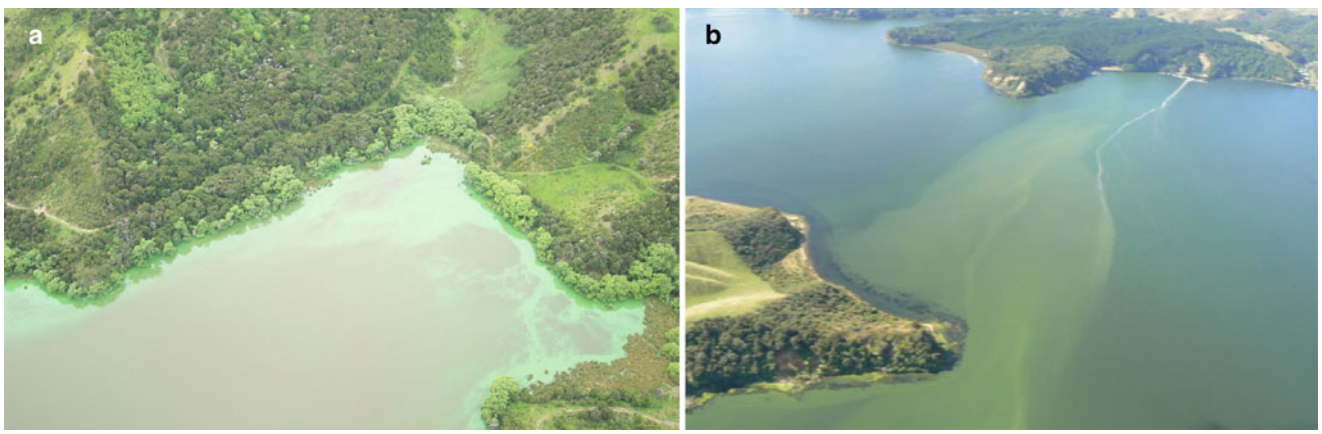


Fig. 6.2 Variability of New Zealand cyanobacteria blooms in space: aerial photos: (**a**) Lake Rotorua, Nelson; (**b**) Lake Rotoehu, Rotorua (Photos with permission: **a** = S.Wood; **b** = P. Scholes)

(LHC), that capture the incident photosynthetically active radiation (PAR) are quite different (Ormerod 1992; Grossman et al. 1995). In the micro-algae the antenna is integral to the thylakoid membrane and comprised largely of accessory chlorophylls. In cyanobacteria, chlorophyll *a*-protein complexes, photosynthetic reaction centres, carotenoids and the electron transport system are all contained within the thylakoids, but the major light-harvesting pigments, the

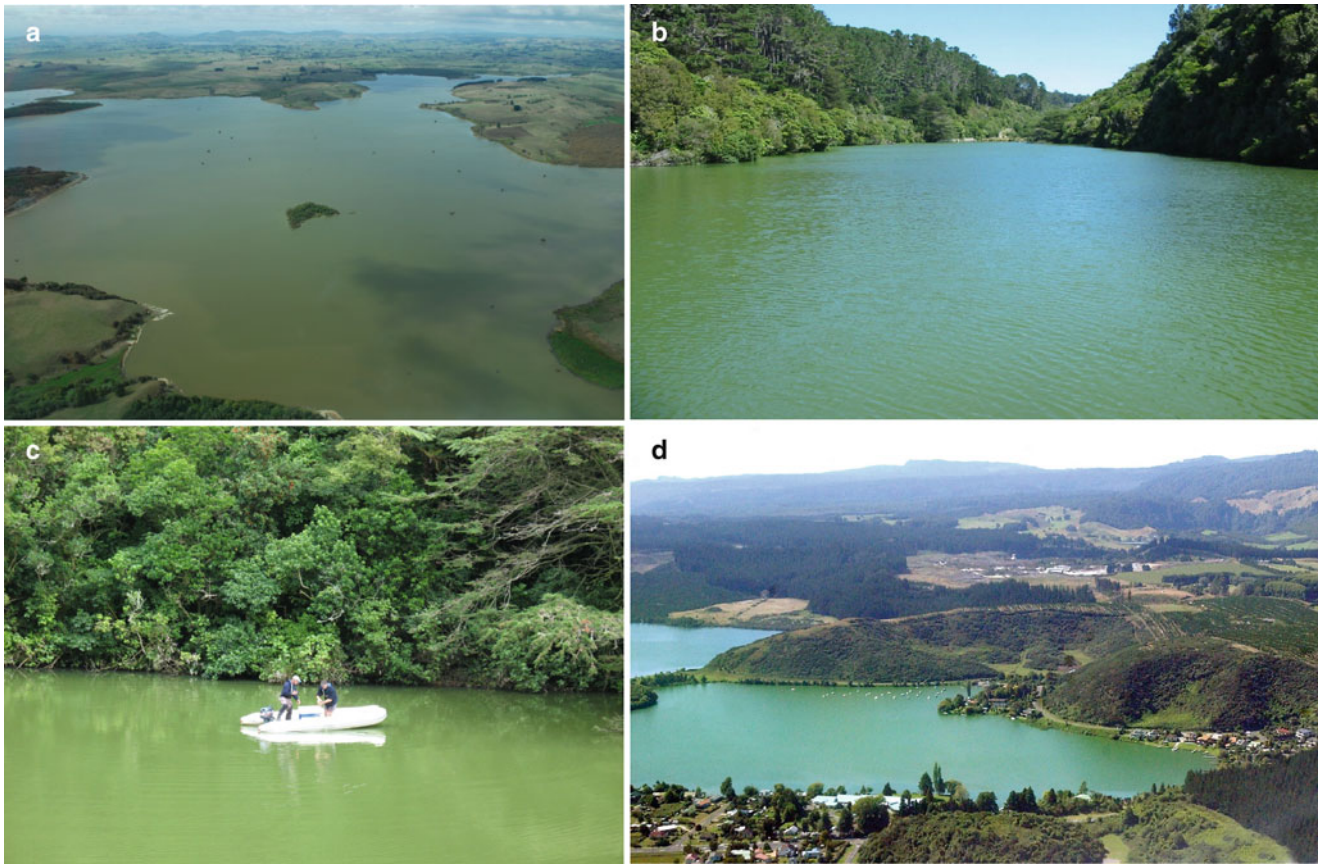


Fig. 6.3 Variability of New Zealand cyanobacteria blooms in space: the whole-lake scale: (**a**) Lake Whangape, Waikato; (**b** and **c**) Karori Reservoir, Wellington; (**d**) Okawa Bay of Lake Rotoiti, Rotorua (Photo of (**a**) by A. Zhang, with permission)

phycobiliproteins, occur within distinct light harvesting complexes called phycobilisomes (PBS) (Adams and Duggan 1999). The PBS form rows of hemidiscoidal structures attached to the surface of the thylakoids (Bryant 1991). Each PBS is made up of pigmented phycobiliproteins that form a series of rods connected to the PBS core. The phycobilin chromophores associated with the rods are phycocyanin (A_{max} 620 nm) and the red pigmented phycoerythrin (A_{max} 560 nm), and these, in conjunction with the PBS core of allophycocyanin (A_{max} 650 nm), determine the light absorption spectra. Whereas all PBS contain allophycocyanin and phycocyanin, only some contain phycoerythrin. It is in species with such PBS that alterations in pigment composition can have their greatest effect, changing blue-green cells to red as they adapt to different light spectra, the process of chromatic adaptation (Tandeau de Marsac and Houmard 1993; Stomp et al. 2004).

The unidirectional flow of energy in the PBS is from the rods, through the core and then via linker polypeptides and terminal electron acceptors to the photosystem reaction centres (Bailey and Grossman 2008). Electrons are generated from the light dependent oxidation of water by the oxygen evolving complex of PSII, and transported through the electron transport chain to PSI. Plastoquionone (PQ) is first reduced and then electrons are passed via cytochrome b6 f complex and plastocyanin to PSI, resulting in the reduction of ferredoxin and $NADP^+$ (Fig. 6.4). This generates energy and reductant for cellular metabolism and carbon fixation (Singh et al. 2009; Alric et al. 2010). Both of the connected photosystems harvest light energy, but of different wavelengths, as the phycobiliproteins in the PBS are largely associated with PSII, while chlorophylls, that have maximum absorbance wavelengths of 435 and 680 nm, are largely associated with PSI (Singh et al. 2009).

6.2.1 Spectral Influence

The underwater light climate varies in quantity and quality with depth. The intensity decreases exponentially as a result of absorption and scattering by particles and coloured compounds, and the selective removal of wavelengths causes shifts in the spectral distribution (Kirk 1983; Oliver 1990; Oliver and Ganf 2000; Stomp et al. 2007). Water absorbs strongly in the red, so that in marine systems and very clear

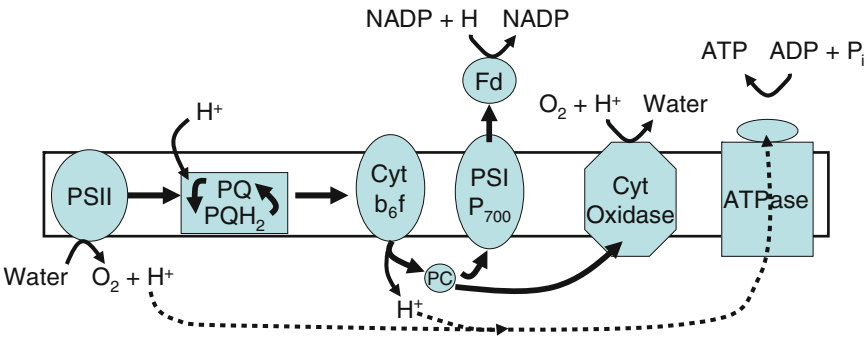


Fig. 6.4 Photosynthetic linear electron transfer pathways in the thylakoids. Photosystem II (PSII), Photosystem I (PSI), Primary electron donor of PSI (P_{700}), Cytochrome b_6f (Cyt b_6f), Plastocyanin (PC), Ferredoxin (Fd), Respiratory terminal oxidase (Cyt Oxidase), Proton pumping for ATP generation (ATPase)

inland waters where the majority of light attenuation is due to the water, the irradiance becomes dominated at depth by shorter wavelengths. In contrast, many inland and coastal waters contain sufficient dissolved organic compounds and suspended particles to absorb strongly in the blue, causing a shift towards longer wavelengths with depth (Kirk 1983; Oliver 1990; Oliver and Ganf 2000; Stomp et al. 2007). Phytoplankton also modifies the spectral distribution of light. For example, green algae use carotenoids and chlorophyll *a* and *b* to absorb light in the red and blue region, whereas they only weakly absorb green and yellow light (525–650 nm range) leaving an orange-green window of irradiance (Kirk 1983; Stomp et al. 2007). These wavelengths are suitable for absorption by the phycobiliproteins and as a result micro-algae may modify the spectral distribution of light at depth to the advantage of cyanobacteria.

The phycobiliproteins absorb PAR over a much wider range of wavelengths than the antennae of the micro-algae (Glazer et al. 1994), particularly in the region between the absorption bands of the accessory chlorophylls b and c, and the carotenoids. This difference between cyanobacteria and eukaryotic micro-algae is likely to be a distinct advantage where either the spectral quality of the underwater light is concentrated in these wavebands, or when there are substantial fluctuations in light quality over time. It might be expected from these comparisons of spectral absorption characteristics that the cyanobacteria would respond quite differently from micro-algae to changes in PAR. This differentiation has not been included in models to distinguish biomass of cyanobacteria from micro-algae in natural systems.

Huisman et al. (1999) measured the critical light intensity required to sustain continuous monocultures of two cyanobacteria (*Microcystis* and *Aphanizomenon*) and two eukaryotic micro-algae (*Chlorella* and *Scenedesmus*). In competition for light the species with the lowest critical light requirement should be the superior competitor. However, in mixed cultures the critical light intensities were different from those measured in monocultures and this altered the respective competitive abilities. This change was attributed to alterations in the spectral distribution of light in the mixed cultures where green algae shifted the light spectrum to green and yellow light that could be absorbed by the phycobilin pigments of the cyanobacteria (Huisman et al. 1999; Stomp et al. 2007). In the competition experiments *Chlorella* displaced all three other species, *Microcystis* displaced both *Aphanizomenon* and *Scenedesmus,* and *Aphanizomenon* only displaced *Scenedesmus.* These findings do not support suggestions that cyanobacteria are better adapted to low light conditions and hence better competitors for light than are green algae (Mur 1983; Richardson et al. 1983). However, in these experiments the light source was white fluorescent tubes and the spectral distribution was modified only by the phytoplankton. It is likely that the outcome of these competition experiments would be different if the water contained particulate and dissolved materials that substantially altered the light spectrum (Oliver 1990; Kirk and Oliver 1995; Ganf et al. 1989; Stomp et al. 2007).

Wyman and Fay (1986) grew eight strains of cyanobacteria under equivalent photon fluxes of red, green, blue and white light and found large differences in the cell concentrations of photosynthetic pigments and in growth rates. In red light there was a decline in chlorophyll and phycobiliprotein content, but all strains grew at a significantly faster growth rate than under an equivalent photon flux of white light. Under green light the pigment composition was similar to that under white light, but only the two phycoerythrin-rich strains (*Oscillatoria agardhii*=*Planktothrix agardhii* and *Gloeotrichia echinulata*) grew significantly faster, all other strains growing at 60–75% of their white light rate. In blue light the pigment composition was again similar to that under white light although a majority of the phycocyanin-rich strains showed a reduction in chlorophyll content. The strains rich in phycocyanin had growth rates <50% of their white light rate, while the phycoerythrin-rich strains, *O. agardhii* and *G. echinulata*, were able to maintain growth rates of 65% and 100% of their white light growth rates, respectively.

Comparison in continuous culture of phycoerythrin-rich species with closely related green species devoid of phycoerythrin has further demonstrated the influence on competition of pigmentation and spectral changes (Stomp et al. 2004; Oberhaus et al. 2007). Stomp et al. (2004) compared a red and green species of *Synechococcus* and showed that although

one would dominate under red light and the other in green light, under white light they could coexist. They then compared these two species with *Tolypothrix tenuis*, a marine filamentous cyanobacterium that can undergo complementary chromatic adaptation by adjusting the ratio of its phycocyanin to phycoerythrin in response to spectral changes. It was found that this species could coexist with either of the *Synecochoccus* species by producing complementary pigments to absorb the alternative colour to that used by the competitor. Under white light *Tolypothrix* coexisted with the green *Synecocchocus* by increasing its phycoerythrin content and turning red. In competition with the red *Synecochcoccus, Tolypothrix* was reduced to low numbers but could not be excluded from the culture because it turned green by increasing its phycocyanin content and utilised the unabsorbed light. Such adaptive pigmentation changes are beyond the process descriptions used in current mechanistic models of phytoplankton dynamics.

6.2.2 Photoacclimation, Photoadaptation and Photoinhibition

To maintain their light harvesting efficiency and to avoid increased risks of photodamage when exposed to high irradiances, phytoplankton have developed mechanisms for adjusting to alterations in the intensity, spectral distribution and periodicity of the PAR supply (Falkowski and La Roche 1991). Photoacclimation describes changes in the overall photosynthetic apparatus to cope with the "average" photon supply that results from prolonged exposure to relatively consistent light conditions. It involves the degradation and synthesis of components of the photosystem including light harvesting pigments, reaction centre components and dark cycle intermediates. Two major strategies are employed for adjusting to irradiance intensity. The first involves alterations in the size of the light harvesting antennae that serve the photosystems, and the second is a change in the total number of photosynthetic units (Wyman and Fay 1986; Falkowski and La Roche 1991). If photosynthesis becomes limited by the rate of delivery of light energy to the photosystems, as under low irradiance, then an increase in antenna size provides one means of increasing the photon supply. If the supply of photons from the antenna approaches the maximum turnover rate of the photosystem, then an increase in the number of photosynthetic units will increase the total supply of energy to the cell for photosynthesis and growth (Falkowski and La Roche 1991). In general lower irradiances result in increased light harvesting and a decrease in electron transport and carbon fixation while higher irradiances have the reverse effect (Bailey and Grossman 2008).

Planktonic cyanobacteria can experience large and rapid changes in their light environment, for example as a result of vertical mixing. Short term light fluctuations are dealt with by short-term, reversible changes to the photosynthetic apparatus that reduce light capture and enhance the release of captured energy as heat rather than through photochemistry. These processes are generally referred to as photoadaptation. Although light is required to drive photosynthesis, excessive light can cause the electron flow to exceed the capacity of the electron transport chain and the downstream utilization of reducing equivalents. This has photo-damaging effects on the photosystem and also results in an imbalance in the redox state of the cell, affecting many other cellular processes including the utilization of nutrients and the activity of metabolic pathways (Aurora et al. 2007; Bailey and Grossman 2008). In cyanobacteria, as in other photoautotrophs, there is a need to regulate the excitation of PSII and PSI in response to light intensity and spectral quality. This is to balance the delivery of energy and maximise the quantum yield of the light reactions, and also to reduce the probability of photodamage (Singh et al. 2009). A number of mechanisms are involved in photoadaptation in cyanobacteria.

High levels of light can cause photoinhibition to PSII reaction centres. At the core of the PSII complex is a heterodimer of two homologous polypeptides D1 and D2. The D1 protein has a more rapid turnover than any other thylakoid or chloroplastic protein and is part of a cycle of damage and repair that is essential for maintenance of PSII function under photoinhibitory conditions (Bouchard et al. 2006). Under illumination the D1 protein degrades and re-synthesizes to limit accumulation of photodamaged PS II reaction centres (Bouchard et al. 2006). When the rate of repair matches the rate of photodamage then photoinhibition is not apparent. However if under increasing light intensity the rate of damage exceeds that rate of repair then photoinhibition occurs (Andersen 1997; Heraud and Beardall 2000; Han et al. 2000; Oliver et al. 2003; Bailey and Grossman 2008). The rate of repair can be negatively influenced by nutrient limitation and UVB light so that the onset of photoinhibition is not just a function of the light intensity (Bouchard et al. 2006).

The PBS of cyanobacteria are highly mobile and can associate or disassociate with PSII or PSI resulting in a process of state-transitions. The state transitions redirect energy between the two photosystems when changing light conditions disturb the energy balance. In cyanobacteria there is more chlorophyll (Chl) associated with PSI than PSII. Under light conditions that predominantly excite Chl (e.g. blue PSI light), cyanobacteria maintain the balance between photosystems by decreasing PBS energy transfer to PSI and increasing energy transfer to PSII. This is a state 2 to state 1 transition.

The state transition is controlled by the redox poise of the plastoquinone pool (PQ) which develops an increased oxidation level under conditions where photons are being directed to PSI. Conversely, when excessive energy is being directed to PSII (red PSII light) and the PQ is more reduced, a State 1 to State 2 transition occurs as the PBS increases energy transfer to PSI (Bailey and Grossman 2008; Singh et al. 2009).

In cyanobacteria, there appear to be at least two photoprotective mechanisms that reduce the transfer of excitation energy from the light-harvesting complexes to the photosynthetic reaction centres through the active dissipation of absorbed energy. The first mechanism is through a blue light induced soluble orange carotenoid protein (OCP) that is widely distributed among cyanobacteria species (Kerfeld 2004). It mediates photoprotective energy dissipation through interaction with the phycobilisome core (Bailey and Grossman 2008; Latifi et al. 2009). The second energy dissipation mechanism is related to the high light-inducible proteins (HLIPs), also designated small CAB-like proteins (SCPs) that may play a critical role in photoprotection by associating with Photosystem II and dissipating excess absorbed energy (Latifi et al. 2009).

6.3 Photosynthesis and Cellular Metabolism

The growth of cyanobacteria depends on light absorption, temperature, and the uptake of a range of nutrients (Behrenfeld et al. 2008) and these cellular functions need to be regulated in a coordinated manner (Aurora et al. 2007). Mechanisms to maximise growth must optimise the allocation of energy (ATP) and reductant (NADPH) between the various cellular processes in order to coordinate resource supply and demand. Assimilation of atmospheric CO_2 is through the Calvin Cycle and requires three ATP per two NADPH molecules, i.e., a ratio of 9:6 to form its product glyceraldehyde-3-phosphate (GAP) (Behrenfeld et al. 2008; Alric et al. 2010). Strictly linear electron transport from PSII to PSI produces these molecules in the ratio of 9:7, which although matching closely the Calvin Cycle requirements means that there is no ATP or NADPH left for other cellular activities. Conversely, the demand for ATP by other metabolic pathways unbalances the ATP to NADPH ratio required for CO_2 fixation by the Calvin Cycle. Photosynthesis is the source of ATP and reductant, and cyanobacteria have evolved multiple methods for enhancing ATP generation to balance overall supply and demand, generally through the conversion of reductant.

At night the ATP to reductant ratio is balanced by respiratory electron transport which uses NADPH to form ATP. But in cyanobacteria this balance is more difficult to maintain during the daylight hours due to a unique characteristic of these organisms. In cyanobacteria, components of the electron transport chain that reside in the thylakoid membrane are shared by both respiratory and photosynthetic electron transport. Consequently, during daylight hours, the electron transport chain is less available for respiration due to photo-reduction of some of its components and this can be further exacerbated by a lack of $NADP^+$ substrate for reduction. Under these conditions PSII activity is curtailed as the oxidation rates of the electron chain intermediaries are reduced due to the respiratory electron transport, a situation that can cause PSII energy absorption to exceed photochemistry, leading to photoinhibition. In order to overcome the imbalances in ATP and NADPH that occur under these conditions cyanobacteria use several mechanisms to regulate the balance between linear and cyclic electron flows (Alric et al. 2010).

Linear electron flow produces reducing power and ATP, while cyclic electron flow around PSI only produces ATP (Fig. 6.5). Low cellular requirements for ATP to NADPH favour coordinated linear electron flow through PSII and PSI. In this case, any requirement for extra ATP is produced through thylakoid water to water cycles like the Mehler reaction and the respiratory terminal oxidase that generate ATP by pumping protons across the thylakoid membrane. In comparison, high ATP to NADPH demands are thought to decouple PSII and PSI to create two distinct pathways (Behrenfeld et al. 2008). One pathway involves cyclic electron flow around PSI in which ferredoxin, reduced by PSI,

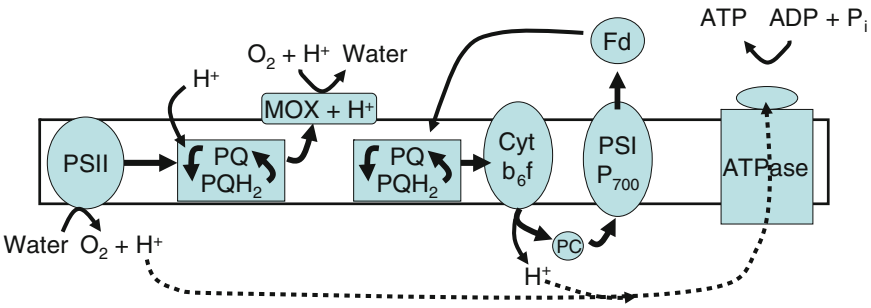


Fig. 6.5 Alternative photosynthetic electron transfer pathways that alter the balance of ATP and NADPH production (After Behrenfeld et al. 2008). Symbols additional to Fig. 6.1: Midstream terminal oxidases (MOX)

transfers electrons to the PQ pool which then transfers them to cyctochrome b6 f, plastocyanin and back to PSI to support ATP production. Secondly it has been suggested that cyclic electron flow around PSI is supported by 'midstream' terminal oxidases (MOXs) that augment ATP synthesis using electron flow from PSII, and this has the extra advantage of helping to reduce PSII photoinhibitory stress (Behrenfeld et al. 2008).

