<u>Appendix D Cyanobacterial growth in</u> <u>constructed water bodies</u>

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Introduction

Algal growth can occur rapidly under favorable conditions in open water bodies. Nuisance growths (blooms) of cyanobacteria (Blue-green algae) can occur in both natural and constructed water bodies. In constructed water bodies it is important to ensure that designs include measures to restrict cyanobacterial growth. Cyanobacterial blooms can have adverse effects on aquatic ecosystem function, Aesthetics and public amenity. Some species of cyanobacteria are of particular concern because of their potential to produce toxins.

D.1 Light

In Australian climatic conditions surface light is rarely a limiting factor for algal growth. Cyanobacterial responses to various light conditions differ between species. Turbidity and mixing conditions within a water body can determine the light environment that algal cells are exposed to by circulating them in and out of the euphotic zone. Typically, cyanobacterial growth rates are reduced under fluctuating light conditions such as those found in well mixed water columns (Mitrovic *et al*, 2003).

Some cyanobacterial species can regulate cell buoyancy and migrate vertically, increasing their exposure to optimum light intensities. Cell buoyancy regulation offers cyanobacteria considerable advantage over other phytoplankton that are distributed evenly throughout the water column (Mitrovic *et al*, 2001). However, this buoyancy advantage is dependent on the mixing regime and degree of turbulence that the cells are exposed to within the water column (Brookes *et al*, 2003).

Depth of light penetration can be reduced by turbidity and therefore limit biomass development. The extent to which turbidity will reduce light availability to cells, and therefore reduce biomass development, depends on the mixing patterns of the water body and the degree of cell buoyancy regulation.

D.2 Temperature

Temperature is an important factor in many cyanobacterial bloom occurrences in Australia. In temperate zones cyanobacterial blooms commonly occur in the warmer months. Cyanobacteria tend to have high optimal growth temperatures compared to green algae and diatoms and achieve maximum growth rates at around 25°C (Chorus and Bartram, 1999).

D.3 Nutrients

Many cyanobacterial blooms are associated with elevated nutrient levels. However, nutrient availability in many aquatic environments is generally adequate to achieve cyanobacterial growth of bloom proportions when other factors such as temperature and hydrodynamics are also favourable. Many of the nuisance species of cyanobacteria are capable of fixing atmospheric nitrogen however, this process requires considerable amounts of energy and may be limited in turbid environments (Chorus and Bartram, 1999).

D.4 Hydrodynamics

A key parameter of aquatic ecosystems is hydraulic detention time (Harris, 1996; Jorgensen, 2003). Long detention times during warm weather in poorly mixed water bodies often leads to persistent stratification of the water column. Periods of stratification of a water body can also facilitate the release of nutrients from the sediments which can act to support algal growth. In lowland rivers and lakes, cyanobacterial blooms are more prevalent during periods of persistent stratification, a condition associated with low flows (Sherman *et al*, 1998). Cyanobacterial species that can regulate their buoyancy, and migrate vertically through the water column, have a competitive advantage over other phytoplankton under stratified conditions (Atlas and Bartha, 1998). Buoyancy regulation allows cell movement between the nutrient rich hypolimnetic waters and the euphotic zone so as to access both high nutrient and optimal light conditions.

In deep water bodies, hydraulic mixing and the breakdown of stratification can slow the growth of cyanobacteria and reduce the prevalence of excessive growth. Hydraulic mixing reduces growth rates by circulating cells below the euphotic zone for long enough to limit light availability, reducing carbohydrate accumulation and exhausting the energy supply required for growth and replication (Brookes *et al*, 2003).

In shallow water bodies, where the ratio of mixing depth: euphotic zone depth is < 3-5, mixing is typically insufficient to reduce growth (Oliver *et al*, 1999). Under such conditions, hydraulic detention time becomes a crucial factor in the control and prevention of excessive algal growth. When the hydraulic detention time is reduced the biomass becomes regulated by the rate at which it is removed from the lake by flushing (Reynolds, 2003).

