Growth of *Anabaena circinalis* in the Lower Murray River, South Australia

by

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Summary

An increase in the incidence and severity of *Anabaena circinalis* blooms in the Murray-Darling Basin has reduced water quality over recent decades. The aim of this thesis was to examine the growth of *A. circinalis* and discern the factors that are primarily responsible for control. The project concentrated on the highly regulated lower Murray River that provides a vital water supply to urban and country areas in the state of South Australia. From current knowledge of *A. circinalis* and the attributes of the lower Murray River, it was hypothesised that phosphorus storage, nitrogen-fixation and buoyancy regulation are important for dominance.

The response of *A. circinalis* to nutrient availability in the river was examined using *in situ* diffusion chambers. Phosphorus and nitrogen additions had no influence on growth, although the heterocyst/vegetative cell ratio increased suggesting nitrogen-fixation. Laboratory bioassays on water taken from the lower Murray River suggested that the wider phytoplankton community was nitrogen limited. In contrast to previous years, a significant volume of water was diverted from the turbid Darling River into the lower Murray River and caused phosphorus concentrations to be elevated. As high inputs from the Darling River were sustained over the course of the project, the importance of phosphorus storage for dominance of *A. circinalis* was unable to be determined.

The lower Murray River is a turbid system and thermal structure may have a strong influence on light availability. Growth of *A. circinalis* was compared under persistent stratification, diurnal stratification and complete mixing to determine whether artificial destratification would be effective. The mixing patterns were simulated using *in situ* diffusion chambers that were adjusted to different positions in the water column throughout the day. A strong relationship between growth and average daily light dose was established and a model developed to examine how changes in vertical light attenuation (K_d), maximum mixed depth (Z_m), incubation time and cell flotation velocity may influence the outcome. Growth of *A. circinalis* was fastest under persistently stratified conditions with the possibility of bloom development within one week, assuming no loss factors. However, growth of neutrally buoyant populations under diurnally stratified conditions was slower than mixed populations as the majority of the population was trapped in darkness during the stratified period. Blooms of *A. circinalis* may only occur under diurnally stratified conditions in the lower Murray River if the flotation velocity of colonies is > 0.5 m h^{-1}. The time for bloom development under these conditions is dependent on Z_m, but K_d had little influence.

Reasons for the decline of *A. circinalis* and dominance of the diatom *Aulacoseira granulata* under high flow conditions in the lower Murray River were also investigated. Unexpectedly, *in situ* growth of *A. circinalis* was faster than *A. granulata* at all Z_{eu}/Z_m ratios. Furthermore, growth of *A. circinalis* was not altered when grown in the same diffusion chambers as *A. granulata* suggesting lack of competition. However, the river was nitrogen limited during experimentation and *A. granulata* is unable to fix nitrogen. Under nitrogen replete conditions in the laboratory, photosynthesis of *A. granulata* was slower than *A. circinalis* under light saturation, but faster at sub-saturating irradiance (< 93.3 μmol m^{-2} s^{-1} or 4.0 mol m^{-2} day^{-1}). Regardless, no definite conclusions regarding the transition from *A. circinalis* to *A. granulata* could be made as nitrogen is rarely abundant in the lower Murray River. Interestingly, growth of *A. granulata* was stimulated when grown with *A. circinalis* in diffusion chambers. It is possible that *A. circinalis* contributes an important source of nitrogen to the lower Murray River through fixation of atmospheric sources.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any other university or other tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text. I consent to the thesis being available for copying and loan, if accepted for the award of the degree.

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Karen Westwood

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CHAPTER 1. General Introduction

The cyanobacterium *Anabaena circinalis* Rabenhorst (1852) is a dominant species of the phytoplankton assemblage in the Murray-Darling Basin, Australia (Baker *et al.* 1993). The catchment is highly managed and the rivers regulated to ensure an adequate water supply. However, the development of blooms under favourable conditions poses a significant threat to water quality. *A. circinalis* has the ability to produce neurotoxins and has been implicated previously in the deaths of livestock (McBarron *et al.* 1975; Negri *et al.* 1995; Baker and Humpage 1994). Water quality is also reduced through the production of geosmin; a chemical exudate with a musty/earthy taste and odour and therefore unwanted in domestic water supplies (Bowmer *et al.* 1992). *A. circinalis* may also have a negative impact on human recreation, causing skin irritations and appearing aesthetically displeasing. As far back as 1830, the explorer Charles Sturt (who named the Murray River) noted that Darling River water had a taste of vegetable decay as well as a slight tinge of green (Sturt 1833). This suggests that blooms in the Murray-Darling are not a new phenomenon. However, algal monitoring programs were established in the catchment in 1978 and there has been some suggestion that the frequency and intensity of cyanobacterial blooms has increased over recent years (MDBMC 1994). In 1991, particular alarm was caused by an extensive toxic bloom of *A. circinalis* in the Darling-Barwon river system that caused the New South Wales government to declare a state of emergency. The bloom was the largest ever recorded inland on an international scale, extending over 1000 km and lasting four months (Bowling and Baker 1996).

Blooms of *A. circinalis* also occur in numerous other parts of Australia, ranging from the cold climate of Tasmania, to the tropical climate of Queensland and the Northern Territory

(Velzeboer *et al.* 2000). They have also been documented overseas, e.g. in Crose Mere, UK, during spring (Reynolds 1975), in Lake Okeechobee, Florida, USA (Havens *et al.* 1998), and in freshwater lakes in Finland (Sivonen *et al.* 1990; Ekman-Ekebom *et al.* 1992). From international research, various hypotheses have emerged regarding the success of cyanobacteria such as *A. circinalis* in aquatic systems. These include the ability to regulate buoyancy (Ganf and Oliver 1982), fix atmospheric nitrogen (Shapiro 1990), store nutrients (Sommer 1985) and cope with low light availability (Mur *et al.* 1977; Zevenboom and Mur 1984; Tilzer 1987). In addition, cyanobacterial blooms are often associated with low nitrogen:phosphorus ratios (Smith 1983; Blomqvist *et al.* 1994), elevated water temperatures (Robarts and Zohary 1987) and low CO_2 availability (Shapiro 1990). However, no single factor has been able to explain the dominance of cyanobacteria and it is more likely that the main factor, or combination of factors, responsible for controlling cyanobacterial growth is species and site specific. This chapter provides a brief overview of the current knowledge of the ecology and physiology of *A. circinalis*. The attributes of the Murray-Darling Basin and possible factors that lead to the dominance of *A. circinalis* in the region are then examined. Specific attention is given to the lower Murray River where blooms are particularly unwanted as water is pumped for irrigation and domestic supplies to urban and country areas in the state of South Australia.

1.1 Current Knowledge of *A. circinalis*

A. circinalis is a gas-vacuolate, nitrogen-fixing cyanobacterium and cells are 6 to 8.5 μm in diameter (Baker *et al.* 1993). The cells form spirally coiled filaments up to 220 μm in length, and 50 to 200 μm in breadth, and the filaments may aggregate to form macroscopic colonies visible to the naked eye (Baker *et al.* 1993; Brookes *et al.* 1999; Oliver and Ganf 2000). *A. circinalis* is included within the order Nostocales due to its ability to produce heterocysts where nitrogen fixation occurs. These thick-walled cells are sites of enhanced respiration,

thus protecting the nitrogen-fixing enzyme complex, nitrogenase, from oxygen which destroys it. *A. circinalis* may also produce akinetes (or cysts) which serve as a resting stage until environmental conditions are more favourable for growth (van Dok and Hart 1996; van Dok and Hart 1997). Akinetes are sausage shaped cells and develop remotely from the heterocysts (Baker *et al.* 1993). A thorough description of the taxonomy of Nostocales is provided by Baker (1991).

1.1.1 Buoyancy Regulation

One of the most generally accepted hypotheses for the success of cyanobacteria is the ability to regulate buoyancy (Ganf and Oliver 1982; Kromkamp *et al.* 1986; Walsby 1994). The migration of colonies through the water column may allow access to optimal light and nutrient availability (Ganf and Oliver 1982), and flotation may reduce sedimentation losses (Kalff and Knoechel 1978; Reynolds 1984). Buoyancy is regulated by the overall balance between cell mass and the gas vesicles which are filled with air and provide lift. However, for buoyancy to be effective, the water column must be relatively calm (Burch *et al.* 1994; Sherman *et al.* 1998; Maier *et al.* 2001).

Photosynthesis, and therefore light availability, has a significant effect on buoyancy in two ways. First, photosynthesis produces carbohydrate molecules which are heavy and may counteract the lift provided by the gas vesicles. The amount of cellular carbohydrate depends both on the rate of photosynthesis and the rate of carbohydrate metabolism. For *A. circinalis*, cells may float when exposed to light doses < 6 mol m^{-2} day^{-1} (Brookes *et al.* 1999). Under higher light doses, or under nutrient limited conditions which hinders the metabolism of carbohydrate, cells may lose buoyancy (Brookes and Ganf 2002). Second, photosynthesis may cause an increase in cell turgor which exerts a pressure on the gas vesicles and causes them to collapse irreversibly (Grant and Walsby 1977; Thomas and Walsby 1985). In *Anabaena*,

typical collapse pressures for the gas vesicles are 0.4 to 0.8 MPa (Hayes and Walsby 1986).

Photosynthetic rates as low as 1.8 mg O_2 mg (chlorophyll a)$^{-1}$ h^{-1} were found to be sufficient

to affect buoyancy in natural populations of *A. circinalis* in Crose Mere, with cell turgor

ranging from 0.5 to 0.57 MPa (Reynolds 1975). Internal cellular pressure may also be

augmented through hydrostatic pressure which increases at 0.011 MPa m^{-1} (Oliver and Ganf

2000).

Buoyancy is also affected by factors other than photosynthesis. Polyphosphate and

cyanophycin are associated with nutrient storage in cyanobacteria and these heavy molecules

may also contribute to buoyancy loss. In addition, the number of gas vesicles per cell may be

influenced by cell division which causes a dilution effect (Oliver 1994; Brookes and Ganf

2002). New gas vesicles are synthesised from new or recycled proteins (Hayes and Walsby

1984) and are produced on the time-scale of one generation time, or approximately one day

(Reynolds 1987). Given the requirement for protein, gas vesicle synthesis may be severely

restricted under nitrogen limited conditions.

Sinking velocities of *A. circinalis* are in the order of -0.2 m h^{-1} (Reynolds *et al.* 1987).

Flotation velocities typically range from 0.01 to 0.40 m h^{-1} (Reynolds *et al.* 1987; Brookes *et

al.* 1999; Mitrovic *et al.* 2001). Brookes *et al.* (1999) also found in Chaffey Dam, New South

Wales, that under light limited conditions, *A. circinalis* may form large filament aggregations

which float as fast as 2.0 m h^{-1}. The ability for *A. circinalis* to float under calm conditions

may be particularly important in turbid systems where light is severely limiting (Mitrovic *et

al.* 2001). For example, Sherman and Webster (1994) showed using a model that the potential

growth of populations with a flotation velocity of 0.036 m h^{-1} exposed to persistent

stratification, is at least double that of mixed populations over a 60 day period.

The flotation of *A. circinalis* may lead to the accumulation of a scum at the water surface, thus making blooms particularly unsightly. Possible benefits for *A. circinalis* include enhanced access to carbon dioxide and gaseous nitrogen (for fixation), and shading of other phytoplankton in the water column (Paerl 1988; Klemer and Konopka 1989). A scum may disappear when cells lose buoyancy (Ganf 1974), or when colonies are entrained back into the water column. Webster and Hutchinson (1994) showed using experimentation and models that colonies are only entrained from the surface when wind speed is > 2 to 3 m s^{-1}. Strong winds may also distribute scums horizontally across the surface to the downwind end of a lake (Webster *et al.* 1997). If buoyancy loss or re-entrainment do not occur, a scum may persist indefinitely (Sherman and Webster 1994).

1.1.2 Nitrogen:Phosphorus Ratios

Fogg (1969) noted the paradoxical observation that although cyanobacteria are associated with eutrophic conditions, they are most successful when certain inorganic nutrients are present in minimal concentrations. Another hypothesis to explain cyanobacterial dominance is that they are favoured by low nitrogen to phosphorus (N:P) ratios (Shapiro 1990; Blomqvist *et al.* 1994). Some cyanobacteria, including *A. circinalis*, are able to fix atmospheric nitrogen sources, thus requiring low dissolved inorganic concentrations (Harris 1986). Tilman *et al.* (1982) also suggest that cyanobacteria, in general, only dominate under low N:P ratios as they are inferior competitors to diatoms for phosphorus. Many studies have shown strong evidence that cyanobacterial development is associated with the N:P ratio and dominance generally occurs when the ratio (by weight) is less than 29 (Schindler 1977; Smith 1982, 1983). However, other studies have found no correlation (Pick and Lean 1987; Elser *et al.* 1990; Levine and Schindler 1999). The N:P ratio is unlikely to be important if nitrogen and phosphorus are in excess of limiting concentrations (Horne and Commins 1987; Reynolds 1992), or the physical stability of the water column is unsuitable for growth (Steinberg and

Hartmann 1988). The optimum N:P ratio of phytoplankton may also be influenced by light availability (Rhee and Gotham 1980; Healey 1985; Wynne and Rhee 1986). Overall, a low N:P ratio is not an absolute requirement for dominance of cyanobacteria. For example, *Microcystis aeruginosa* may dominate when TN:TP (not N:P) is > 80 (Harris 1986). Furthermore, Reynolds (1986) found no correlation between the TN:TP ratio and growth of the nitrogen-fixers *Anabaena* and *Aphanizomenon* in enclosure experiments.

In studies where the N:P ratio was found to have an influence on the dominance of *Anabaena*, the ratio (by weight) was generally < 10 (Schindler 1977; Sakshaug *et al.* 1983; Harris 1986; Stockner and Shortreed 1988). Under these conditions, nitrogen is severely limiting and the ability for *Anabaena* to dominate is likely to be the result of fixation of atmospheric nitrogen sources. For example, in the Experimental Lakes Area, Ontario, *A. circinalis* grew to bloom proportions in mesocosms where N:P = 4. Nitrogen fixation occurred at rates of up to 42 mg N m^{-2} day^{-1} and caused the biomass to increase to levels similar to (but different species) the treatment where N:P = 33 (Levine and Schindler 1992, 1999). Evidence for the success of *A. circinalis* under nitrogen limited conditions also comes from Crose Mere, United Kingdom, where depletion over spring and early summer caused a switch from *Eudorina* to *Anabaena* dominance (Reynolds and Reynolds 1985). The ability for *A. circinalis* to fix atmospheric nitrogen may provide this species with a distinct ecological advantage under nitrogen limited conditions. It may also infer that populations are more susceptible to phosphorus limitation given that this nutrient must be obtained solely from the water-column (Sherman *et al.* 1998; Train and Rodrigues 1998).

1.1.3 Nutrient Storage

The ability for cells to store nutrients may provide an effective mechanism to cope with a limited nutrient environment (Shapiro 1990; Pettersson *et al.* 1993; Blomqvist *et al.* 1994).

Cyanobacteria generally have slow growth rates relative to nutrient uptake rates and have been classified as storage specialists (Sommer 1984). Phosphorus is stored in the form of polyphosphate and nitrogen in the form of cyanophyin, and both are known to be produced by *Anabaena* (Stewart *et al.* 1978; Allen and Hutchinson 1980; Gotham and Rhee 1981). The ability to store large amounts of nitrogen is unique to the cyanobacteria, although phosphorus and nitrogen storage may also be found in other groups (e.g. diatoms).

Nutrient storage may be particularly advantageous when the ambient nutrient supply fluctuates across limiting thresholds on a spatial or temporal scale (Turpin and Harrison 1979; Lehman and Scavia 1982; Sommer 1985; Kromkamp *et al.* 1989). Competitive outcomes within a phytoplankton community may depend on both the frequency and strength of nutrient pulses (Goldberg and Novoplansky 1997). For example, long intervals between nutrient pulses may allow species that are able to cope with low nutrient conditions to competitively exclude those that are intolerant (Goldberg and Novoplansky 1997). When a nutrient pulse occurs, only some species may benefit given that the half-saturation for nutrient uptake varies among species (Tilman *et al.* 1982). Nutrient availability can fluctuate over time-scales ranging from minutes (e.g. zooplankton excretion) to years (e.g. climatic events). On a spatial scale, nutrient patches may range from millimeters to kilometers, with fluxes arising from various sources including release from sediments, release from plants and animals, or anthropogenic runoff. Even microscale nutrient fluxes (e.g. millimeters) may have a large influence on nutrient budgets in aquatic systems, particularly in oligotrophic waters. For example, Lehman (1980) found in Lake Washington that zooplankton supplied ten times more inorganic phosphorus to surface waters during summer than entered the lake from all sources. Furthermore, Carrillo *et al.* (1996) calculated that zooplankton released phosphorus at rates between 0.2 to 2.9 μg mg dry weight^{-1} h^{-1} in a Spanish lake - a significant quantity

when considering that phytoplankton cells require only ηg to ρg of phosphorus for active growth (Reynolds 1984).

The effectiveness of nutrient storage in the success of cyanobacteria was demonstrated in a classic experiment by Sommer (1985). Phosphorus pulses were administered to mixed phytoplankton assemblages at weekly intervals (35 µg L^{-1} P $week^{-1}$) over a 5 to 8 week period. Many species responded by proliferating immediately after a nutrient pulse and then declining once the nutrient was again limiting. These were termed 'oscillating' species and included both green algae and diatoms. Other species, including the cyanobacteria, maintained relatively constant numbers over the time of the experiment despite the pulses. These were classed as 'stable' species and the response was likely to have been enabled through nutrient storage. Finally, a number of species were unable to utilise the nutrient pulses and were excluded completely from the experiment. It is interesting that when nutrients were supplied continuously rather than as discrete pulses, it was the cyanobacteria that were excluded completely from the experiment. This raises the question as to why cyanobacteria are associated with eutrophic conditions, given that they are better competitors under pulsed nutrient conditions. However, cyanobacteria are large and may require strong nutrient pulses to enable effective uptake and storage (Reynolds 1984).

A study that provides evidence for the success of *Anabaena* under pulsed nutrient supply was conducted by Reinertsen *et al.* (1986). Competition for phosphorus between *Anabaena* and the green alga *Staurastrum* was examined in enclosure experiments. When zooplankton were introduced into the enclosures, *Anabaena* biomass did not increase, whereas *Staurastrum* did. However, when fish were introduced into the enclosures, both species increased in abundance. The observed difference was due to the patchy release of phosphorus by the fish

and a more homogeneous release by the zooplankton. *Anabaena* was able to rapidly take up the temporary pulses of phosphate excreted by the fish and thus co-exist with *Staurastrum*.

1.1.4 Nutrient Limitation

To gain an understanding of the interaction between nutrient availability and cyanobacterial growth, consideration needs to be given to limiting thresholds of phosphorus and nitrogen availability. The association between eutrophic conditions and cyanobacterial dominance has long been recognised (Vollenweider 1968; Hecky and Kilham 1988; Steinberg and Hartmann 1988; Sas 1989; Oliver and Ganf 2000). It should follow that nutrient removal reduces the occurrence of blooms. However, studies have shown variable effects and that the nutrient-biomass interaction is complex (Oliver and Ganf 2000).