The requirement for a high or low ATP to NADPH ratio is determined by the metabolic activity of a cell so that balancing the relative supplies of energy and reductant involves not only adjustments in the photosystem, but also changes in cellular metabolism. Molecular and biochemical studies have provided insight to the homeostatic interactions between light capture, cellular metabolism and cell growth. Measurement of transcript abundance by DNA microarray shows that approximately 33% of genes in *Synechocystis* are regulated in response to changes in light quality (Singh et al. 2009). Analysis of these genes during changes in light quality that induced state transitions led to the identification of cellular processes that enable *Synechocystis* to circumvent reduced production of energy and reductant (Singh et al. 2009). That most cellular processes responded immediately to the imbalance in the excitation of reaction centres suggests that state transitions and adjustments of photosystem structure are not sufficient by themselves to reverse the effects of excitation imbalance (Singh et al. 2009).

The supply and demand for energy and reductant are determined by the extent to which ATP and NADPH from the photosystem are used in different metabolic pathways (Fig. 6.6). The synthesis of amino acids utilises GAP produced by photosynthesis, but the pathway releases carbon with a net production of ATP and NADPH to support other metabolic activities. So a significant proportion of energy and reductant from the photosystem can be channelled through this pathway when it is operating. In contrast, if GAP is used for carbohydrate synthesis and storage which does not release ATP and reductant, then only a fraction of the energy and reductant from the photosystem can be invested in this process as the rest is required to directly support other cellular metabolic functions (Behrenfeld et al. 2008).

The activity of these various metabolic pathways will also be influenced by nutrient availability. One example is nitrogen assimilation, where energetically NH_4^+ is preferential to NO_3^-, as the former requires far less energy for assimilation into glutamate (Coruzzi and Last 2000). The form of nitrogen used will influence the ATP:reductant demand ratio and alter the activity of different nitrogen assimilatory pathways (Behrenfeld et al. 2008).

Singh et al. (2009) found in *Synechocystis* that photodamage from high light decreased the output of products from the light reactions with the reduction in energy reducing CO_2 fixation. This in turn caused a reduction in N transport and assimilation. The reduced assimilation of C and N had consequences for various pathways, including those involved in transcription, translation, DNA replication, fatty acid metabolism, and biosynthesis of amino acid and nucleotides. They also found significant changes in response to light quality. Under PSII light *Synechocystis* was limited for NADPH and preferentially utilised ammonia over nitrate under these conditions. This was supported by experiments that showed the growth of *Synechocystis* increased significantly in the presence of ammonium under PSII light compared with white light (Singh et al. 2009). These connections mean that alterations in photosynthesis ramify through the cell, causing changes in stoichiometric composition and metabolic activity in a connected and coordinated way.

6.3.1 Modelling of Photosynthesis

Modelling of photosynthesis in cyanobacteria has utilised the same general functions that are applied to phytoplankton (Baklouti et al. 2006). In most cases empirical relationships have described the dependency of photosynthesis (P) on light in terms of the maximum rate of photosynthesis (P_{max}) and a function of irradiance (E) and a light saturation parameter (E_k):

$$P = P_{max} f\left(\frac{E}{E_k}\right)$$

Numerous different equations of this basic form have been fitted to experimental data (Table 1 in Baklouti et al. 2006). Some equations include additional parameters to describe photoinhibition, but these are also empirical formulations that provide "best fits" to the data (Jassby and Platt 1976). Even when describing the same data these various equations provide different estimates of the characteristics of the P-E curve including the initial slope (α), and the light saturation parameter (E_k) (MacIntyre et al. 2002; Baklouti et al. 2006). These parameters reflect the underlying biophysical and physiological processes that regulate photosynthesis (MacIntyre et al. 2002), but the standard empirical P-E data sets cannot explicitly and quantitatively describe these processes. As the equations usually describe empirical data from incubations under fixed or average conditions they are limited in their ability to describe photo-physiological adjustments or to predict changes in the P-E relationship under changing environmental conditions (Walsby et al. 2001).

Progress in modelling these interactions has come from advances in biochemical and molecular understanding, and also improvements in the measurement of cellular photophys-

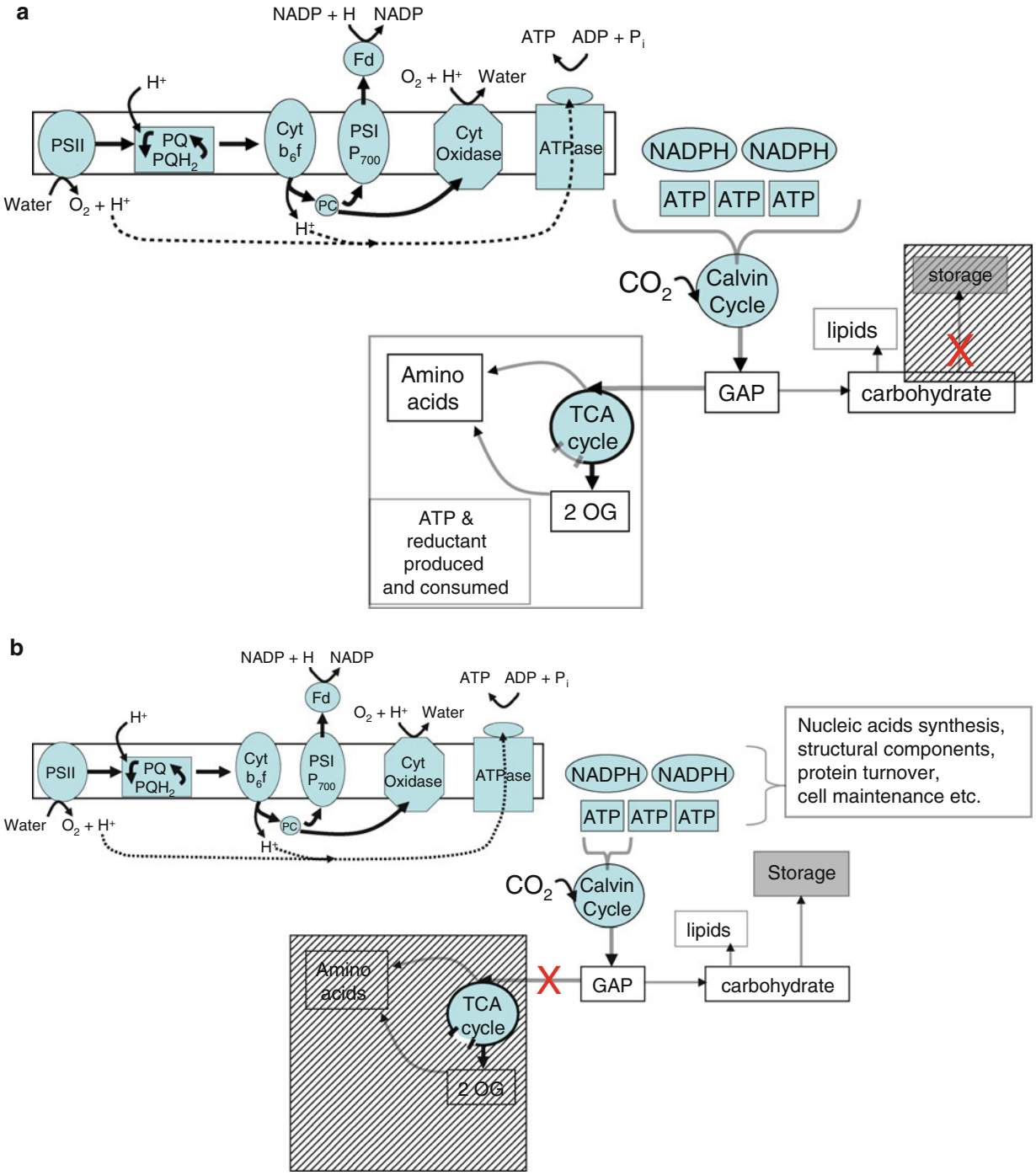


Fig. 6.6 Dominant metabolic pathways during (**a**) amino acid synthesis and (**b**) carbohydrate synthesis (After Behrenfeld et al. 2008). Symbols additional to Fig. 6.4: Glyceraldehyde-3-phosphate (GAP), Tricarboxylic acid cycle (TCA), 2-oxoglutarate (2-OG)

iology, especially through active fluorometry (Schreiber et al. 1995; Kolber and Falkowski 1993; Oliver and Whittington 1998). These techniques have led to process based models that include photophysiological characteristics such as the absorption cross-section of the photosynthetic units, electron transfer rates, and photo-acclimation rates (Falkowski and Kolber 1993; Han et al. 2000; Oliver et al. 2003). An equation for photosynthesis based on fluorescence measurements was derived by Falkowski and Kolber (1993):

$$P = E\sigma q_p \frac{\Phi'}{\Phi_m} \phi_e \eta_{PSII}$$

Here P is the rate of photosynthesis (mol O_2 mol chla^{-1} h^{-1}), E is the irradiance intensity (mol photons m^{-2} h^{-1}), σ is the effective absorption cross-section of the antennae which determines the photons actually reaching the PSII reaction centre, (m^2 mol quanta^{-1}), Φ'/Φ_m is the fraction of functional reaction centres, ϕ_e the mol of oxygen evolved per photon processed by the reaction centres, and η_{PSII} the number of functional PSII reaction centres per mole of chlorophyll-a (Falkowski and Kolber 1993). The effective absorption cross-section has a spectral dependence and this can be built into the equation though in general a spectrally integrated absorption coefficient is used. It will be important to improve the modelling of spectral effects to better estimate photosynthesis under changing light conditions and to improve the modelling of competition between species (Sathyendranath et al. 2007). Photoinhibition has also been included in some models of this type (Han et al. 2000; Oliver et al. 2003).

6.4 Nutrients

Nutrient limitation of cyanobacteria elicits both general and specific responses. The general responses are the result of the stresses imposed by arrested anabolism while specific responses are acclimation processes to particular nutrient limitations. Specific responses lead to modification of metabolic and physiological activities to compensate for the restriction (Schwarz and Forchhammer 2005). Nutrient limitation is frequently considered the cause of reduced growth of phytoplankton in natural environments and an important driver of competition that determines community composition. Considerable attention has been focused on nutrient limitation by phosphorus versus that by nitrogen, and the ratios of these nutrients have been used at whole lake scales to predict the relative abundance of cyanobacteria amongst lake phytoplankton (Sect. 6.8).

General responses of phytoplankton to nutrient limitation include: carbohydrate accumulation, a reduction in the cell-specific quantum yield of photosynthesis (Turpin 1991), a reduction in the cellular content of the limiting nutrient (Droop 1973; Riegman and Mur 1984) and an increase in the specific uptake rate of the limiting nutrient (Gotham and Rhee 1981; Riegman and Mur 1984; Kromkamp 1987). Nutrient limitation stimulates the storage of non-limiting nutrients as a result of their relative excess compared to the reduced requirements of the cell. Nutrient storage is a valuable attribute, enabling cells to utilise pools of nutrients that are spatially and temporally separated so that growth is maintained during periods of nutrient scarcity. Phytoplankton must match their energy input to their cellular metabolic capacity, and as nutrient limitation slows down the reoxidation of the final electron acceptors, electron transfer activity must be down-regulated and cellular metabolic pathways adjusted to minimise possible photodamage and to maximise energy efficiency.

6.4.1 Phosphorus

Under phosphorus limiting conditions cellular phosphorus concentrations decline as the phosphorus limited growth rate declines, while phosphorus uptake potential increases. As a consequence, a pulse of phosphorus delivered to P-limited cells results in substantial formation of polyphosphate reserves, the polyphosphate 'overplus' phenomenon, with cellular P levels able to exceed those under steady state maximum growth rates (Allen 1984; Riegman and Mur 1984). Most phytoplankton can store surplus phosphorus, usually in the form of polyphosphate (PP), and these reserves can be sufficient for several cell doublings. There do not seem to be any consistent phylogenetic differences between microalgae and cyanobacteria in the range of values for phosphate uptake, and the kinetics appear to be species specific (Healey 1982; Tilman et al. 1982; Kromkamp 1987; Reynolds 1993).

Despite its importance in eutrophication and its role in cellular energy dynamics surprisingly little detailed molecular or biochemical data are available on the cellular metabolism of P in cyanobacteria compared to that for N. This probably reflects the dominance of marine phytoplankton research in this area and the focus on N limitation in marine systems. This deficiency needs to be addressed.

6.4.2 Nitrogen

Cyanobacteria are able to utilise a range of N sources including ammonium, nitrate, nitrite, urea, and in some cases arginine or glutamine (Flores and Herrero 2005). Certain groups can fix atmospheric N_2, a trait that distinctly separates them from the autotrophic eukaryotes. The order of preference amongst the commonly available inorganic sources is $NH_4^+ > NO_3^- > N_2$ (Tandeau de Marsac and Houmard 1993). Energetically, NH_4^+ is preferential to NO_3^- as the former requires only one NAD(P)H or ferredoxin and one ATP for assimilation into glutamate, while the latter requires nine reductants and one ATP (Coruzzi and Last 2000). During the day, these substrates are provided directly from photosynthesis and thus the form of nitrogen used influences both the ATP:reductant demand ratio and the photosynthesis:carbon fixation ratio (Behrenfeld et al. 2008). In nutrient replete cells carbohydrate stores are small and assimilation of combined inorganic nitrogen is strongly dependent on recent CO_2 fixation (Guerrero and Lara 1987; Turpin 1991). Under these conditions reductions in photosynthesis, for example due to darkness or CO_2 deprivation, will reduce nitrogen assimilation.

In contrast, nitrogen limited cells accumulate carbohydrate reserves that can be utilised through glycolysis as a source of energy and carbon skeletons for nitrogen assimilation both in the dark and the light (Guerrero and Lara 1987; Turpin 1991; Garcia-Gonzalez et al. 1992; Tapia et al. 1996).

The uptake of nitrate/nitrite, urea and most amino acids usually involves permeases, while the uptake of ammonium involves secondary transporters. Within the cell, nitrate is converted to nitrite by nitrate reductase and then nitrite is converted to ammonium by nitrite reductase. Arginine is catabolized by a combination of the urea cycle and arginase pathway, while urea is degraded by a Ni^{2+}-dependent urease; both these pathways also produce ammonium (Flores and Herrero 2005).

Ammonium, derived from direct uptake or produced from conversion of other nitrogen sources, is incorporated into carbon skeletons through the glutamine synthetase–glutamate synthase cycle (GS-GOGAT). When NH_4^+, the preferred N source is available, its presence represses the genes encoding permeases and enzymes for the assimilation of alternative nitrogen sources and cyanobacteria and micro-algae do not assimilate these other forms of nitrogen. This process is known as 'nitrogen control' (Turpin 1991; Ochoa de Alda et al. 1996; Flores and Herrero 2005). Induction of ammonium inhibition of nitrate uptake requires that ammonium has first been metabolised by the initial glutamine synthetase step of the GS-GOGAT system (Herrero et al. 2001). The subsequent glutamate synthase step requires carbon skeletons and these are supplied as 2-oxo-glutarate, which is the final compound in the oxidative TCA cycle in cyanobacteria as they lack 2-oxo-glutarate dehydrogenase. Ammonium depletion limits GS-GOGAT activity and results in the accumulation of 2-oxo-glutarate, while large supplies of ammonium or restrictions to photosynthesis may lead to reduced concentrations of 2-oxo-glutarate (Schwarz and Forchhammer 2005). This metabolic arrangement where 2-oxo-glutarate consumption through GOGAT is directly linked to ammonium assimilation (Fig. 6.7), integrates the N and C assimilatory pathways of cyanobacteria and provides the basis of an important regulatory system where 2-oxo-glutarate is an indicator of the C to N ratio of the cells (Schwarz and Forchhammer 2005; Flores and Herrero 2005).

Two proteins responsive to 2-oxo-glutarate are the signal transducer protein PII and the nitrogen-control transcription factor NtcA. The protein PII binds both ATP and 2-oxo-glutarate in a synergistic manner to alter its reactivity (Forchhammer 2004). PII can exist in four different forms, non-phosphorylated and increasingly phosphorylated at one, two or three of its subunits. In high concentrations of 2-oxo-glutarate the PII is liganded to both ATP and 2-oxo-glutarate and in this form it can be phosphorylated while in reduced concentrations of 2-oxo-glutarate the PII is bound only to ATP and can be dephosphorylated. In ammonium-grown cells, where the high demand for carbon skeletons lowers the 2-oxo-glutarate concentration, PII is non-phosphorylated. The level of PII phosphorylation is increased in nitrate-grown cultures and higher again in nitrogen-starved cells, but this is not a simple monotonic progression as the level of phosphorylation increases when cells are incubated in the presence of CO_2 enriched air (Tandeau de Marsac et al. 2001; Schwarz and Forchhammer 2005; Kolodny et al. 2006). As a result, the degree of phosphorylation of PII is a function of the N and C supply of the cell such that phosphorylation is inversely correlated with nitrogen availability, but directly correlated with carbon availability (Herrero et al. 2001). The degree of phosphorylation is also expected to be influenced by the energy status of the cell as determined by the balance of ATP and reductant.

Depending on its conformational state, the PII protein interacts with various target proteins, most of which regulate the nitrogen assimilatory pathways (Forchhammer 2008). This includes nitrate/nitrite permeases, bicarbonate uptake and gene expression through the global nitrogen control factor NtcA (Forchhammer 2008). The non-phosphorylated form inhibits nitrate/nitrite uptake while in the phosphorylated form this inhibition is relieved (Tandeau de Marsac et al. 2001).

The nitrogen-control transcription factor NtcA is the major mediator of global nitrogen control at the level of gene expression (Luque et al. 1994; Schwarz and Forchhammer 2005). The activity of NtcA is subject to metabolic regulation, such that under conditions of nitrogen excess (low 2-oxoglutarate levels), the NtcA protein is inactive (Luque et al. 2004) while increased 2-oxoglutarate concentrations and the absence of ammonium lead to stimulation of NtcA activity (Vázquez-Bermúdez et al. 2003; Schwarz and Forchhammer 2005). NtcA regulates the expression of genes encoding for the assimilation of ammonium, or alternative nitrogen sources when cells are incubated under limiting concentrations of ammonium but with adequate carbon to give the cells a high C to N ratio. When NtcA is activated there is a high expression of the *glnB* gene that produces the PII protein. The PII protein that is synthesised, ligands with 2-oxoglutarate which is in high concentrations and ATP. This negatively controls the affinity uptake of bicarbonate and releases inhibition of the nitrate/nitrite transporters. This research has suggested a complex interaction between PII and NtcA where phosphorylated PII activates NtcA and in turn the activated NtcA augments the levels of NtcA and PII as well as stimulating PII phosphorylation (Schwarz and Forchhammer 2005) (Fig. 6.8).

The extent of the link between PII and 2-oxo-glutarate was also found to affect the metabolism of carbon. Whatever its phosphorylated state, PII was found to negatively influence the high affinity uptake system for bicarbonate under high inorganic carbon (*Ci*) conditions when 2-oxo-glutarate presence is elevated (Tandeau de Marsac et al. 2001). This sug-

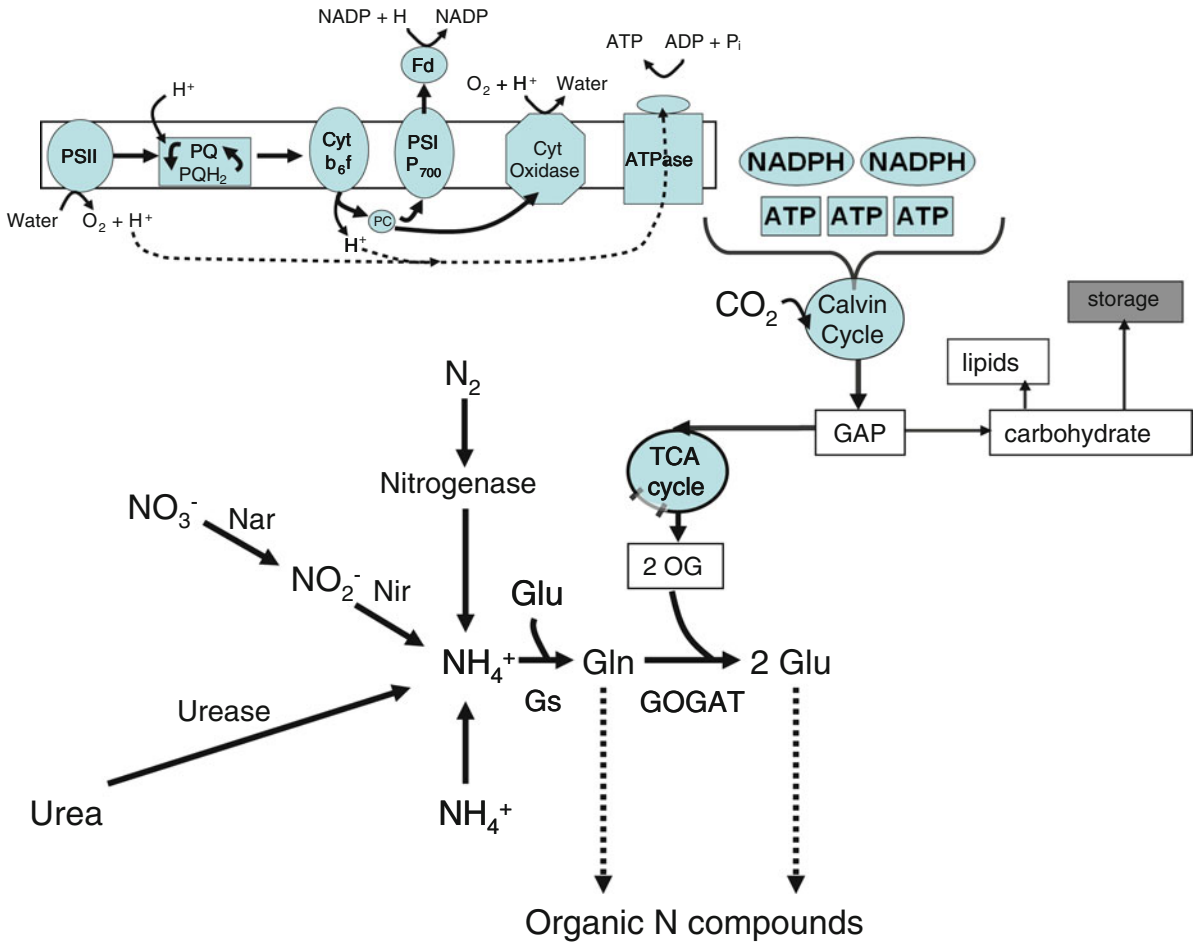


Fig. 6.7 Major nitrogen assimilation pathways in cyanobacteria. Additional symbols: Nitrate reductase (Nar), Nitrite reductase (Nir), Glutamine synthetase (GS), Glutamate synthase (GOGAT), Glutamine (Glm), Glutamate (Glu)

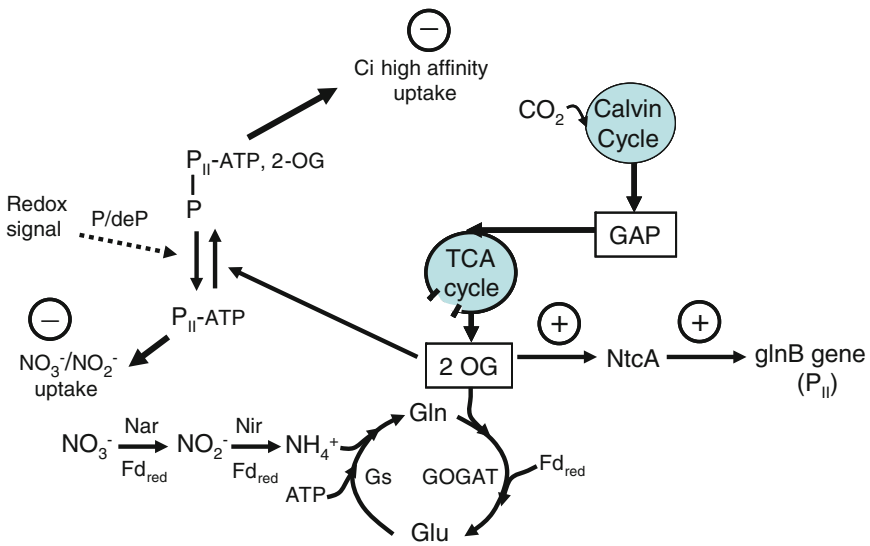


Fig. 6.8 Interactions between nitrogen and carbon metabolism in cyanobacteria with positive and negative influences indicated by plus and minus (Redrawn from Tandeau de Marsac et al. 2001). Additional symbols: Nitrogen-control transcription factor (NtcA), gene encoding the PII protein (glnB), Reduced ferredoxin (Fd_{red}), Inorganic carbon (C_i)

gests that the extent to which PII is linked with 2-oxo-glutarate has opposite effects on the nitrate/nitrite and the high affinity *Ci* uptake system (Fig. 6.8). Such a system would allow an intracellular N/C balance to be maintained in response to changes in the nitrogen and/or carbon regimes of the cells (Tandeau de Marsac et al. 2001).