D.5 Growth Rates

Assuming adequate light and nutrient availability, a model of algal growth can be developed using a simple relationship between time and growth rate at various temperatures. The exponential growth rate equation is $\mu = (1/t) \times \ln(N_t/N_0)$, where μ is the growth rate, t is the number of days, N_t is the final cell concentration and N_0 the starting cell concentration. This simple model can be used to determine how long it will take for an algal population to reach bloom proportions (15,000 cells/mL) and hence inform the development of guidelines on water body hydraulic detention time.

D.6 Common growth rate range

Under favorable growth conditions (20°C and light saturation) laboratory cultures of planktonic cyanobacteria have growth rates of between 0.21 and 0.99 day⁻¹, or 0.3 to 1.4 doublings per day respectively (Chorus and Bartram, 1999). Figure 1 illustrates theoretical growth curves based on growth rates of laboratory grown cultures that have been adjusted to account for a slower growth rate (0.5 normal growth rate) at night (12 out of 24 hours). The graphs are indicative of the range of growth rates both between species and between individual populations of the same species grown in laboratory cultures.



Figure D.1. Common cyanobacterial growth rate range illustrated using theoretical growth curves based on growth rates of laboratory grown cultures (20°C and light saturation) adjusted for a 12h:12h light dark cycle. Growth curves were constructed using an initial algal cell concentration of 50 cells/mL.

These results illustrate the wide range of growths rates that have been recorded for cyanobacteria and suggest that, under ideal conditions at 20°C, laboratory cultured cyanobacteria can achieve bloom conditions in 9 – 41 days depending on the species.

D.7 Laboratory cultures v in situ growth rates

Physiological characteristics such as maximum photosynthetic capabilities, photoinhibition levels and flotation rates (speeds of vertical movement) vary considerably between cyanobacterial species and between individual populations within species. Growth rates also decrease with increasing cell or colony sizes (Reynolds 1984). Environmental variables, such as those discussed earlier, influence which species will dominate and the maximum growth rate. Typically, slower *in situ* growth rates occur as a result of these environmental variables. The relationship between laboratory growth rates and *in situ* growth rates is poorly understood. For example, *Microcystis* rarely grows in colonial form when grown in laboratory cultures however, successful growth of colonies in culture have shown much slower growth rates than those recorded previously from unicellular cultures (Reynolds, 1984). As a result, *in situ* growth rates are more desirable to use in models attempting to predict *in situ* conditions.

D.8 Mixing conditions

Westwood and Ganf (2004) measured the *in situ* growth of *Anabaena circinalis* in the Murray River at Morgan (Table D.1). Growth was measured under well mixed and persistently stratified conditions and also under conditions that take into account a range of typical flotation velocities (or mixing conditions) recorded for *A. circinalis* populations (0.01 to 0.40 m h⁻¹).

Species	Hydrodynamic Treatment	Growth rates (day-1)	Reference	
Anabaena circinalis	Persistent stratification	0.43		
	1.0 m h ⁻¹ mixing rate (diurnal stratification)	0.23		
	0.5 m h ⁻¹ mixing rate	0.15	Westwood and Ganf (2004)	
	(diurnal stratification)			
	Well mixed	0.19		

Table D.1. In situ growth rates for Anabaena circinalis under various mixing conditions

Figure D.2 has been constructed based on the *in situ* growth rates of *A. circinalis* recorded by Westwood and Ganf (2004). With starting cell concentrations of 50 cells/mL, the measured growth rates of neutrally buoyant populations under well-mixed conditions suggested the population would take approximately 31 days to reach bloom proportions. Under persistently stratified conditions, bloom proportions would be reached within 14 days. *A. circinalis*

populations with flotation velocities of 0.5 and 1.0 m h^{-1} , and under diurnally stratified conditions, would take longer than 25 days to reach bloom proportions.

Water bodies incorporating best practice design features are assumed to be relatively shallow (< 2.5 - 3.0 m), have a flat bottom and be subject to wind mixing. These design features are assumed to prevent persistent stratification and create systems that are well mixed or only diurnally stratified. Where diurnal stratification occurs, mixing rates during the non-stratified period are expected to be relatively fast due to the shallow nature of the water body. As a result, *in situ* growth rates for a fully mixed system and *in situ* growth rates for a partially mixed system with a relatively fast mixing rate, have been adopted. Figure D.2 shows the expected mixing conditions for water bodies that incorporates best management practice design features.