Early studies of eutrophication from the northern hemisphere established quantitative relationships between total phosphorus availability and chlorophyll biomass (Sakamoto 1966; Vollenweider 1968; Dillon and Rigler 1974). The models were based on a log-log linear relationship with regression slopes ranging from 0.4 to 2.2 (Reynolds 1992) and it was later established they were generally applicable to examples from the southern hemisphere provided that turbid systems (> 10 NTU) were excluded from the analysis (Ferris and Tyler 1985). However, the confidence intervals of the models were broad, indicating that factors other than phosphorus availability are also important in the control of growth. Sakamoto (1966) and Smith (1979, 1982) correctly inferred that both nitrogen and light availability must also play important roles in controlling biomass. When total nitrogen (TN) and total phosphorus (TP) were considered in quantitative log-log models, the relationship with chlorophyll was significantly improved (Smith 1982). In light limited systems, various models incorporated the use of Z_{eu}/Z_m:TP, turbidity:TP (Verduin *et al.* 1978; Hoyer and Jones 1983), or a ratio that considers the maximum photosynthetic rate per unit volume of the euphotic

zone (Smith 1979). As Australia has a large proportion of turbid rivers and lakes (Bowling 1989; Oliver 1990), light availability may have an important influence on the response of a phytoplankton community to nutrient removal.

From Vollenweider's (1968) study on eutrophication, it was concluded that the probability of cyanobacterial blooms was significantly increased if the loading of nutrients to lakes is sufficient to cause spring concentrations to exceed 10 μg L^{-1} phosphorus and 200 - 300 μg L^{-1} nitrogen. Sas (1989) also examined data from 18 lakes and reservoirs in Europe that ranged in depth and retention time. The analysis showed that growth was unlikely to be limited if filterable reactive phosphorus was > 10 μg L^{-1}, or inorganic nitrogen > 100 μg L^{-1}. For nutrients to be limiting, concentrations of phosphorus and nitrogen had to be below these threshold values either on average over the entire growing season, or absolutely for at least half the growing season. Steinberg and Hartmann (1988) also suggest that at phosphorus concentrations > 10 μg L^{-1}, the physical environment becomes more important for the control of cyanobacterial growth. For the purposes of this thesis, 10 μg L^{-1} inorganic phosphorus and 100 μg L^{-1} inorganic nitrogen are accepted as the threshold values below which cyanobacterial growth is restricted.

1.1.5 Light Availability

The hypothesis that cyanobacteria are favoured by low light availability (Shapiro 1990; Blomqvist *et al.* 1994) was likely to be derived from two observations. First, that cyanobacteria have a low maintenance energy requirement and may outcompete other species under low light (Mur *et al.* 1977). Second, that cyanobacteria are characterised by intracellular phycobilins that are efficient in light harvesting (Zevenboom and Mur 1984). However, other studies have demonstrated that cyanobacteria have a high light requirement and grow better under continuous compared to fluctuating light (Fogg *et al.* 1973; Nicklisch 1998). The

response to light may depend on the genus of cyanobacteria being considered. For example, *Oscillatoria* is favoured by low light conditions and may promote its own dominance through shading of high light requiring species (Scheffer *et al.* 1997).

In contrast to *Oscillatoria*, *Anabaena* is known to have a high light requirement (Reynolds and Walsby 1975). This was clearly demonstrated in Crose Mere, United Kingdom, where *A. circinalis* was confined to conditions where the minimum average light period was greater than 5.8 h day^{-1} (Reynolds and Reynolds 1985), but favoured conditions where Z_{eu}/Z_m was greater than 1 (Reynolds and Walsby 1975). Furthermore, in Lake Okeechobee, Florida *A. circinalis* abundance was found to be positively correlated with secchi depth and the secchi depth/total depth ratio (Havens *et al.* 1998). From these examples it is not unexpected that rapid growth of *A. circinalis* is generally associated with stratified conditions. The high light requirement of *Anabaena* may be linked with nitrogen-fixation which is an energetically costly process (Lewis and Levine 1984). A possible danger with high light availability is that cells may become photoinhibited if exposed to high intensities for extended periods. However, Paerl and Kellar (1979) have shown that *Anabaena* may cope with oxygen toxicity through sequential optimisation of carbon dioxide and nitrogen fixation. In addition, the oxygen-evolving pigment phycocyanin is decreased, and the carotenoid content is increased to protect the cell.

As phytoplankton cells are small enough for entrainment within water flow, turbulence may have a strong influence on the light available for growth (Spigel and Imberger 1987; MacIntyre 1998). The degree of turbulence in the surface mixed layer is dependent on wind-induced mixing and surface heat fluxes. When solar energy input is high, and wind speed is low, strong resistance to mixing processes may occur and stratification may develop. This is particularly so in waters with high attenuation coefficients where steep temperature gradients

are formed from rapid solar absorption with depth. Under these conditions there is some difficulty in mixing the surface mixed layer as more energy is required. For example, in North Lake, Western Australia, at least 4 hours of fast wind speeds (> 6 m s^{-1}) were required for the 2.6 m water column to fully mix during the day (MacIntyre and Melack 1998). Shallow turbid lakes typically undergo diurnal cycles of stratification and mixing (MacIntyre 1993). Mixing generally occurs from overnight cooling with the surface mixed layer deepening to below the euphotic zone or penetrating to the bottom (Bormans *et al.* 1997). In deeper systems, the maximum mixed depth (Z_m) may occur above a seasonal or parent thermocline (Sherman *et al.* 1998; Brookes *et al.* 1999).

High resolution temperature profiles have demonstrated that turbulence can vary on extremely fine scales (Imberger 1985; MacIntyre 1993). For example, in North Lake, Western Australia, overturns were found to vary from a few centimetres to 1.5 m. In turbid Lake Biwa, Japan, the average size of overturns was in the order of 0.2 cm (MacIntyre 1996). At this site, the most significant overturn suggested that an algal cell can circulate from 90% to 5% surface irradiance within three to four minutes. In the case of *Anabaena*, turbulence may have a negative influence as growth is better under continuous compared to fluctuating light when average irradiance is low (Litchman 1998; Sherman *et al.* 1998). However, if the floating or sinking velocities of cyanobacteria are greater than the mixing velocity of the water, the colonies may disentrain from the flow. For disentrainment to occur, the velocity of the cells needs to be at least an order of magnitude faster than the flow velocity (MacIntyre 1993). This may be achieved through the aggregation of colonies which allows faster flotation or sinking velocities to occur (Brookes *et al.* 1999).

1.1.6 Cyanobacteria in Rivers

The environment that phytoplankton populations experience in rivers is different to lakes given that retention times are less and cells are subject to advection downstream. Temporal fluctuations in the number of species and phytoplankton densities in rivers are generally higher than lakes (Izaguirre *et al.* 2001). Flow rate has a large influence on species composition with buoyancy-regulating cyanobacteria tending only to dominate under low flows in association with stratified conditions (Reynolds 1995; Sherman *et al.* 1998; Train and Rodrigues 1998; Izaguirre *et al.* 2001). Under high flows, green algae tend to dominate the upper reaches of rivers, whereas diatoms tend to dominate downstream (Reynolds 1995; Köhler and Bosse 1998). Generally, species diversity is also higher under high flows but biomass is less (Train and Rodrigues 1998).

Reynolds *et al.* (1991) and Reynolds (1995) suggested that it is impossible for cell densities of phytoplankton in downstream stretches of rivers to be the result of an increase in inocula from upstream. This was based on the observation that species composition changes downstream and that the increase of a single species would need to be faster than the maximum possible growth rate. It was hypothesised that 'dead-zones' are important contributors to biomass in the main channel and are sites of significant phytoplankton growth. Flow in river-channel cross-sections is highly variable, with some areas that are very slow moving or essentially stationary, as demonstrated in the River Severn (Reynolds *et al.* 1991). The influence of these dead-zones may explain the spatial variability of phytoplankton and typically include near-bank eddies, backwaters (including floodplain lagoons) and side-arms. As cyanobacteria prefer calm conditions, dead-zones may provide an important environment for the development of populations that later seed the main river channel (Burch *et al.* 1994).

The dominance of cyanobacteria, and more specifically *Anabaena*, under low flow conditions in rivers has been documented extensively both in Australia and overseas (Burch *et al.* 1994; Bowling and Baker 1996; Train and Rodrigues 1998; Izaguirre *et al.* 2001; Mitrovic *et al.* 2001). One of the best examples comes from Maud Weir, on the Murrumbidgee River, Australia (Sherman *et al.* 1988). At flows less than 1000 ML day^{-1}, *Anabaena* increased at a rate of 0.37 day^{-1}, and became dominant after 2 weeks. Low flow allowed the development of strong persistent stratification ($Z_{eu}/Z_m =1$), effectively becoming a two-layered system with colder water from upstream plunging below the thermocline and discharging through the underflow weir. There was little dilution of epilimnetic waters and buoyant colonies of *Anabaena* accumulated in the top 2 m where growth occurred. Under diurnally stratified (flow 1000 - 3000 ML day^{-1}) or completely mixed conditions (flow > 4000 ML day^{-1}) *Anabaena* declined and the filamentous diatom *A. granulata* became dominant.

Train and Rodrigues (1998) also documented the dominance of *Anabaena* under low flow conditions in the Baía River, Brazil. The river was nitrogen limited with a maximum total nitrogen concentration of 77 µg L^{-1} and oxidised nitrogen concentrations below 20 µg L^{-1}. The dominant species were *Anabaena circinalis, Anabaena spiroides* and *Anabaena solitaria* that together comprised 96% of the total biovolume. Under high flows, associated with rain, there was a transition to centric diatoms mainly represented by *Urosolenia eriensis* var. *morsa* and *Aulacoseira granulata* var. *granulata*. The transition to diatoms was the result of turbulence and an increase in nitrate caused through sediment re-suspension.

1.1.7 Artificial Mixing

Given the association between cyanobacterial abundance and river flow, the latter may be used as a management tool to artificially mix a regulated river and reduce growth (Webster *et al.* 2000; Maier *et al.* 2001). The reduction of nutrient load in rivers may take a number of

years due to the time required to implement effective land practices and to reduce nutrient stores that have accumulated in sediments (Webster *et al.* 2000). Furthermore, as demonstrated earlier, the relationship between nutrient availability and chlorophyll biomass is based on a $\log_{10}$ relationship so that large reductions in nutrient availability are required to be effective (Vollenweider 1968; Smith 1982; Canfield *et al.* 1983). The use of river flow provides a short-term option for preventing cyanobacterial blooms. However, an increase in river flow requires serious consideration due to the problem of wasted resource.

There have been varying degrees of success with artificial mixing to reduce cyanobacterial growth. Visser *et al.* (1996a) studied the effect on growth of *Microcystis aeruginosa* in Lake Nieuwe Meer, the Netherlands. The mean depth of the lake was 18 m with a maximum depth of 30 m. The artificial mixers were designed to produce a water velocity of 1 m h^{-1}, to overcome a mean floating velocity of *Microcystis* colonies of 0.11 m h^{-1}, with a maximum of 2.6 m h^{-1}. In years without mixing when blooms occurred, Z_{eu}/Z_m ranged from 0.59 to 0.67 ($Z_m < 7$ m). Under a year of continually mixed conditions Z_{eu}/Z_m decreased to 0.16 ($Z_m = 27$ m) and there was a successful transition from cyanobacteria to flagellates, green algae and diatoms. The euphotic depth was similar during years and nutrient availability did not alter significantly with phosphorus and nitrogen in excess of limiting concentrations.

Visser *et al.* (1996b) also studied the De Gijster storage reservoir in the Netherlands but found that artificial destratification was unsuccessful in completely eliminating *Microcystis* blooms. The mean depth of the reservoir was 13 m with a maximum depth of 28 m. However, the deep central region was surrounded by relatively shallow regions of 4 to 6 m (35% of the total area). The bubble plumes produced by the artificial mixers were efficient in entraining cyanobacteria within the whole lake. Therefore, insufficient mixing velocity was not the cause of failure. Rather, in the deep section, the maximum mixed depth (Z_m) ranged from 9 to 18 m

and Z_{eu}/Z_m ranged from 0.45 to 0.66. In the shallow areas the surface mixed layer reached the bottom and Z_{eu}/Z_m ranged from 0.98 to 2.40. From calculations of light availability and measurement of buoyancy losses, Visser *et al.* (1996b) concluded that the shallow mixed areas provided sufficient light for significant growth of *Microcystis* and that this was the cause of the sustained high cell densities. However, even in the deep region, a Z_{eu}/Z_m ratio > 0.5 may allow sufficient light for growth (Reynolds and Walsby 1975). The relationship between the Z_{eu}/Z_m ratio and growth of cyanobacteria is important when considering the necessity for artificial destratification as the ratio approximates the light dose received. From the literature, *Anabaena* generally dominates at Z_{eu}/Z_m ratios ranging from 0.5 to 3.3 (Reynolds and Walsby 1975).

The necessity to use artificial destratification to reduce cyanobacterial growth when diurnal (rather than persistent) stratification is the predominant mixing pattern is unclear. Under diurnally stratified conditions, it is possible that rapid growth may occur when Z_{eu}/Z_m is < 0.5 provided that colonies have sufficient flotation velocities for a significant proportion of the populations to reach the euphotic zone during the stratified period (Humphries and Lynne 1988). In this case, use of the Z_{eu}/Z_m ratio may be misleading as the surface mixed layer only reaches the maximum mixed depth (Z_m) during the night, but is restricted nearer to the surface during the day when stratification develops. An example of successful growth of *A. circinalis* under diurnally stratified conditions comes from Chaffey Dam, New South Wales where blooms are common (Brookes *et al.* 1999). The dam was characterised by a persistent thermocline between 11 and 14 m with the diurnal mixed layer ranging from 0 to 8 m (Z_m), depending on the time of day. The vertical light attenuation was 1.02 m^{-1} so that maximum Z_{eu}/Z_m was 0.30. In the early morning, *A. circinalis* was distributed evenly throughout the diurnal surface mixed layer down to 8 m, but by midday had become concentrated in the top 2

m with the development of stratification and the flotation of aggregated colonies at a rate of 1.32 m h^{-1}.

1.2 The Murray-Darling Basin

The Murray-Darling Basin (24-37°S, 138-151° E) extends over most of south-east Australia and drains the slopes of the Great Dividing Range. The catchment area is 1.073 million km^2, however the majority of the basin is arid or semi-arid and significant run-off occurs in only a small section (Walker and Thoms 1993). The mean annual evaporation (1200 mm) generally exceeds the mean annual rainfall of only 450 mm (Thoms and Walker 1993).

The Murray and Darling Rivers are the two major drainage systems within the catchment (Figure 1.1). The Murray River lies near the southern perimeter with headwaters rising in the Snowy Mountains in south-east New South Wales. The water flows in a general westerly direction some 2560 km to the mouth in South Australia. The Darling River enters the Murray River at Wentworth, 827 km from the mouth. Darling River water contains high levels of very fine clay particles that do not settle under normal flow conditions (Oliver *et al.* 1993). Turbidity in the Darling River is therefore high and inputs to the Murray River may significantly affect water clarity.

The section of the Murray River below the Murray-Darling confluence is termed the lower Murray River (Figure 1.1) and 80 % of its length is comprised of valley and gorge (Walker and Thoms 1993). In the valley section, the river meanders and there are extensive floodplains up to 10 km wide. In the gorge section, the river is channelled through limestone cliffs and the floodplain is constrained to 2 to 3 km. Associated with the floodplains along the lower Murray are shallow lagoons (also known as billabongs) that either adjoin the river channel permanently, or are isolated until overbank flow occurs from adequate discharge upstream.

The lower Murray has a small gradient of only 4.5 cm km^{-1} (Sullivan *et al.* 1988), river flow is generally slow and water at the Murray-Darling confluence may take approximately 2 weeks to reach the sea (Walker 1992).

Given the semi-arid nature of the Murray-Darling, the system was historically subject to wide fluctuations in discharge associated with floods and drought (Walker 1992). However, for the purposes of irrigation and river navigation, the system is now highly regulated to ensure an adequate water supply. The Murray River is impounded by Hume Dam and 13 weirs with adjacent locks that were constructed between 1922 and 1935. Ten of the weirs are located on the lower Murray (Figure 1.1), and as the reaches are nearly overlapping the entire lower section is often a continuous pool environment. The Darling River is comparatively unregulated with a sole water storage at Menindee Lakes. Lake Victoria lies adjacent to the Murray River and is also used as an off river storage until water in the lower Murray River is required. Lake Victoria is filled by water transferred from Menindee Lakes or from winter flushes in the Murray River. The overall effect of regulation in the Murray is that the extremes in river flow have been removed. This has caused a decrease in the frequency of overbank flows and an increase in the frequency of flows at or near channel capacity.

Given the extensive area of the Murray-Darling Basin, the catchment lies within four states of Australia, *i.e.* New South Wales, Victoria, Queensland and South Australia. The Murray-Darling Basin Commission (MDBC) administers the *Murray-Darling Basin Agreement (1987)* on behalf of each of the state governments, and the Commonwealth government. A complex set of operating rules are used to determine flow allocations to each of the states. As irrigation accounts for about 90% of annual water consumption, this largely determines the pattern of regulation. The lower Murray River also provides between 50 and 90% of vital water supply to urban and country areas in the arid regions of South Australia. The MDBC

guarantees South Australia an average yearly entitlement flow of 1850 GL to ensure that requirements are met. Annual flow is significantly different between years in the lower Murray River. For example, between 1950 and 1980 it ranged between 0.617 and 49 272 GL (Walker 1986). However, entitlement flow is generally exceeded with an average annual flow of 6570 GL (Burch *et al.* 1994).

1.3 *A. circinalis* Abundance in the lower Murray River

The incidence of *Anabaena* blooms in the lower Murray River is generally restricted to the summer period of December to March (Burch *et al.* 1994; Figure 1.2). Over this time, solar input is high, water temperature is warm (>20° C), and discharge is generally low. The lock and weir system that regulates the water supply, combined with the small gradient of the lower Murray River, means that the river essentially becomes a continuous pool environment when discharges are low, and flows are generally < 6000 ML day^{-1} (Figure 1.2). These coincident characteristics over summer may allow the development of stratification (Burch *et al.* 1994).

Light and nutrient availability in the lower Murray River are largely influenced by turbidity levels (Figure 1.3). Turbidity causes a decrease in light availability through increasing the vertical attenuation coefficient (Walmsley *et al.* 1980), whereas nutrient availability increases (Figure 1.3). Total phosphorus concentrations are particularly well correlated with turbidity which can possibly be attributed to the phosphate buffer mechanism where phosphorus molecules are able to absorb and desorb from suspended particulate matter (Fox 1993). Turbidity also appears to have a strong influence on total Kjeldahl nitrogen (TKN) and a smaller influence on inorganic phosphorus and oxidised nitrogen (Figure 1.3). Trends in ammonia availability appear unrelated.

Turbidity levels are highly fluctuating in the lower Murray River, ranging from 4 to 304 NTU with an average of 51.6 ± 38.9 NTU between 1990 and 1996 (Figure 1.3). River flow has a small influence on turbidity with high flows causing a slight increase (Figure 1.2). However, Darling River water has the largest influence with turbidity levels becoming greatly elevated when significant proportions are diverted to the Murray. Average turbidity at Morgan, below the Darling-Murray confluence, was 70 NTU between 1978 and 1986. At Merbein, just above the Darling-Murray confluence, the average turbidity was only 25 NTU (Sullivan *et al.* 1988). The influence of Darling River water on turbidity in the lower Murray is also shown in 1990 and 1996 (Figure 1.3) when there was a high input. The overall effect of Darling River water entering the Murray River is that light availability decreases, whereas nutrient availability increases. This trade-off may have interesting implications for growth of the phytoplankton community

1.3.1 Nutrients

An increase in the incidence and severity of cyanobacterial blooms in the lower Murray River has been partly attributed to eutrophication from agricultural run-off and point-source inputs (Baker *et al.* 1993). However, before expensive management options for nutrient removal are adopted, a more thorough evaluation of the effect of nutrients on growth is required. Examination of nutrient availability at Morgan suggests that the lower Murray river may not be as eutrophic as assumed (Figure 1.3). During summer, inorganic nutrient concentrations are generally low and fluctuate across limiting thresholds (Figure 1.3), yet this is the main period of *A. circinalis* growth (Burch *et al.* 1994). Inorganic phosphorus concentrations were below 10 μg L^{-1} on 56% of sampling occasions, and inorganic nitrogen (oxidised nitrogen plus ammonia) below 100 μg L^{-1} on 83% of sampling occasions over summer from 1990 to 1996 (Figure 1.3). The growth of *A. circinalis* at times of limited nutrient availability is further evidenced by Baker *et al.* (2000) who tracked a parcel of water downstream in the

lower Murray River. They found that advected populations were able to grow at 0.176 day^{-1}, yet both inorganic phosphorus and nitrogen were undetectable. Furthermore, in Maud Weir, within the Murray-Darling Basin, *Anabaena* abundance increased eight-fold despite an apparent absence of available phosphorus (Sherman *et al.* 1998). These examples demonstrate that *A. circinalis* can adequately cope with a limited nutrient environment and that biomass may increase under these conditions.