The key enzyme of the arginine synthesis pathway, N acetylglutamate kinase (NAGK), was also identified to

be a receptor for PII (Heinrich et al. 2004; Burillo et al. 2004; Schwarz and Forchhammer 2005) influencing the degree of cellular nitrogen storage (Heinrich et al. 2004; Maheswaran et al. 2006). Complex formation and catalytic activation of NAGK by PII was shown to depend both on the phosphorylation state of PII and on its binding of effector molecules (Sect. 6.6).

Nitrogen assimilation influences the rate of CO_2 fixation, the fate of newly fixed carbon, and the level of carbohydrate reserves (Guerrero and Lara 1987; Turpin 1991; Garcia-Gonzalez et al. 1992; Tapia et al. 1996) with major effects expected on cell growth, cell turgor pressure and cell density (Sect. 6.11).

6.5 Nitrogen Fixation

When other sources of inorganic nitrogen have become limiting, cyanobacteria equipped with the nitrogenase enzyme complex can utilise N_2 gas through the process of nitrogen fixation. In contrast there are no known micro-algae that can fix molecular nitrogen. This provides the nitrogen fixing cyanobacteria with a major advantage when sources of combined inorganic nitrogen are depleted from the water. However, the fixation of nitrogen requires a substantial amount of energy, using 16 ATP molecules as well as eight electrons to produce two molecules of ammonium and one molecule of H_2, the H_2 being respired under oxygenic conditions (Stal 2009). In addition to this the production and maintenance of the nitrogenase enzyme is a significant energy cost as it comprises about 10% of total cellular protein.

Cyanobacteria are the only nitrogen fixing organisms that also produce oxygen through photosynthesis, and this creates difficulties as the nitrogenase complex is inhibited by oxygen. This has resulted in an array of morphological, metabolic and behavioural adaptations within the different cyanobacteria to protect nitrogenase activity and enable nitrogen fixation (Berman-Frank et al. 2003; Stal 2009). Separation of nitrogen fixation from photosynthesis is achieved either by spatial segregation, temporal separation, or a mixture of both, and to some extent reflects the habitat to which the cyanobacteria are adapted (Berman-Frank et al. 2003). These structural and metabolic patterns were classified into four groups by Berman-Frank et al. (2003) as shown in Fig. 6.9. These range from colony types with nitrogenase distributed evenly across all cells to those with specialised cells called heterocysts that are specifically differentiated for nitrogen fixation. Marked differences occur between taxa in response to diel changes in light flux and there will indeed be many subtle variations of these idealised profiles, particularly given the variability of light flux induced by position in the water column and temporal variation in the light field at sub-daily (e.g. from clouds, wind-waves), daily and seasonal time scales.

In the two groups of cyanobacteria that have nitrogenase activity evenly distributed across cells (eg. *Plectonema*, *Lyngbya*), nitrogen fixation is separated in time from photosynthesis and occurs largely at night, supported by the catabolism of C reserves that were synthesized during the previous light period. In the first of these two groups nitrogen fixation only occurs under microaerobic conditions. This protects nitrogenase from oxygen while still supplying sufficient oxygen to support the production of ATP and reductant through respiration. In the second group nitrogen fixation can occur in oxygenic conditions with peak nitrogenase activity coinciding with high respiration rates 12 h after the peak of photosynthetic activity. In this case the regular pattern continues even under continuous light suggesting circadian control (Berman-Frank et al. 2003).

In the filamentous, non-heterocystous, marine cyanobacterium *Trichodesmium,* there is a complex interaction between spatial and temporal segregation of the photosynthetic, respiratory and nitrogen fixing activities. Measurements of photosynthesis and nitrogen fixation show a temporal separation during the photoperiod with photosynthesis peaking in the morning and afternoon offset by ca. 6 h from the peak of nitrogen fixation. During the night nitrogenase is inactivated and turned over under the control of a circadian clock (Berman-Frank et al. 2007). Activation of nitrogenase in cells is linked to a reduction in gross photosynthesis and increased respiratory activity that results in a negative net production of oxygen which creates the conditions suitable for nitrogenase activity. There is no evidence that any cells undergo unidirectional differentiation as occurs in the formation of heterocysts within some filamentous cyanobacteria (Stal 2009). Active photosynthetic components are found in all cells but it is uncertain whether all cells have the capacity for nitrogen fixation, or whether nitrogenase occurs in all cells or in a fraction of cells arranged consecutively along the trichome (Berman-Frank et al. 2007; Stal 2009). Whatever the arrangement, nitrogen fixation involves cells switching between photosynthesis and nitrogen activity to give a combined temporal and spatial response. This mode of nitrogen fixation has not been reported for freshwater species.

In some filamentous cyanobacteria, N_2 fixation takes place in specialized cells called heterocysts that differentiate irreversibly from vegetative cells 12–20 h after combined nitrogen sources are removed from the medium. This occurs when ambient nitrate concentrations are depleted to ca. 0.3–1.6 μmol L^{-1} but there is considerable variation amongst different cyanobacteria in the duration of persistence of heterocysts when nitrate concentrations return to levels exceeding this range (Holl and Montoya 2005; Agawin et al. 2007). Maximum in situ abundance of heterocysts amongst vegetative cells therefore appears to be closely aligned with very low concentrations of dissolved inorganic nitrogen but has been shown by Wood et al. (2010) to precede

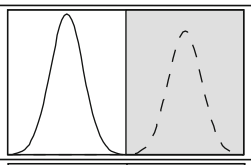
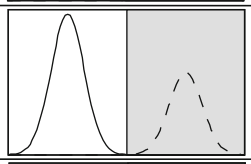
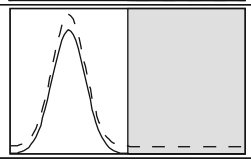
Class	Morphology: nitrogenase	Examples	Nitrogen fixation
Microaerobic Type I	Filaments: Evenly distributed	*Plectonema*	
Aerobic Type II	Filaments or unicells: Evenly distributed	*Cyanothece* *Lyngbya*	
Some cellular differentiation Type III	Filaments: Specialised or localised cells	*Trichodesmium* *Katagnymene*	
Cellular differentiation Type IV	Filaments: Heterocysts	*Anabaena* *Nostoc*	

Fig. 6.9 Classes of cyanobacteria derived by Berman-Frank et al. (2003), based on morphology, location of nitrogenase activity and behavioural adaptations enabling nitrogen fixation. The graphs show idealised representation of day (unshaded) and night (shaded) periods and rates of photosynthesis (solid lines) and nitrogen fixation (dashed lines)

the peak in vegetative cell biomass often associated with blooms of *Anabaena planktonica*. Heterocyst differentiation is strictly controlled by the nitrogen transcription factor NtcA linking differentiation to nitrogen deficiency (Flores and Herrero 2005). The heterocysts have a modified metabolism to maintain a microaerobic environment for nitrogenase expression while providing ATP and electrons for nitrogenase function. The heterocysts do not contain PSII and so do not generate O_2, but consequently cannot fix CO_2. Instead they rely on a supply of fixed carbon from adjacent cells for respiratory substrate to provide reducing equivalents, vegetative cells in return receiving fixed nitrogen from the heterocysts. The heterocysts generate ATP in the light by cyclic electron flow around PSI but the extent of this depends on the light conditions (Berman-Frank et al. 2007; Stal 2009). In the dark, respiration is the only source of energy.

Heterocystous cyanobacteria fix N_2 during the day in parallel with photosynthesis in the vegetative cells. In some cases nitrogen fixation continues into the night and this continued activity is supported by the transfer of fixed carbon from stores accumulated in the vegetative cells. However, at non-saturating light intensities and in the dark, nitrogenase activity can become energy limited (Stal 2009). In order to optimise energy generation the O_2 influx into the heterocysts needs to be as large as possible, but without exceeding the respiration capacity, so that anoxic conditions can be maintained. As the cyanobacteria occur in dynamic environments they need to respond quickly to changes in conditions in order to perform optimally. The heterocysts have a thick cell wall that carries extra glycolipid and polysaccharide layers to reduce gas permeability (Walsby 1985), but this influences both O_2 and N_2 fluxes and is unlikely to change quickly (Stal 2009). It has been suggested (Walsby 2007) that the diffusion properties of the cell wall may slowly respond to growth conditions, and that dynamic gas exchange is controlled by the pores that connect the heterocyst with neighbouring vegetative cells.

N_2 fixation confers a significant competitive advantage for cyanobacteria but its contribution to nitrogen inputs at a system scale may vary. *Nodularia spumigena* contributed more than 81% of the annual N inputs to a high altitude hyposaline lake in the United States (Horne and Galat 1985). Schindler et al. (2008) generalised that N inputs from fixation could overcome deficiencies of N in lake phytoplankton assemblages and advocated a phosphorus control paradigm to manage cyanobacterial blooms. Scott and McCarthy (2010) subsequently refuted the capacity for N fixation to provide for N replete conditions in Lake 227, Canada, when they showed that phytoplankton biomass decreased in response to a reduction in total nitrogen concentration. Translating conceptual models of N-fixation (e.g., Oliver and Ganf 2000) into numerical formulations is challenged by the temporal and spatial variability of N-fixation. Levine and Lewis (1987) developed a model of N-fixation by heterocystous

cyanobacteria for Lake Valencia, Venezuela, to estimate the importance of fixation for lake N inputs. Their model is closely linked to rates of photosynthesis to reflect a baseline rate of N fixation at night which is ramped up with photosynthesis during the day:

$$N_{fh} = N_s(1-e^{-a})e^{-b} + D$$

where N_s is the maximum rate of N_2 fixation per heterocyst in the absence of light, $a = \alpha E N_s^{-1}$ and $b = \beta E N_s^{-1}$ where I is light intensity, α is a parameter for the slope of the rising limb of the light response curve and β is a photoinhibition parameter, and D is the rate of N_2 fixation per heterocyst in the dark. Values for N_s given by Levine and Lewis (1987) are up to a maximum of nearly 0.04 µmol $(10^6$ heterocyst$)^{-1}$ h^{-1} which compares with a value of 7×10^{-13} g N heterocyst^{-1} h^{-1} (or 0.05 µmol $(10^6$ heterocyst$)^{-1}$ h^{-1}) given by Howarth et al. (1993). For more general models of ecosystem processes that include cyanobacteria, Howarth et al. (1999) present the first known model that differentiates N fixing from non-N fixing cyanobacteria. They use a multiplier term (N_{mult}) to enhance growth rates under low DIN concentrations:

$$N_{mult} = \frac{\text{DIN}}{\text{DIN} + Km_N}$$

where Km_N is the half-saturation constant for uptake of DIN, taken to be 20 µM by Howarth et al. (1999) (see also Zevenboom and Mur 1978). The corresponding N uptake from fixation is assumed to meet all of the N demand for growth and there is no DIN assimilation from the water column. Another multiplier term (Mo_{mult}) is used to account for the availability of molybdenum (Mo):

$$Mo_{mult} = 4.2\frac{\text{Mo}}{\text{Mo} + Km_{Mo}\left(1+\frac{\text{S}}{Ki}\right)}$$

where Km_{Mo} is the half-saturation constant for uptake of Mo, Ki is an inhibition constant for the effect of sulphate (S) on Mo uptake and 4.2 is a scaling factor where Mo_{mult} is assigned a value of one for concentrations of Mo and S encountered in freshwaters. Few ecosystem models deal with potential for micronutrient limitation or explicitly include micronutrients as state variables, but limitation by iron or other trace elements such as molybdenum may potentially be alleviated by periods when these micronutrients are present in high concentrations (Donnelly et al. 1997), even in freshwater systems where their chelation by salts is greatly reduced compared with estuarine or marine systems.

The N derived from fixation is assumed in the Howarth et al. (1999) model to be in direct proportion to P assimilated, at a molar ratio of 15:1, to maintain constant nitrogen content per cell of 3.3×10^{-12} mol N.

An alternative approach to modelling N_2 fixation has been used by Stal and Walsby (1998) in which the rate of fixation N_{fc} is expressed as a chlorophyll-specific value:

$$N_{fc} = N_{sc}(1-e^{-c}) + D + d$$

where N_{sc} is the maximum rate of N fixation at light saturation, D is the rate of N fixation in darkness, $c = -\alpha E / N_{sc}$ and d (negative) is a phtoinhibition parameter ($d = \beta E^2$). This formulation is similar to that used by Levine and Lewis (1987) except for an expression for the effects of photosynthesis on N fixation. Stal and Walsby (1998) used values for N_{sc} of 6.5 µmol N_2 (mg chl-a)$^{-1}$ h^{-1} and D of 1.48 µmol N_2 (mg chl-a)$^{-1}$ h^{-1}. Other values of N_{sc} include up to 6.48 µmol N_2 (mg chl a)$^{-1}$ h^{-1} (Ohlendiek et al. 2000), and 4.59, 1.34–4.64 and 6.5 for *Aphanizomenon* sp., *Nodularia* spp. and a Baltic Sea sample (Stal and Walsby 2000). A conversion between these values and those given above by Levine and Lewis (1987) and Howarth et al. (1993) would require several assumptions including the ratio of heterocysts amongst the vegetative cells, considered by Levine and Lewis (1987) to be rarely more than 1:10, as a chlorophyll specific mass per vegetative cell.

A different approach is used by Hense and Beckmann (2006), recognising a threefold increase in energy requirements for growth using fixed N, to reflect the utilisation of at least 16 ATP molecules per N_2 molecule reduced. According to their model the heterocystous form of a four-stage cyanobacterial life cycle occurs when there is low DIN, but adequate energy reserves in the cell. The fixation rate is given by

$$N_{fx} = \omega_{fx}\sigma_E C$$

where N_{fx} represents the accumulation rate of organic nitrogen in the cell as a result of fixation, ω_{fx} is the growth rate defined in terms of change in nitrogen, σ_E is a limitation function for the internal energy store, represented by:

$$\sigma_E = 1 - \left(\frac{En}{En_{\max}}\right)^n$$

Where En and En_{max} are equivalent to internal energy storages and n is an assigned exponent. In this model an internal energy quota regulates partitioning between growth (when the internal N quota is large) and uptake (as the quota decreases). Hense and Burchard (2009) analysed the sensitivity of their four-compartment cyanobacterial life cycle model, which included akinetes, recently germinated vegetative cells, and heterocystous and non-heterocystous stages,

against simpler alternative models. They showed a 30% variation in simulated annual N_2 fixation rates for the dominant diazotroph, *Nodularia spumigena*, in the Baltic Sea. The model of Hense and Burchard (2009) is one of the few to include a benthic stage (akinetes), but in general mechanistic modelling of overwintering (e.g. in *Microcystis* – see Chap. 7) has either been ignored or is implicitly included in the parameterisation of planktonic processes.

6.6 Nitrogen Storage

Cyanobacteria have the capacity to store significant amounts of N in excess of their immediate requirements. The two storage components are phycocyanin, a phycobiliprotein, and cyanophycin, a co-polymer of aspartate and arginine. Whereas phycocyanin is also a major pigment component of the light harvesting antenna, the primary function of cyanophycin is to store nitrogen and perhaps energy (Allen 1984; Kolodny et al. 2006). Cyanophycin and phycocyanin are both at low concentrations in N-limited cells. Cyanophycin occurs in low concentrations during balanced growth of non-diazotrophic cyanobacteria, but it can occur in large concentrations in diazotrophic cyanobacteria and acts as a transient store for newly fixed nitrogen in heterocysts. Cyanophycin accumulates in cyanobacteria when they are grown under all unbalanced nutrient conditions except nitrogen starvation, and it is used as a nitrogen source before other sources during N-starvation (Kolodny et al. 2006). In a manner reminiscent of the P luxury storage phenomenon, cyanophycin accumulates on the addition of a useable nitrogen source to N-limited cells (Simon 1987). Cyanophycin synthesis peaks sometime after the addition of N and then decreases to the levels found in typical exponentially growing cells (Mackerras et al. 1990a, b; Kolodny et al. 2006). In response to nitrogen starvation the cyanophycin granules are first degraded, followed by cell bleaching due to degradation of components of the phycobilisome including phycocyanin (Tandeau de Marsac and Houmard 1993). Nitrogen stores are also utilised when low-light cells are shifted to high light, with cyanophycin and phycocyanin both decreasing.

Control of cyanophycin formation is through the enzyme for arginine biosynthesis, N-acetylglutamate kinase (NAGK). NAGK is linked to cell N status through the signalling protein PII and its interaction with 2-oxo-glutarate and ATP/ADP (Llácer et al. 2008). NAGK activity is strongly enhanced by complex formation with the non-phosphorylated form of PII that is produced when N is abundant and 2-oxo-glutarate levels are reduced. This binding also releases NAGK from arginine feedback inhibition. Under these conditions high levels of arginine build up and cyanophycin synthetase can produce cyanophycin stores (Flores and Herrero 2005; Maheswaran et al. 2006; Llácer et al. 2008). When nitrogen is scarce, 2-oxo-glutarate accumulates and binds to PII in the presence of ATP and this promotes dissociation of the PII-NAGK complex decreasing activity and enabling arginine inhibition. Under these conditions arginine levels are below those required for cyanophycin formation by cyanophycin synthetase (Maheswaran et al. 2006).

6.6.1 Nitrogen Starvation

Prolonged nitrogen starvation causes a series of cellular changes in cyanobacteria. Firstly, a rapid degradation of phycobilisomes occurs before other proteins and pigments are utilized. The cells finally become almost completely depigmented and enter a survival mode (Schwarz and Forchhammer 2005). These changes are reversible, and following the addition of a combined nitrogen source the cells return to vegetative growth within a few days. Sauer et al. (2001) analysed *Synechococcus* cells that were kept in nitrogen depleted conditions for more than 2 months. The cells retained residual PSI and PSII activity at about 0.1% of growing cells. Using protein labelling techniques it was shown that the apparently dormant cells turned over proteins associated with photosynthesis and redox homeostatis, but not proteins involved in the translational machinery.

6.7 Cellular Elemental Stoichiometry

The Redfield ratio of 106C:16N:1P is the average cellular mole ratio of carbon, nitrogen and phosphorus originally derived from measurements of marine phytoplankton. It has been used widely in aquatic studies to determine the nutrient status of systems, to link biogeochemical models of these elements, and to estimate cellular production (Geider and La Roche 2002). For example, the N:P ratio of 16:1 is frequently used to identify whether systems are more likely to be phosphorus or nitrogen limited assuming that N:P < 16 is indicative of N limited conditions. The reliability of these assumptions has been questioned frequently in view of the large elemental fluctuations that have been observed in phytoplankton (Hecky et al. 1993). Geider and La Roche (2002) reviewed the data on C:N:P ratios in cultures grown under nutrient replete, nutrient limited and optimal growth conditions. They also estimated elemental ratios based on the likely biochemical composition of physiologically competent cells in order to constrain the elemental ranges measured in cultures. They concluded that the laboratory data do not support the idea of a biochemically fixed C:N:P ratio in the proportions defined as the Redfield ratio. They found the N:P mole ratio in cultures to range from <5 to >100. Even under optimal growth conditions the range was from 5 to 19 N:P with most measurements below the Redfield ratio.

Biochemical calculations suggested likely N:P compositions between 15 and 30. The transition between N and P limitation was estimated from limited data to be more likely in the range of 20–50 N:P, substantially higher than the Redfield value of 16.

In contrast, the C:N mole ratio, although still variable, was much more constrained, especially in optimally growing, nutrient-replete cultures where it was on average close to the Redfield ratio of 6.6. These observations support findings from integrated studies of nitrogen assimilation and carbon fixation suggesting that a range of mechanisms appear to be targeted at maintaining the cellular C:N ratio within narrow bounds during growth.

The variability in ratios, particularly of N:P and C:P, reflects the known physiological plasticity of phytoplankton and also the phenomenon of luxury consumption where non-limiting nutrients can form intra-cellular stores. Cyanobacteria store nitrogen as cyanophycin when phosphorus and other growth requirements are limiting, and store phosphorus as polyphosphate when nitrogen and other growth requirements are limiting. Phosphorus storage as polyphosphate markedly reduces the N:P and C:P ratios so broadening the range of possible values. In contrast, nitrogen storage as cyanophycin has a N:C mass ratio of ca. 0.5, which is not greatly different from the typical values observed in cells, so it has a lesser effect on overall cellular N:C stoichiometry. Carbohydrate accumulation under nutrient-limited conditions will also influence the carbon ratios.

6.8 Whole Lake Nutrient Influences

The classical work of a number of authors in the 1960s (e.g. Sakamoto 1966; Vollenweider 1968) led to the recognition of the importance of increased phosphorus loadings in the process of eutrophication of lakes. These studies on phosphorus, and later studies on the interaction between nitrogen and phosphorus (e.g. Smith 1983), led to ecological research focused on the manipulation of whole lakes or portions of them to explore the responses of phytoplankton abundance and community structure to nutrient conditions (Schindler 1971; Lund and Reynolds 1982). Many of these studies have focused on total nutrients rather than bioavailable forms. The bioavailable forms are commonly considered to be in dissolved inorganic form but in some mesotrophic lakes dissolved organic nutrients (e.g. for *Aphanizomenon flos-aquae* in Lake Kinneret) can also constitute an important component of the cellular nutrient uptake, particularly in the presence of alkaline phosphatise to mobilise organic phosphorus (Berman 1997).