Figure D.2. Growth of *Anabaena circinalis* under various mixing conditions illustrated using growth curves constructed from data collected *in situ* (Westwood and Ganf, 2004) and assuming starting cell concentrations of 50 cells/mL. Area of shading represents the range of mixing conditions likely to be found in best practice design systems.

D.9 Temperature effects

Provided that other factors (e.g. light, nutrients) remain non-limiting, maximum growth rates of cyanobacteria respond directly to changes in temperature. Specific responses to temperature changes differ between species but, typically, growth rates increase with increasing temperature (Reynolds, 1984). The effect of temperature can be accounted for by adjusting growth rates using Q₁₀ values⁶. Data presented in Table D.2 indicate that Q₁₀ values can vary significantly between species.

 $^{^{6}}$ Q₁₀ is the temperature coefficient (Q_v) that represents the increase in growth rate that occurs with a 10°C increase in temperature.

Genus	Q10 Range	Temperature Range (°C)	Reference	
Asterionella, Anabaena, Aphanizomenon and Oscillatoria	1.8 - 2.9	10 - 20	Reynolds (1984)	
<i>Microcystis, Merismopedia</i> and <i>Oscillatoria</i>	1.97 - 4.16	15 - 25	Coles and Jones (2000)	

D.10 Starting concentration

The theoretical growth rate curves are constructed using initial cell counts of 2 cells/mL and 50 cells/mL which represent typical natural background levels. Webster *et al* (2000) found blooms in the Maude Weir pool forming from initial concentrations of 10 cells/mL. It is clear that the initial starting concentration can influence the time required to reach bloom proportions (although the degree of influence will be depend on the growth rate). For instance for *Anabaena circinalis* in well mixed conditions and 20°C, starting concentrations of 2 and 50 cells/mL result in bloom proportions of 15,000 cells/mL after approximately 33 and 51 days respectively, as illustrated in Figure D.3.



Figure D.3. Cyanobacterial growth curves at starting concentrations of 2 and 50 cells/mL. Constructed using growth rates of *A. circinalis* measured *in situ*, under well mixed conditions (Westwood and Ganf, 2004), adjusted for 20°C (Q₁₀ 2.9). The number of days taken to reach bloom proportions varies from 33 to 51 days depending on the starting cell concentration.

D.11 Detention time

Reynolds (2003) recommends that the sensitivity of lakes to eutrophication, in relation to changes in external phosphorus loads, can be classified according to hydraulic detention time. Short detention times weaken the response of lakes to changes in external phosphorus loads. The weakened response of lakes, to changes in phosphorus loads, is due to the biomass becoming regulated by the rate at which it is removed from the lake by flushing, rather than the availability of phosphorus (Reynolds, 2003). The most sensitive lakes being those with a detention time of greater than 30 days. Lakes with a detention time of 3 - 30 days are only slightly sensitive to changes in external phosphorus loads, while lakes with a detention time of less than 3 days are not at all sensitive to changes in phosphorous loads (Reynolds, 2003).

In the Australian climate, designing constructed water bodies with a detention time of less than 3 days is neither practical nor achievable. An upper limit of 30 days may be applied as a general precaution to ensure that water bodies do not lie within the 'very sensitive' category of >30 days detention time. Wagner-Lotkowska *et al* (2004) recommend a hydraulic detention time of less than 30 days for the control of algal blooms in medium sized reservoirs.

Wastewater treatment ponds could be viewed as ideal environments for algal growth (shallow, adequate light, high nutrients). However, experience has shown (e.g. Breen, 1983) that it is rare to get cyanobacteria dominating the phytoplankton community in wastewater treatment ponds with detention times below 30 days.

D.12 Model parameters

From the information presented in previous sections, the values presented in Table D.3 have been adopted to create a model appropriate for water bodies with best management practice design. These systems are assumed to be shallow, have a flat bottom and are generally well mixed. A reasonable assumption is that the hydrodynamic conditions in a best management practice design varies somewhere between fully mixed and diurnally, partially mixed as represented by the shaded zone in Figure D.2.