As suggested earlier, the ability for *A. circinalis* to grow under nitrogen limited conditions may be enabled through nitrogen-fixation and this may play an important role in success in the lower Murray River (Lewis and Levine 1984; Horne and Commins 1987; Blomqvist *et al.* 1994). Growth during phosphorus limited conditions may be enabled through the utilisation of phosphorus pulses that are stored and utilised when required. When a phosphorus pulse is encountered under phosphorus limited conditions, it may be immediately taken up by cells so that a change in availability would not be detected through measuring concentrations on a weekly basis. For this reason, it would be more appropriate to examine the response of *A. circinalis* to phosphorus fluxes over time. Oliver *et al.* (1993) also suggest that a significant percentage of total phosphorus associated with suspended particulate matter, in the size range 0.2 to 25 µM, may be available for phytoplankton use in the Murray-Darling system.

1.3.2 The Physical Environment

Another important aspect of the development of *A. circinalis* in the lower Murray River is that blooms coincide with conditions that are conducive to the development of stratification (Burch *et al.* 1994; Sherman *et al.* 1998). The association between persistent stratification and cyanobacterial dominance has long been recognised (Sherman *et al.* 1998). However, Bormans *et al.* (1997), measured temperature profiles over an extended period in the lower Murray River and showed that river flow and wind speed have a significant effect on the

physical environment. Persistent stratification only developed when flow was < 4000 ML day^{-1} and when average daily wind speed was < 1.2 m s^{-1}. Diurnal stratification was the predominant mixing pattern and occurred when flow was < 4000 ML day^{-1} and average daily wind speed was between 1.2 and 2.9 m s^{-1}, or at flows > 4000 ML day^{-1} ranging to 10 000 ML day^{-1}. Under diurnal stratification, the surface mixed layer was restricted to the euphotic zone during the day ($Z_{eu}/Z_m = 1$) but deepened to the bottom during the night ($Z_{eu}/Z_m < 0.5$) due to surface cooling. Complete mixing occurred at flows < 4000 ML day^{-1} and average daily wind speeds > 3.0 m s^{-1}, or when flow was > 4000 ML day^{-1}, ranging to 10 000 ML day^{-1}, in association with weather fronts. Bormans et $al.$ (1997) suggested that cyanobacterial blooms are unlikely in the lower Murray River given the low incidence of persistently stratified conditions. However, this was based purely on physical data and biological information of the response of $A.$ $circinalis$ to different mixing patterns is required. Numerous other studies have demonstrated that buoyancy-regulating cyanobacteria, including $Anabaena$ and $Microcystis$, benefit from diurnal stratification due to the flotation of colonies into the euphotic zone when stratification develops each day (Ganf 1974; Reynolds et $al.$ 1981; Zohary and Robarts 1989; Ibelings et $al.$ 1991a; Brookes et $al.$ 1999).

A number of shallow lagoons that adjoin the main river channel along the length of the lower Murray River may also provide an important source of biomass to the main channel population (Burch et $al.$ 1994). The lagoons are essentially dead-zones with long retention times and are often associated with high cyanobacterial cell densities. The lagoons may provide a favourable environment for $A.$ $circinalis$ growth due to high light availability (Z_{eu}/Z_m 0.5 to > 1.0), low dilution, or high nutrient availability through significant sediment-water interactions from turbulence in the shallow water-column (0.5 to 1 m). Webster et $al.$ (1997) demonstrated significant water exchange between a lagoon and the main river channel of the lower Murray River with flow out of the lagoon sufficient to empty it in nine days.

1.3.3 Transitions between Anabaena and Aulacoseira

While low flow conditions may lead to dominance of *Anabaena* in the lower Murray River, the abundance of the diatom *Aulacoseira granulata* declines. However, when flow increases to allow completely mixed conditions, *Anabaena* decreases and the dominance of *A. granulata* resumes. Consequently, there is an inverse relationship between the two species (Figure 1.2). Transitions between *Anabaena* and *Aulacoseira* under alternating states of turbulence are common in both rivers and lakes (Lewis 1978; Miyajima *et al.* 1994; Reynolds and Reynolds 1985; Izaguirre *et al.* 2001). Sherman *et al.* (1998) clearly demonstrated in Maud Weir, within the Murray Darling Basin, that the reason for the transition from *A. granulata* to *Anabaena* under low flow conditions is due to the development of stratification. As *A. granulata* is a heavy diatom species, it sinks rapidly out of the euphotic zone and cells are unable to be re-advected due to calm conditions. In contrast, *A. circinalis* is able to maintain position within the euphotic zone and receive adequate light through buoyancy regulation. Bormans and Condie (1998) successfully modelled the increase of *Anabaena* and a decrease in *Aulacoseira* in Maud Weir under low flow conditions, based on surface heat fluxes that were calculated using physical data recorded at the site. However, the underlying reasons for a decrease in *Anabaena* when stratification breaks down and mixed conditions resume are currently unknown.

1.4 Objective of Study

The objective of this thesis is to examine the growth of *A. circinalis* in the lower Murray River and to discern the factors that are primarily responsible for control. Given the attributes of the river, it is hypothesised that phosphorus storage, nitrogen-fixation and buoyancy regulation may play integral roles in successful growth. Chapter 2 assesses techniques to investigate the effect of phosphorus fluxes on growth. Baseline laboratory data on the response of *A. circinalis* and the diatom *A. granulata* to phosphorus availability are also

examined to assist with the interpretation of field results. Chapter 3 examines the response of *A. circinalis* and the wider phytoplankton community to phosphorus and nitrogen availability in the river and the effect of enrichment. As nutrient availability is dynamic, the technique of *in situ* diffusion culturing is used to enable a realistic representation of the ambient nutrient environment. Chapter 4 investigates possible reasons for the decline of *A. circinalis* when stratification breaks and mixed conditions resume. Competition with *A. granulata*, the diatom that becomes dominant under mixed conditions, is examined. Growth and photosynthetic responses to light availability are also compared between the two species. Chapters 5 and 6 examine the relationship between growth of *A. circinalis* and the physical environment. Specific attention is given to growth under diurnally stratified conditions as this is the predominant mixing pattern in the lower Murray River. The benefit provided by positive buoyancy is also examined and a model demonstrates how changes in vertical light attenuation (e.g. though inputs from the turbid Darling River) and maximum mixed depth (Z_m) may influence the outcome. Chapter 7 examines trends across all field experiments, providing a synthesis of the factors that control growth of *A. circinalis* in the lower Murray River. A summary of the conditions that may allow bloom development is also provided. It should be noted at this stage that this thesis only deals with factors that affect growth of *A. circinalis* and loss factors are ignored. Therefore, any predictions regarding the possibility of bloom development should be treated as a worst case scenario.
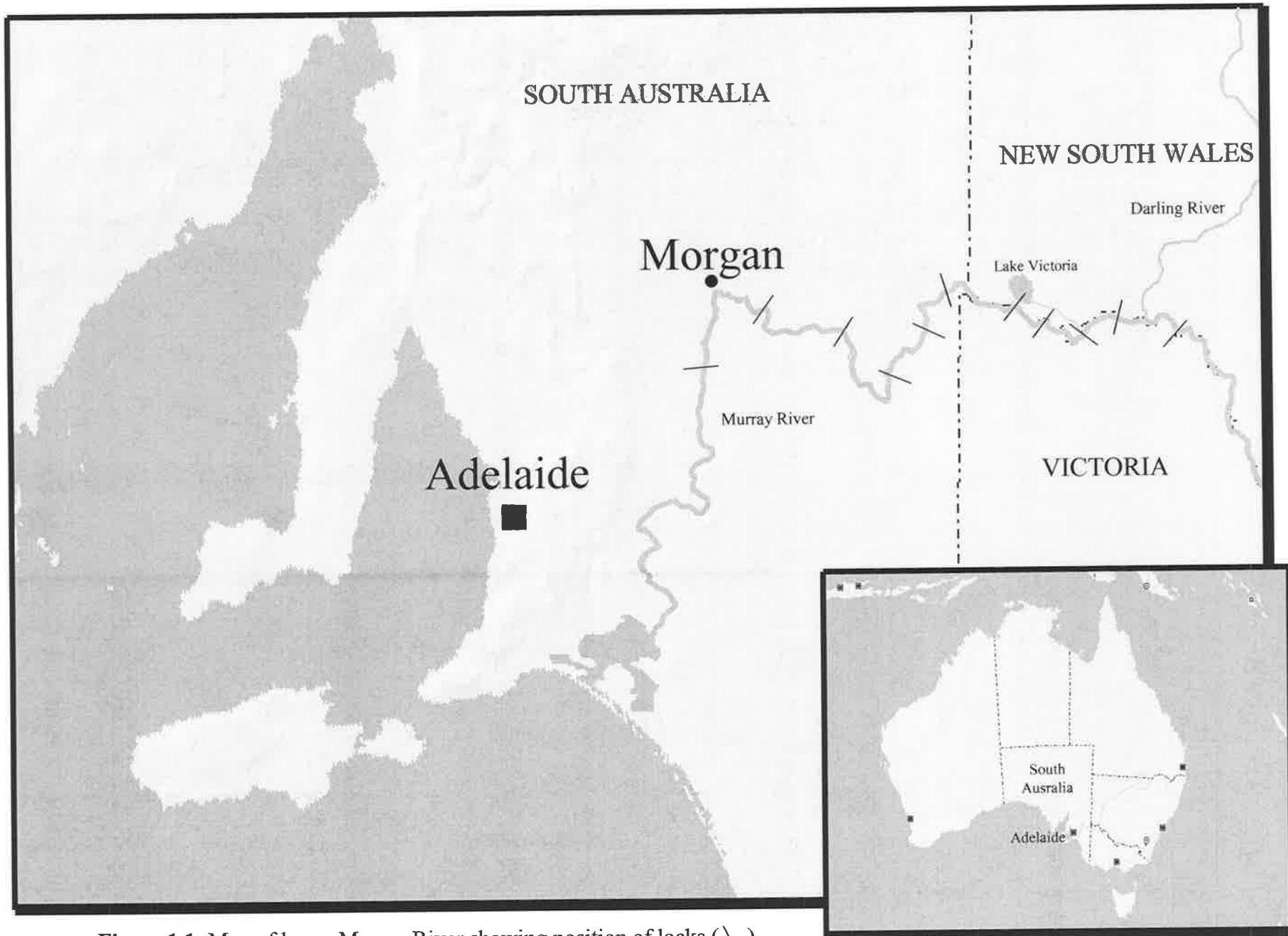
Figure 1.1: Map of lower Murray River showing position of locks (\ ) and location of field site at Morgan.
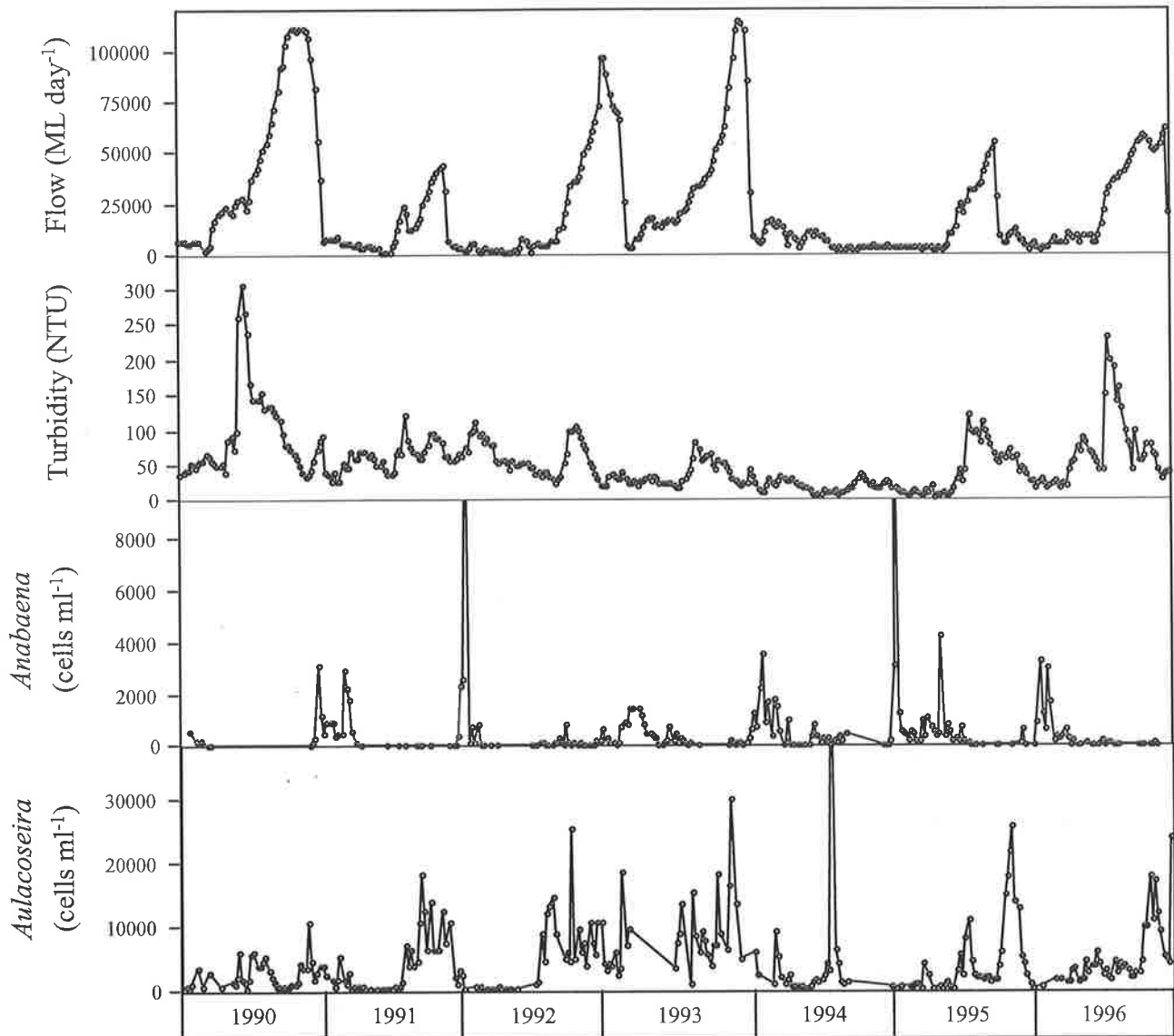
Figure 1.2: Monitoring data taken at Morgan on the lower Murray River

(data from the Murray-Darling Basin Commission).
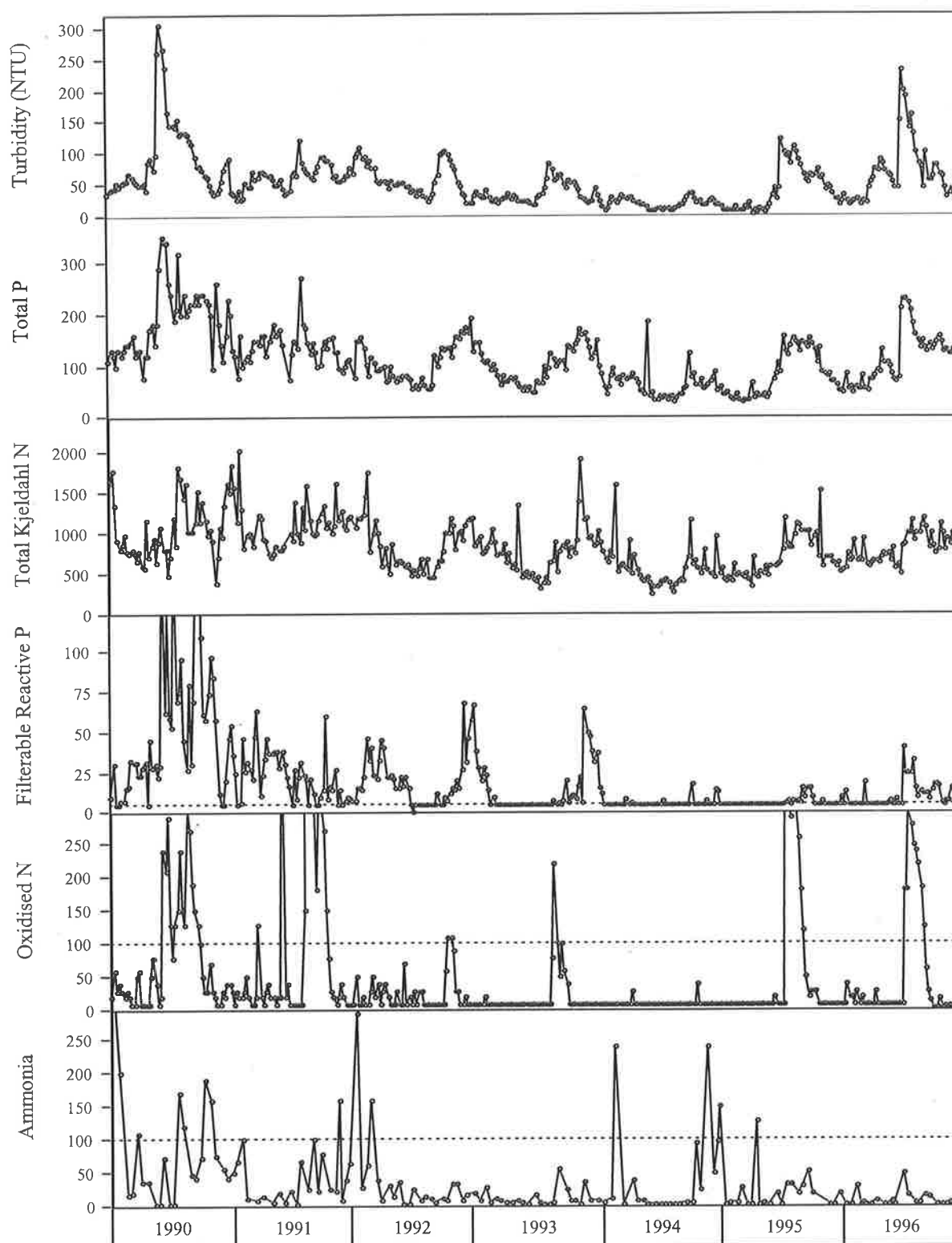
Figure 1.3: Monitoring data taken at Morgan on the lower Murray River (data from the Murray Darling Basin Commission). Dashed lines indicate limiting nutrient concentrations.

CHAPTER 2. An Assessment of Techniques to Investigate the Relationship Between Nutrient Availability and Growth of *Anabaena circinalis* in the Lower Murray River.