On the basis of the Redfield ratio, and the fact that many of the bloom-forming cyanobacteria can fix nitrogen, it was suggested that cyanobacteria should dominate in waters with low N:P ratios (Smith 1983; Downing et al. 2001). This was supported by an analysis of 20 lakes largely from northern Europe (Smith 1983), but the conclusion was questioned because some samples were not considered to be independent or representative of the lakes. Further analysis of these data by Trimbee and Prepas (1987) suggested that the individual nutrient concentrations, either total phosphorus or total nitrogen, provided more reliable estimates of average cyanobacterial dominance than their ratio. Similarly, Downing et al. (2001) carefully collected and prepared information from published reports to create a dataset of 269 observations from 99 lakes around the world. This was used to investigate further the influence of nutrient stoichiometry (i.e. N:P ratio) on cyanobacterial dominance, compared to nutrient concentrations (N or P), or algal biomass. They concluded that the risk of water quality degradation by cyanobacteria blooms was more strongly correlated with variation in total P, total N, or standing algal biomass than the ratio of N:P. They suggested that correlations between N:P and cyanobacterial dominance were the result of a strong negative correlation between nutrient enrichment and the N:P ratio due to nutrient sources often being depleted in N relative to P (Downing et al. 2001). These findings are in accord with doubts about the reliability of the Redfield ratio for drawing conclusions about system scale responses.

These outcomes are important to water quality management as the notion that a TN:TP molar ratio above c. 15 indicates a switch from a high to a low potential for cyanobacterial dominance has been misinterpreted with major implications for catchment scale nutrient management. Schindler et al. (2008) were concerned that many studies in lakes and estuaries were still concluding that N must be controlled as well as, or instead of, P to reduce eutrophication. They described a 37-year whole lake experiment testing the effect of N reduction and concluded that to reduce eutrophication the focus of management must be on decreasing P inputs. Downing et al. (2001) concluded from their analyses that the most potentially useful of the relationships was that based on total P, which predicts phytoplankton biomass and clearly discriminates lakes dominated by cyanobacteria. They suggest that the risk of dominance by cyanobacteria is only 0–10% between 0 and 30 μg L^{-1} total P, rising abruptly to about 40% between 30 and 70 μg L^{-1}, and reaching an asymptote at around 80% near 100 μg L^{-1}. This does not mean that management of N enrichment should be ignored, but that the principle focus should be on phosphorus control (Carpenter 2008).

The relationship between eutrophication and an increased biomass of gas-vacuolate cyanobacteria has been attributed to the requirement that sufficient nutrients need to be available, either in the water or from internal recycling, when physical conditions eventually become suitable to provide the cyanobacteria with a competitive advantage. In temperate systems, if nutrients are depleted by phytoplankton growth during

spring and early summer, then the bloom-forming cyanobacteria are faced with depauperate nutrient conditions when the physical environment is most suitable for their growth. Similar arguments can be made for tropical waters, but on cycles driven by daily to weekly meteorological events as well as seasonal conditions (Lewis 1978a, b). It is under these conditions that resting stages such as akinetes or an overwintering phase (*Microcystis*; Chap. 7) can be highly advantageous and allow for rapid recolonisation of the water column when conditions become favourable again.

6.9 Modelling Nutrient-Dependent Growth Rates

Flynn (2003b) identified three modelling strategies to describe nutrient uptake by phytoplankton. In order of increasing complexity these are the Monod, quota and mechanistic modelling approaches. The Monod model makes growth rate a direct function of the external nutrient concentration. The quota model makes the internal nutrient content (the quota) the controlling factor, although the quota is itself a function of the external nutrient supply. The more complex mechanistic models, with feedback processes and perhaps multiple internal pools, seek to simulate more closely biochemical reality (Baird and Emsley 1999; Flynn 2003b).

The Monod model relates the nutrient specific steady-state growth rate (μ_i) to the external concentration of the nutrient (x_i) and a half saturation constant for growth (k_i) according to a rectangular hyperbolic function:

$$\mu_i = \frac{x_i}{x_i + k_i}$$

When multiple nutrients are being considered a threshold approach is generally used with the nutrient giving the slowest growth rate considered to be controlling. This is often determined according to the Redfield ratio. Once a nutrient is selected as limiting then others are ignored, or assumed to respond in a fixed proportion which is again frequently set by the Redfield ratio. This type of modelling approach cannot account for alterations in internal nutrient reserves or the changes in nutrient ratios that occur when cells modify their responses to changing environmental conditions.

In comparison the quota model (Droop 1968) relates growth to the internal concentration of a nutrient, which means that growth can continue at the expense of previously accumulated nutrient stores. In the quota model the nutrients can be described in relation to cellular carbon levels so that the model can also simulate carbon biomass or it can be formulated on a cellular basis. One form of the model is that described by Caperon and Meyer (1972):

$$\mu_c = \mu_{mX} \frac{X_c - X_{co}}{X_c - X_{co} + k_{qX}}$$

Where μ_c is the carbon-specific growth rate, X_c the carbon based nutrient quota (nutrient:C ratio), X_{c0} the minimum carbon based cell quota of the nutrient at which growth is zero, k_{qX} is a curve fitting constant similar to the half saturation constant, and μ_{mX} the maximum theoretical growth rate when using nutrient X. As with the Monod equation, the usual method to address multiple nutrient influences has been the threshold approach, although multiplicative interactions have been investigated. Neither the multiplicative or threshold formulations have a firm mechanistic basis.

Both the Monod and quota models are only valid under conditions of balanced growth, when the specific rates of change of all cellular components are equal. This occurs when nutrient uptake, light harvesting and carbon fixation are directly coupled giving a fixed cell stoichiometry. Consequently these models are restrictive when modelling multi-nutrient interactions, or light nutrient interactions. The models cannot describe the uncoupled rates of nutrient assimilation or carbon fixation that occur in response to rapid fluctuations in environmental conditions (Geider et al. 1998). As the elemental composition of phytoplankton does not necessarily conform to the Redfield ratio (Geider and La Roche 2002), it is no longer acceptable to assume that knowledge of one element enables the concentration of other elements to be calculated (Baklouti et al. 2006). As a result it has become necessary to develop multi-nutrient models and to explore the possibilities of multi-nutrient limitation and co-limitation.

6.10 Modelling Metabolic Interactions and Cellular Growth

Whereas many models disaggregate the functions of photosynthesis, nutrient uptake and cellular composition, it is apparent that these are closely linked, and a major improvement in modelling has come from the consideration of integrated energy and material fluxes through the cellular machinery (Shuter 1979; Behrenfeld et al. 2008; Singh et al. 2008). This considers that various cellular mechanisms are involved in trying to maximise cellular growth through linked homeostatic interactions (Behrenfeld et al. 2008). It is suggested that the pigment concentration is adjusted to optimise delivery of ATP and reductant under the available light conditions, but modified in response to the overall cellular demand created by the particular growth environment. The required supply of ATP and reductant is influenced by the balance between the demand for carbon fixation to provide new carbon components in metabolic pathways, the necessity to support other cellular metabolic activities directly from

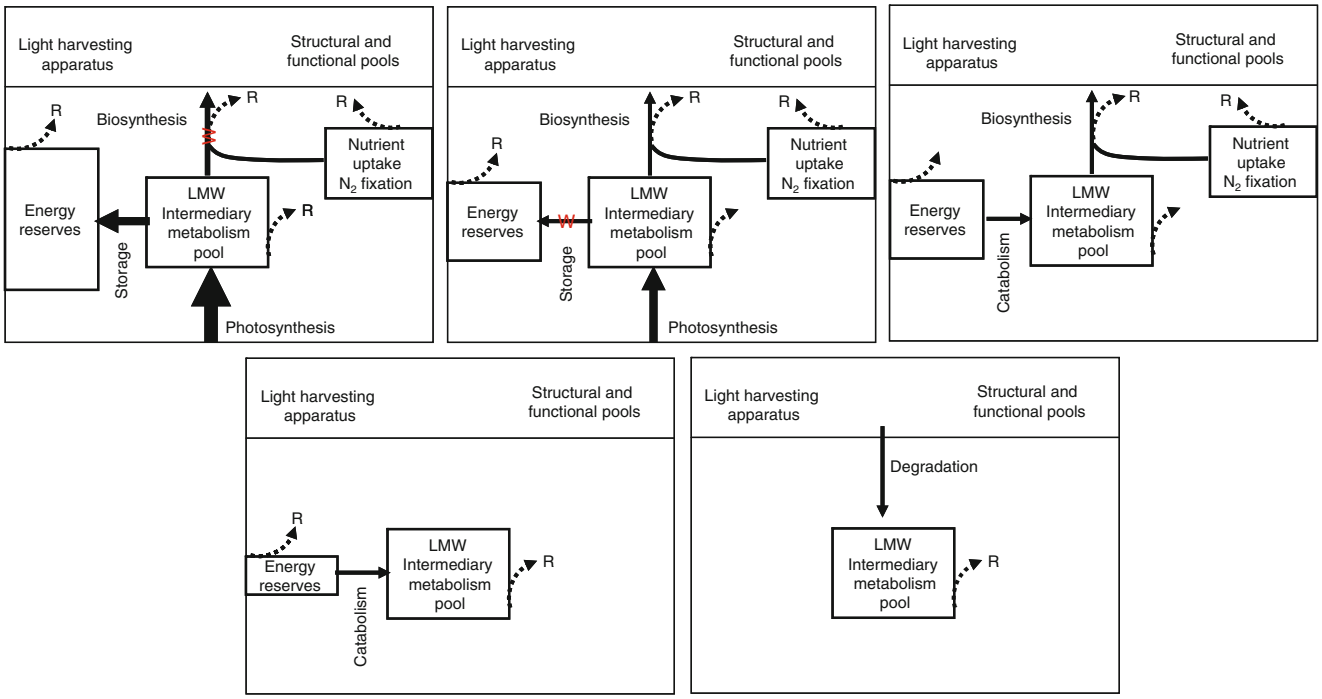


Fig. 6.10 Major cellular components and fluxes that have been included in compartment models to describe fluctuations in cellular composition during unbalanced growth. The series of figures depict the effects of decreasing light intensity followed by increasing periods of dark. Respiration (R), Low molecular weight carbohydrates (LMW), resistance to pathway flux (W)

the photosystem, and the requirement for storage products. These alternatives are influenced by alterations in the dominating metabolic pathways and their ATP and reductant requirements. There is a constant adjustment to minimise differences in the production and demand ratios for ATP and reductant (Behrenfeld et al. 2008). Such physiologically based models that describe cell function can include feedback mechanisms that more closely reflect reality.

If the variability in cell stoichiometry is considered to be largely due to stored nutrients while the structural components remain relatively constant (Klausmeier et al. 2004; Baklouti et al. 2006), then distinguishing between cellular pools is important. This concept identifies the need for more physiological modelling to describe these components pools. This approach has been explored in several models (Shuter 1979; Geider et al. 1996; Rabouille et al. 2006; Ross and Geider 2009) and appears to be promising (Fig. 6.10).

In the various cellular compartment models the components have been identified in different ways depending on the purpose of the model. Rabouille et al. (2006) in developing a physiological growth model to describe nitrogen fixation in *Trichodesmium* recognised four compartments. Cellular carbon was divided into three pools; the low molecular weight carbohydrates from photosynthesis, the internal carbon reserves, and the structural biomass. In this formulation, the low molecular weight pool has a short turnover time and is the intermediate for all metabolic processes. It is consumed either to build carbon reserves, or for incorporation into structural components, or to supply energy through respiration for maintenance and biosynthesis. The low molecular weight pool is supplied by photosynthesis or by catabolism of carbohydrate reserves. The presence of a carbohydrate reserve enables a cell to continue biosynthesis for some time into the night using carbon fixed during the day. In this model cellular nitrogen was also described in terms of an internal storage pool that grouped glutamine and the nitrogen reserve components cyanophycin and phycobiliproteins and was fed by the uptake of external nitrogen. This provided the intracellular nitrogen pool available for the synthesis of structural biomass through biosynthesis with carbon (Fig. 6.8). By incorporating two separate reserves of carbon and nitrogen with unbalanced dynamics the fluctuations in cellular composition could be investigated and the effects of these on nitrogen fixation assessed (Rabouille et al. 2006).

Ross and Geider (2009) used a similar but simpler approach to model photosynthesis and photoacclimation under nutrient replete conditions. Their model contains only two carbon pools, a functional and a reserve pool. Their model alludes to, but does not describe, the dynamics of a low molecular weight component. The Ross and Geider (2009) model has nitrogen in the functional pool occurring at a fixed ratio with carbon and alterations in the cellular N:C ratio are attributed

to the accumulation or catabolism of carbohydrate reserves. This model also incorporates consideration of the light harvesting apparatus so that photosynthesis and photoacclimation can be included.

These cellular compartment models are in their infancy, but provide a framework for integrating the new molecular and biochemical information that more clearly describes the interactions in cellular photochemistry and metabolism that operate to maximise cell growth while minimising damage. The models have further attributes that will be useful in considering the growth and ecology of cyanobacteria as well as phytoplankton generally. They are likely to be of particular value to the modelling of planktonic cyanobacteria because of: (i) the roles that different forms of carbohydrate play in buoyancy regulation and the interactions with nutrients and light (Oliver 1990; Oliver and Ganf 2000); (ii) the cellular differentiation, often associated with nitrogen fixation, that requires individual cell conditions to be modelled within the context of colonial responses (Adams and Duggan 1999); (iii) the complex lifestyles of different groups that can include resting stages that overwinter in the sediments (Hense and Beckmann 2006). As they are cell based the physiological functions can be connected with cell size and geometry, a powerful link that has been used in empirical models simulating light-nutrient interactions (Reynolds et al. 2001). This approach can be taken further with a more theoretical consideration of the geometrically dependent physical processes influencing cellular functions as in the biomechanical approach described in Baird and Emsley (1999).

6.11 Buoyancy and Its Regulation

The success of gas-vacuolate cyanobacteria is often attributed to their ability to regulate buoyancy in response to changing environmental conditions (Reynolds and Walsby 1975; Ganf and Oliver 1982; van Rijn and Shilo 1985; Walsby 1987, 1994; Reynolds et al. 1987). Buoyancy and its regulation confers a competitive advantage for cyanobacteria over the sedimentation losses of many other negatively buoyant phytoplankton (Reynolds 1984), increased access to irradiance (Humphries and Lyne 1988; Walsby et al. 1997), and an ability to bridge the vertical separation that develops in stratified waters between higher availability of nutrients at depth and greater illumination in surface layers (Ganf and Oliver 1982).

6.11.1 Gas Vacuole Structure

Gas vacuoles are comprised of numerous cylinders known as gas vesicles (Bowen and Jensen 1965). These structures are hollow but rigid, proteinaceous cylinders capped at either end by a cone. Specific gas vacuole genes encode for the various proteins used in their synthesis (Walsby 1994; Oliver 1994). Gas vesicles are permeable to gas and therefore contain air of composition similar to that of the surrounding liquid, but the air is not required to maintain the hollow space as the gas vesicle walls are rigid. However, the gas vesicle wall is impermeable to water due to the presence of hydrophobic amino acids on the inner side.

Gas vesicles are ordered into gas vacuoles to occupy minimal space and provide maximum buoyancy. The cylindrical gas vesicles are stacked in hexagonal arrays with the cones interdigitating. In *Anabaena* where the gas vesicle density is 120 kg m^{-3}, if the intervening space (15%) is filled with water at a density of 1,000 kg m^{-3}, then the overall gas vacuole density will be 252 kg m^{-3}, one-fourth the density of water and an efficient mechanism to provide lift (Walsby 1994).

6.11.2 Pressures Acting on Gas Vesicles

The wall of gas vesicles is subjected to hydrostatic and turgor pressures. If the combination of these pressures exceeds the strength of the wall then the gas vesicle will collapse and the cell will lose buoyancy (Walsby 1971). Hydrostatic pressure increases at a rate of 0.1 MPa per 10 m water depth. Episodic deep mixing can potentially entrain cells to greater depths where, in order to retain buoyancy, these cells will need to have gas vesicle walls with sufficient strength to withstand the increase in hydrostatic pressure. Increases in turgor pressure result from increased levels of soluble organic intermediates from photosynthesis (Grant and Walsby 1977), coupled with light-dependent uptake of potassium salts (Allison and Walsby 1981).

Turgor pressure can be measured as the difference in the critical applied pressure (ρ_c) required to collapse vesicles of cells suspended in 0.5 M sucrose which removes the turgor pressure, compared with the pressure (ρ_a) required for turgid cells suspended in filtered lake water or culture medium: $\rho_t = \rho_c - \rho_a$. Turgor pressures can vary from 0 to 0.5 MPa in different organisms (Walsby 1994) and increases can lead to gas vesicle collapse.

The collapse of gas vesicles is dependent on their strength, which may be described by the pressure exerted on them (Walsby 1994):

$$\rho_c = 275(r)^{-1.67}$$

where ρ_c is the critical collapse-pressure (MPa) and r is the cylinder radius (nm). The balance between efficient provision of buoyancy and capacity to withstand hydrostatic pressures appears to be the basis for variations in r amongst species (Hayes and Walsby 1986; Walsby and Bleything 1988; Brookes et al. 1994).

6.11.3 Buoyancy Regulation

Buoyancy regulation occurs in cyanobacteria in response to different environmental stimuli and in turn, allows cyanobacteria to regulate these environmental gradients through vertical movement in the water column (Reynolds and Walsby 1975; Walsby and Reynolds 1980; Reynolds 1987; Oliver 1994; Walsby 1994; Wallace and Hamilton 1999). Buoyancy can be adjusted through changes in the extent of gas vacuolation or by the balance of cellular constituents of different density such as carbohydrates (density, $\rho \sim 1{,}600$ kg m^{-3}) and proteins ($\rho \sim 1{,}300$ kg m^{-3}). Metabolic processes that rapidly alter the size of carbohydrate reserves will be of major significance to buoyancy regulating cyanobacteria as these reserves provide ballast to offset the lift due to gas vesicles. The accumulation of cellular carbohydrate reserves through photosynthesis, or depletion through respiration and conversion to less dense constituents, is one of the most important short-term influences on buoyancy. Changes in other cellular constituents of varying density, including storage materials such as polyphosphate granules, can also affect buoyancy (Romans et al. 1994).

Gas vacuolation can play an important role in buoyancy regulation through changing the extent to which gas vesicles counteract the density of other cellular constituents. Gas vacuolation may be altered through increased turgor pressure acting to collapse gas vesicles, or through the diluting effect of growth and cellular replication (Oliver 1994; Walsby 1994).

N limitation can affect gas vesicle assembly by restricting the production of essential proteins resulting in a loss of buoyancy (Klemer et al. 1982; Chu et al. 2007). The results of laboratory studies (Turpin 1991; Garcia-Gonzalez et al. 1992; Tapia et al. 1996) suggest that where cyanobacteria move between the well illuminated, nutrient-poor surface layers and nutrient-rich aphotic zones, the source of available nitrogen at depth can have a significant effect on rates of buoyancy regulation. For example, the increased availability of ammonium common in the hypolimnion of stratified lakes may cause a reduction in the carbohydrate reserves of sedimenting cyanobacteria leading to a quicker reversal of cell buoyancy and a reduction in the extent of vertical migration. Detailed studies of this interaction are required.

The interplay of limitation by light and macronutrients strongly regulates buoyancy regulation (Konopka et al. 1987b). For example, the accumulation of carbohydrate as polysaccharide occurs in response to excess photosynthate production (Gibson 1978; Kromkamp and Mur 1984), leading to sinking. By contrast carbon limitation leading to depletion of carbohydrate reserves may lead to periods of buoyancy though prolonged carbon limitation and will result in a reduction in buoyancy with reductions in gas vesicle synthesis. This balance between carbohydrate accumulation and incorporation into other compounds is regulated by light availability and cellular nutrient status (Healey 1978).

When light and nutrient supply rates are balanced then the relative growth rate given by μ/μ_{max} is high even if the specific growth rate is low. Under these conditions carbohydrate use is optimised for growth and storage is reduced. If growth is restricted by a limiting nutrient then energy capture exceeds utilisation and carbohydrate is stored when the light supply exceeds that required to achieve the maximum relative growth rate for the nutrient limited growth rate, RGR_{max}. Konopka and Schnur (1980) obtained carbohydrate to protein ratios four to seven times higher in cultures limited by nitrogen, phosphorus or sulphur, than in non-limited cultures or those limited by carbon. In general, when major nutrients such as phosphorus or nitrogen limit cell growth, buoyancy decreases because carbohydrate accumulates, resulting in greater diurnal changes in buoyancy compared with nutrient replete cells (Chu et al. 2007). However associated turgor pressure increases can also collapse gas vesicles, especially if there is an accompanying rise in hydrostatic pressure due to sedimentation of cells to greater depths (Reynolds and Walsby 1975; Klemer 1978, 1991; Walsby 1987). The degree of gas vacuolation may also be reduced through molecular controls on gas vesicle production but this is species-specific. If nutrient limitation greatly depresses growth rate, then carbohydrate accumulation and buoyancy loss can occur even at low light intensities. Under severe and sustained nitrogen limitation reduced gas vesicle synthesis results in reduced buoyancy.

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

When nutrient replete cells are exposed to irradiances close to saturating then they will grow at rates close to maximum and cellular elemental composition will approach the Redfield ratio (106C:16N:1P by atoms) (Hecky and Kilham 1988; Hecky et al. 1993). Under these conditions *Aphanizomenon flos-aquae* is generally negatively or neutrally buoyant (Konopka et al. 1987a; Kromkamp et al. 1988) whereas

Microcystis aeruginosa is positively buoyant (Kromkamp et al. 1988; Chu et al. 2007).

When nutrient sufficient cells are exposed to irradiance, carbohydrate stores increase with irradiance up to a maximum when photosynthesis is saturated. The enlarged carbohydrate store increases cell density, but molecular controls may decrease the rate of gas vesicle synthesis in some species. Gas vesicle collapse can occur from the resulting buildup of turgor pressure under light exposure, although this most often occurs synergistically with other buoyancy reducing mechanisms under normal light:dark cycles (Oliver and Walsby 1984; Kromkamp et al. 1986).

One of the first models to consider buoyancy was developed by Okada and Aiba (1983a, b). This model related density to rates of change of cell turgor pressure as follows:

$$\frac{dP}{dt} = \alpha(P_{max} - P)Q_{O_2} - \beta(P - P_{min})$$

where P is the turgor pressure of the cells (kN m^{-2}), P_{max} and P_{min} are the maximum and minimum assigned values of the turgor pressure, respectively, Q_{O2} is the photosynthetic activity of the cells (ml O_2 (g cell)$^{-1}$ h^{-1}), and α and β are proportionality constants for the respective rates of increase (g cell (ml O_2)$^{-1}$) or decrease (h^{-1}) in turgor pressure. Okada and Aiba (1983a) give values of P_{max} and P_{min} of 760 and 360 kN m^{-2}, respectively. While turgor pressure generated by osmotically active photosynthates or potassium ions is strong enough to collapse gas vesicles in some species (Allison and Walsby 1981; Oliver and Walsby 1984) cell density is altered most rapidly by the accumulation or loss of dense polysaccharides during photosynthesis and respiration (Kromkamp and Mur 1984; Utkilen et al. 1985; Kromkamp and Walsby 1990).

6.11.4 Sinking or Floating

The change in cell, colony or filament polysaccharide concentration alters cell density which then changes the rate of sinking or floating. In quiescent waters, i.e. under laminar flow conditions, the sinking or floating rate of phytoplankton can be calculated from the Stokes equation which describes sinking as the balance between the downward gravitational force from excess density and the buoyant viscous force provided by water:

$$v_s = \frac{2gr^2(\rho - \rho_w)}{9\eta\varphi}$$

where the terminal velocity of the organism (v_s) is dependent on gravitational acceleration (g), the size of the organism estimated as the radius (r) of a sphere of equal volume, the density of the organism (ρ), and the density (ρ_w) and viscosity (η) of the medium. A 'form factor' (ϕ) is included to adjust for the non-spherical shape of some organisms. This is defined as v_s/v_ϕ where v_ϕ is the terminal velocity of a sphere of equal volume and density to that of the organism. For some shapes the form factor is known from empirical relationships (McNown and Malaika 1950; Davey and Walsby 1985) but generally it has to be determined after measuring all other variables (Oliver et al. 1981). The Stokes equation is suitable for calculating sinking and floating velocities if the assumption of laminar flow is not violated, with particle-Reynolds number ($Re = 2rv_s\rho/\eta$) not exceeding 0.5 (Walsby and Reynolds 1980; Reynolds 1987). As a consequence the equation will not be reliable for large phytoplankton colonies where $r > 300$ µm (Reynolds 1987).