Variable	Value	Comment	Reference
Hydrodynamics	Well mixed to 1.0 m h ⁻¹ with diurnal stratification	Water bodies incorporating best practice design are assumed to be relatively shallow, have a flat bottom and be easily mixed by wind. As a result, <i>in situ</i> growth rates for a fully mixed system and a partially mixed system with a relatively fast mixing rate, have been adopted. From Figure 2 this approach is considered conservative.	Mixing values from Westwood and Ganf (2004)
Growth rate	0.19 to 0.23 day-1	Adoption of <i>in situ</i> growth rate of a common nuisance cyanobacterial species (<i>Anabaena circinalis</i>) is considered reasonable given the frequency of <i>Anabaena</i> in blooms.	Westwood and Ganf (2004)

Table D.3. Summary of model parameters

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Variable	Value	Comment	Reference
Q10	2.9	Adoption of the upper limit of the range of Q ₁₀ values recorded for various genera including <i>Anabaena</i> is considered a conservative assumption.	Reynolds (1984)
Temperature range	15-25°C	Likely temperature ranges of surface waters in Victoria	
Starting concentrations	50 cells/mL	Conservative, or likely upper limit, of background cell concentrations for cyanobacteria in water bodies without chronic bloom problems.	

D.13 Modelling Results

Model results are shown in Figures D.4 and D.5 for partially and well mixed systems respectively. The temperature ranges can be broadly interpreted in Victoria as follows:

- 15°C Use for upland sites in the Eastern and Western Ranges.
- 20°C Use for lowland sites south of the Great Dividing Range.
- 25°C Use for lowland sites north of the Great Dividing Range.

The values represent summer water temperatures. Local water body temperature will clearly vary from site to site and within different years. Where local water temperature data are available they should be used to guide the selection of the critical detention time.



Figure D.4. Growth curves illustrating modelled times for cyanobacterial populations to reach bloom proportions under different temperature conditions and 1 m h⁻¹ mixing conditions with diurnal stratification. Based on growth rates of *A. circinalis* measured *in situ* (Westwood and Ganf, 2004) adjusted for temperature, Q_{10} 2.9, and assuming 50 cells/mL starting concentrations.

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Figure D.5. Growth curves illustrating modelled times for cyanobacterial populations to reach bloom proportions under different temperature conditions and well mixed conditions. Based on growth rates of *A. circinalis* measured *in situ* (Westwood and Ganf, 2004) adjusted for temperature, Q₁₀ 2.9, and assuming 50 cells/mL starting concentrations.

Target detention times for the modelled temperature ranges are summarized in Table D.4 for both partially and well mixed systems. It is likely that the hydrodynamic state of best practice design water bodies would move between the proposed mixing conditions.

Table D.4. Modelled times for	cyanobacterial po	pulations to reach bl	oom proportions	under different tem	perature conditions.
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Variables	Iles Partially Mixed Fully Mixed					
Temperature (°C)	15	20	25	15	20	25
Time (days)	46	27	19	55	33	21

The modeling approach taken is considered to be reasonably conservative. For example it adopts:

- Non-limiting conditions for nutrient and light availability
- Growth rates for a known nuisance species (Anabaena circinalis)
- Summer temperature values (the main risk period)
- High starting population concentrations (50 cells/mL)

As a result, a probabilistic approach to the use of detention time criteria is recommended. A 20% exceedance is suggested as an acceptable risk to compensate for the occurrence of all other risk factors being favorable for algal growth. The 20% exceedance of a specific detention time objective does not indicate that a bloom will occur; just that detention time

(for a given temperature range) is long enough for exponential growth to achieve a bloom alert level of 15,000 cells/mL if all other risk factors were favourable. The 20% exceedance value is an interim value chosen as a relatively conservative estimate of the general variation in ecological factors in the Australian environment.

D.14 Recommended design criteria

The following guideline detention times are recommended. For water bodies with summer water temperatures in the following ranges, the 20% tile detention times should not exceed:

- 50 days (15°C)
- 30 days (20°C)
- 20 days (25°C)

These values are broadly consistent with literature detention time values considered to be protective against the risk of cyanobacterial blooms (Reynolds 2003, Wagner-Lotkowska *et al* 2004) and consistent with current industry experience.

D.15 Acknowledgements

Thank you to Professor Barry Hart, Water Studies Centre, Monash University, for providing a helpful review of the first draft of this technical note and also to Melbourne Water for supporting this work.

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