ABSTRACT

Techniques to examine the relationship between nutrient availability and growth of *Anabaena circinalis* were assessed as part of a pilot study in the lower Murray River. Diffusion chambers were found to be a suitable means of examining the nutrient-growth relationship. The diffusion of nutrients into the chambers was faster than the uptake rate of the enclosed cells so that the internal nutrient environment realistically represented the ambient conditions. To allow optimal diffusion rates, the chambers required rotation in the water column. The suitability of iron-oxide impregnated strips to detect phosphorus fluxes was also assessed. Unfortunately, the strips were not highly efficient in absorbing all available phosphorus and the amount absorbed tended to represent ambient concentrations rather than to detect fluxes with time. The iron-oxide strips were not considered a viable technique to use for future work. Finally, base-line data on the response of *A. circinalis* and the diatom *Aulacoseira granulata* to phosphorus availability were measured in the laboratory. Both species showed a clear decrease in growth in response to phosphorus limitation. Fluorescence measurements also detected phosphorus limitation effectively and it may be possible to use this technique for equating growth of *A. circinalis* in the field, thus removing the necessity for time-consuming cell counts.

2.1 Introduction

The initial focus of this thesis was to examine the response of *A. circinalis* to phosphorus availability in the lower Murray River. Given that growth may occur under nutrient limited conditions (Baker *et al.* 2000, Chapter 1), it was thought that cells may take up fluxes from the river which are stored and later utilised during intervals of low external availability. To examine this possibility and to adequately represent the dynamic nutrient environment of the lower Murray River, the technique of *in situ* diffusion culturing was tested. Diffusion culturing was originally developed by Schultz and Gerhardt (1969) for bacteria and was first used for phytoplankton by Jensen *et al.* in 1972. Cells are enclosed in a porous membrane which prevents them from escaping. However, the pores are large enough for soluble nutrients (e.g. nitrate and orthophosphate) and gases (e.g. carbon dioxide and oxygen) to

diffuse through. This eliminates the problems often encountered with the incubation of phytoplankton in closed bottles where wall effects, nutrient limitation and pH shifts may occur. The open exchange between the enclosed phytoplankton and external environment means that ambient nutrients can affect growth, thus providing a realistic response. Initial studies using diffusion cultures employed inexpensive dialysis tubing as the diffusion interface; a transparent, semi-permeable membrane made of regenerated cellulose. However, there may be problems with fouling and degradation of the membrane by cellulytic bacteria (e.g. Vargo *et al.* 1975). In a pilot study for this thesis it was found that dialysis tubing was unsuitable for the Murray River with leakage from bacterial degradation occurring within 4 days of *in situ* incubation (personal observation). Owens *et al.* (1977) developed a more robust diffusion culturing technique whereby filter papers are used as the porous membrane. Polysulphone filter papers are not readily degraded by bacteria (Phillip Ford personal communication, CSIRO Land and Water) and diffusion is faster due to a larger pore size (Köhler 1997). A modified version of the robust chambers described by Owens *et al.* (1977) was designed for this project. To facilitate the diffusion of solutes and gases across the terminal membranes, the cultures may require slow agitation or rotation in the water column (Jensen *et al.* 1972). This reduces the boundary layer that surrounds each chamber allowing faster exchange of molecules.

As *A. circinalis* can fix atmospheric nitrogen, it was considered that phosphorus availability may be more important in controlling growth of this species in the lower Murray River (Schindler 1977; Blomqvist *et al.* 1994). To gain a high resolution of phosphorus availability, the use of *in situ* iron-oxide strips (Fe-strips) to absorb phosphorus was tested. The Fe-strip technique was originally used for the detection of soil phosphorus available to plants (van der Zee *et al.* 1987; Robinson and Sharpley 1994; Chardon *et al.* 1996; Menon *et al.* 1997) and was later adopted for phytoplankton studies (Sharpley 1993 a, b, c; Shalders *et al.* 1998).

Strips of filter paper are covered with iron oxy-hydroxides and when immersed in water, absorb the phosphorus molecules that are present. As the strips do not release the phosphorus once it is absorbed, they behave in a similar manner to a phytoplankton cell in that they act as a sink, until saturation is reached.

The use of Fe-strips was developed as a means of measuring bioavailable phosphorus for phytoplankton (Shalders *et al.* 1998). From bioassays, it now seems that phytoplankton have access to more phosphorus than is indicated by inorganic phosphorus concentrations. Oliver *et al.* (1993) estimated that in the Murray-Darling Basin between 18 and 48% of total phosphorus is desorbable and available for phytoplankton use. The Fe-strips provide a reasonable indication of 'true' phosphorus availability and this is simply determined through incubating the strips in a known volume of water and measuring the concentration of the phosphorus absorbed. Another idea for the Fe-strip that was conceived for this thesis, is that the technique may provide an integrated measurement of phosphorus availability over time. Therefore, a flux rate rather than a concentration can be measured. Essentially, the Fe-strip may 'see' the same phosphorus molecules that a phytoplankton cell would encounter and the amount accumulated on the strip can be determined. This would be particularly useful if phosphorus concentrations are below detection. It is possible under these conditions that phytoplankton may still encounter phosphorus for growth, particularly if there is a temporary pulse. In a phosphorus impoverished environment, a temporary pulse would be immediately taken up by the phytoplankton so that no change in concentration would be detected. The incubation of Fe-strips *in situ* for a number of days also allows the amplification of available phosphorus, as the accumulated phosphorus would increase with time, thus ensuring that concentrations are above detection limits.

Base-line data on the physiology of *A. circinalis* and the diatom *Aulcoseira granulata* measured in the laboratory are also presented in this chapter to enable a better interpretation of field results. Growth and uptake rates in response to phosphorus availability are measured. The possibility of using fluorescence to detect phosphorus limitation is also explored for *A. circinalis* (Wood and Oliver 1995). Stress in plants and phytoplankton can be measured using the F_v/F_m ratio. Minimal fluorescence (F_o) is measured on raw samples, and maximal fluorescence (F_m) measured once all the reaction centres are closed. From these, the variable fluorescence ($F_v = F_m-F_o$) and the F_v/F_m ratio can be calculated. The F_v/F_m ratio emperically approximates photosynthetic efficiency. Theoretically, the maximum ratio should be equal to 1. However, this does not occur in nature as a proportion of light energy absorbed is always given back off as heat or fluorescence. From the literature, the maximum F_v/F_m for healthy phytoplankton is approximately 0.65 (Bolhár-Nordenkampf and Öquist 1993). In phosphorus limited populations of *A. circinalis* the F_v/F_m ratio should be significantly lower, indicating stress.

This chapter aims to assess the various techniques described above. It does not include all methods contained within the thesis as many are chapter specific. Further details of methods are provided within each appropriate chapter.

2.2 Methods

2.2.1 Diffusion Culturing

Diffusion chambers were constructed from 9.5 cm lengths of UV transmitting polycarbonate tubing (50.8 mm outside diameter, 44.5 mm inside diameter). The chambers were completed by the addition of 0.45 μm polysulphone filters (Activon 47 mm) attached at either end with a tightly-fitting hollow screw lid (Figure 2.1). The total volume of the chambers was 140 ml. A hole was cut in the middle of each chamber and a rubber septum (Becton and Dickinson)

fitted to allow repetitive sampling with a syringe and needle. The polycarbonate tubing, filter papers and septums were able to be sterilised in an autoclave at 118°C for 20 minutes, prior to use. Therefore, cultures may remain axenic when incubated in the chambers. To facilitate diffusion across the membranes, two submersible rotating machines (100 * 80 cm) were constructed which rotated at 1 revolution per minute (Figure 2.2). Replicate chambers were tied between two of five wheels arranged along the axle. Each machine supported thirty diffusion cultures.

A comparison of phosphorus flux rates across polysulphone filters was made between rotated and static diffusion chambers incubated in the Murray River. The diffusion chambers were filled with 140 ml millipore water and immersed in the river for up to 50 hours. Triplicates were removed at regular intervals over the course of the experiment and frozen until phosphorus analysis at a later date. The initial millipore water was also sampled to determine if there was any background phosphorus. Both filterable reactive phosphorus (FRP) and total phosphorus (TP) analysis was conducted by the Australian Water Quality Centre. As total phosphorus entered the diffusion chambers via a 0.45 μm membrane, this measurement essentially represented filterable reactive phosphorus plus dissolved organic phosphorus and was termed DOP+FRP. It is possible that a small amount of phosphorus associated with colloidal material could also cross the membrane, although this was not quantified. Temperature and flow were measured three times throughout the experiment.

2.2.2 Iron-oxide Strips

Iron-oxide strips (Fe-strips) were made according to the protocol of Procedure I outlined in Shalders et al. (1998). For this project, the Fe-strips were made using Whatman No. 542 filter paper rather than Whatman No. 1 as the former is hardened and therefore more appropriate to use for long exposure periods in the river. To make the Fe-strips, the filter papers (24 cm

diameter) were cut into 7.5 * 4 cm strips (total area of one side = 30 cm^2) and immersed in a solution of 65 g L^{-1} FeCl$_3$ (0.24 M). After one minute, each strip was pulled through a container of distilled water to remove excess iron solution, and then placed into an ammonia solution which was made by diluting 94 ml of 25% ammonia solution (AnalaR sp. gr. 0.91) to 500 ml with distilled water. After immersion in the ammonia solution, both sides of the filter paper were rinsed with a spray of distilled water so that excess iron particles were removed. The strips were then air-dried and stored in an air-tight plastic bag until required. To determine the amount of phosphorus adsorbed onto Fe-strips after experimental incubation, each was placed into a 50 ml flask and the iron oxy-hydroxide dissolved using 25 ml H$_2$SO$_4$ (0.04 M). Seven additional strips were immersed in acid and used as blanks for construction of a phosphorus standard curve. After 3 hours, the strips were removed from each flask and ortho-phosphate analysis conducted on the remaining solution using the procedure of Mackereth *et al.* 1978 which is a modified version of Murphy and Riley (1962) and Stephens (1963). The phosphorus adsorbed onto the strips was termed Fe-strip phosphorus.

To determine the appropriate incubation time for Fe-strips in the Murray River, a series were incubated *in situ* in cylindrical canisters suspended at 0.1 m. The canisters were 450 ml with fourteen 0.7 cm diameter holes drilled at each end. The experiment was conducted over 50 hours with triplicate strips removed throughout the experiment. A time series of accumulated phosphorus (P) was plotted and modelled using an equation based on Michaelis-Menten enzyme kinetics (Monod 1942);

$$P \text{ accumulated} = (\text{maximum P accumulated}*\text{time})/(P_s + \text{time}) \qquad \mu g \qquad (1)$$

where;

maximum P accumulated = phosphorus amount that saturates Fe-strip, μg

time = period of Fe-strip incubation, h

P_s = time at which half saturation of Fe-strip occurs, h

To determine the efficiency of phosphorus uptake by the Fe-strips, a laboratory experiment was conducted. Strips were exposed to phosphorus amounts ranging from 0 to 139.5 µg in the form of K_2HPO_4 dissolved in distilled water. To ensure adequate uptake, the strips were gently agitated. After 24 hours of immersion, the strips were removed and the amount of phosphorus that had been adsorbed analysed.

A pilot study of the Fe-strip technique was conducted in the Torrens River, close to the University of Adelaide. Similar to the Murray, previous work on the Torrens had suggested that *Microcystis aeruginosa* was able to bloom despite a seeming absence of available phosphorus for growth. It was hypothesised that cells were utilising fluxes of phosphorus from the sediments caused through anaerobic conditions during stratification. The experiment was a factorial design with three factors; "Flux", "Site" and "Depth". Within the factor "Flux", there were two levels; flux and no-flux. Within the factor "Site" there were three levels; Albert Bridge, Stormwater Drain and Weir. "Depth" had two levels; surface and bottom (1.5 m Albert Bridge; 1.5 m Stormwater Drain; 4.5 m Weir).

For the flux treatment, strips were incubated *in situ* for three days in cylindrical canisters (see above), then removed and frozen until phosphorus analysis at a later date. For the no-flux treatment, discrete water samples were taken at the appropriate site and depth in the river using a Freidinger sampler. An aliquot of the water (45 ml) was placed in a 50 ml centrifuge tube and incubated with an Fe-strip for 24 hours whilst being gently agitated. The remainder of the sampled water was frozen and later analysed for filterable reactive and total phosphorus. All Fe-strip data from specific sites and depths was analysed using 1-way ANOVA and a post-hoc Tukey-Kramer HSD test (JMP, SAS Institute Inc. Version 3.0.2. 1989-94).

2.2.3 Baseline Data

To examine the physiological response of *A. circinalis* and *A. granulata* to phosphorus limitation, separate cultures of each species were grown in WC media (Guillard and Lorenzen 1972) at 50 to 60 μmol m^{-2} sec^{-1} under a 14 h:10 h light/dark cycle at 25 °C. Six days prior to the commencement of the experiment the cultures were starved of phosphorus. For starvation the cells were concentrated using a centrifuge at 3000 rpm's for 10 minutes. The gas vesicles of *A. circinalis* were first collapsed in a pressure bomb at 1100 kPa for 3 minutes, using nitrogen gas as the pressure source. After centrifugation of each species, the supernatant was drawn off and the cells resuspended in phosphorus-free WC media. Complete WC media normally contains phosphorus in the form of K_2HPO_4. The ensure that cells had sufficient K in phosphorus-free media, an equimolar solution of KCl was added. The cells were centrifuged and rinsed twice more before being transferred to culture vessels and diluted to approximately 8 * 10^3 cells ml^{-1} (with phosphorus-free media). At the start of experimentation the cell density of each species was adjusted back to 8 * 10^3 cells ml^{-1} given that some growth had occurred since initial starvation, due to internal phosphorus reserves.

To conduct the experiment, 150 ml aliquots of either *A. circinalis* or *A. granulata* culture were placed into 250 ml Ehrlenmeyer flasks. A stock solution of K_2HPO_4 was added to triplicate flasks to give final phosphorus concentrations of 0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 or 10 μM. The flasks were incubated in a growth cabinet at 80 to 100 μmol m^{-2} sec^{-1} at 25 °C on a 12 h:12 h light/dark cycle for 9 days. Samples were taken from each flask and preserved using Lugol's iodine on days 0, 3, 7 and 8 of the experiment. Cell numbers were later enumerated using a Sedgewick-Rafter chamber and inverted microscope.

On day 8, phosphorus uptake rates were examined for each species using carrier-free ^{32}phosphorus (Bresatec) which has a half life of 14 days. Prior to the measurement of uptake

rates, 50 ml of each culture was removed from its experimental flask and washed three times in phosphorus-free WC media (see above) so the ambient phosphorus from the growth treatment was removed. Triplicates of each species were then transferred to plastic vials and uptake rates measured at eight phosphorus concentrations; 0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 μM. To measure uptake, stock solutions of 10, 100 and 1000 μM of phosphorus in the form of K_2HPO_4 were used and had specific activities of 26.98, 17.59 and 32.86 CPM pmol^{-1}, respectively. After the addition of the appropriate volume of stock solution, the cells were incubated for 10 min on an orbital shaker set at low speed and then filtered onto a 25 mm GF/C (Whatman) filter. The filters were placed in 4 ml scintillation fluid and the radioactivity measured using a Beckman LS3801 (Fullerton, California) scintillation counter. Adjustments for natural decay of the ^{32}phosphorus were calculated manually.

A second experiment on the response of *A. circinalis* to phosphorus availability was conducted to investigate the use of the F_v/F_m ratio as an indicator of phosphorus limitation, and to examine phosphorus uptake at higher concentrations than the first experiment. Cultures of *A. circinalis* were exposed to the same concentrations of phosphorus as for the first growth experiment (see above). Cell counts were taken on days 0, 4 and 8 of the experiment and uptake rates at phosphorus concentrations of 0.1, 0.5, 1, 2, 5, 10, 20, 40, 100 and 185 μM were measured on day 9. On days 4 and 8, fluorescence measurements were taken using a Turner Fluorometer (Model 111). Minimum fluorescence (F_o) was measured on raw 4 ml samples. To determine maximum fluorescence (F_m), 8 μL of 1 g L^{-1} 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) dissolved in 100 % ethanol was added to each sample to give a final concentration of 10 μM. Measurements were made after 1 minute. Variable fluorescence ($F_v = F_m - F_o$) and F_v/F_m were later calculated from the F_o and F_m readings.

Data on the growth (G) and uptake (V) rates of *A. circinalis* and *A. granulata* according to phosphorus availability were modelled using the Monod (1942) equation;

$$G = G_{max} \times [P] / K_s + [P] \quad \mu g \quad (2)$$

where;

G_{max} = maximum growth rate, day^{-1}

V_{max} = maximum phosphorus uptake rate, pmoles 10^7 cells h^{-1}

P = phosphorus concentration, μM

K_s = phosphorus concentration where half saturation of either growth (G) or

uptake (V) occurs, μM

The data were fitted according to least squares regression of modelled and predicted values.

2.3 Results

2.3.1 Diffusion Culturing

Phosphorus diffusion across polysulphone membranes was linear over the first 10 hours and approached equilibrium after this time (Figure 2.3). The amounts of phosphorus that diffused across the membrane per unit time (μg h^{-1}, 0-10 hours) were; FRP static = 0.315, FRP+DOP static = 0.364, FRP rotated = 0.378 and FRP+DOP rotated = 0.588 μg h^{-1}. Therefore, diffusion was more rapid for the rotated cultures compared to the static cultures which was expected as rotation reduces the boundary layer surrounding the membrane. The external FRP and TP concentrations in the river were 37.3 and 145.3 μg L^{-1}, respectively (Table 2.1). The equilibrium concentration of FRP+DOP inside the rotated diffusion chambers was 45 μg L^{-1} indicating that a large proportion (69%) of TP was unable to diffuse across the membrane; presumably because it was associated with particulate matter, or dissolved organic molecules

that were too large to cross the membrane. However, internal and external FRP concentrations were similar at equilibrium, demonstrating effective exchange of inorganic phosphorus.

2.3.2 Iron-oxide Strips

The absorption of phosphorus by Fe-strips when incubated in the Murray River consisted of 2 phases (Figure 2.4). Initially, the strips accumulated phosphorus rapidly but started to saturate by 10 hours. Between 10 and 50 hours there was further phosphorus accumulation but at a slower rate. The model fitted to the data between 0 and 50 hours showed that the maximum phosphorus amount that may be accumulated by the strips is 300 µg and that the half-saturation time is 27.5 h ($r^2 = 0.989$). Ambient FRP was 62 µg L^{-1} on the first day of experimentation and 59 µg L^{-1} on the second. TP concentrations were 207 and 163 µg L^{-1} on the first and second days. Fe-strip phosphorus measured in a known volume of river water (rather than accumulated *in situ*) was 146.9 µg L^{-1} on the first day of experimentation.

Results from the pilot study on Fe-strips incubated in the Torrens River were unclear (Figure 2.5). Analysis of each separate site and depth indicated that in the majority of cases, there was no significant difference in the amount of adsorbed phosphorus between flux and no-flux treatments. The exception was at the Weir surface where the amount of phosphorus adsorbed onto Fe-strips in the flux treatment was significantly higher than in the no-flux treatment. A puzzling result was from the Stormwater Drain at the surface where the amount of phosphorus adsorbed onto Fe-strips in the no-flux treatment was significantly higher than the flux treatment. The total amount of phosphorus absorbed by Fe-strips at the Weir, at both the surface and bottom, was significantly higher than at Albert Bridge or the Stormwater Drain suggesting that ambient phosphorus concentrations at the Weir were higher. This was supported by the nutrient data measured in the river over the course of the experiment with approximately 40% more FRP at the Weir compared to the other sites (Table 2.2). At Albert

Bridge and the Stormwater Drain, the concentrations of FRP and TP were higher at the surface compared to the bottom (Table 2.2). This was also reflected by Fe-strip phosphorus in the flux treatments. Higher concentrations at the surface compared to the bottom do not suggest that significant amounts of phosphorus were being released from the sediments.