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

Kromkamp and Walsby (1990) conceived a model to explain buoyancy regulation in a culture of *Oscillatoria* which had previously been incubated at an irradiance flux E of 10 µmol m^{-2} s^{-1}:

$$\frac{d\rho}{dt} = c_1 \frac{E}{K_E + E} - c_3$$

where $d\rho/dt$ is the rate of density change (kg m^{-3} min^{-1}), c_1 is the rate coefficient for rate of increase in density with time (given as 0.132 kg m^{-3} min^{-1}), c_3 is the minimum rate of decrease in density with time (given as 0.023 kg m^{-3} min^{-1}) and K_E is the half saturation constant for the density response to irradiance (kg m^{-3}). The Michaelis-Menten form of this equation produces a hyperbolic response of rate of change of density with increasing irradiance up to a maximum assigned rate of density increase ($c_1 = 0.109$ kg m^{-3} min^{-1}). When transferred to the dark the change in density of the culture can be described by:

$$\frac{d\rho}{dt} = c_2 E_a - c_3$$

where E_a is the previous irradiance (μmol m^{-2} s^{-1}) and c_2 is the light dependent rate coefficient for density change.

Howard et al. (1996) and Visser et al. (1997) included intracellular carbon dynamics to connect irradiance to carbohydrate accumulation and density fluctuations. Visser et al. (1997) hypothesised that the Kromkamp and Walsby (1990) model did not adequately describe density change at high light intensities because photoinhibition was not considered. Further experimentation by Visser et al. (1997) involving exposure of *Microcystis* to light intensities up to 1,500 μmol m^{-2} s^{-1} revealed photoinhibition of carbohydrate accumulation at $E > 1{,}374$ μmol m^{-2} s^{-1}, described by:

$$\frac{\mathrm{d}\rho}{\mathrm{d}t} = \left(\frac{N_0}{60}\right) I \mathrm{e}^{(-E/E_0)} - \ell$$

where N_0 is a 'normative factor' (0.0945 kg m^{-3} (μmol photon)$^{-1}$ m^2), E_0 is the value of E where $\rho(E)$ is maximal (μmol m^{-2} s^{-1}) and c_3 has been defined above but was assigned a value of 0.0165 kg m^{-3} min^{-1} to represent density change at $E = 0$ (Visser et al. 1997) compared with the value of c_3 of 0.023 kg m^{-3} min^{-1} used for *Oscillatoria* by Kromkamp and Walsby (1990). The above equation of Visser et al. (1997) was applied for values of photon irradiance above compensation levels (E_c, assigned as 10.9 μmol m^{-2} s^{-1}) while for $E < E_c$, the rate of density change was given as:

$$\frac{\mathrm{d}\rho}{\mathrm{d}t} = f_1 \rho_i + f_2$$

where ρ_i is the initial density (kg m^{-3}), f_1 regulates the rate of decrease in density (kg m^{-3} h^{-1}) and f_2 is a value corresponding to the rate of density change with no carbohydrate storage in the cells (kg m^{-3} h^{-1}). This equation takes into account that the rate of carbohydrate loss, and hence density decrease, is dependent upon the previous light history and the concentration of carbohydrate stored within cells (Stone and Ganf 1981). Beardall et al. (1994) observed that an increase in dark respiration following light exposure was dependent upon both the photon flux and the duration of light exposure; processes that are implicitly included in the model of Visser et al. (1997). Howard et al. (1996) used the change in density equations of Kromkamp and Walsby (1990) coupled with a simple hydrodynamic model as the basis for a model of cyanobacterial bloom formation known as SCUM. They considered the generation of ballast in response to "excess" carbohydrate accumulation (B) as:

$$B = P_{qi} - R - K$$

where P_{qi} is the photosynthetic rate for a given light intensity (mol C (mol C)$^{-1}$ s^{-1}), R is the respiration rate (assigned a value of 0.55×10^{-6} mol C (mol C)$^{-1}$ s^{-1} according to Reynolds (1990)) and K is growth (assigned a maximum value of 5.5×10^{-6} mol C (mol C)$^{-1}$ s^{-1} from Reynolds (1990)). Once B is assigned it is possible to determine the change in cell density resulting from the addition of carbohydrate as ballast:

$$\frac{\mathrm{d}\rho_{cell}}{\mathrm{d}t} = \frac{(B_g C_{cell})}{67}$$

where ρ_{cell} is cell density (g C μm^{-3} s^{-1}), B_g is $2.38B$ (mol C (mol C)$^{-1}$), C_{cell} is the carbon content per cell (14×10^{-6} g C) and 67 (μm^3) represents an assigned cell volume for *Microcystis*. If there is not sufficient photosynthetic carbohydrate generated then ballast is not accrued to increase density, and the carbohydrate is used to support growth and offset losses from respiration.

The increased rate of respiration following high irradiance can lead to cells with different rates of density change and productivity at a given irradiance, depending upon prior light history (Beardall et al. 1994; Ferris and Christian 1991). Patterson et al. (1994) used a particle tracking model to attempt to include previous light history in a model of cyanobacterial growth but they did not explicitly include a dynamic component of buoyancy regulation in their model.

Wallace and Hamilton (1999) extended the previous buoyancy models of Kromkamp and Walsby (1990), Howard et al. (1996), Visser et al. (1997) by including two additional terms; a transient response term τ_r and a dependence on the previous rate of density change ($\mathrm{d}\rho(0)/\mathrm{d}t$). The induced density changes represent a physiological adjustment time for cells to respond to variations in irradiance, observed to be c. 20 min ($\tau_r = 20$ min). Their equation for change in density with time for the case where $D(0) = 0$ (i.e., starting conditions in which density is constant) took the form:

$$\frac{\mathrm{d}\rho}{\mathrm{d}t} = \left(c_1 \frac{E}{K_E + E} - c_3\right)\left(1 - \mathrm{e}^{-(\mathrm{t}/\tau_r)}\right)$$

Their model was calibrated with measured data to give $c_1 = 0.0427$ kg m^{-3} min^{-1}, $c_3 = 4.6 \times 10^{-6}$ kg m^{-3} min^{-1} and $K_E = 530$ μmol m^{-2} s^{-1}.

One of the major difficulties with the idealised representations of buoyancy regulation presented above is the ability to include a light history term for cells as they are advected and diffused between the layers or compartments that are typically used in hydrodynamic models. To begin with, a model is required to describe the irradiance at a given water depth:

$$E(z) = E(0)\mathrm{e}^{-\varepsilon z}$$

Where $E(0)$ is the surface irradiance corrected for albedo, z is water depth (positive downward) and ε is the vertical attenuation coefficient for broadband photosynthetically available radiation (400–700 nm). Various formulae

(e.g., Luo et al. 2010) are commonly used to distribute both total daily irradiance and albedo in a sinusoidal pattern as a function of time of day, as well as to construct irradiance from measurements based on, for example, global horizontal radiation, cloud cover and an assigned fraction of photosynthetically active radiation (0.46; Kirk 1991).

The vertical attenuation coefficient, ε, is affected by five constituents of natural water, that is water itself, dissolved organics, phytoplankton, detritus and inorganic solids. The way in which cyanobacteria affect ε can be described by:

$$\varepsilon = \varepsilon_O + K_C C$$

where ε_O is the vertical attenuation contributed by water and other natural constituents including other phytoplankton, C is a variable describing biomass of cyanobacteria and K_C is a specific attenuation coefficient normalised for the cyanobacterial biomass unit. The literature on specific light attenuation coefficient values for cyanobacteria and other phytoplankton is characterised by both a wide range of units and values.

Litchman (2003) gives species-specific light attenuation coefficients derived in the laboratory of 5.2×10^{-6} cm^2 filament^{-1} for *Phormidium luridum* and 1.7×10^{-6} cm^2 filament^{-1} for *Anabaena flos-aquae*. Different units have been used in modelling studies; 3.4 mm^2 cell^{-1} for *Microcystis* (Jöhnk et al. 2008) and 10 m^2 g^{-1} phytoplankton phosphorus for filamentous cyanobacteria (Scheffer et al. 1997). Values of K_c vary not only between species but also within species. Ganf et al. (1989) found that values of K_C when *Microcystis aeruginosa* was the dominant species in an artificial lake varied at 0.2 m depth from 0.0138 to 0.0106 m^2 mg^{-1} chlorophyll *a* for individual cells and colonies, respectively. At greater depths they found K_C values of 0.14 and 0.11 m^2 mg^{-1} for cells and colonies, and 0.029 and 0.020 m^2 mg^{-1} for non-vacuolate cells and colonies, respectively. They attributed gas vacuoles to be responsible for around 80% of the light scattering arising from their observed *Microcystis* cells and colonies.

6.11.5 Colony Formation and the Effect on Buoyancy

Sinking/floating velocity increases with the square of the radius, according to Stokes Law, and so colony size plays an important role in determining the vertical position of colonies and filaments in the water column. While carbohydrate accumulation and loss occur in response to light this may not be expressed as large diurnal migrations in small colonies that barely move in the water column (Visser et al. 1997). As colony size increases, the amplitude of migration increases and in a quiescent water body colonies may oscillate between the surface and depth several times within a single day (Kromkamp and Walsby 1990; Visser et al. 1997). Slow vertical migration has several implications for small colonies. Cells stranded near the surface can experience photoinhibition which decreases the ability for vertical migration as carbohydrate ballast accumulation is impaired (Ibelings and Maberly 1998; Brookes et al. 2000; Wallace and Hamilton 1999). Cells at depth may experience extended light limitation. In both cases there is an inability to overcome the vertically separated resources of light and nutrients (Ganf and Oliver 1982).

Typical vertical migration velocities for cyanobacteria range from 7 μm s^{-1} for *Planktothrix rubescens* up to 3,000 μm s^{-1} for *Microcystis aeruginosa* (Reynolds et al. 1987), influenced strongly by the size of filaments or colonies. In filamentous forms, the length of filaments can increase with growth and the production of new cells within the trichome. This mechanism of increasing filament size is limited by the rate of cell division and is relatively slow. The aggregation of filaments to produce larger units offers the potential to rapidly change the size of the unit and significantly increase the floating velocity. Filaments of *Anabaena circinalis* have been observed to aggregate at low light intensities (Brookes et al. 1999). The greatest axial linear dimension increased in the dark from 90.3 to 202.9 μm which effectively increased the floating velocity from 0.39 to 202.9 m h^{-1} (108–56,360 μm s^{-1}).

The aggregation of cells into colonies may result from modification of charge or hydrophocity and increases in colony size are generally associated with increased exudation of polysaccharides (Yang et al. 2008). Studies have demonstrated that microcystin and lectins (Kehr et al. 2006) or the microcystin-related protein MrpC (Zilliges et al. 2008) may play a role in inter-cellular aggregation in *Microcystis*. Not only does colony formation enable large vertical migrations, but it may also be a mechanism by which to avoid grazing losses (Sect. 6.12).

6.11.6 Within-Population Variability

In populations where buoyancy loss is observed often a significant proportion of the population retains buoyancy as some cells have sufficient gas vesicles to counteract the accumulation of dense polysaccharide. Persistently buoyant *Anabaena lemmermannii* and *Anabaena minutissima* filaments were observed in Lake Windermere (Walsby et al. 1991) and Lake Rotongaio, respectively (Walsby et al. 1987, 1989). It is interesting to note that the majority (88.9%) of *Anabaena minutissima* suspended in bottles at the water surface retained buoyancy following 9 h of daylight, while at the end of the light period on the second day of incubation 44.9% of filaments still remained buoyant (Walsby et al. 1991).

Persistent buoyancy is often associated with nutrient enrichment. Brookes et al. (1999) found that nutrient enriched *Anabaena circinalis* displayed significantly attenuated buoyancy loss relative to a treatment with no nutrients added. Similarly, *Aphanizomenon* with nutrients added maintained buoyancy after a 5 h light incubation (Klemer et al. 1995). Brookes et al. (2000) used flow cytometry to investigate gas vesicle volume and metabolic activity of individual cells of *Microcystis aeruginosa*. Even in culture populations, which are generally considered to be homogeneous, there was considerable variability of both gas vesicle volume and photosynthetic rate, presumably a function of cell age and stage of cell division.

The variation in buoyancy response highlights the considerable physiological variability in individuals in natural populations. Accounting for this variability in models is challenging because the attributes of individual colonies need to be predicted through time. This is possible with particle tracking models. Attributes such as photosynthesis, buoyancy regulation, nitrogen-fixation and growth can be predicted for the individual cells or colonies using this method, not only for an 'average population'. Furthermore these models enable the modelling of vertical particle trajectories in turbulent aquatic environments where the turbulent mixing is non-uniform (Ross and Sharples 2004). Beckman and Hense (2004) have developed a model of vertical migration that is dependent upon internal nutrient stores which when linked to a particle tracking model could predict the buoyancy response of individuals based on the cellular nutrient status. Advances with this modelling strategy would satisfy the need to move toward a more physiologically based approach rather than assigning rate constants for populations irrespective of cellular nutrient status.

Buoyancy may also play a role in recruitment and loss of colonies from sediments. Temperature controls on the respiration rate can lead to more accumulation of carbohydrate in cooler water bodies. The accumulation of glycogen results from a lower rate of protein synthesis at low temperatures and has been implicated in the loss of colonies from the water column in autumn as water temperatures decrease (Visser et al. 1994). The recruitment of cells from sediments may also be facilitated by buoyancy although Verspagen et al. (2004) suggest that recruitment is more likely to result from wind-driven resuspension.

6.11.7 Effects of Turbulence

Buoyancy maintains cyanobacterial cells in suspension while its regulation enables them to move vertically through the water in response to changing growth conditions. However the extent to which gas-vacuolate cyanobacteria can control their vertical distribution is also a function of the turbulent mixing regime. Steinberg and Hartmann (1988) analysed cyanobacterial distributions across a number of waterbodies and concluded that turbulence in lakes and rivers should be regarded as a special quasi-resource that can be differentially exploited by various phytoplankton in a manner analogous to nutrients or light (Reynolds and Walsby 1975; Harris 1986). Consequently, research into hydrodynamics has significantly advanced our understanding of the effects of turbulent mixing on the growth and distribution of phytoplankton, and particularly in selecting for gas-vacuolate cyanobacteria.

6.12 Population Losses

Factors promoting algal blooms are relatively well documented, but bloom collapse is often less clear. For example, Thompson et al. (2003) were able to document the bloom formation of *Anabaena* spp. in the Canning River, Western Australia, but were unable to ascertain the reasons behind the collapse of the bloom except to say that low dissolved inorganic phosphorus, low inorganic carbon, low light and high oxygen concentrations were probable causes. Such outcomes are not uncommon where investigations focus on the abiotic factors that promote blooms in anticipation that reversal of these factors will cause the demise of a bloom. Grazing by zooplankton and protozoans impact upon phytoplankton populations (Canter and Lund 1968; Oliver and Ganf 2000; Sommer et al. 2003; Reichwaldt and Stibor 2005) whilst the impact of infection by cyanophages, whether by bacteria, bacteriophages, fungi, or viruses can also be significant. As noted also in Chap. 7, copepods, cladoerans and rotifers can graze phytoplankton, but many cyanobacteria have specific features that make them less palatable to grazing than other phytoplankton. Large filamentous or colonial cyanobacteria may mechanically interfere with grazers as a result of their size or mucilage, while toxins produced by some cyanobacteria have also been implicated in having an allelopathic function against grazers (Chap. 7). Yang et al. (2006) propose that colony formation in *M. aeruginosa* is a defense strategy against grazing flagellates due to a mismatch in size between the colonies and the grazers and may be considered as an inducible defense against flagellate grazing. It appears that while toxins may be effective against metazoan zooplankton (Yang et al. 2006; Liu et al. 2005; Ghadouani et al. 2003) colony size may also play a role. The palatability and capacity for grazers to exert significant controls of very dense, potentially toxic blooms in which cells are mostly aggregated into large colonies, such as *Microcystis* blooms in Lake Taihu, China (Fig. 6.11), is questionable. There is, however, some indication that *Microcystis* cells can also respond to grazers by aggregating into colonies in the presence of purified microcystin toxin (Sedmak and Elersek 2006), and in cultures exposed to spent *Daphnia* media and disrupted *Microcystis* cells (Becker 2010).

Fig. 6.11 Bloom of *Microcystis* spp. in Lake Taihu, Jiangsu Province, China, in October 2007. This followed a massive lake-wide spring-summer (May–July) bloom, which severely interfered with the water supply for several million residents in nearby Wuxi City. Lake Taihu is well known for such *Microcystis* blooms at this time of year

Shilo (1970, 1971) drew attention to biological agents that cause cell lysis in cyanobacteria and Daft and Stewart (1971) suggested that over 40 cyanobacterial strains, including bloom forming species, were susceptible to bacterial isolates that cause cell lysis. A combination of abiotic factors and pathogens may elicit cellular responses such as differentiation, cell death, cell-cycle arrest, formation of resting stages and asymmetric cell division leading to death of the older cell (Franklin et al. 2006; Chap. 21).

Several researchers (e.g. Mann 2006; Raven 2006; Middelboe et al. 2008) have recognised recently that bloom collapse may be due to biotic as well as abiotic factors or a combination of both. Biotic factors include fungal infection, viruses, bacteriophages, programmed cell death and glycogen accumulation that decrease rates of photosynthesis but increase respiration. There are a myriad of terms to describe cell death but Franklin et al. (2006) provide a useful set of definitions: natural cell death, programmed cell death, apoptosis, lysis, necrosis, paraptosis and senescence.

6.12.1 Chytrid Infections

Chytrids, mainly aquatic fungi, belong to the phylum Chytridiomycota, class Chytridiomycetes, order Chytridiales. Two groups of zoosporic fungi once included among chytrids are now classified as separate phyla, Blastocladiomycota and Neocallimastigomycota. The importance of these fungi is illustrated by the discovery of the frog chytrid (*Batrachochytrium dendrobatidi*) that has caused the widespread death of amphibians in Australia (Berger et al. 1998). Chytrids typically have a haploid (n) and a diploid (2n) phase. It is the flagellated diploid zoospore that attacks algal cells. Kagami et al. (2007) have reviewed fungal parasitism on phytoplankton and provide some interesting insights into identification, visualisation and culturing of chytrids, as well as information on the likelihood of infection, host specificity and fungal epidemics. They describe how fungal parasitism may influence food chains and introduce a new pathway termed the 'Mycoloop'. This explains how large and often inedible phytoplankton are infected by parasitic fungi (particularly Chytrids) whose zoospores consume nutrients from their phytoplankton hosts which are subsequently eaten by zooplankton, thus extending our knowledge of both algal death and nutrient cycling. They conclude that estimates of zoospore abundance and the prevalence to infection of phytoplankton are urgently needed if the collapse of algal blooms is to be understood, and advocate the adoption of the model parameters described by Brunning et al. (1992).

Anabaena flos-aquae is a common bloom-forming cyanobacteria of freshwaters and is susceptible to infection by a chytrid. Sigee et al. (2007) used a simple dye (Evans Blue) to ascertain whether cells present in a eutrophic lake (Rostherne Mere, UK) were alive, senescent or dead. Vegetative cells and akinetes were susceptible to infection with filamentous colonies disintegrating and 30% of the akinetes died. In the same paper the authors investigated the death of *Microcystis aeruginosa* and came to the conclusion that death was primarily a response to adverse conditions with no evidence of fungal infection, but moribund cells underwent programmed cell death.

Although chytrids may infect various algae their presence does not always lead to significant decreases in cell numbers. Takano et al. (2008) showed that in Lake Shumarinai, Japan, two chytrid types infected *Anabaena smithii,* one on akinetes and the other on heterocysts. Although maximum parasitism of filaments was 20.6%, the abundance of *A. smithii* did not decrease. They suggested that nitrogen fixation could be affected but the concentration of available nitrogen was too high to detect any effect.

6.12.2 Programmed Cell Death

Marine cyanobacteria of the genera *Trichodesmium* often form extensive blooms that may disappear abruptly within 1–2 days as cell lysis and decomposition occur. Ohki (1999) has suggested that this is due to bacteriophage infection. However, Berman-Frank et al. (2004) have implicated autocatalytic cell death triggered by nutrient stress (Segovia et al. 2003) as a likely mechanism. Autocatalytic cell death is analogous to programmed cell death (PCD) in multi-cellular organisms and refers to an active, genetically controlled, cellular self-destruction mechanism driven by a series of biochemical events. They showed that in cultures consisting

primarily of *Trichodesmium erythraeum* programmed cell death was initiated by a combination of Fe and P limitation at high irradiances.

Franklin et al. (2006) suggest that freshwater environments are ideal habitats for the investigation of PCD because phytoplankton are relatively abundant and blooms are frequent and more accessible than in the marine environment. They found that the death of *Anabaena flos-aquae* appeared to be related to oxidative stress and nutrient limitation leading to PCD. It appears as though PCD is correlated with oxidative stress; an imbalance between the synthesis of reactive oxygen and the cell's ability to maintain a normal internal redox potential. This can be caused by nutrient deficiency, viral infection or other stressors. For example in Saint Lucie River Estuary, Florida, a population of *Microcystis aeruginosa* became stressed due to an increase in salinity with detectable toxin levels rising by 90%. This was accompanied by a significant increase in the production of hydrogen peroxide compared with unstressed cells, with levels similar to those that have induced PCD elsewhere (Ross et al. 2006). Franklin et al. (2006) suggest that free radicals such as hydrogen peroxide may act as internal signals promoting PCD.

6.12.3 Cyanophages

Viruses are very abundant in freshwater and can reach concentrations of 10^6–10^9 cells mL^{-1}. They are known to cause the death of both eukaryotic and prokaryotic, marine and freshwater phytoplankton as well as macroalgae (Brussaard 2004; Chap. 21). They were thought to be host specific, but Deng and Hayes (2008) have shown that cyanophages isolated from Lake Zurich, Switzerland and Cotswold Water Park, U.K., had a very broad host range and were able to infect *Anabaena, Microcystis* and *Planktothrix*. Viruses can control cyanobacterial populations. Tucker and Pollard (2005) isolated *Microcystis aeruginosa* from Lake Baroon, Queensland, Australia. The growth rate of *M. aeruginosa* under optimal conditions of light and nutrients was 0.023 h^{-1} (0.552 day^{-1}) and reached a density of 1.09×10^7 cells mL^{-1} after 6 days. When *M. aeruginosa* was incubated with a series of tenfold dilutions of the natural viral community the final concentration of *M. aeruginosa* cells fell. At the natural lake viral concentration the final biomass of cells was reduced by 95% and this was correlated ($r^2 = 0.95$) with the final number of viral like particles (10^7 mL^{-1}) present.

However, not all viral infections produce the anticipated result. Cell numbers of *Cylindrospermopsis raciborskii* from Lake Samsonvale in southeast Queensland, Australia, incubated with natural viral like particles (VLP) decreased by 85% after 5 days and this was correlated with a step-wise increase in VLP (Pollard and Young 2010). However, as the cells lysed the filaments of cells split into smaller units which still had viable cells. They suggest that this process may aid in the re-distribution of *C. raciborskii* throughout the lake. Further, phytoplankton may have a defence mechanism against parasitic viruses that is dependent upon the release of dissolved organic matter which attracts flagellates that eat the parasitic viruses (Murray 1995).

Viral ecology is an expanding science (Chap. 21) and viruses can be a major agent for the death of host phytoplankton. However, whether or not viral ecology can be used to manage cyanobacterial blooms is still problematic because of the rapid selection of resistant host strains (Wilhelm and Matteson 2008) and the only examples of artificial control of cyanobacteria by phages is at an experimental system scale (Chap. 21). Similarly, models of effects of cyanophages are still in their infancy, but have yielded useful hypotheses about the dependence of viral impacts on the physiological status of cells (Gons et al. 2006; Chap. 21).

6.13 Water Movement and Blooms

It is possible to separate three main categories of models that characterise the effects of water movement on cyanobacteria populations. One category includes the application of hydrodynamic models to explicitly quantify the effects of advective transport and turbulent mixing processes on cyanobacteria populations. The resulting models are considered in more detail in Sect. 6.14. Another category uses various numerical indices to denote, in combination with phytoplankton physiological attributes, when water stability may be conducive to formation of blooms. The last uses conceptual models as simple as asking are hydrodynamic/light conditions 'correct' for a noxious cyanobacteria bloom (Elser 1999). This type of conceptual model has been used as part of a decision support tree approach to help step through a set of prequisite environmental conditions that might promote a bloom (Elser 1999; Oliver and Ganf 2000). Exploration of the 'correctness' of the hydrodynamic environment and light climate for blooms leads naturally to the approach used in category two of seeking numerical indicators.

A process based model of bloom potential of cyanobacteria is given by Humphries and Lyne (1988). Their model was the first to consider positive buoyancy (i.e. rising particles) in a growth-diffusion model application to the vertical dimension. The model examines the interplay of turbulence in the surface mixed layer and positively buoyant cells or colonies of *Microcystis.* At short time scales buoyancy plays a far more important role in determining the vertical concentration profile. If turbulent mixing is intense then populations of both positively-buoyant and negatively-buoyant phytoplankton will be homogenised through the surface mixed layer. If turbulent mixing is weak, then populations can become disentrained from the predominant turbulent eddies and

strong concentration discontinuities ensue. Humphries and Lyne (1988) used a non-dimensional time scale for diffusion in the mixed layer relative to the sinking or floating time scales of populations. This diffusion or mixing time scale T_{mix} was given as:

$$T_{mix} = \frac{z_m^2}{K}$$

where z_m is the depth of the surface mixed layer (m) and K is the vertical coefficient of eddy diffusivity ($m^2\ s^{-1}$). The characteristic time scale T_v (s) for cells sinking or rising over the water depth is:

$$T_v = \frac{z_m}{\nu_s}$$

where ν_s is the sinking or rising velocity ($m\ s^{-1}$). The variable *Pe*, known as the Péclet number, gives a value for the rate of diffusion relative to sinking or rising:

$$Pe = \frac{T_{mix}}{T_v}$$

A value of $Pe > 1$ defines when the influence of sinking or floating begins to dominate the vertical distribution of cells, as opposed to the homogenising effect of turbulent diffusion. Huisman and Hulot (2005) suggested that at $Pe > 10$ *Microcystis* will completely disentrain from the turbulence and can then form surface blooms. Values of the Péclet number also have relevance to artificial mixers or destratification for control of cyanobacterial blooms (see Reynolds et al. (1984) and Visser et al. (1996), for applications of artificial mixing for this purpose). Increasing the rates of vertical mixing through artificial mixing or destratification effectively reduce T_{mix} and therefore *Pe*, with the outcome that *Pe* will remain sufficiently low so as to prevent cells or colonies from accumulating at the surface.

Another approach to assessing the effect on cyanobacteria distributions of water movement in the surface mixed layer is used by Howard et al. (1996) in the "SCUM" model. This model uses the Wedderburn number (W) which reflects the balance between surface wind stress and the energy inherent in the water density gradient (Imberger and Hamblin 1982), to attempt to capture the temporal dynamics of the depth of the surface mixed layer (h) using the equation:

$$h^2 = \frac{W \rho_w v_* L}{g \Delta\rho}$$

where ρ_w is a standard reference density for water, $\Delta\rho$ is the density gradient that separates water of uniform density at the water surface from the water density at the base of the gradient, L is the fetch length (the length of the lake or reservoir at the density gradient in the direction of the wind), g is the gravitational acceleration constant and v_* is the average turbulent water velocity given by:

$$v_*^2 = \frac{C_d \rho_a U_{10}^2}{\rho_w}$$

where C_d is the drag coefficient (1.3×10^{-3}), ρ_a is the density of air ($1.2\ kg\ m^{-3}$) and U_{10} is the wind velocity at 10 m elevation ($m\ s^{-1}$). When the calculated value of W falls below 1, the mixed layer depth is recalculated with $W = 1$ (i.e. wind stress and buoyancy forces are approximately equal). Within this mixed layer the cyanobacteria colonies are redistributed with a random-walk model to reflect the dominant influence of turbulent mixing over the rising velocities of colonies within this layer. A random walk model for cyanobacteria motion in the surface mixed layer has been used in a similar way by Patterson et al. (1994) and Wallace and Hamilton (2000) but for those cases the transport and mixing of water was accomplished with the hydrodynamic model DYRESM (Imberger and Patterson 1981). Wallace and Hamilton (1999) examined other theoretical cases of cyanobacteria movement within the surface mixed layer represented by coherent paths of Langmuir circulation, using a model by Buranathanitt et al. (1982), as well as a case with no water movement at all. Each of these cases demonstrated the critical role of colony size where larger colonies were more easily able to become disentrained from the turbulent mixing to form surface blooms. The entrainment of cyanobacteria can also be related more directly to wind speed (Webster and Hutchinson 1994), with values above a critical value of 2–3 $m\ s^{-1}$ defining when colonies are entrained into the turbulent surface layer rather than floating to the surface. Otherwise, colonies can float within a surface film and marked horizontal gradients ensue that can lead to blooms on leeward shores under continuous directional forcing by wind (Figs. 6.1c and 6.1h). This exponential accumulation of colonies downwind has a length scale that is inversely proportional to the colony flotation speed (Hutchinson and Webster 1994).

Turbulent mixing itself can also play a role in colony size, as shown in grid-stirred tank experiments with *Microcystis aeruginosa* by O'Brien et al. (2004). They obtained a significant relationship ($r^2 = 0.85$, $p < 0.01$) between turbulent dissipation and colony size corresponding to occurrence of smaller colonies with increasing turbulence. The variability of, and interactions amongst wind speed, water turbulence, and cyanobacteria buoyancy (Sect. 6.11) and colony size, make it highly challenging to adapt process based models for prediction of blooms and hence the application of simpler indicators of bloom potential discussed above.

6.14 Ecosystem Models That Include Water Movement

In a review of lake ecosystem models Mooji et al. (2010) indicate the large number and wide diversity of approaches that have been used to model phytoplankton biomass at the ecosystem scale. A smaller subset of these models has been used to model explicitly the biomass or chlorophyll *a* associated with particular species of cyanobacteria or of the phylum collectively. Many of these models have an underpinning hydrodynamic model to account for the effects of advection and diffusion in transporting cyanobacteria. The most complex of these models is three-dimensional (3-D) and generally represents a lake ecosystem by Cartesian coordinates (x, y, z) in an orthogonal coordinate system where the coordinates are perpendicular to each other. In a curvilinear coordinate system the orthogonal coordinate system is modified by transforming lines to curves. Several applications of 3-D hydrodynamic models (e.g. ELCOM) have used mathematical formulae to transform between orthogonal and curvilinear coordinate systems (Hodges and Imberger 2001; Venkatesh et al. 2005). A non-Cartesian coordinate system allows for flexibility of coordinate positions so that the model can be adapted to better resolve specific areas within a model domain and reduce distortion of the physical domain, but with considerable increase in mathematical complexity. Dispersion of a pollutant or flow in spatially constricted areas such as channels provide examples where non-Cartesian coordinates can better capture the dynamics than a Cartesian coordinate system for a given computational effort. In the case of cyanobacteria it would be expected that these models may be more suitable for capturing fine-scale shoreline accumulations often characterising blooms in lentic waters.

Most 3D models have a set of governing equations for transport of mass and momentum, and surface thermodynamics. Commonly the transport terms are based on numerical solutions of the unsteady Reynolds-averaged Navier-Stokes (RANS) equations which describe the velocity of a fluid in 3D space. The numerical approximation in the RANS equations commonly requires closure via a turbulence model in order to include the turbulent properties of the flow. With increasing computational power applications of these models for hindcasting or predicting cyanobacterial blooms are becoming more routine (Robson and Hamilton 2004) and circumvent some of the issues associated with models of reduced dimension which have used vertical or horizontal averaging and will not capture horizontal wind transport of colonies (Burger et al. 2007) or surface blooms associated with short-term stratification events (MacIntyre 1993), respectively. Nevertheless these models of reduced dimension have yielded important insights into bloom dynamics including applications with the 1D (horizontally averaged) DYRESM model to understand interactions amongst water movement and cyanobacteria colony size and density change (Wallace and Hamilton 1999, 2000; Wallace et al. 2000) and a 1D model to examine competition amongst cyanobacteria and negatively buoyant phytoplankton (Huisman et al. 2004).

It is impossible to do justice to the many coupled hydrodynamic-ecological models that could potentially be used for the purpose of modelling cyanobacteria. A few of the better known models are discussed briefly below, with limited examples of applications for cyanobacteria populations. ELCOM-CAEDYM is a 3D model that has been used to simulate a major bloom of *Microcystis aeruginosa* in the Swan River estuary in Western Australia (Robson and Hamilton 2004). The model was used to capture a transition following a major rainfall event of brackish water displacement by freshwater and the resulting bloom. The seven-compartment phytoplankton model used in CAEDYM allows for specific species or groups of cyanobacteria to be simulated concurrently with other phytoplankton groups, to capture seasonal patterns of succession (Bruce et al. 2006; Burger et al. 2007) or changes that may be expected with a warming climate (Trolle et al. 2010). Figure 6.12 shows an application of the ELCOM-CAEDYM model to Lake Rotorua, New Zealand, with daily simulation output of surface concentrations of cyanobacteria (represented as equivalent chlorophyll *a* concentration) changing rapidly. These rapid changes are induced mostly by wind acting on the surface mixed layer where buoyant populations of cyanobacteria accumulate. Some evidence of a mid-lake concentration is also sometimes observed within this lake (Fig. 6.13). In this figure cyanobacteria (*Microcystis* sp.) were numerically dominant in the phytoplankton population and chlorophyll fluorescence readings across the lake (vertically and horizontally) were interpreted as representative of the relative biomass of cyanobacteria. PROTECH (Phytoplankton RespOnses To Environmental CHange) is a phytoplankton model that has most commonly been used with a one-dimensional model of mixing and transport to examine the seasonal succession of different phytoplankton groups in lakes (Reynolds et al. 2001). Simulations have demonstrated the likelihood of increasing relative abundance of cyanobacteria in a warmer climate (Elliot et al. 2005, 2006), which has subsequently been reinforced in modelling by Trolle et al. (2010). PROTECH can simulate up to 10 phytoplankton species or groups but has the most extensive phytoplankton parameter library that includes over 100 phytoplankton species. Many of these parameters make use of a morphological trait of surface area to volume of individual cells or colonies in order to assign key physiological characteristics such as growth and respiration rates to individual species (Kruk et al. 2010). Delft3D-ECO is a 3-D hydrodynamic-ecological model that can incorporate an additional model known as BLOOM (Los et al.

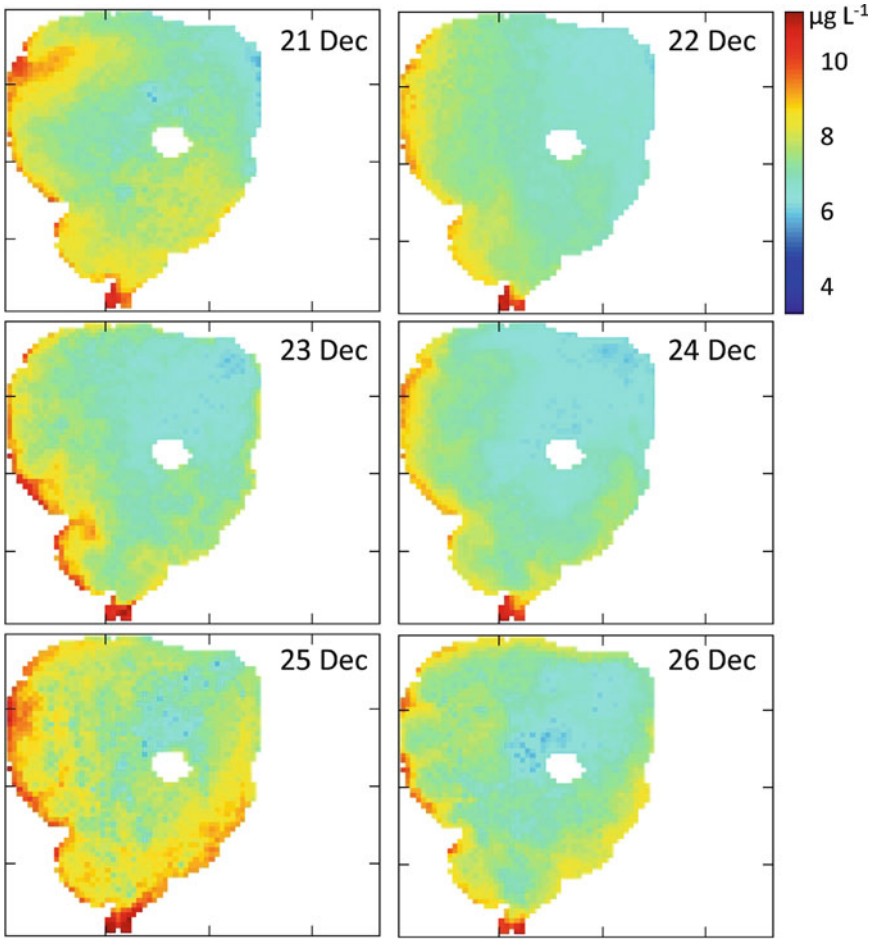


Fig. 6.12 Daily output (midday, 21–26 December 2007) of cyanobacteria (as chlorophyll *a* concentration) derived from a 3-dimensional hydrodynamic-ecological model (DYRESM-CAEDYM) of Lake Rotorua, North Island, New Zealand. A colour scale is on the right and represents cyanobacteria as equivalent chlorophyll *a* concentration. The lake has an area of 80 km^2

2008; Los 2009) which explicitly includes state variables of *Microcystis, Aphanizomenon* and *Planktothrix*. SALMO (Simulation of an Analytical Lake Model)-HR is a 1D (horizontally averaged) model that includes at least three functional phytoplankton groups (Recknagel et al. 2008). There are many models of various levels of complexity and resolution which have different levels of parameterisation either inherent in the model (e.g. PROTECH) or provided by a user as part of the calibration process based on assigned ranges. Many of the current models seek some balance between lumping phytoplankton into one or a few biomass categories and seeking to capture dynamics of species that are of intense interest; for example particular species of cyanobacteria that may form blooms and be toxic. As noted by Harris (1994), however, these two models can be of very different types, pointing to the difficulty of generalising phytoplankton populations as a group for ease of parameterisation versus attempting mechanistic descriptions to capture the characteristics of the many different species and their interactions found in an ecosystem. Thus capturing the seasonal succession of cyanobacteria through a growing season still remains a challenge for the foreseeable future for aquatic ecosystem models attempting quantitative predictions of cyanobacterial blooms. Furthermore, life cycle strategies of overwintering and akinete formation and germination are mostly poorly captured and yet are critical to the survival strategy of many cyanobacteria (Chap. 7). Quantitative modelling of the environmental triggers for benthic life cycle stages is largely unexplored and should be addressed for a more complete understanding of the triggers leading to cyanobacterial blooms.

6.15 Conclusions

Cyanobacteria have a number of key attributes that enable them to be able to accumulate in large concentrations and form surface blooms under certain conditions. A coalescence of favourable conditions for bloom formation includes, but is not limited to: adequate nutrients to support cell replication including nitrogen fixation in a subset of cyanobacteria when cellular nitrogen demands are not met; buoyancy imparted by gas vacuoles; alterations in photosynthetically-fixed carbohydrate that acts as ballast; aggregations of cells as colonies

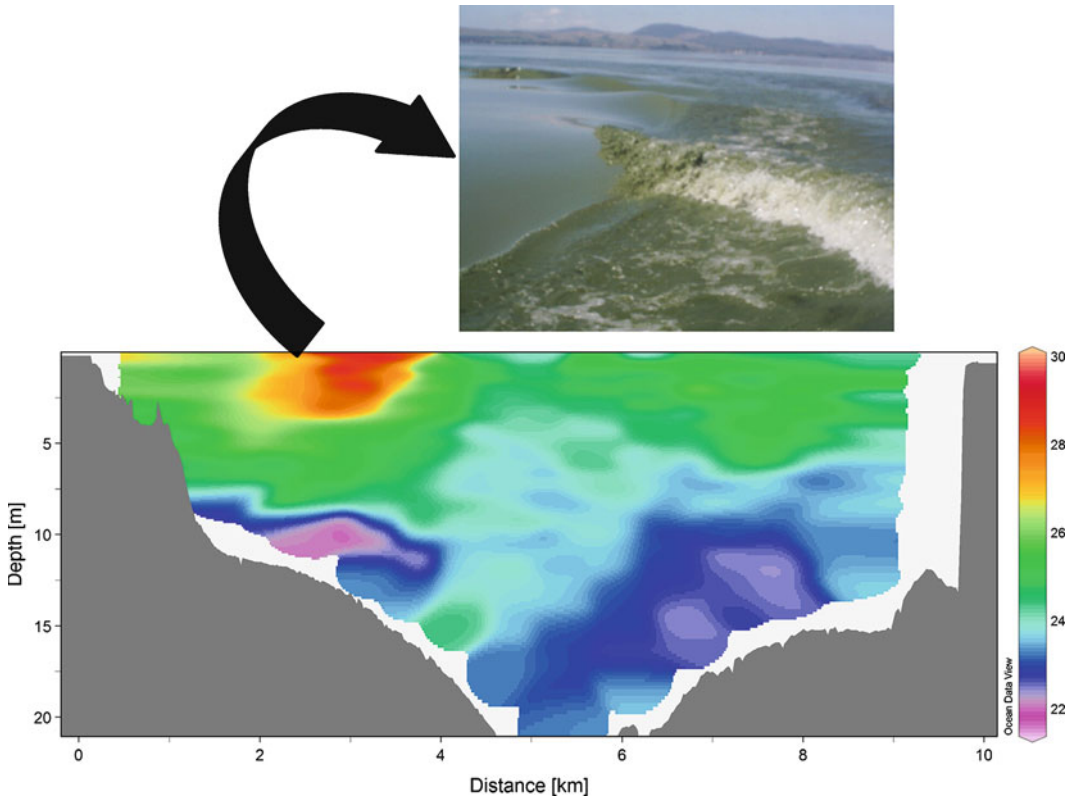


Fig. 6.13 Cross-sectional profile (depth 21 m, distance across lake c. 10 km) of chlorophyll fluorescence in Lake Rotorua, North Island, New Zealand on 10 March 2004 (lower). *Anabaena* sp. was strongly dominant amongst the phytoplankton assemblage and the colour scale is an indicative concentration in µg chlorophyll *a* L^{-1}. Note the high concentration (bloom) at a horizontal location of 2.5 km (The photo (upper) was taken at this location (D. Burger, with permission))

or filaments to enhance buoyancy; and disentrainment from turbulent motions in the surface mixed layer of waterbodies. Furthermore the cyanobacteria are characterised by substantial capacity for photoacclimation and photoadaptation that enables them to maintain light harvesting efficiency and to avoid increased risk of photodamage associated with high surface irradiances that may occur in blooms. The complexity and diversity of these processes and their variability within and between species represents an enormous challenge for models attempting to forecast the occurrence of cyanobacterial blooms at the scale of whole waterbodies. At best these models can capture only a subset of the processes and species parameters necessary to predict successional sequences within and between cyanobacterial species and other phytoplankton. Nevertheless, as knowledge of cyanobacterial physiology grows, and with increases in computational power, models will extend beyond tools to test theoretical concepts and single response factors, and yield integrated, quantitative estimates of the timing, frequency and magnitude of cyanobacterial blooms. Models that incorporate the collapse of blooms when mediated by biotic factors (e.g. infection and or grazing) will be particularly useful. This degree of realism will allow these models to be used more routinely for quantitative predictions of cyanobacterial biomass with applications for strategies that could be used to influence the timing and magnitude of populations.

Acknowledgements This work has been partially funded through the Foundation of Research, Science and Technology (Contract UOWX0505) and the University of Waikato Lakes Chair supported by Environment Bay of Plenty.