The amount of phosphorus accumulated on the Fe-strips at the Weir ranged between 200 and 400 µg. However, the data from the Murray River (see above) suggested a maximum of only 300 µg that is able to absorb onto the strips. As ambient FRP concentrations at the Weir were 40% higher than in the Murray River, the uptake by the Fe-strips was likely to have been more rapid due to the theoretically faster diffusion of molecules towards the binding sites. However, the upper saturation point should have been the same regardless of the ambient concentration of phosphorus available. Another problem with the Fe-strips is that they were not highly efficient in absorbing all the available phosphorus, as shown by the laboratory experiment (Figure 2.6). The regression of the amount supplied v the amount absorbed gave rise to a slope of only 0.7. However, it may be possible to use the technique for phosphorus amounts lower than 50 µg as the relationship was 1:1.

2.3.3 Baseline Data

A. circinalis and *A. granulata* showed clear responses to phosphorus limitation in the laboratory (Figure 2.7). Over the first 3 days of experimentation, the growth rates of both species increased with phosphorus availability up to a maximum, then decreased at higher concentrations suggesting an inhibitory effect. For *A. circinalis*, a maximum growth rate of 0.47 day^{-1} was obtained at 0.5 µM and there was a sharp decline at higher concentrations. For *A. granulata*, maximum growth rate was 0.51 day^{-1} at 2 µM. Growth of both species between days 3 and 7 was slower than over the initial days of the experiment, but there was no longer an inhibitory effect caused by high phosphorus concentrations. Growth of *A. granulata* was

particularly slow between day 3 and day 7 with a maximum rate of 0.33 day^{-1} at 10 μM, compared to 0.52 day^{-1} for *A. circinalis*. Consequently, overall growth between days 0 and 7, was slower for *A. granulata* compared to *A. circinalis*. The modelled maximum growth rate for *A. circinalis* over 7 days was 0.4 day^{-1} (r^2=0.932) compared to 0.37 day^{-1} for *A. granulata* (r^2=0.912; Table 2.3). Phosphorus concentrations for half-saturated of growth of *A. circinalis* and *A. granulata* corresponded to 0.10 and 0.36 μM (Table 2.3). The maximum growth rates of both species fell within the normal range for phytoplankton growth (Table 2.3). Half-saturated growth of *A. circinalis* was similar to that determined for *Microcystis aeruginosa* by Holm and Armstrong (1981). *A. granulata* had a similar half-saturated growth rate to *Cyclotella meneghiniana* (Tilman *et al.* 1982).

Phosphorus uptake rates of *A. circinalis* and *A. granulata* were dependent on the preconditioning phosphorus treatments that the cells had received in the growth experiment (Figures 2.8 and 2.9). The higher the phosphorus preconditioning treatment, the slower the uptake, presumably because phosphorus cell quotas were higher. In all cases, the uptake rates of *A. circinalis* were significantly faster than *A. granulata*. Maximum modelled uptake rates were 8.0 x 10^6 (r^2=0.833) and 1.1 x 10^6 (r^2=0.771) ρmoles 10^7 cells h^{-1}, respectively. For comparison with the literature, these values correspond to 800 x 10^{-9} and 110 x 10^{-9} μmol cell^{-1} h^{-1} (Table 2.3). The uptake rate for *A. circinalis* is considerably higher than the range 0.78 x 10^{-9} to 332.3 x 10^{-9} μmol cell^{-1} h^{-1} determined for other phytoplankton species (Table 2.3). *A. granulata* is also in the upper range. The rapid uptake of phosphorus by *A. circinalis* and *A. granulata* may have been due to the extended period of phosphorus starvation in the cultures that received no phosphorus during experimentation. The length of time between starvation and measurement of phosphorus uptake in the 0 μM phosphorus cultures was 13 days.

The uptake of phosphorus by *A. circinalis* may be characterised by two systems. At concentrations between 0 and 2 μM, it appears that a high affinity uptake system was operating which started to saturate. At concentrations higher than 2μM, phosphorus uptake rate continued to increase, but at a slower rate, indicating that the phosphorus was possibly entering the cells either through diffusion, or by a second low affinity system (Mimura 1998; Reid *et al.* 2000). As 20 μM of phosphorus was insufficient to saturate the uptake rate of *A. circinalis*, a second experiment was conducted which showed that the maximum rate was at 50 μM (Figure 2.9). However, at higher concentrations the uptake rate sharply declined. The half-saturation for uptake (K_s) of *A. circinalis* was 10.0 μM compared to 3.0 μM for *A. granulata* (Table 2.3). *A. circinalis* has a significantly higher K_s than other phytoplankton species, with the exception of *Euglena gracilis* (Table 2.3). The high V_{max} and K_s for uptake of phosphorus by *A. circinalis*, compared to the lower values of G_{max} and K_s for growth, suggests that this species has a large capacity for phosphorus storage.

The F_v/F_m ratio successfully detected phosphorus limitation in *A. circinalis* (Figure 2.10). On day 4, F_v/F_m reached a maximum of 0.50 arbitrary units at 0.5 μM phosphorus but there was a sharp decline at higher concentrations. On day 8, the F_v/F_m ratio reached a maximum of 0.45 arbitrary units at 2 μM and there was only a slight decline at higher concentrations. Overall, the F_v/F_m ratios suggested that *A. circinalis* was phosphorus limited when measurements were less than 0.4. In general, the F_v/F_m ratios reflected the growth rates measured in the cultures and there was a significant relationship on day 8 ($r^2 = 0.709$) (Figure 2.11). However, the correlation on day 4 was not significant ($r^2 = 0.523$).

2.4 Discussion

2.4.1 Assessment of Diffusion Chambers

A comparison of the flux rate of phosphorus across polysulphone membranes and the measured uptake rates of phosphorus by *A. circinalis* suggests that the diffusion cultures designed for this project provide a suitable method for examining the effect of nutrient availability on growth. Flux rates of phosphorus across polysulphone membranes were 0.378 $\mu g\ h^{-1}$ FRP and 0.588 $\mu g\ h^{-1}$ FRP+DOP for rotated chambers and 0.315 FRP $\mu g\ h^{-1}$ and 0.364 $\mu g\ h^{-1}$ FRP+DOP for static chambers. At the time of experimentation, the external FRP concentration in the river was 37.3 $\mu g\ L^{-1}$ (1.2 μM). The phosphorus uptake rate in starved cells of *A. circinalis* at a concentration of 2 μM was $7.3 * 10^5$ pmoles P 10^7 cells^{-1} h^{-1} which is equal to 22.6 $\mu g\ L^{-1}$ P 10^7 cells^{-1} h^{-1}. Given an average starting cell density of 8×10^3 cells ml^{-1} in the 140 ml diffusion chambers, the total number of cells is approximately 1×10^6 and the expected uptake is 2.26 $\mu g\ L^{-1}$ P hr^{-1} or 0.32 μg P 140 ml^{-1} hr^{-1}. Therefore, the uptake of phosphorus by the cells is similar to the supply rate through the polysulphone membranes. Diffusion was faster in rotated cultures compared to static. This agrees with Köhler (1997) who used conductivity to examine diffusion rates across 2 μm polycarbonate membranes. When chambers were static, conductivity increased at 0.05 $\mu S\ h^{-1}$. In chambers moved at 0.5 m min^{-1}, conductivity increased at 0.12 $\mu S\ h^{-1}$.

It should be noted that when sampling the diffusion chambers using a syringe and needle through the rubber septum, an additional needle must also be placed through the septum. This allows the equalisation of pressure inside the chambers as the sample is being removed. Without the additional needle, it was found that the filter papers at either end of the chamber ruptured due to the low pressure created inside the chamber.

2.4.2 Assessment of Iron-oxide Strips

The data examining the use of Fe-strips to measure phosphorus fluxes suggests that this aspect of using the technique is too complicated to allow easy interpretation of results. Incubation of Fe-strips in canisters in the Murray River showed that phosphorus accumulation occurred in two phases. There was an initial rapid accumulation over the first 10 hours, followed by a slower rate between 10 and 50 hours. The decrease in accumulation rate was possibly caused by a decrease in phosphorus concentration with time, as indicated by the measurements of ambient availability. However, the decrease was not significant with FRP concentrations decreasing from only 62 to 59 μg L^{-1} and TP decreasing from 207 to 163 g L^{-1}. Another possibility for rapid uptake over the initial 10 hours is that the inorganic phosphorus component of the water was being absorbed. This was then followed by a slower uptake of additional phosphorus as it desorbed from the total phosphorus component between 10 and 50 hours (e.g. from particulate matter). The limiting step for the absorption process of Fe-strips is not the absorption rate of the strip, but the rate of movement of phosphorus molecules towards it (Shalders *et al.* 1998).

The same experiment suggested that saturation of the Fe-strip occurs at 300 μg of phosphorus. However, when Fe-strips were incubated in canisters in the Torrens River, amounts up to 400 μg were measured. One of the problems that can occur with the Fe-strip technique is that suspended particulate matter may adhere to the strip and occupy binding sites (Robinson and Sharpley 1994). There are two possible outcomes if this occurs. First, if the strip is near saturation, the particulate matter will prevent further phosphorus from being adsorbed. Second, acid digestion of Fe-strips during phosphorus analysis may cause organic phosphorus to slowly hydrolyse leading to an overestimation of Fe-strip phosphorus (Robinson and Sharpley 1994). Given that the lower Murray and Torrens rivers are highly

turbid, it is possible that suspended particulate matter played a role in the difference in Fe-strip saturation between sites.

The laboratory experiment on the efficiency of phosphorus uptake by Fe-strips showed that at amounts greater than 50 µg, the amount absorbed was significantly lower than the amount supplied. This would suggest that the Fe-strip was becoming saturated. However, supplies were well within the limits of saturation indicated by the field experiments. Shalders et $al.$ (1998) encountered a similar problem (85% phosphorus recovery) and determined that high iron concentrations during phosphorus analysis may interfere with the rate of reduced phosphomolybdate formation, thus causing inaccurate phosphorus measurements. In their study, further work showed that the effectiveness of the detection chemistry is not severely affected when iron concentrations are below 6.9 mM, but that the reaction rate is slowed to 65 minutes as concentrations approach this value. To improve the reaction time, the ascorbic acid concentration in the reaction mixture may be increased. When using 8.28 mM ascorbic acid in the reaction mixture, Shalders et $al.$ (1998) suggested 40 minutes incubation to allow for adequate development of colour. In this study, the final concentration of ascorbic acid in the samples was 10.2 mM and 60 minutes was allowed for colour development. Furthermore, the total surface area of the Fe-strips used in this study was only 30 cm^2, compared to 54.6 cm^2 in the study of Shalders et $al.$ (1998) where six Fe-strips per sample were incubated. Given the smaller surface area of the Fe-strips in this study, the final ferric ion concentrations should have been less than 3.9 mM. Overall, iron interference should not have caused a problem with the detection chemistry in this study and the low efficiency of phosphorus uptake by the Fe-strips is unable to be explained.

At phosphorus amounts lower than 50 µg the Fe-strips were efficient in absorbing the available phosphorus with a 1:1 relationship between the amount supplied and the amount

absorbed. It was unfortunate that ambient phosphorus concentrations were significantly elevated and in the eutrophic to hyper-eutrophic range for the field experiments in this study, and all subsequent experiments in the lower Murray River. The high phosphorus conditions in the lower Murray River in this study meant that 50 μg had absorbed onto the Fe-strips within 4 hours. This defeats the purpose of using the Fe-strips if incubated over such a short time period as there is not sufficient time for useful information of phosphorus fluxes to be gained. However, the Fe-strip technique may still be suitable for situations where the external phosphorus concentrations are low. Further field testing is required under these conditions.

The Fe-strips were unsuccessful in detecting phosphorus fluxes in the Torrens River with no difference between flux and no-flux treatments across all sites and depths. Two weeks prior to the experiment, concentrations in the river were undetectable. However, a significant rainfall event occurred a week later causing phosphorus to be elevated to as high as 112 $\mu g \, L^{-1}$ FRP at the Weir. The lack of difference between flux and no-flux treatments is puzzling given that the Fe-strips in the flux treatment were incubated for 3 days, allowing sufficient time for significant quantities of phosphorus to accumulate on the strips. A possible reason for no difference is that for the no-flux treatment, the Fe-strips were incubated in small volumes of water (45 ml) and the solutions were shaken for 24 hours to ensure that all the available phosphorus was absorbed. However, for the flux treatment, the Fe-strips were incubated in large 450 ml canisters with holes drilled at either end and there was no agitation. The water movement inside the canisters would have been minimal, causing a low probability of phosphorus molecules coming into contact with the binding sites on the Fe-strips. To examine the difference between flux and no-flux treatments, it would have been more appropriate to incubate the no-flux treatment in a canister the same size as the flux treatment without agitation, or *vice versa*.

Comparison of the Fe-strip data with phosphorus measured in the Torrens River showed that the strips detected a similar pattern in concentration between sites. This was expected as a larger number of phosphorus molecules would be present near the binding sites of the Fe-strips at higher ambient concentrations. Consequently, more phosphorus can be adsorbed onto the strip per unit time. Unfortunately, when the amounts of phosphorus adsorbed onto the Fe-strips in the no-flux treatments are converted to concentrations, they are significantly higher than the TP concentrations in the river which is impossible. For example, at Albert Bridge, the surface FRP and TP concentrations were 64 μg L^{-1} and 186 μg L^{-1}, respectively. The amount of phosphorus absorbed by the Fe-strips in the no-flux treatment was 155.5 μg per 450 ml or 345.5 μg L^{-1} which is considerably higher than the TP concentration. FRP and TP concentrations were determined by the Australian Water Quality Centre (South Australia) which is accredited by the National Association of Testing Authorities (NATA). However, Fe-strip phosphorus analysis was conducted at the University. There should be no reason that there were differences in phosphorus detection between the two locations. However, this is currently the only explanation that can be suggested. Extensive tests on possible background contamination by the incubation or analysis vessels did not show that this was the cause.

Overall, the use of Fe-strips to examine nutrient fluxes or bioavailable phosphorus in the lower Murray River is associated with problems. Differences were found with the saturation point when the strips were incubated at two difference locations, the uptake efficiency of the Fe-strips was inadequate at amounts greater than 50 μg, and the measurements of Fe-strip phosphorus gave rise to higher concentrations than total phosphorus. Unfortunately, these discrepancies mean that with current knowledge of the Fe-strip technique, an accurate representation of the phosphorus that phytoplankton cells encounter is unable to be discerned for the lower Murray River. For future work, growth of *A. circinalis* will be examined solely in relation to FRP and TP concentrations.

2.4.3 Physiological Responses to Phosphorus Limitation

Three responses of *A. circinalis* to phosphorus limitation were measured in the laboratory *i.e.* growth, uptake and fluorescence (F_v/F_m ratio). Under severely starved conditions (*i.e.* the initial days of experimentation), growth and fluorescence of *A. circinalis* increased with increasing external phosphorus concentrations up to 0.5 μM, but decreased at higher concentrations suggesting an inhibitory effect. In the latter stages of the experiment when cells would have acquired some phosphorus, no inhibition was detected with increases in growth and fluorescence with phosphorus concentrations up to 10 μM. This shows the importance of the nutrient preconditioning history of *A. circinalis*. For example, if cells are in phosphorus limited conditions and encounter a flux of high phosphorus concentration, they will not necessarily be in optimal conditions. Rather, the internal cell quota needs to increase before the external flux can be utilised effectively.

The uptake rate of phosphorus by *A. circinalis* consisted of 2 stages; an initial high affinity system that saturated at 2 μM, and an additional phase which allowed uptake rates to increase until a saturation point of 50 μM. Uptake rates were faster for *A. circinalis* compared to the diatom *A. granulata* suggesting that *A. granulata* dominance over *A. circinalis* under mixed conditions in the lower Murray River is unlikely to be related to phosphorus availability (see also Chapter 4). The inhibition of phosphorus uptake in *A. circinalis* at concentrations greater than 50 μM may be the result of the limitation of another nutrient at high phosphorus concentrations which would indicate a symport system of uptake (Reid *et al.* 2000). Ullrich and Glaser (1982) found that phosphorus uptake by the green alga *Ankistrodesmus* was strongly stimulated by sodium and proposed that uptake occurred via a P/Na symport. A similar system could also be present in *A. circinalis*.

Phosphorus uptake rates were higher for *A. circinalis* and *A. granulata* compared to other studies (Table 2.3). From the literature, the values for uptake range from 0.78×10^{-9} to 332.30×10^{-9} μmol cell^{-1} h^{-1}. For *A. circinalis* and *A. granulata*, uptake was 800.00×10^{-9} and 110.00×10^{-9} μmol cell^{-1} h^{-1}, respectively. This may have been the consequence of severe phosphorus limitation as cells had been starved of phosphorus for 13 days. The half-saturation (K_s) for growth of *A. circinalis* was also significantly higher than other phytoplankton species (10 μmol). Although, K_s for *A. granulata* (3 μmol) was similar to the diatom *Asterionella formosa* (Tilman and Kilham 1976). The high V_{max} and K_s for phosphorus uptake by *A. circinalis*, compared to lower values for G_{max} and K_s for growth, suggests that this species has a large capacity for phosphorus storage. This supports the suggestion that phosphorus storage may be an important characteristic for dominance of *A. circinalis* in the lower Murray River.