References

Adams DG, Duggan PS (1999) Tansley review No. 107. Heterocyst and akinete differentiation in cyanobacteria. New Phytol 144:3–33

Agawin NSR, Rabouille S, Veldhuis MJW, Servatius L, Hol S, van Overzee HMJ, Huisman J (2007) Competition and facilitation between unicellular nitrogen-fixing cyanobacteria and non-nitrogen-fixing phytoplankton species. Limnol Oceanogr 52:2233–2248

Ahn C-Y, Joung S-H, Park C-S, Kim H-S, Yoon B-D, Oh H-M (2008) Comparison of sampling and analytical methods for monitoring of cyanobacteria-dominated surface waters. Hydrobiologia 596:413–421

Allen MM (1984) Cyanobacterial cell inclusions. Annu Rev Microbiol 38:1–25

Allison EM, Walsby AE (1981) The role of potassium in the control of turgor pressure in a gas-vacuolate blue-green alga. J Exp Bot 32:241–249

Alric J, Lavergne J, Rappaport F (2010) Redox and ATP control of photosynthetic cyclic electron flow in *Chlamydomonas reinhardtii* (I) aerobic conditions. Biochim Biophys Acta Bioenerg 1797:44–51

Andersen T (1997) Pelagic nutrient cycles. Herbivores as sources and sinks. Springer, Berlin/Heidelberg/New York, 280 pp

Arhonditsis GB, Qian SS, Stow CA, Lamon EC, Reckhow KH (2007) Eutrophication risk assessment using Bayesian calibration of process-based models: application to a mesotropic lake. Ecol Model 208:215–229

Aurora R, Hihara Y, Singh AK, Pakrasi HB (2007) A network of genes regulated by light in cyanobacteria. OMICS J Integr Biol 11(2): 166–185

Azevedo SMFO, Carmichael WW, Jochinsen EM, Rinehart KL, Lau S, Shaw G, Eaglesham GK (2002) Human intoxication by microcystins during renal dialysis treatment in Caruaru – Brazil. Toxicology 181–182:441–446

Bailey S, Grossman A (2008) Photoprotection in cyanobacteria: regulation of light harvesting. Photochem Photobiol 84(6):1410–1420

Baird ME, Emsley SM (1999) Towards a mechanistic model of plankton population dynamics. J Plankton Res 21:85–126

Baklouti M, Diaz F, Pinazo C, Faure V, Quéguiner B (2006) Investigation of mechanistic formulations depicting phytoplankton dynamics for models of marine pelagic ecosystems and description of a new model. Prog Oceanogr 71(1):1–33

Baumert HZ, Petzoldt T (2008) The role of temperature, cellular quota and nutrient concentrations for photosynthesis, growth and light-dark acclimation in phytoplankton. Limnologica 38:313–326

Beardall J, Burger-Wiersma T, Rijkeboer M, Sukenik A, Lemoalle J, Dubinsky Z, Fontvielle D (1994) Studies on enhanced post-illumination respiration in microalgae. J Plankton Res 16(10): 1401–1410

Becker S (2010) Biotic factors in induced defense revisited: cell aggregate formation in the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806 is triggered by spent *Daphnia* medium and disrupted cells. Hydrobiologia 644:159–168

Beckmann A, Hense I (2004) Torn between extremes: the ups and downs of phytoplankton. Ocean Dyn 54:581–592

Behrenfeld MJ, Halsey KH, Milligan AJ (2008) Evolved physiological responses of phytoplankton to their integrated growth environment. Philos Trans R Soc Lond B 363:2687–2703

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H (1998) *Chytridiomycosis* causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proc Natl Acad Sci USA 95:9031–9036

Berman T (1997) Dissolved organic nitrogen utilization by an *Aphanizomenon* bloom in Lake Kinneret. J Plankton Res 19:577–586

Berman-Frank I, Lundgren P, Falkowski P (2003) Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. Res Microbiol 154:157–164

Berman-Frank I, Bidle KD, Haramaty L, Falkowski PG (2004) The demise of the marine cyanobacterium, *Trichodesmium* spp., via an autocatalyzed cell death pathway. Limnol Oceanogr 49:997–1005

Berman-Frank I, Quigg A, Finkel ZV, Irwin AJ, Haramaty L (2007) Nitrogen-fixation strategies and Fe requirements in cyanobacteria. Limnol Oceanogr 52(5):2260–2269

Bhaya D, Schwarz R, Grossman AR (2000) Molecular responses to environmental stress. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 397–442, 669 pp

Bouchard JEN, Roy S, Campbell DA (2006) UVB effects on the photosystem II-D1 protein of phytoplankton and natural phytoplankton communities. Photochem Photobiol 82(4):936–951

Bowen CC, Jensen TE (1965) Blue-green algae: fine structure of the gas vacuoles. Science 147:1460–1462

Brookes JD, Carey CC (2011) Resilience to Blooms. Science 334:46–47

Brookes JD, Ganf GG, Burch MD (1994) The separation of forms of *Microcystis* from *Anabaena* in mixed populations by the application of pressure. Aust J Mar Freshw Res 4:133–138

Brookes JD, Ganf GG, Green D, Whittington J (1999) The influence of light and nutrients on buoyancy, filament aggregation and flotation of *Anabaena circinalis*. J Plankton Res 21:327–341

Brookes JD, Ganf GG, Oliver RL (2000) Heterogeneity of cyanobacterial gas vesicle volume and metabolic activity. J Plankton Res 22:1579–1589

Bruce LC, Hamilton DP, Imberger J, Gal G, Gophen M, Zohary T, Hambright KD (2006) A numerical simulation of the role of zooplankton in C, N and P cycling in Lake Kinneret, Israel. Ecol Model 193:412–436

Bruning K, Lingeman R, Ringelberg J (1992) Estimating the impact of fungal parasites on phytoplankton populations. Limnol Oceanogr 37:252–260

Brussaard CPD (2004) Viral control of phytoplankton populations – a review. J Eukaryot Microbiol 51:125–138

Bryant DA (1991) Cyanobacterial phycobilisomes: progress toward complete structural and functional analysis via molecular genetics. In: Bogorad L, Vasil IK (eds) Cell culture and somatic cell genetics of plants, vol 7B. Academic, New York, pp 255–298

Buranathanitt T, Cockrell DJ, John PH (1982) Some effects of Langmuir circulation on the quality of water resource systems. Ecol Model 15:49–74

Burger DF, Hamilton DP, Pilditch CA (2007) Modelling the relative importance of internal and external nutrient loads on water column nutrient concentrations and phytoplankton biomass in a shallow polymictic lake. Ecol Model 211:411–423

Burillo S, Luque I, Fuentes I, Contreras A (2004) Interactions between the nitrogen signal transduton protein PII and N-acetylglutamate kinase in organisms that perform oxygenic photosynthesis. J Bacteriol 186:3346–3354

Canter HM, Lund JWG (1968) The importance of protozoa in controlling the abundance of planktonic algae in lakes. Proc Linn Soc Lond 179:203–219

Caperon J, Meyer J (1972) Nitrogen-limited growth of marine phytoplankton – I. Changes in population characteristics with steady-state growth rate. Deep Sea Res 19:601–618

Carey CC, Ibelings BW, Hoffmann EP, Hamilton DP, Brookes JD (2012) Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate, Water Research doi:10.1016/j.watres.2011.12.016

Carmichael WW (1992) Cyanobacteria secondary metabolites – the cyanotoxins. J Appl Bacteriol 72:445–459

Carpenter SR (2008) Phosphorus control is critical to mitigating eutrophication. Proc Natl Acad Sci USA 105:11039–11040

Chu Z, Jin X, Yang B, Quigru Z (2007) Buoyancy regulation of *Microcystis flos-aquae* during phosphorus-limited and nitrogen-limited growth. J Plankton Res 29:739–745

Codd GA (1995) Cyanobacterial toxins: occurrence, properties and biological significance. Water Sci Technol 32(4):149–156

Codd GA, Steffensen DA, Burch MD, Baker PD (1994) Toxic blooms of cyanobacteria in Lake Alexandrina, South Australia – learning from history. Aust J Mar Freshw Res 45:1–6

Coruzzi G, Last R (2000) Amino acids. In: Buchannan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists Textbook, Rockville, Chapter 8, pp 358–410

Daft MJ, Stewart WDP (1971) Bacterial pathogens of freshwater blue-green algae. New Phytol 70:819–829

Damerval T, Castets A-M, Houmard J, Tandeau de Marsac N (1991) Gas vesicle synthesis in the cyanobacterium *Pseudanabaena* sp.: occurrence of a single photoregulated gene. Mol Microbiol 5:657–664

Davey MC, Walsby AE (1985) The form resistance of sinking algal chains. Br Phycol J 20:243–248
Deng L, Hayes PK (2008) Evidence for cyanophages active against bloom-forming freshwater cyanobacteria. Freshw Biol 53:1240–1252
Donnelly TH, Grace MR, Hart BT (1997) Algal blooms in the Darling-Barwon River Australia. Water Air Soil Pollut 99:487–496
Downing JA, Watson SB, McCauley E (2001) Predicting cyanobacteria dominance in lakes. Can J Fish Aquat Sci 58:1905–1908
Droop MR (1968) Vitamin B_{12} and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. J Mar Biol Assoc UK 48:689–733
Droop MR (1973) Some thoughts on nutrient limitation in algae. J Phycol 9:264–272
Elliott JA, Thackeray SJ, Huntingford C, Jones RG (2005) Combining a regional climate model with a phytoplankton community model to predict future changes in phytoplankton in lakes. Freshw Biol 50:1404–1411
Elliott JA, Elliot JA, Thackeray SJ (2006) Testing the sensitivity of phytoplankton communities to changes in water temperature and nutrient load, in a temperate lake. Hydrobiologia 559:401–411
Elser JJ (1999) The pathway to noxious cyanobacteria blooms in lakes: the food web as the final turn. Freshw Biol 42:537–543
Falconer IR, Beresford AM, Runnegar MTC (1983) Evidence of liver damage by toxin from a bloom of the blue-green alga *Microcystis aeruginosa*. Med J Aust 1:511–514
Falkowski PG, Kolber K (1993) Estimation of phytoplankton photosynthesis by active fluorescence. Int Counc Explor Sea Symp 197:92–103
Falkowski P, La Roche J (1991) Acclimation to spectral growth irradiance in algae. J Phycol 25:8–14
Faithful CL, Burns CW (2006) Effects of salinity and source of inocula on germination of Anabaena akinetes from a tidally influenced lake. Freshwat Biol 51:705–716
Ferguson AJD (1997) The role of modelling in the control of toxic blue-green algae. Hydrobiologia 349:1–4
Ferris JM, Christian R (1991) Aquatic primary production in relation to microalgal responses to changing light: a review. Aquat Sci 53:187–217
Flores E, Herrero A (2005) Nitrogen assimilation and nitrogen control in cyanobacteria. Biochem Soc Trans 33:164–167
Flynn KJ (2003a) Do we need complex mechanistic photoacclimation models for phytoplankton? Limnol Oceanogr 48(6):2243–2249
Flynn KJ (2003b) Modelling multi-nutrient interactions in phytoplankton; balancing simplicity and realism. Prog Oceanogr 56(2):249–279
Flynn KJ (2005) Castles built on sand: dysfunctionality in plankton models and the inadequacy of dialogue between biologists and modellers. J Plankton Res 27(12):1205–1210
Forchhammer K (2004) Global carbon/nitrogen control by P_{II} signal transduction in cyanobacteria: from signals to targets. FEMS Microbiol Rev 28:319–333
Forchhammer K (2008) P_{II} signal transducers: novel functional and structural insights. Trends Microbiol 16(2):65–72
Francis G (1878) Poisonous Australian lake. Nature (London) 18:11–12
Franklin DJ, Brussaard CPD, Berges JA (2006) What is the role and nature of programmed cell death in phytoplankton ecology. Eur J Phycol 41:1–14
Ganf GG, Oliver RL (1982) Vertical separation of light and available nutrients as a factor causing replacement of green algae by blue-green algae in the plankton of a stratified lake. J Ecol 70:829–844
Ganf GG, Oliver RL, Walsby AE (1989) Optical properties of gas-vacuolate cells and colonies of *Microcystis* in relation to light attenuation in a turbid, stratified reservoir (Mount Bold reservoir, South Australia). Aust J Mar Freshw Res 40:595–611
Garcia-Gonzalez M, Sivak MN, Guerrero MG, Preiss J, Lara C (1992) Depression of carbon flow to the glycogen pool induced by nitrogen assimilation in intact cells of *Anacystis nidulans*. Physiol Plant 86:360–364
Geider RJ, La Roche J (2002) Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. Eur J Phycol 37:1–17
Geider RJ, MacIntyre HL, Kana TM (1996) A dynamic model of photoadaptation in phytoplankton. Limnol Oceanogr 41:1–15
Geider RJ, MacIntyre HL, Kana TM (1998) A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. Limnol Oceanogr 43:679–694
Ghadouani A, Pinel-Alloul B, Prepas EE (2003) Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. Freshw Biol 48:363–381
Gibson CE (1978) Carbohydrate content as an ecological tool in the study of planktonic blue-green algae. Verh Int Ver Theor Angew Limnol 20:630–635
Glazer AN, Gindt YM, Chan CF, Sauer K (1994) Selective disruption of energy flow from phycobilisomes to photosystem I. Photosynth Res 40:167–173
Gons HJ, Hoogveld HL, Simis SGH, Tijdens M (2006) Dynamic modelling of viral impact on cyanobacterial populations in shallow lakes: implications of burst size. J Mar Biol Assoc UK 86:537–542
Gotham U, Rhee G-Y (1981) Comparative kinetic studies of nitrate-limited growth and nitrate uptake in phytoplankton in continuous culture. J Phycol 17:309–314
Grant NG, Walsby AE (1977) The contribution of photosynthate to turgor pressure rise in the planktonic blue-green alga *Anabaena flos-aquae*. J Exp Bot 28:409–415
Grossman A, Bhaya D, Apt K, Kehoe D (1995) Light-harvesting complexes in oxygenic photosynthesis: Diversity, control, and evolution. Ann Rev Genet 29:231–287
Guerrero MG, Lara C (1987) Assimilation of inorganic nitrogen. In: Fay P, Van Baalen C (eds) The cyanobacteria. Elsevier, Amsterdam, pp 163–186, 543 pp
Guven B, Howard A (2006) A review and classification of the existing models of cyanobacteria. Prog Phys Geogr 30:1–24
Hamilton GS, Fielding F, Chiffings AW, Hart BT, Johnstone RW, Mengersen K (2007) Investigating the use of a Bayesian Network to model the risk of *Lyngbya majuscula* bloom initiation in Deception Bay, Queensland, Australia. Hum Ecol Risk Assess 13:1271–1287
Han B-P, Virtanen M, Koponen J, Straskraba M (2000) Effect of photoinhibition on algal photosynthesis: a dynamic model. J Plankton Res 22:865–885
Harris GP (1986) Phytoplankton ecology. Structure, function and fluctation. Chapman & Hall, London, 384 pp
Harris GP (1994) Pattern, process and prediction in aquatic ecology. A limnological view of some general ecological problems. Freshw Biol 32:143–160
Hayes PK, Walsby AE (1986) The inverse correlation between width and strength of gas vesicles in cyanobacteria. Br Phycol J 21:191–197
Healey FP (1978) Physiological indicators of nutrient deficiency in algae. Mitt Int Ver Theor Limnol 21:34–41
Healey FP (1982) Phosphate. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 105–124, 676 pp
Hecky RE, Kilham P (1988) Nutrient limitation of phytoplankton in freshwater and marine environments; a review of recent evidence on the effects of enrichment. Limnol Oceanogr 33:796–822
Hecky RE, Campbell P, Hendzel LL (1993) The stoichiometry of carbon, nitrogen and phosphorus in particulate matter of lakes and oceans. Limnol Oceanogr 38:709–724
Heinrich A, Maheswaran M, Ruppert U, Forchhammer K (2004) The *Synechococcus elongatus* PII signal transduction protein controls arginine synthesis by complex formation with N-acetyl-l-glutamate kinase. Mol Microbiol 52(5):1303–1314
Hense I, Beckmann A (2006) Towards a model of cyanobacteria life cycle-effects of growing and resting stages on bloom formation of N_2-fixing species. Ecol Model 195(3–4):205–218
Hense I, Burchard H (2009) Modelling cyanobacteria in shallow coastal seas. Ecol Model 221(2):238–244

Heraud P, Beardall J (2000) Changes in chlorophyll fluorescence during exposure of *Dunaliella tertiolecta* to UV radiation indicate a dynamic interaction between damage and repair processes. Photosynth Res 63:123–134

Herrero A, Muro-Pastor AM, Flores E (2001) Nitrogen control in cyanobacteria. J Bacteriol 183:411–425

Hodges BR, Imberger J (2001) Simple curvilinear method for numerical methods of open channels. J Hydraul Eng ASCE 127:949–958

Hodges BR, Imberger J, Saggio A, Winters KB (2000) Modelling basin-scale internal waves in a stratified lake. Limnol Oceanogr 45:1603–1620

Holl CM, Montoya JP (2005) Interactions between nitrate uptake and nitrogen fixation in continuous cultures of the marine diazotroph *Trichodesmium* (Cyanobacteria). J Phycol 41:1178–1183

Horne AJ, Galat DL (1985) Nitrogen fixation in an oligotrophic, saline desert lake: Pyramid Lake, Nevada. Limnol Oceanogr 30:1229–1239

Howard A, Irish AE, Reynolds CS (1996) A new simulation of cyanobacterial underwater movement (SCUM '96). J Plankton Res 18:1375–1385

Howarth RG, Butler T, Lunde K, Swaney D, Chu CR (1993) Turbulence and planktonic nitrogen fixation: a mesocosm experiment. Limnol Oceanogr 38:1696–1711

Howarth RW, Chan F, Marino R (1999) Do top-down and bottom-up controls interact to exclude nitrogen-fixing cyanobacteria from the plankton of estuaries: explorations with a simulation model. Biogeochemistry 46:203–231

Huisman J, Hulot FD (2005) Population dynamics of harmful cyanobacteria. Factors affecting species composition. In: Huisman J, Matthijs HCP, Visser PM (eds) Harmful cyanobacteria. Springer, Dordrecht, pp 143–176

Huisman J, Jonker RR, Zonneveld C, Weissing FJ (1999) Competition for light between phytoplankton species: experimental test of mechanistic theory. Ecology 80:211–222

Huisman J, Sharples J, Stroom J, Visser PM, Kardinaal WEA, Verspagen JMH, Sommeijer B (2004) Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology 85:2960–2970

Humphries SE, Lyne VD (1988) Cyanophyte blooms: the role of cell buoyancy. Limnol Oceanogr 33:79–81

Hutchinson PA, Webster IT (1994) On the distribution of blue-green algae in lakes: Wind-tunnel tank experiments. Limnol Oceanogr 39:374–382

Ibelings BW, Maberly SC (1998) Photoinhibition and the availability of inorganic carbon restrict photosynthesis by surface blooms of cyanobacteria. Limnol Oceanogr 43:408–419

Ibelings BW, Marijke V, Los HFJ, van der Molen DT, Mooij WM (2003) Fuzzy modeling of cyanobacterial surface waterblooms: validation with NOAA-AVHRR satellite images. Ecol Appl 13:1456–1472

Imberger J, Hamblin JF (1982) Dynamics of lakes, reservoirs, and cooling ponds. Annu Rev Fluid Mech 14:153–187

Imberger J, Patterson JC (1981) A dynamic reservoir simulation model-DYRESM 5. In: Fischer HB (ed) Transport models for inland and coastal waters. Academic Press, New York, pp 310–361

Imberger J, Patterson JC (1990) Physical limnology. In: Hutchinson JW, Wu T (eds) Advances in applied mechanics, vol 27. Academic, Boston, pp 303–475

Imboden D (2004) The motion of lake waters. In: O'Sullivan P, Reynolds CS (eds) The lakes handbook: limnology and limnetic ecology. Blackwell Science Ltd, Oxford, pp 115–152

Izaguirre G, Hwang CJ, Krasner SW, McGuire MJ (1982) Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems. Appl Environ Microbiol 43:708–714

Jassby AD, Platt T (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol Oceanogr 21:540–547

Jöhnk KD, Huisman J, Sharples J, Sommeijer B, Visser PM (2008) Summer heatwaves promote blooms of harmful cyanobacteria. Glob Change Biol 14(3):495–512

Kagami M, de Bruin A, Ibelings BW, van Donk E (2007) Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. Hydrobiologia 578:113–129

Kehr JC, Zilliges Y, Springer A, Disney MD, Ratner DD, Bouchier C, Seeberger PH, Tandeau de Marsac D, Dittmann E (2006) A mannan binding lectin is involved in cell–cell attachment in a toxic strain of *Microcystis aeruginosa*. Mol Microbiol 59:893–906

Kerfeld CA (2004) Structure and function of the water-soluble carotenoid-binding proteins of cyanobacteria. Photosynth Res 81(3):1573–1579

Kirk JTO (1983) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge, 401 pp

Kirk JTO (1991) Volume scattering function, average cosines, and the underwater light field. Limnol Oceanogr 36:455–467

Kirk JTO, Oliver RL (1995) Optical closure in an ultraturbid lake. J Geophys Res 100:13221–13225

Klausmeier CA, Litchman E, Daufresne T, Levin SA (2004) Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature 429:171–174

Klemer AR (1978) Nitrogen limitation of growth and gas vacuolation in *Oscillatoria rubescens*. Verh Int Ver Theor Angew Limnol 20:2293–2297

Klemer AR (1991) Effects of nutritional status on cyanobacterial buoyancy, blooms and dominance with special reference to inorganic carbon. Can J Bot 69:1133–1138

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

Klemer AR, Hendzel LL, Findlay DL, Hedin RA, Mageau MT, Konopka A (1995) Carbon availability and the pattern of cyanobacterial dominance in enriched low-carbon lakes. J Phycol 31:735–744

Kolber Z, Falkowski PG (1993) Use of active fluorescence to estimate phytoplankton photosynthesis *in situ*. Limnol Oceanogr 38:1646–1665

Kolodny NH, Bauer D, Bryce K, Klucevsek K, Lane A, Medeiros L, Mercer W, Moin S, Park D, Petersen J, Wright J, Yuen C, Wolfson AJ, Allen M (2006) Effect of nitrogen source on cyanophycin synthesis in *Synechocystis* sp. strain PCC 6308. J Bacteriol 188:934–940

Konopka A, Schnur M (1980) Effect of light intensity on macromolecular synthesis in cyanobacteria. Microb Ecol 6:291–301

Konopka A, Kromkamp J, Mur LR (1987a) Regulation of gas vesicle content and buoyancy in light or phosphate-limited cultures of *Aphanizomenon flos-aquae* (Cyanophyta). J Phycol 23:70–78

Konopka A, Kromkamp JC, Mur LR (1987b) Buoyancy regulation in phosphate-limited cultures of *Microcystis aeruginosa*. FEMS Microbiol Ecol 45:135–142

Kosten S, Huszar VLM, Becares E, Costa LS, van Donk E, Hansson LA, Jeppesenk E, Kruk C, Lacerot G, Mazzeo N, De Meester L, Moss B, Lurling M, Noges T, Romo S, Scheffer M (2012) Warmer climates boost cyanobacterial dominance in shallow lakes. Glob Change Biol 18(1):118–126

Kromkamp J (1987) Formation and functional significance of storage products in cyanobacteria. N Z J Mar Freshw Res 21:457–465

Kromkamp JC, Mur LR (1984) Buoyant density changes in the cyanobacterium *Microcystis aeruginosa* due to changes in the cellular carbohydrate content. FEMS Microbiol Lett 25:105–109

Kromkamp JC, Walsby AE (1990) A computer model of buoyancy and vertical migration in cyanobacteria. J Plankton Res 12:161–183

Kromkamp J, Konopka A, Mur LR (1986) Buoyancy regulation in a strain of *Aphanizomenon flos-aquae* (Cyanophyceae): the importance of carbohydrate accumulation and gas vesicle collapse. J Gen Microbiol 132:2113–2121

Kromkamp J, Konopka A, Mur LR (1988) Buoyancy regulation in light-limited continuous cultures *Microcystis aeruginosa*. J Plankton Res 10:171–183

Kruk C, Huszar VM, Peeters ETHM, Bonilla S, Coasta L, Rling MLU, Reynolds CS, Scheffer M (2010) A morphological classification capturing functional variation in phytoplankton. Freshw Biol 55:614–627

Laanemets J, Lilover M-J, Raudsepp U, Autio R, Vahtera E, Lips I, Lips U (2006) A fuzzy logic model to describe the cyanobacteria *Nodularia spumigena* blooms in the Gulf of Finland, Baltic Sea. Hydrobiologia 554:31–45

Latifi A, Ruiz M, Zhang CC (2009) Oxidative stress in cyanobacteria. FEMS Microbiol Rev 33(2):258–278

Levine SN, Lewis WM (1987) A numerical model of nitrogen fixation and its application to Lake Valencia, Venezuela. Freshw Biol 17:265–274

Lewis WM (1978a) Dynamics and succession of the phytoplankton in a tropical lake: Lake Lanao, Philippines. J Ecol 66:849–880

Lewis WM (1978b) Analysis of succession in a succession in a tropical phytoplankton community and a new measure of succession rate. Am Nat 112:401–418

Litchman E (2003) Competition and coexistence of phytoplankton under fluctuating light: experiments with two cyanobacteria. Aquat Microb Ecol 31:241–248

Liu Y, Xie P, Chen F, Wu X (2005) Effect of combinations of the toxic cyanobacterium *Microcystis aeruginosa* PCC7820 and the green alga *Scenedesmus* on the experimental population of *Daphnia pulex*. Bull Environ Contam Toxicol 74:1186–1191

Llácer JL, Fita I, Rubio V (2008) Arginine and nitrogen storage. Curr Opin Struct Biol 18:673–681

Los FJ (2009) Eco-hydrodynamic modelling of primary production in coastal waters and lakes using BLOOM. PhD thesis, Wageningen University, Wageningen. Available at http://edepot.wur.nl/1249

Los FJ, Villars MT, Van der Tol MWM (2008) A 3-dimensional primary production model (BLOOM/GEM) and its application to the southern North Sea (coupled physical-chemical-ecological model). J Mar Syst 74:259–274

Lund JWG, Reynolds CS (1982) The development and operation of large limnetic enclosures in Blelham Tarn, English Lake District, and their contribution to phytoplankton ecology. Prog Phycol Res 1:1–65

Luo L, Hamilton D(P), Han B (2010) Estimation of total cloud cover from solar radiation observations at Lake Rotorua, New Zealand. Solar Energy 84(3):501–506

Luque I, Flores E, Herrero A (1994) Nitrate and nitrite transport in the cyanobacterium *Synechococcus* sp. PCC 7942 are mediated by the same permease. Biochim Biophys Acta 1184:296–298

Luque I, Vazquez-Bermudez MF, Paz-Yepes J, Flores E, Herrero A (2004) In vivo activity of the nitrogen control transcription factor NtcA is subjected to metabolic regulation in Synechococcus sp strain PCC 7942. Fems Microbiol Lett 236(1):47–52

MacIntyre S (1993) Vertical mixing in a shallow, eutrophic lake: possible consequences for the light climate of phytoplankton. Limnol Oceanogr 38:798–817

MacIntyre HL, Kana TM, Anning T, Geider RJ (2002) Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. J Phycol 38(1):17–38

Mackerras AH, De Chazal NM, Smith GD (1990a) Transient accumulations of cyanophycin in *Anabaena cylindrica* and *Synechocystis* 6308. J Gen Microbiol 136:2057–2065

Mackerras AH, Youens BN, Weir RC, Smith GD (1990b) Is cyanophycin involved in the integration of nitrogen and carbon metabolism in the cyanobacteria *Anabaena cylindrica* and *Gloeothece* grown on light/dark circles? J Gen Microbiol 136:2049–2056

Maheswaran M, Ziegler K, Lokau W, Hagemann M, Forchhammer K (2006) PII-Regulated arginine synthesis controls accumulation of cyanophycin in *Synechocystis* sp. Strain PCC 6803. J Bacteriol 188(7):2730–2734

Mann NH (2006) Phages of cyanobacteria. In: Calendar R (ed) The bacteriophages. Oxford University Press, New York, pp 517–532, 746 pp

McNown JS, Malaika J (1950) Effects of particle shape of settling velocity on low Reynolds numbers. Trans Am Geophys Union 31:74–82

Middelboe M, Jacquet S, Weinbauer M (2008) Viruses in freshwater ecosystems: an introduction to the exploration of viruses in new aquatic habitats. Freshw Biol 53:1069–1075

Mooji WM, Trolle D, Jeppesen E, Arhonditsis G, Belolipetsky PV, Chitamwebwa DBR, Degermendzhy AG, DeAngelis DL, De Senerpont Domis LN, Downing AS, Elliott JA, Fragoso CR Jr, Gaedke U, Genova SN, Gulati RD, Håkanson L, Hamilton DP, Hipsey MR, 't Hoen J, Hülsmann S, Los FJ, Makler-Pick V, Petzoldt T, Prokopkin IG, Rinke K, Schep SA, Tominaga K, Van Dam AA, Van Nes EH, Wells SA, Janse JH (2010) Challenges and opportunities for integrating lake ecosystem modelling approaches. Aquat Ecol 44(3):633–667

Mur LR (1983) Some aspects of the ecophysiology of cyanobacteria. Ann Microbiol 134B:61–72

Murray AG (1995) Phytoplankton exudation: exploitation of the microbial loop as a defence against algal viruses. J Plankton Res 17:1079–1094

O'Brien KR, Meyer DL, Waite AM, Ivey GN, Hamilton DP (2004) Disaggregation of *Microcystis aeruginosa* colonies under turbulent mixing: laboratory experiments in a grid-stirred tank. Hydrobiologia 519:143–152

Oberhaus L, Briand JF, Leboulanger C, Jacquet S, Humbert JF (2007) Comparative effects of the quality and quantity of light and temperature on the growth of *Planktothrix agardhii* and *P. rubescens*. J Phycol 43(6):1191–1199

Ochoa de Alda JAG, Tapia MI, Fabrice F, Llama MJ, Serra JL (1996) Changes in nitrogen source modify distribution of excitation energy in the cyanobacterium *Phormidium laminosum*. Physiol Plant 97:69–78

Ohki K (1999) A possible role of temperate phage in the regulation of *Trichodesmium* biomass. Bull Inst Oceanogr (Monaco) 19:287–291

Ohlendiek U, Stuhr A, Siegmund H (2000) Nitrogen fixation by diazotrophic cyanobacteria in the Baltic Sea and transfer of the newly fixed nitrogen to picoplankton organisms. J Mar Syst 25:213–219

Okada M, Aiba S (1983a) Simulation of water bloom in a eutrophic lake. II: reassessment of buoyancy, gas vacuole and turgor pressure of *Microcystis aeruginosa*. Water Res 17:877–882

Okada M, Aiba S (1983b) Simulation of water-bloom in a eutrophic lake. III: modeling the vertical migration and growth of *Microcystis aeruginosa*. Water Res 17:883–893

Oliver R (1990) Optical properties of waters in the Murray-Darling Basin, south-eastern Australia. Aust J Mar Freshw Res 41:581–601

Oliver RL (1994) Floating and sinking in gas-vacuolate cyanobacteria. J Phycol 30:161–173

Oliver RL, Ganf GG (2000) Freshwater blooms. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 149–194, 669 pp

Oliver RL, Walsby AE (1984) Direct evidence for the role of light-mediated gas vesicle collapse in the buoyancy regulation of *Anabaena flos-aquae* (cyanobacteria). Limnol Oceanogr 29:879–886

Oliver RL, Whittington J (1998) Using measurements of variable chlorophyll-a fluorescence to investigate the influence of water movement on the photochemistry of phytoplankton. In: Imberger J (ed) Physical processes in lakes and oceans, Coastal and Estuarine Studies, Vol 54,American Geophysical Union, Washington, DC, pp 517–534, 668pp

Oliver RL, Kinnear AJ, Ganf GG (1981) Measurements of cell density of three freshwater phytoplankters by density gradient centrifugation. Limnol Oceanogr 26:285–294

Oliver R, Whittington J, Lorenz Z, Webster I (2003) The influence of vertical mixing on the photoinhibition of variable chlorophyll *a*

fluorescence and its inclusion in a model of phytoplankton photosynthesis. J Plankton Res 25(9):1107–1129
Ormerod JG (1992) Physiology of the photosynthetic prokaryotes. In: Mann NH, Carr NG (eds) Photosynthetic prokaryotes. Plenum Press, New York, pp 93–120
Patterson JC, Hamilton DP, Ferris J (1994) Modelling of cyanobacterial blooms in the mixed layer of lakes and reservoirs. Aust J Mar Freshw Res 45:829–845
Pollard P, Young LM (2010) Lake viruses lyse cyanobacteria, *Cylindrospermopsis raciborskii*, enhances filamentous-host dispersal in Australia. Acta Oecol 36:114–119
Rabouille S, Staal M, Stal LJ, Soetaert K (2006) Modeling the dynamic regulation of nitrogen fixation in the cyanobacterium *Trichodesmium* sp. Appl Environ Microbiol 72(5):3217–3227
Raven JA (2006) Aquatic viruses: the emerging story. J Mar Biol Assoc UK 86:449–451
Recknagel F, Cetin L, Zhang B (2008) Process-based simulation library SALMO-OO for lake ecosystems. Part 1: object-oriented implementation and validation. Ecol Inform 3:170–180
Reichwaldt ES, Stibor H (2005) The impact of diel vertical migration of *Daphnia* on phytoplankton dynamics. Oecologia 146:50–56
Reynolds CS (1984) The ecology of freshwater phytoplankton. Cambridge Univ. Press, Cambridge, 384 pp
Reynolds CS (1987) Cyanobacterial water blooms. In: Callow JA (ed) Advances in botanical research, vol 13. Academic, London, pp 67–143
Reynolds CS (1990) Temporal scales of variability in pelagic environments and the response of phytoplankton. Freshw Biol 23:25–53
Reynolds CS (1993) Scales of disturbance and their roles in plankton ecology. Hydrobiologia 249:151–171
Reynolds CS, Walsby AE (1975) Water blooms. Biol Rev 50:437–481
Reynolds CS, Wiseman SW, Clarke MJO (1984) Growth- and loss-rate responses of phytoplankton to intermittent artificial mixing and their potential application to the control of planktonic algal biomass. J Appl Ecol 21:11–39
Reynolds CS, Oliver RL, Walsby AE (1987) Cyanobacterial dominance: the role of buoyancy regulation in the billowing environment. N Z J Mar Freshw Res 2:379–390
Reynolds CS, Irish AE, Elliott JA (2001) The ecological basis for simulating phytoplankton responses to environmental change (PROTECH). Ecol Model 140(3):271–291
Richardson K, Beardell J, Raven JA (1983) Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phycol 93:157–191
Riegman R, Mur LC (1984) Regulation of phosphate uptake kinetics in *Oscillatoria agardhii*. Arch Microbiol 139:28–32
Robson BJ, Hamilton DP (2004) Three-dimensional modelling of a *Microcystis* bloom event in the Swan River estuary. Ecol Model 174:203–222
Romans KM, Carpenter EJ, Bergman B (1994) Buoyancy regulation in the colonial diazotrophic cyanobacterium *Trichodesmium tenue*: ultrastructure and storage of carbohydrate, polyphosphate and nitrogen. J Phycol 30:935–942
Ross ON, Geider RJ (2009) New cell-based model of photosynthesis and photo-acclimation: accumulation and mobilisation of energy reserves in phytoplankton. Mar Ecol Prog Ser 383:53–71
Ross ON, Sharples J (2004) Recipe for 1-D Langrangian particle tracking models in space-varying diffusivity. Limnol Oceanogr Method 2:289–302
Ross C, Santiago-Vazquez L, Paul V (2006) Toxin release in response to oxidative stress and programmed cell death in the cyanobacterium *Microcystis aeruginosa*. Aquat Toxicol 78:66–73
Sakamoto M (1966) Primary production by phytoplankton community in some Japanese lakes and its dependence upon lake depth. Arch Hydrobiol 62:1–28
Sathyendranath S, Platt T, Forget MH (2007) Oceanic primary production: comparison of models. Oceans 2007 – Europe international conference, Aberdeen, Scotland, IEEE
Sauer J, Schreiber U, Schmid R,Volker U, Forchhammer K (2001). Nitrogen starvation-induced chlorosis in Synechococcus PCC 7942. Low-level photosynthesis as a mechanism of long-term survival. Plant Physiol 126:233 –243
Scheffer M, Rinaldi S, Gragnani A, Mur LR, van Ness EH (1997) On the dominance of filamentous cyanobacteria in shallow, turbid lakes. Ecology 78:72–282
Schindler DW (1971) Carbon, nitrogen and phosphorus and the eutrophication of freshwater lakes. J Phycol 7:321–329
Schindler DW, Hecky RE, Findlay DL, Stainton MP, Parker BR, Paterson MJ, Beaty KG, Lyng M, Kasian SEM (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proc Natl Acad Sci USA 105(32):11254–11258
Schopf JW, Packer BM (1987) Early Archean (3.3 billion to 3.5 billion-year-old) microfossils from Warrawoona Group, Australia. Science 237:70–73
Schreiber U, Endo T, Mi H, Asada K (1995) Quenching analysis of chlorophyll fluorescence by the saturation pulse method: particular aspects relating to the study of eukaryotic algae and cyanobacteria. Plant Cell Physiol 36:873–882
Schwarz R, Forchhammer K (2005) Acclimation of unicellular cyanobacteria to macronutrient deficiency: emergence of a complex network of cellular responses. Microbiology 151:2503–2514
Scott JT, McCarthy MJ (2010) Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. Limnol Oceanogr 55(3):1265–1270
Sedmak B, Elersek T (2006) Microcystins induce morphological and physiological changes in selected representative phytoplanktons. Microb Ecol 51:508–515
Segovia M, Haramaty L, Berges JA, Falkowski PG (2003) Cell death in the unicellular chlorophyte *Dunaliella tertiolecta*: an hypothesis on the evolution of apoptosis in higher plants and metazoans. Plant Physiol 132:99–105
Shilo M (1970) Lysis of blue-green algae by myxobacter. J Bacteriol 104:453–461
Shilo M (1971) Biological agents that cause lysis of blue-green algae. Mitt Int Ver Theor Limnol 19:206–213
Shuter B (1979) A model of physiological adaptation in unicellular algae. J Theor Biol 78(4):519–552
Sigee DC, Selwyn A, Gallois P, Dean AP (2007) Patterns of cell death in freshwater colonial cyanobacteria during the late summer bloom. Phycologia 46:284–292
Simon RD (1987) Inclusion bodies in the cyanobacteria: cyanophycin, polyphosphate, polyhedral bodies. In: Fay P, van Baalen C (eds) The cyanobacteria. Elsevier, Amsterdam, pp 199–225
Singh AK, Elvitigala T, Bhattacharyya-Pakrasi M, Aurora R, Ghosh B, Pakrasi HB (2008) Integration of carbon and nitrogen metabolism with energy production is crucial to light acclimation in the cyanobacterium *Synechocystis*. Plant Physiol 148(1):467–478
Singh AK, Bhattacharyya-Pakrasi M, Elvitigala T, Ghosh B, Aurora R, Pakrasi HB (2009) A systems-level analysis of the effects of light quality on the metabolism of a cyanobacterium. Plant Physiol 151:1596–1608
Smith VH (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green in lake phytoplankton. Science 221:669–671
Sommer U, Sommer F, Santer B, Zellner E, Jergens K, Jamieson C, Boersma M, Gocke K (2003) *Daphnia* versus copepod impact on summer phytoplankton: functional compensation at both trophic levels. Oecologia 135:639–647
Stal LJ (2009) Is the distribution of nitrogen-fixing cyanobacteria in the oceans related to temperature? Environ Microbiol 11:1632–1645
Stal LJ, Walsby AE (1998) The daily integral of nitrogen fixation by planktonic cyanobacteria in the Baltic Sea. New Phytol 139:665–671
Stal LJ, Walsby AE (2000) Photosynthesis and nitrogen fixation in a cyanobacterial in the Baltic Sea. Eur J Phycol 35:97–108

Steinberg CEW, Hartmann HM (1988) Planktonic bloom-forming cyanobacteria and the eutrophication of lakes and rivers. Freshw Biol 20:279–287

Stomp M, Huisman J, de Jongh F, Veraart AJ, Gerla D, Rijkeboer M, Ibelings BW, Wollenzien UIA, Stal LJ (2004) Adaptive divergence in pigment composition promotes phytoplankton biodiversity. Nature 432(7013):104–107

Stomp M, Huisman J, Stal LJ, Matthijs HCP (2007) Colorful niches of phototrophic microorganisms shaped by vibrations of the water molecule. ISME J 1(4):271–282

Stone S, Ganf G (1981) The influence of previous light history on the respiration of four species of freshwater phytoplankton. Arch Hydrobiol 91:435–462

Takano K, Ishikawa Y, Mikami H, Igarashi S, Hino S, Yoshioka T (2008) Fungal infection for cyanobacterium *Anabaena smithii* by two chytrids in eutrophic region of large reservoir Lake Shumarinai, Hokkaido, Japan. Limnology 9:213–218

Tandeau de Marsac N, Houmard J (1993) Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol Rev 104:119–190

Tandeau de Marsac N, Lee HM, Hisbergues M, Castets AM, Bédu S (2001) Control of nitrogen and carbon metabolism in cyanobacteria. J Appl Phycol 13:287–394

Tapia MI, Ochoa de Alda JAG, Llama MJ, Serra JL (1996) Changes in intracellular amino acids and organic acids induced by nitrogen starvation and nitrate or ammonium resupply in the cyanobacterium *Phormidium laminosum*. Planta 198:526–531

Thompson PA, Waite AM, McMahon K (2003) Dynamics of a cyanobacterial bloom in a hypereutrophic, stratified weir pool. Mar Freshw Res 54:27–37

Tilman D, Kilham SS, Kilham P (1982) Phytoplankton community ecology: the role of limiting nutrients. Annu Rev Ecol Syst 13:349–372

Trimbee AM, Prepas EE (1987) Evaluation of total phosphorus as a predictor of the relative biomass of blue-green algae with emphasis on Alberta lakes. Can J Fish Aquat Sci 14:1337–1342

Trolle D, Hamilton DP, Pilditch CA, Duggan IC (2010) Predicting the effects of climate change on trophic status of three morphologically varying lakes: implications for lake restoration and management. Environ Model Softw 26:354–370

Tucker S, Pollard P (2005) Identification of cyanophage Ma-LBP and infection of the cyanobacterium *Microcystis aeruginosa* from an Australian subtropical lake by the virus. Appl Environ Microbiol 71:629–635

Turpin DH (1991) Effects of inorganic N availability on algal photosynthesis and carbon metabolism. J Phycol 27:14–20

Utkilen HC, Oliver RL, Walsby AE (1985) Buoyancy regulation in a red *Ocillatoria* unable to collapse gas vacuoles by turgor pressure. Arch Hydrobiol 102:319–329

van Rijn J, Shilo M (1985) Carbohydrate fluctuations, gas vacuolation, and vertical migration of scum-forming cyanobacteria in fishponds. Limnol Oceanogr 30(6):1219–1228

Vázquez-Bermúdez MF, Herrero A, Flores E (2003) Carbon supply and 2-oxoglutarate effects on expression of nitrate reductase and nitrogen-regulated genes in *Synechococcus* sp. strain PCC 7942. FEMS Microbiol Lett 221(2):155–159

Venkatesh MM, Maidment DR, Hodges BR (2005) Geospatial representation of river channels. J Hydrol Eng ASCE 10:243–251

Verspagen JMH, Snelder EOFM, Visser PM, Huisman J, Mur LR, Ibelings BW (2004) Recruitment of benthic *Microcystis* (Cyanophyceae) to the water column: internal buoyancy changes or resuspension? J Phycol 40:260–270

Vincent WF (2009) Cyanobacteria. In: Likens GE (ed) Encyclopedia of inland waters, vol 3. Elsevier, Oxford, pp 226–232

Visser PM, Ibelings BW, Mur LR (1994) Autumnal sedimentation of *Microcystis* spp. as result of an increase in carbohydrate ballast at reduced temperature. J Plankton Res 17:919–933

Visser PM, Ibelings BW, Van Der Veer B, Koedoods J, Mur LR (1996) Artificial mixing prevents nuisance blooms of the cyanobacterium *Microcystis* in Lake Nieuwe Meer, The Netherlands. Freshw Biol 36:435–450

Visser PM, Passarge J, Mur LR (1997) Modelling vertical migration of the cyanobacteria *Microcystis*. Hydrobiologia 349:99–109

Vollenweider RA (1968) Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Technical report DA 5/SCI/68.27. OECD, Paris, 250 pp

Wallace BB, Hamilton DP (1999) The effect of variations in irradiance on buoyancy regulation in *Microcystis aeruginosa*. Limnol Oceanogr 44:273–281

Wallace BB, Hamilton DP (2000) Simulation of water-bloom formation in the cyanobacterium Microcystis aeruginosa. J Plankton Res 22(6):1127–1138

Wallace BB, Bailey MC, Hamilton DP (2000) Simulation of vertical position of buoyancy regulating *Microcystis aeruginosa* in a shallow eutrophic lake. Aquat Sci 62:320–333

Walsby AE (1971) The pressure relationships of gas vacuoles. Proc R Soc Lond B 178:301–326

Walsby AE (1985) The permeability of heterocysts to the gases nitrogen and oxygen. Proc R Soc Lond B 226:345–366

Walsby AE (1987) Mechanisms of buoyancy regulation by planktonic cyanobacteria with gas vesicles. In: Fay P, Van Baalen C (eds) The cyanobacteria. Elsevier Science Publishers, Amsterdam, pp 377–392, 543 pp

Walsby AE (1994) Gas vesicles. Microbiol Rev 58:94–144

Walsby AE (2007) Cyanobacterial heterocysts: terminal pores proposed as sites of gas exchange. Trends Microbiol 15:340–349

Walsby AE, Bleything A (1988) The dimensions of cyanobacterial gas vesicles in relation to their efficiency in providing buoyancy and withstanding pressure. J Gen Microbiol 134:2635–2645

Walsby AE, Reynolds CS (1980) Sinking and floating. In: Morris I (ed) The physiological ecology of phytoplankton. Blackwell Scientific Publications, Oxford, pp 371–412

Walsby AE, Reynolds CS, Oliver RL, Kromkamp J, Gibbs MM (1987) The role of buoyancy in the distribution of *Anabaena* sp. in Lake Rotongaio. N Z J Mar Freshw Res 21:525–526

Walsby AE, Reynolds CS, Oliver RL, 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. Arch Hydrobiol Ergeb Limnol 32:1–25

Walsby AE, Kinsman R, Ibelings BW, Reynolds CS (1991) Highly buoyant colonies of the cyanobacterium *Anabaena lemmermannii* form persistent surface waterblooms. Arch Hydobiol 121:261–280

Walsby AE, Hayes PK, Boje R, Stal LJ (1997) The selective advantage of buoyancy provided by gas vesicles for planktonic cyanobacteria in the Baltic Sea. New Phytol 136:407–417

Walsby AE, Dubinsky Z, Kromkamp JC, Lehmann C, Schanz F (2001) The effects of diel changes in photosynthetic coefficients and depth of *Planktothrix rubescens* on the daily integral of photosynthesis in Lake Zürich. Aquat Sci 63:326–349

Webster IT, Hutchinson PA (1994) Effects of wind on the distribution of phytoplankton cells in lakes-revisited. Limnol Oceanogr 39:365–373

Wilhelm S, Matteson AR (2008) Freshwater and marine virioplankton: a brief overview of commonalities and differences. Freshw Biol 53:1076–1089

Wood SA, Prentice MJ, Smith K, Hamilton DP (2010) Low dissolved inorganic nitrogen and increased heterocyte frequency: precursors to *Anabaena planktonica* blooms in a temperate, eutrophic reservoir. J Plankton Res 32:1315–1325

Wyman M, Fay P (1986) Underwater light climate and the growth and pigmentation of planktonic blue-green algae (Cyanobacteria) I. the influence of light quality. Proc R Soc Lond B 227:381–393

Yang Z, Kong F, Shi X, Cao H (2006) Morphological response of *Microcystis aeruginosa* to grazing by different sorts of zooplankton. Hydrobiologia 563:225–230

Yang Z, Kong F, Shi X, Zhang M, Xing P, Cao H (2008) Changes in the morphology and polysaccharide content of *Microcystis aeruginosa* (Cyanobacteria) during flagellate grazing. J Phycol 44:716–720

Zevenboom W, Mur LR (1978) On nitrate uptake by *Oscillatoria agardhii*. Verh Int Ver Limnol 20:2302–2307

Zhao J, Ramin M, Cheng V, Arhonditsis B (2008) Competition patterns among phytoplankton functional groups: how useful are the complex mathematical models? Acta Oecol 33(3): 324–344

Zilliges Y, Kehr JC, Mikkat S, Bouchier C, Tandeau N, de Marsac T, Borner E, Dittmann E (2008) An extracellular glycoprotein is implicated in cell-cell contacts in the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806. J Bacteriol 190:2871–2879