The success of the F_v/F_m ratio in detecting phosphorus limitation of *A. circinalis* may provide an easy means of detection in the lower Murray River. However, this requires testing in the field as many other factors may alter the fluorescence signal, including light availability and limitation by nutrients other than phosphorus (Krause and Weis 1991; Campbell *et al.* 1998). The significant relationship between growth of *A. circinalis* and the F_v/F_m ratio may also provide a means of estimating growth without the need to count tangled coils which is difficult and time-consuming. Given the relationship was not significant until after 8 days growth, the ratio could only be used in long-term (>1 week) experiments. The relationship between F_v/F_m and growth also requires further investigation.
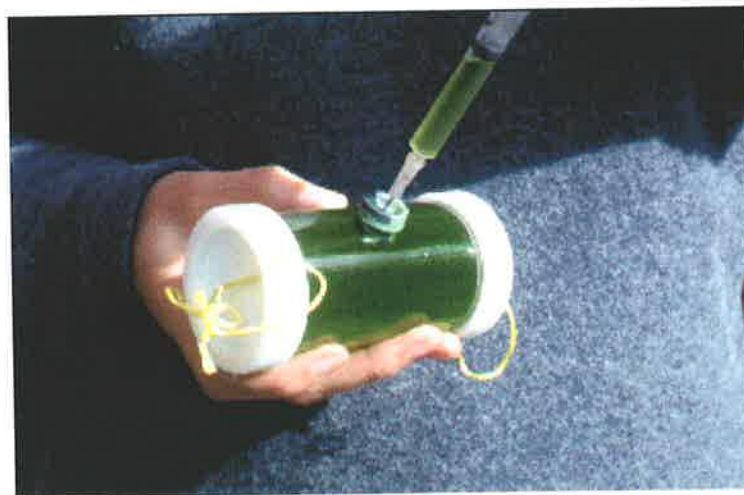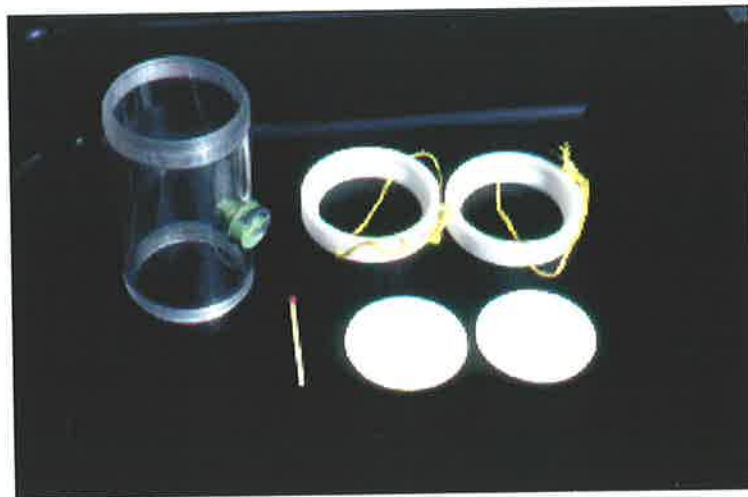
Figure 2.1: Components and sampling of a diffusion chamber.
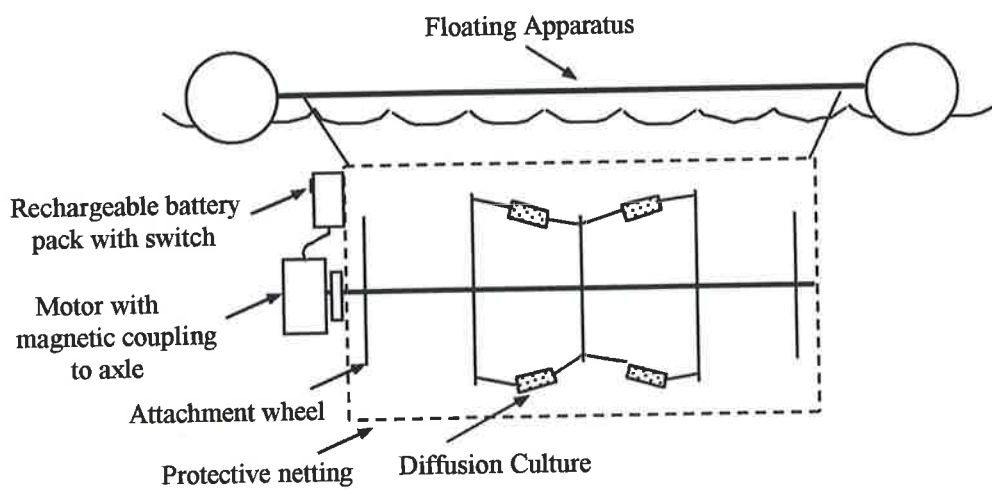
Figure 2.2: Sketch of rotating machine and method of suspension in the lower Murray River at Morgan.
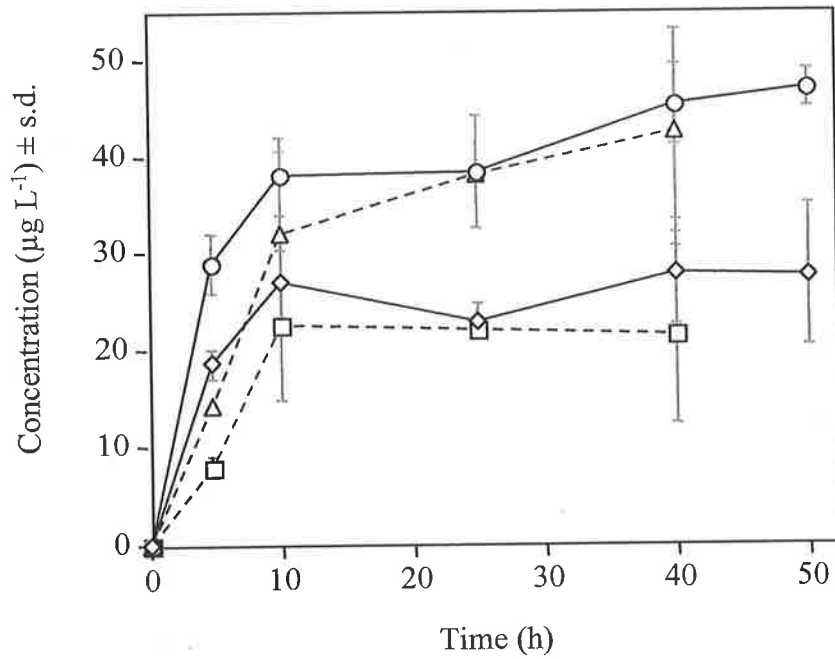
Figure 2.3: Flux of ambient filterable reactive phosphorus (FRP) and filterable reactive phosphorus plus dissolved organic phosphorus (FRP+DOP) across polysulphone membranes into experimental diffusion chambers. Cultures were either rotated (diamond FRP; circle FRP+DOP, solid line) or static (square FRP; triangle FRP+DOP, broken line).

Table 2.1: Ambient nutrient availability (μg L^{-1}) during incubation of diffusion chambers in the lower Murray River to determine phosphorus flux rates across polysulphone membranes. Temperature (°C) and flow data (m s^{-1}) are also given.

Day	FRP	TP	NOx	NH$_4$	TKN
1	43	159	<0.005	34	860
2	36	130	<0.005	25	780
3	33	147	<0.005	30	970
Average	37.3	145.3	<0.005	29.7	870.0
S.D.	4.2	11.9	0	3.7	77.9
Temperature	23.2 °C				
Flow Rate	0.04 m s^{-1}				
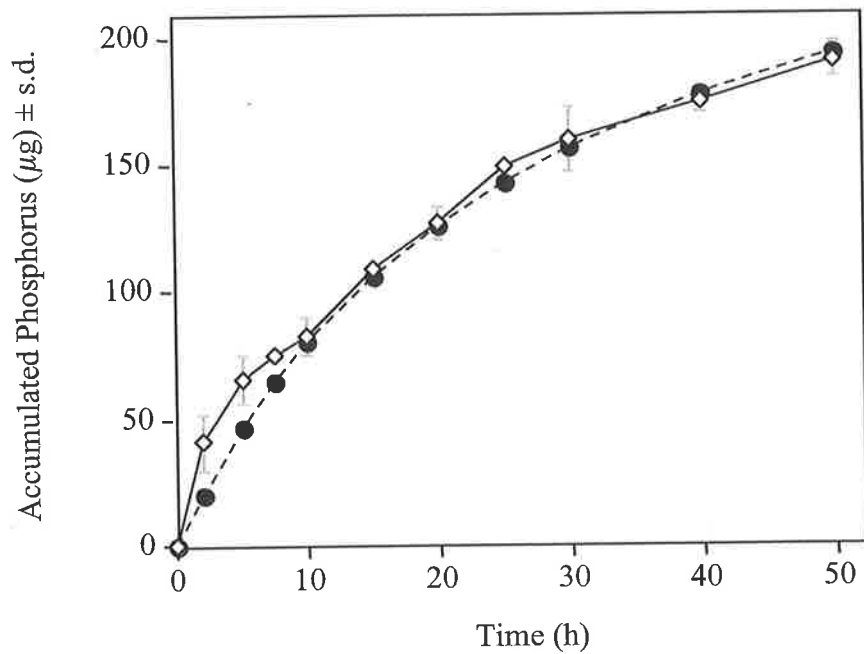
Figure 2.4: Accumulated phosphorus on Fe-strips when incubated in the lower Murray River (diamond, solid line). Data from 0 to 50 hours was modelled to determine the saturation point of Fe-strips (circle, broken line). See methods for equation.
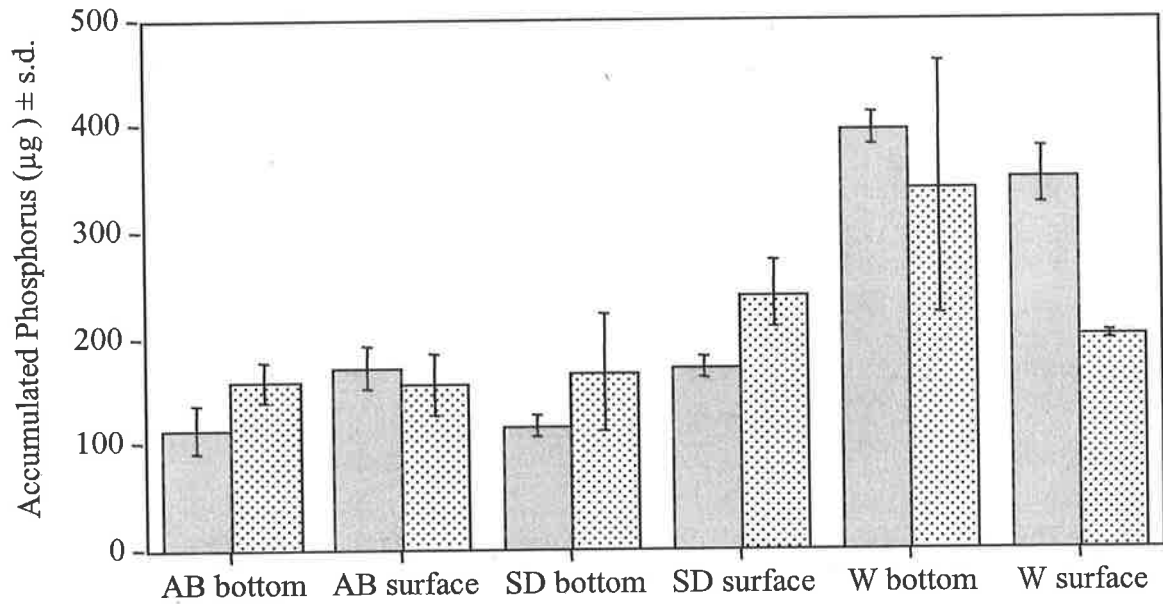
Figure 2.5: Fe-strip phosphorus in flux (■) and no-flux (☒) treatments in the

Torrens River at Albert Bridge (AB), Stormwater Drain (SD) and Weir (W).

Table 2.2: Ambient nutrient concentrations ($\mu g\ L^{-1}$) in the Torrens River during Fe-strip incubations to examine phosphorus availability in flux and no-flux treatments. For integrated nutrient measurements, water was sampled from the surface with a 1.5 m hosepipe. Surface and bottom water samples were taken with a Freidinger sampler.

	FRP	TP
Stormwater Drain		
integrated	64	140
surface	65	158
bottom	46	123
Weir		
integrated	112	227
surface	106	205
bottom	101	197
Albert Bridge		
integrated	62	165
surface	64	186
bottom	36	105
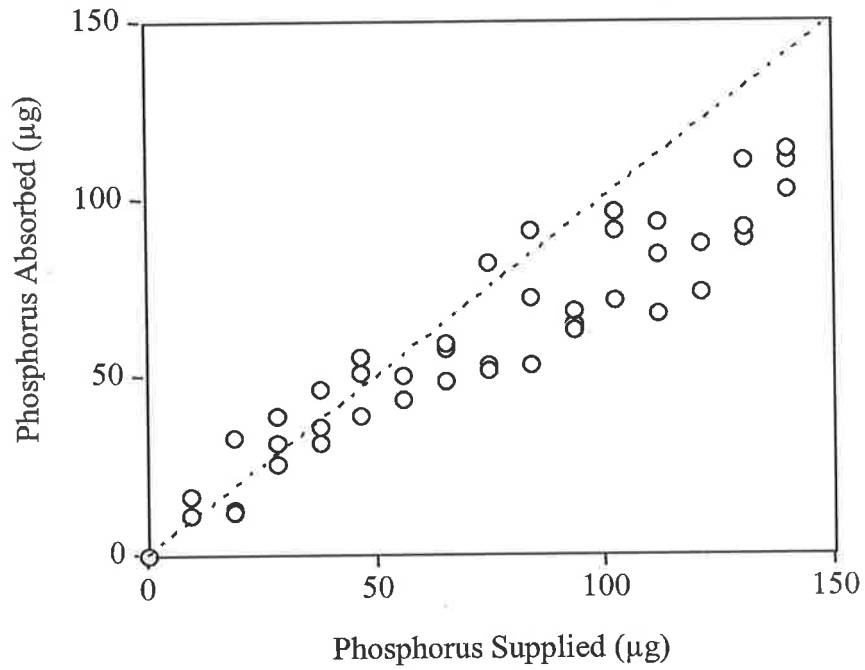
Figure 2.6: Efficiency of phosphorus uptake by Fe-strips incubated in the

laboratory
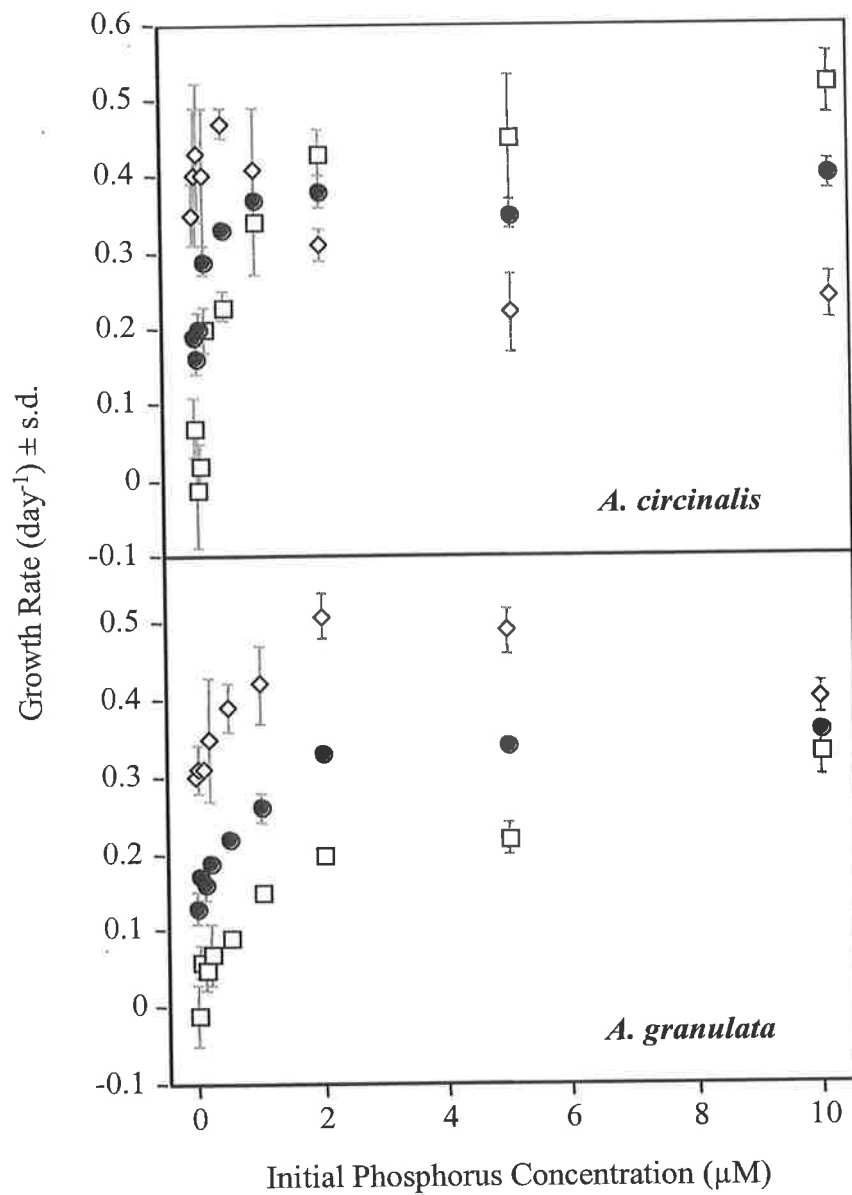
Figure 2.7: Growth of phosphorus-starved *A. circinalis* and *A. granulata*

(diamond days 0-3; square days 3-7; circle days 0-7) under different

phosphorus concentrations.

Table 2.3: Comparison of values derived from modelling of growth (G) and uptake rates (V) of *A. circinalis* and *A. granulata* under different phosphorus concentrations, with values from the literature. G_{max}, day^{-1}; V_{max}, 10^{-9} µmol cell^{-1} h^{-1}; K_s, µmol. Data of Riegman (1985) and Kromkamp *et al.* (1989) converted assuming the following dry weights per cell, from Reynolds (1984); *Anabaena flos-aquae* = 45.0 pg cell^{-1}, *Aphanizomenon flos-aquae* = 3.9 pg cell^{-1}, *Microcystis aeruginosa* = 32.0 pg cell^{-1}.

Species	G_{max}	K_s (growth)	V_{max}	K_s (uptake)	Reference
Anabaena circinalis	0.40	0.100	800.00	10.00	This study
Aulacoseira granulata	0.37	0.360	110.00	3.00	This study
Cyanobacteria					
Anabaena flos-aquae			47.30	1.54	Riegman (1985)
Anabaena planctonica			73.50	0.06	Smith and Kalff (1982)
Aphanizomenon flos-aquae			0.78	0.23	Riegman (1985)
			57.10	0.06	Smith and Kalff (1982)
Microcystis aeruginosa	0.25	0.190	8.00	1.23	Holm and Armstrong (1981)
			44.80	3.80	Kromkamp *et al.* (1989)
			20.00	2.00	Brookes (1997)
Oscillatoria agardhii	0.86	0.035			Tilman *et al.* (1982)
Oscillatoria tenuis			332.30	0.04	Smith and Kalff (1982)
Diatoms					
Asterionella formosa	0.67	0.070	15.00	0.70	Holm and Armstrong (1981)
			13.20	1.90	Tilman and Kilham (1976)
			9.80	2.80	Tilman and Kilham (1976)
			18.00	0.06	Smith and Kalff (1982)
Cyclotella meneghiniana	0.25	0.250			Tilman *et al.* (1982)
			5.50	0.80	Tilman and Kilham (1976)
Fragilaria crotonensis	0.80	0.011			Tilman *et al.* (1982)
			132.30	0.03	Smith and Kalff (1982)
Synedra acus			15.60	0.06	Smith and Kalff (1982)
Synedra filiformis	0.65	0.003			Tilman *et al.* (1982)
Others					
Cryptomonas erosa			2.20	0.13	Morgan (1976)
Euglena gracilis				16.00	Healey (1973b)
			150.60	1.90	Chisholm and Stross (1975)
Scenedesmus sp.			4.80	0.60	Rhee (1973)
Volvox aureus	0.69	1.220			Tilman *et al.* (1982)
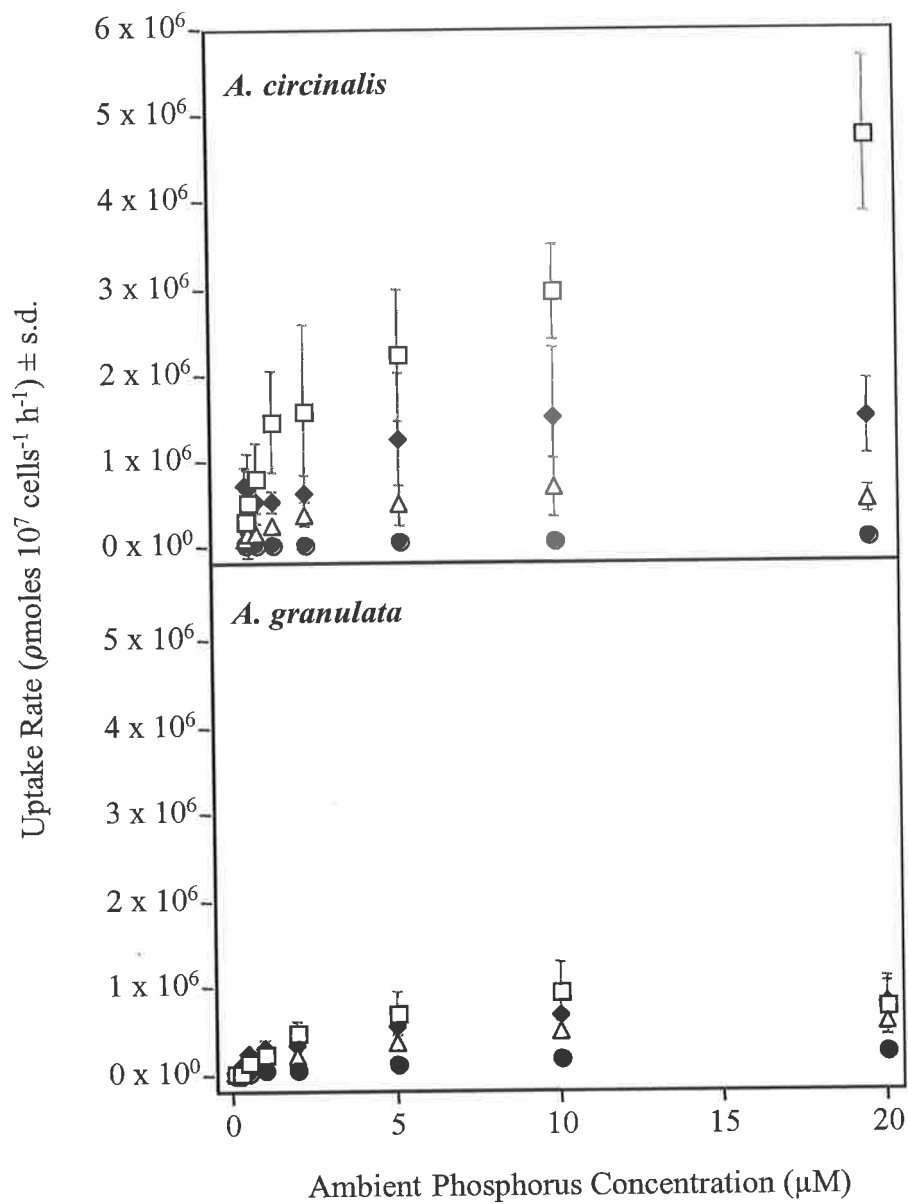
Figure 2.8: Phosphorus uptake rates of *A. circinalis* and *A. granulata* with different pre-conditioning phosphorus treatments (square 0; diamond 0.2; triangle 0.5; circle 10 uM)
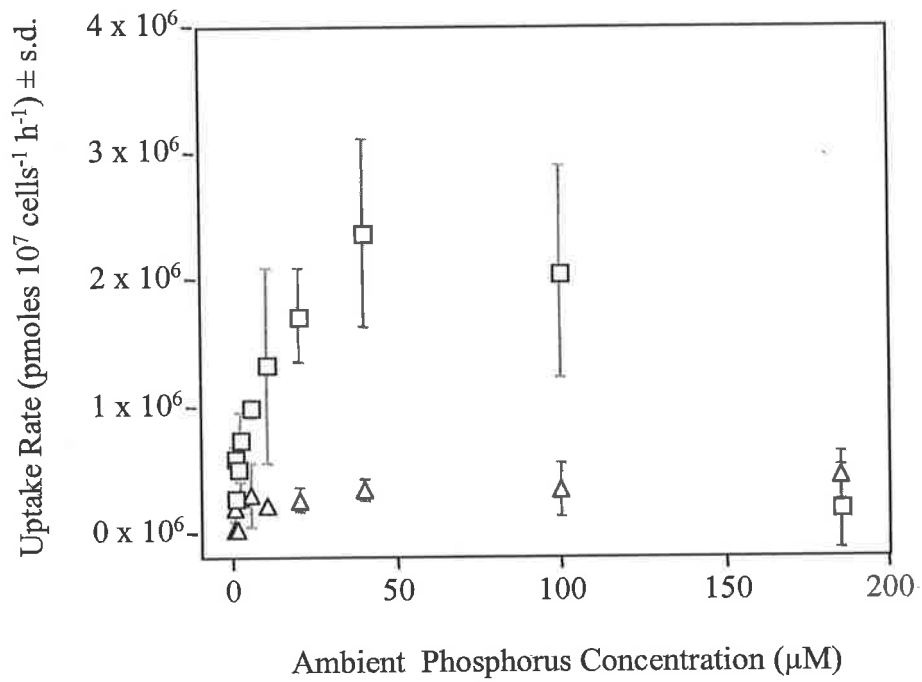
Figure 2.9: Phosphorus uptake rates of *A. circinalis* with different pre-

conditioning phosphorus treatments (square 0; triangle 0.5 uM).
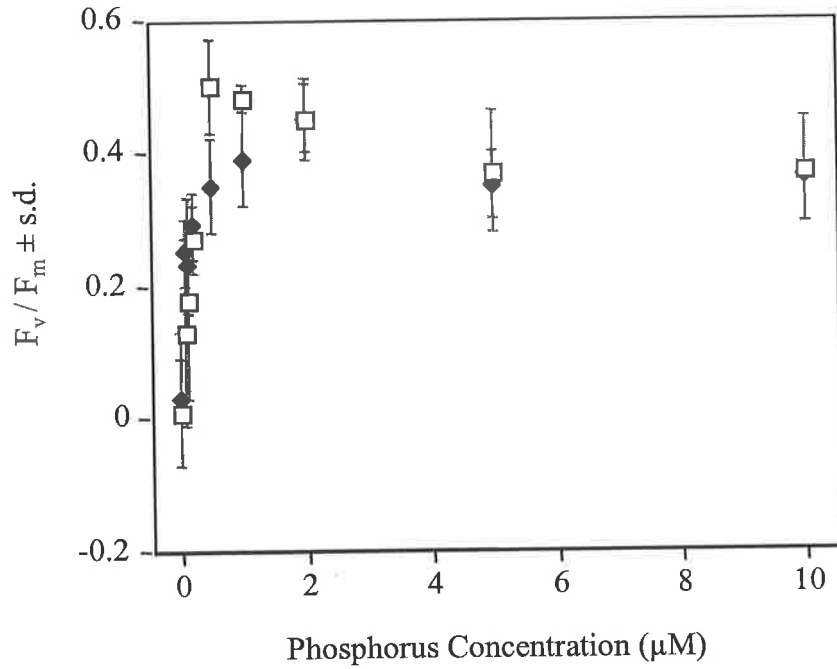
Figure 2.10: Use of fluorescence (F_v/F_m ratio) to detect phosphorus limitation of *A. circinalis* on day 4 (square) and day 8 (diamond) of the growth experiment.
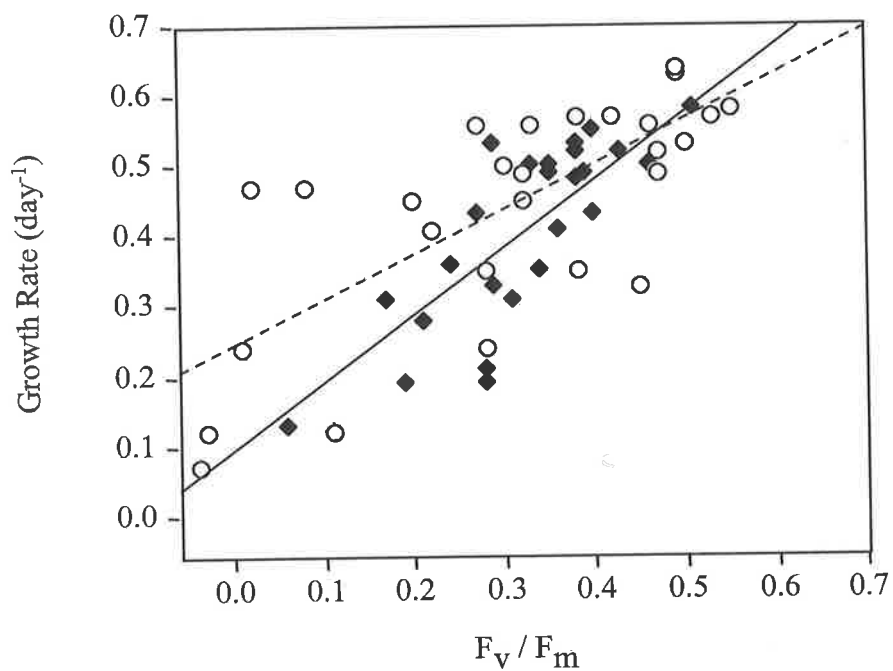
Figure 2.11: Correlation of F_v/F_m and growth rate on day 4 (circle, broken

line, y = 0.640x + 0.243) and day 8 (diamond, solid line, y = 0.965x +

0.095) of growth experiment.

CHAPTER 3. Eutrophication in the Lower Murray River: Effect of Phosphorus and Nitrogen Enrichment on Cyanobacterial Growth.

ABSTRACT

The importance of nutrients for the stimulation of cyanobacterial blooms in the lower Murray River was investigated *in situ* over two consecutive growth seasons. Over both seasons, *Anabaena circinalis* was incubated in diffusion chambers and the effect of phosphorus and nitrogen additions on growth rate assessed. Nutrient additions had no influence on growth, although the heterocyst/vegetative cell ratio increased significantly indicating that this species was fixing atmospheric sources of nitrogen. In the second season, water was taken from the Murray River and incubated *in situ* in both unsealed and sealed diffusion chambers. After 8 days the phytoplankton biomass in unsealed chambers was significantly higher than in sealed chambers indicating that nutrient fluxes are important for sustained growth of the natural assemblage in the river. Nitrogen and phosphorus additions to unsealed chambers did not cause a further increase in biomass and there was no change in community structure regardless of whether nitrogen was administered in the form of ammonium or nitrate. Initial numbers of nitrogen-fixing cyanobacteria in the assemblage were low but growth was not significantly stimulated by either nitrogen or phosphorus. The lack of response to nutrient additions in unsealed diffusion chambers was attributed to elevated concentrations of inorganic phosphorus and nitrogen in the lower Murray River during the time of the experiments. High vertical light attenuation coefficients in the river indicated that elevated nutrients were likely to have been caused through significant proportions of water diverted from the turbid Darling River upstream.

3.1 Introduction

Shifts in phytoplankton species composition towards cyanobacterial dominance are often associated with the process of eutrophication (Reynolds 1987; Steinberg and Hartmann 1988). Phosphorus and nitrogen inputs from allochthonous sources, including sewage and agricultural run-off, may lead to significant increases in biomass, thus reducing water quality (Pick and Lean 1987; Hecky and Kilham 1988). The effect of nutrient enrichment has previously been demonstrated in Australia with blooms of *Nodularia spumigena* in the Peel-Harvey Estuary in Western Australia, and *Anabaena* spp. in the Hawkesbury River, New South Wales (George and Bradby 1993; Cullen 1994; Humphries and Robinson 1995).

Examples from overseas are numerous, including blooms of *Aphanizomenon flos-aquae* in Lake Müggelsee, Germany (Nixdorf 1994), *Microcystis aeruginosa* in Lake McIlwaine, Zimbabwe (Thornton 1982), and *Oscillatoria rubescens* in Lake Washington, USA (Edmondson and Lehman 1981). In the Murray-Darling Basin, intense agricultural land-use and the process of eutrophication have been implicated in an increase in the incidence and severity of *A. circinalis* blooms. However, before expensive management actions are adopted to reduce nutrient load, the importance of nutrient supply in contributing towards bloom development must be evaluated. The response of chlorophyll biomass to nutrient removal is variable (Oliver and Ganf 2000) and large reductions in nutrient loading are required to be effective (Sakamoto 1966; Vollenweider 1968; Dillon and Rigler 1974; Smith 1979, 1982).

In contrast to the assumption that the Murray-Darling Basin is eutrophic, inorganic phosphorus and nitrogen concentrations are often below the limit of detection and fluctuate across limiting thresholds (Chapter 1). Regardless, growth of cyanobacteria can still occur. For example, *A. circinalis* grew at 0.176 day^{-1} in a parcel of water tracked down the lower Murray River when both inorganic nitrogen and phosphorus were undetectable (Baker *et al.* 2000). Sufficient nitrogen may have been obtained by *A. circinalis* through fixation of atmospheric sources (Lewis and Levine 1984). However, phosphorus must be obtained solely from the water-column. Oliver *et al.* (1993) estimated that in the Murray-Darling Basin, between 18 and 48% of total phosphorus is desorbable and available for phytoplankton use (bioavailable phosphorus) and it is possible that *A. circinalis* had access to this phosphorus source. Nutrient storage may also provide a distinct advantage when external concentrations are low (Grillo and Gibson 1979; Gotham and Rhee 1981; Konopka *et al.* 1993). Cyanobacteria are particularly efficient in storing phosphorus and nitrogen and are considered storage specialists (Sommer 1985; Kromkamp 1987; Mackerras *et al.* 1990; Pettersen *et al.* 1993). As cells encounter fluxes of nutrients, e.g. from zooplankton recycling or sediment

release, the nutrients may be immediately taken up and stored so that a change in concentration would not be detected. This may have been the reason that Sherman *et al.* (1998) found an eight-fold increase in *Anabaena* in Maude Weir, within the Murray-Darling Basin, when bioavailable phosphorus was below the limit of detection. With rapid cycling of nutrients, true availability for phytoplankton is missed. This problem may be overcome through incubating cells in diffusion chambers that allow enclosed cells to access the external fluctuating nutrient environment.

Nitrogen availability is also important in the control of cyanobacterial growth. Blomqvist *et al.* (1994) reviewed data from temperate lakes and conducted enclosure experiments to demonstrate that the phytoplankton community responds differently to nitrogen availability according to the concentration and form of nitrogen available. Their data suggests that the development of cyanobacteria is restricted if the concentration of nitrate is greater than 20 µg L^{-1}. During times of nitrogen deficiency (no nitrate or ammonia), nitrogen-fixing cyanobacteria develop. When nitrate is available, eukaryotes dominate, but are out-competed by non-nitrogen-fixing cyanobacteria when nitrate is depleted and ammonia is available. Blomqvist *et al.* (1994) proposed that these differential responses to nitrogen availability are an important consideration for the dominance of cyanobacteria. However, few other *in situ* studies have been conducted to support this theory (Elser *et al.* 1990). The lower Murray River is an ideal site for further testing due to low nitrogen availability. For example, oxidised nitrogen concentrations were less than 20 µg L^{-1} on 78% of sampling occasions, and mostly undetectable over summer growth periods between 1990 and 1996 (Chapter 1).

The aim of this study was to examine the *in situ* response of cyanobacteria to phosphorus and nitrogen enrichment in the lower Murray River. Particular attention was given to the toxic cyanobacterium *Anabaena circinalis*, as this species is considered the most abundant and

problematic in the river systems of the Murray-Darling Basin (Baker and Humpage 1994). The response to enrichment at the community level was also examined. From the work of Blomqvist *et al.* (1994), it was hypothesised that nitrogen-fixing cyanobacteria would dominate in cultures with no nitrogen additions, that non-nitrogen-fixing cyanobacteria would dominate in cultures with ammonia additions, and that eukaryotes would dominate in cultures with nitrate additions. The effect of nutrient fluxes on community structure was also examined.

3.2 Methods

3.2.1 Incubation of Anabaena circinalis

Two *in situ* experiments examining the response of *Anabaena circinalis* to nutrients were conducted in the lower Murray River at Morgan (see Chapter 1). The first was conducted in December 1997 and the second in March 1998. For both experiments, cells of *A. circinalis* (ANA271B, isolated from Wongulla Lagoon, Murray River 1992) were grown in WC media (Guillard and Lorenzen 1972) at 50-60 μmol m^{-2} s^{-1} under a 14 h:10 h light/dark cycle at 25 °C. Six days prior to the start of each experiment the cells were rinsed and transferred to phosphorus-free (P-free) WC media to deplete cells of internal phosphorus reserves (see Chapter 2). Three days prior, the same procedure was adopted using nitrogen-free (and P-free) WC media to deplete internal nitrogen reserves. Cells were starved of phosphorus longer than nitrogen as cyanobacteria are more efficient in storing the former through polyphosphate cell inclusions (Allen 1984).

A day prior to each experiment the starved cultures were made up to 10 L with N and P-free WC media and the cell number adjusted to approximately 8 x 10^3 cells ml^{-1}. The cultures were then transported to the field laboratory and left in the dark overnight. At the start of experimentation, diffusion cultures were labelled, filled with 140 ml of culture and immersed

in buckets containing Murray River water for transportation to the field site. Samples were taken from the remaining laboratory cultures and preserved in Lugol's iodine for enumeration at a later date. In addition, fluorescence measurements (F_0 and F_m) were made using a Turner fluorometer (Chapter 2).

At the field site, the diffusion cultures were tied onto the rotating machines (Chapter 2) and deployed between approximately 16:00 and 17:00 h. In December 1997, the rotating machines were suspended at 0.70 m (depth of mid-axle) so that midday light availability (1800 μmol m^{-2} s^{-1}) ranged from 133.7 μmol m^{-2} s^{-1} at the top of the cage (0.53 m) to 23.2 μmol m^{-2} sec^{-1} at the bottom (0.87 m). For the March 1998 experiment, the rotating machines were suspended at 0.43 m and midday light availability ranged from 328.8 μmol m^{-2} s^{-1} at the top of the cage (0.26 m) to 30.4 μmol m^{-2} s^{-1} at the bottom (0.60 m).

For both experiments, diffusion cultures containing *A. circinalis* were spiked with nutrients every two days throughout an 8 day incubation (day 0 inclusive). In December 1997 there were five spiking treatments, each with five replicates; Control, 0.1, 1 and 50 μM K_2HPO_4 and 1000 μM $NaNO_3$. In the March 1998 experiment there were two nutrient treatments; Control and 30 μM $NaNO_3$. Since *A. circinalis* can fix atmospheric nitrogen, it was considered that phosphorus may be more important in controlling growth. The series of phosphorus concentrations tested in December 1997 was used with the intent of producing a saturation curve.

For both experiments, 6 ml samples were collected from diffusion chambers every 2 days between 8:00 and 11:00 h. To determine cell and heterocyst numbers, 2 ml of sample were preserved in Lugol's iodine and later enumerated in the laboratory using a Sedgwick-Rafter

chamber and inverted microscope. The remainder of the sample was taken back to the field

laboratory for fluorescence measurements to be determined between 13:00 and 17:00 h.

3.2.2 Incubation of Murray River Water

The second objective of the field work conducted in March 1998 was to investigate the

response of the wider phytoplankton community to nutrient enrichment, and test the theory of

Blomqvist *et al.* (1994). The importance of nutrient fluxes for sustained growth of the

community was also examined. Diffusion chambers were filled with water taken from the

Murray River containing the natural algal assemblage, rather than with pure culture grown in

the laboratory. To collect the water for incubation, integrated samples were taken from the

river using a 1.5 m hose-pipe at a number of locations around the field site. The samples were

spatially separated as far as possible in order to represent the area effectively. All samples

were placed in a 10 L carboy and mixed thoroughly to form one homogenous culture.

The diffusion cultures containing the river water were exposed to one of six nutrient

treatments every 2 days throughout the 8 day experiment; Control, 15 μM NO_3, 30 μM NO_3,

15 μM NH_4, 30 μM NH_4 and 2 μM P. In addition, there was a 'No Flux' treatment where five

replicate cultures were incubated in diffusion chambers that were sealed with plastic discs so

that no additional nutrients could diffuse in. The 'No Flux' treatment was included as a

comparison to the Control which was the 'Flux' treatment. There were no additional nutrients

administered to either of these treatments throughout the experiment.

The deployment of diffusion cultures and subsequent sampling was the same as that described

for the incubation of *A. circinalis* in March 1998, described above. To determine the effect of

the nutrient treatments on community structure the species were grouped into four

phytoplankton types; greens, diatoms, non-nitrogen-fixing cyanobacteria and nitrogen-fixing

cyanobacteria. Increases in both cell number and biovolume were examined. Biovolumes were calculated from the shape and average measured dimensions of each species.

3.2.3 Laboratory Bioassays

Laboratory bioassays were conducted by Dr Justin Brookes at the University of Adelaide, in parallel to both field experiments. Integrated water samples were collected at the field site on the first day of each experiment and transported back to the university. The water was then placed in a series of Ehrlenmeyer flasks and triplicate samples spiked with nutrients to give one of the following treatments; Control, +P (50 μM K_2HPO_4), +N (1000 μM $NaNO_3$) or N+P (both nutrients). The cultures were incubated at 90 to 100 μmol m^{-2} s^{-1} on a 12 h:12 h light/dark cycle at 25 °C. On day 0 and day 6 (December 1997) or day 7 (March 1998), samples (150 ml) were concentrated onto GF/C filter papers and analysed for chlorophyll a following overnight extraction in 95 % ethanol.

For the March 1998 experiment, additional bioassays were conducted to examine the effect of two different forms and concentrations of nitrogen on growth. The river water was spiked with either 15 μM NH_4, 15 μM NO_3, 30 μM NH_4 or 30 μM NO_3. An additional treatment, +WC(-N-P), was also included to determine if nutrients other than nitrogen or phosphorus were limiting. For the +WC(-N-P) treatment, N and P-free WC media was added to water samples to give final concentrations of elements used in normal laboratory culturing procedures (Guillard and Lorenzen 1972). The +WC(-N-P) treatment therefore contained the macronutrients carbon and silicon as well as trace metals and vitamins, but no nitrogen or phosphorus. Sampling for these bioassays was the same as that described above.

3.2.4 Environmental Monitoring

Nutrient concentrations were measured three times over the course of each experiment. Integrated water samples were taken with a 1.5 m hose-pipe at three locations around the field site and mixed to form a composite sample. For total phosphorus (TP) and total Kjeldahl nitrogen (TKN) analysis, 500 ml of sample was placed directly into PET-bottles and frozen until analysis at a later date. For analysis of filterable reactive phosphorus (FRP), oxidised nitrogen (nitrate + nitrite; NOx) and ammonia (NH_4), the water was pre-filtered using a Whatman GF/C due to high levels of suspended particulate matter. Final filtering was conducted using a 0.22 μm Supor membrane (Gelman 47 mm). Nutrient analysis of all samples was conducted by the Australian Water Quality Centre in South Australia which is accredited by the National Association of Testing Authorities (NATA), and conducts analysis according to standard procedures (Anon. 1995).

It should be noted that 0.45 μm membranes are normally used to filter samples for analysis of inorganic nutrients, rather than 0.22 μm membranes. However, recent evidence has shown that 0.45 μm membranes do not remove a significant number of water column bacteria. The bacteria can comprise up to 50 % of the total water column phosphorus when there is significant phytoplankton growth occurring (Phillip Ford personal communication, CSIRO Land and Water). Furthermore, Oliver et al. (1993) showed evidence that between 6 and 12% of particle bound phosphorus is in the < 0.2 μm fraction, whereas up to 70 % is contained in the > 0.2 μm fraction, with the greatest proportion in the 0.2 to 1 μm fraction. Overall, water filtered through a 0.45 μm membrane may contain a significant amount of phosphorus that is associated with the total phosphorus component rather than the dissolved inorganic component.

Additional environmental parameters measured throughout both experiments included water-temperature and light availability. Water-temperature was logged using Optic StowAways (Hasting Data Loggers, NSW, Australia) suspended at 0.1, 2.5 and 5.0 m. The loggers recorded data every 10 minutes and were downloaded at the University at a later date. Underwater light measurements were taken daily using a cosine-corrected underwater sensor (LICOR-1000). Vertical light attenuation was calculated from regression of light measurements against depth.

3.2.5 Data Analysis

Data for both experiments were analysed using JMP (SAS Institute Inc. Version 3.0.2 1989-94). The treatments were compared using one-way ANOVA and a post-hoc Tukey-Kramer HSD test. Prior to analysis, the data were checked for normal distribution using the Shapiro-Wilk Test. In cases where the data were skewed, the numbers were transformed using $\log_{10} y$. Data on the proportions of greens, diatoms, non-nitrogen-fixing cyanobacteria and nitrogen-fixing cyanobacteria were transformed using $\arcsin \sqrt{y}$.

3.3 Results

3.3.1 Response of A. circinalis to Nutrient Enrichment

The diffusion culture technique successfully supported growth of *Anabaena circinalis* in the field (Figure 3.1). Trends in growth were similar to the laboratory results determined in Chapter 2. For the December 1997 experiment, growth rates over 4 days ranged from 0.40 to 0.54 day^{-1} and were faster than growth over 8 days with rates of 0.37 to 0.42 day^{-1}. Growth was inhibited under high phosphorus (50 μM) and nitrogen (1000 μM) doses over the initial 4 days of experimentation, but inhibition was overcome by day 8. A problem with the measurement of growth over 4 days is that the growth rates are likely to reflect the growth

conditions prior to experimentation rather than the conditions in the Murray River, as nutrients diffusing in from the river may not have had sufficient time to affect growth. The starvation of cells prior to experimentation was likely to have induced a limitation effect over the initial days of the experiment.

Over 8 days, there was no significant difference in growth between nutrient treatments, suggesting that phosphorus and nitrogen were not limiting for *A. circinalis* in the lower Murray River (Figure 3.1). After an initial lag, the 50 μM phosphorus and 1000 μM nitrogen treatments grew rapidly and achieved cell densities similar to the other treatments. The slow growth of the no nutrient, 0.1 μM and 1.0 μM phosphorus treatments between days 4 and 8 suggests that the cultures were reaching a plateau in growth. However, the maximum cell density was only 2.5×10^5 cells ml^{-1}, and subsequent experiments showed that growth rates in the diffusion chambers does not slow until cell densities greater than 5×10^6 cells ml^{-1} are achieved. At cell densities between 1×10^5 and 1×10^6, the growth rate may start to increase, perhaps due to a shift in pH (personal observation; Shapiro 1990). It is possible that the slower growth rates were caused through adjustments in cell physiology. For example, energy may have been diverted for the production of heterocysts, photosynthetic pigments and mucilage, rather than utilised for growth. The same growth patterns were seen in the laboratory experiments in Chapter 2 for both *A. circinalis* and *A. granulata*, and in all subsequent experiments.

Examination of the ratio of heterocysts to vegetative cells in *A. circinalis* indicates an increase over 8 days for all treatments (Figure 3.2a). This suggests that *A. circinalis* was fixing atmospheric nitrogen in order to meet cellular requirements for this nutrient, and that dissolved inorganic nitrogen was limiting. On day 8, the 1000 μM N treatment had a lower ratio than the phosphorus treatments, although the trend was not significant (P = 0.15).

Therefore, nitrogen additions to diffusion chambers reduced the requirement for *A. circinalis* to utilise atmospheric sources.

There was no significant difference in fluorescence measurements between nutrient treatments on day 4 ($P = 0.21$) or day 8 ($P = 0.23$, Figure 3.3). On day 8, F_v/F_m ranged from 0.35 to 0.44 arbitrary units. Comparisons of values with those determined in the laboratory-based phosphorus limitation experiments in Chapter 2 suggest that the measurements were in the upper range and the cells were therefore in favourable phosphorus conditions. There was no correlation between the growth rates predicted from the F_v/F_m ratios (see Chapter 2 for equations) compared to the measured growth rates in the field (Figure 3.4).

Growth of *A. circinalis* in diffusion chambers in the lower Murray River in March 1998 showed a similar trend to the December 1997 experiment. Growth over the initial 4 days was significantly faster than in the latter 4 days, and there was no significant difference in growth between the control and nitrogen treatment, suggesting that cells had sufficient nitrogen in both treatments (Figure 3.5; $P=0.074$). The average growth rate for the control between days 0 and 8 was 0.68 day^{-1} compared to 0.73 day^{-1} for the nitrogen treatment. These rates were faster than any previously measured in the laboratory indicating that *A. circinalis* was in favourable conditions.

There was a significant increase in the heterocyst/vegetative cell ratio between days 4 and 8 for the control treatment in March 1998, suggesting that ambient inorganic nitrogen was limiting and that *A. circinalis* was fixing atmospheric nitrogen sources (Figure 3.2b). Day 0 data was not analysed due to the large standard deviation associated with the initial samples. On day 8, the heterocyst/vegetative cell ratio in the nitrogen treatment was significantly lower

than the control. Therefore, as with the December 1997 experiment, nitrogen additions reduced the requirement for *A. circinalis* to fix atmospheric gas.

3.3.2 Nutrient Availability in the Lower Murray River

Ambient phosphorus concentrations were in the eutrophic to hypertrophic range in the Murray River throughout both field experiments (Table 3.1). During December 1997, inorganic phosphorus was 28.7 μg L^{-1}, almost 3-times higher than the suggested limiting concentration of 10 μg L^{-1} (Vollenweider 1968; Sas 1989). In March 1998 inorganic phosphorus was 98.7 μg L^{-1}, almost 10-times limiting levels. Inorganic nitrogen (oxidised nitrogen + ammonia) concentrations in December 1997 and March 1998 corresponded to 149.7 and 95.4 μg L^{-1}, respectively. These concentrations greatly exceeded the threshold of 20 μg L^{-1} suggested by Blomqvist *et al.* (1994) for cyanobacterial dominance (Table 3.1). The TN:TP ratio (by weight) of inorganic nutrients was low during both experiments, suggesting that nitrogen was limiting compared to phosphorus; 6.6 in December 1997 and 3.2 in March 1998.

Compared to other years, nutrient concentrations in the lower Murray River were elevated significantly. In a typical year, inorganic phosphorus and nitrogen would be expected to be close to undetectable levels during the summer growth period (Chapter 1). In this study, oxidised nitrogen and inorganic phosphorus concentrations were greater than 75 µg L^{-1} and 28 µg L^{-1}, respectively. Turbidity in the lower Murray River was high during both experiments causing vertical light attenuation to be greater than 5.0 m^{-1} (Table 3.1). Based on previous monitoring data from the Murray River, high turbidity suggests that there were significant diversions of water from the Darling River upstream (Sullivan *et al.* 1988; Mackay *et al.* 1988). Darling River water is associated with elevated concentrations of total phosphorus, total Kjeldahl nitrogen, inorganic phosphorus and oxidised nitrogen (Chapter 1).

3.3.3 The in situ Effect of Nutrients on the Natural Phytoplankton Assemblage

The final cell number (Figure 3.6a) after 8 days growth in the 'flux' treatment was significantly greater than the initial (P = 0.0074), but did not differ from the 'no flux' treatment. In contrast the biovolume of cells (Figure 3.6b) in the 'flux' treatment was significantly higher than both the initial and the 'no flux' treatment (P = 0.0053). This indicates that the assemblage requires a continual flux of nutrients for sustained growth in the Murray River. There was no significant difference in biovolume or total cell number between day 0 and day 8 in the 'no flux' chambers. Given that light and temperature availability were the same for all treatments, growth was limited by nutrient availability. The nutrient concentrations in the river (see above) and the bioassays (see below) suggest that nitrogen was the main limiting nutrient.

The addition of nitrogen to chambers with unsealed ends ('flux') did not cause further growth of the natural phytoplankton assemblage. There was no significant increase in cell density (P = 0.08) or biovolume (P = 0.08) compared to the control for any treatment (Figure 3.6), although there was an upward trend suggesting slight nitrogen limitation. Overall, the concentration and form of nitrogen administered to the unsealed chambers did not have an influence on biomass, suggesting that sufficient nutrients were already diffusing in. The addition of phosphorus to diffusion chambers also failed to elicit a response (Figure 3.6; P = 0.90 cells, P = 0.69 biovolume) but from the bioassay results (see below) phosphorus was not likely to be limiting in any case.

The concentration and form of nitrogen administered to the diffusion chambers also had no significant effect on community structure (Figures 3.7 and 3.8). This was likely to be due to the high ambient oxidised nitrogen concentrations that were greater than 20 μg L^{-1} (Blomqvist et al. 1994; Table 3.1). Statistical analysis showed that the proportions of greens, diatoms,

non-nitrogen-fixing cyanobacteria and nitrogen-fixing cyanobacteria did not alter between nutrient treatments when examining either cells ml^{-1} or total biovolume ($P > 0.05$). Non-nitrogen-fixing cyanobacteria formed a large component of the community when considering cell density. However, the cells were extremely small compared to the other groups and contributed little to the total biovolume. The eukaryotes (greens and diatoms) formed the majority of the biovolume and were dominant.

The main genera that contributed to the total cell number of green algae were *Palmellopsis, Scenedesmus, Miractinium, Ankistrodesmus, Planktonema* and a number of unidentified flagellates. When considering biovolume, *Scenedesmus* was the main contributor to the proportion of greens, followed by *Palmellopsis, Oocystis, Eudorina,* and *Planktonema.* For the diatoms, *Aulacoseira granulata* had the highest total cell number and biovolume. There was also a high component of *Fragilleria* contributing to the cell number, however it was not a large contributor to the biovolume due to the small average dimensions of the cell. Other diatoms present included *Aulacoseira granulata var angutissima, Cyclotella* and a number of unidentified centrics (small sized) and pennates (medium sized). The dominant non-nitrogen-fixing cyanobacteria that contributed to high cell numbers included *Microcystis, Chrococcus* and *Apanothecae,* but these species had significantly less influence on biovolume. The number of nitrogen-fixing cyanobacteria was low for all treatments. *Anabaena circinalis* was the main species present in addition to small numbers of *Aphanizomenon issatschenkoi, Anabaena aphanizomenoides* and *Anabaena affinis.* It is interesting that increases (though insignificant) in nitrogen-fixing cyanobacteria occurred in the control and 2 µM P treatments where the N:P ratio was lowest.

3.3.4 Bioassays

The laboratory-based bioassay experiments provided clear evidence of nitrogen limitation of the phytoplankton community in the Murray River (Figure 3.9a,b). For both field experiments, the addition of phosphorus to Murray River water did not cause a significant increase in chlorophyll, indicating that another nutrient was limiting to growth. The addition of nitrogen caused a significant increase in chlorophyll indicating that this was the main limiting nutrient.

As bioassays were conducted over 6 days in December 1997 and over 7 days in March 1998, increases in chlorophyll were converted to growth rates so the results could be compared between the two years (Table 3.2). The growth rates suggest that in December 1997 there was slight phosphorus as well as nitrogen limitation of the phytoplankton community. The addition of nitrogen allowed a growth rate of 0.18 day^{-1}, whereas the addition of both nitrogen and phosphorus caused the rate to increase to 0.43 day^{-1}. In March 1998, nitrogen additions allowed a growth rate of 0.30 day^{-1}. The addition of nitrogen and phosphorus increased the rate to only 0.35 day^{-1}. Therefore, in March 1998 phosphorus was less limiting to growth of the phytoplankton community which was expected due to the high *in situ* inorganic concentrations.

The form of nitrogen used in March 1998 bioassays had no significant effect on biomass with a similar response when nitrogen was administered as either nitrate or ammonia (Figure 3.10). However, as would be expected from the bioassay results above, the nitrate and ammonia additions caused a significant increase in growth compared to the control for both the 15 μM and 30 μM concentrations ($P < 0.05$). Growth was slightly faster in the 30 μM N treatments compared to the 15 μM N treatments, although the differences were not significant. There was no significant difference between the Control, +P and +WC(-N-P) treatments. The lack of

response in the +WC(-N-P) treatment suggests that nutrients other than nitrogen or phosphorus (*i.e.* carbon, silicon, trace metals and vitamins) were not limiting to growth.

3.4 Discussion

Due to unusually high concentrations of inorganic phosphorus and nitrogen availability in the lower Murray River, *in situ* responses of cyanobacteria to nutrient enrichment were not discernible. Typically, nutrient concentrations are undetectable throughout the summer growth season in the lower Murray River (Chapter 1). During the experiments in this study, a significant proportion of water was sourced from the Darling River upstream which caused oxidised nitrogen and inorganic phosphorus to increase to greater than 75 μg L^{-1} and 28 μg L^{-1}, respectively. Blomqvist *et al.* (1994) suggest that cyanobacterial growth is restricted when nitrate concentration exceeds 20 μg L^{-1}. The high oxidised nitrogen concentrations in this study meant that the theory of Blomqvist *et al.* (1994) was unable to be properly tested with cyanobacteria failing to develop to significant proportions in any nutrient treatment. However, it is interesting that the community was dominated by eukaryotes (greens and diatoms) which would be predicted under high oxidised nitrogen conditions.

Growth of *A. circinalis* as pure culture in diffusion chambers in the Murray River was similar to the community in that it did not respond to nutrient additions. However, there was an increase in the heterocyst/vegetative cell ratio during both experiments suggesting that *A. circinalis* was fixing nitrogen gas and that inorganic nitrogen was limiting for this species. Previous studies on *Anabaena* spp. have shown that the heterocyst/vegetative cell ratio increases from 0.0001 to 0.10 in natural populations under nitrogen limitation (Horne *et al.* 1972; Reynolds 1986). Horne and Commins (1987) also suggest that nitrogenase activity is suppressed at total inorganic nitrogen concentrations > 50-100 μg L^{-1}. In this study, the heterocyst/vegetative cell ratio increased to a maximum of only 0.015 and concentrations of

combined oxidised nitrogen and ammonia were greater than 95 μg L^{-1} during both experiments. These examples suggest that nitrogen limitation of *A. circinalis* was not severe. The heterocyst/vegetative cell ratio was lower in diffusion chambers with nitrogen additions, suggesting that the necessity to fix atmospheric nitrogen was reduced. As the response was not transferred to growth rate, the heterocyst/vegetative cell ratio may be a more sensitive indicator of nitrogen limitation.

In contrast to the results from the diffusion chambers with nutrient fluxes, the laboratory bioassays detected significant nitrogen limitation of the phytoplankton community in the lower Murray River, as nutrient availability was finite. In both December 1997 and March 1988, nitrogen additions caused a significant increase in biomass, whereas phosphorus had no effect. These results differ from early quantitative models of eutrophication from the northern hemisphere that implicate phosphorus as the main limiting nutrient for phytoplankton growth (Vollenweider 1968; Dillon and Rigler 1974; Lund and Reynolds 1982). However, other studies have shown that consideration of nitrogen and light availability may greatly improve the nutrient-chlorophyll relationship (Sakomoto 1966; Smith 1979, 1982). Ferris and Tyler (1985) demonstrated through their data from Lake Burragorang, New South Wales, and the literature, that quantitative phosphorus-chlorophyll relationships in waters from the southern hemisphere generally agree with those of the northern hemisphere, provided that turbid waters (> 10 NTU or secchi depth < 0.4 m) are excluded from the analysis. As the lower Murray River is highly turbid, with measurements as high as 500 NTU (Chapter 1), it would not be expected that this system would fall within the confidence intervals of phosphorus-chlorophyll models.

Growth rates of *A. circinalis* using *in situ* diffusion chambers in this study, are comparable with rates from the literature. In December 1997, the maximum rate over 8 days was 0.42

day^{-1}, and in March 1998 the rate was as fast as 0.73 day^{-1}. Previous studies on growth of

natural populations of *A. circinalis* in the field have found rates of 0.37 day^{-1} (Sherman *et al.*

1998) and 0.176 day^{-1} (Baker *et al.* 2000). In the laboratory, McCausland *et al.* (2002)

measured rates of 0.47 day^{-1} in mixed microcosms ($Z_{eu}/Z_m > 1$), and 0.65 day^{-1} in culture.

Winder and Cheng (1995) measured rates of 0.63 day^{-1} in cultures exposed to an optimal light

intensity of 120 μmol m^{-2} s^{-1}. Growth rates of *A. circinalis* determined in Chapter 2 in culture

ranged from 0.15 to 0.40 day^{-1}, depending on phosphorus availability. The rates measured for

A. circinalis in the diffusion chambers are more comparable with the laboratory studies than

the field studies. However, this is expected as the cells were enclosed and there were no loss

factors affecting population balance.

The faster growth of *A. circinalis* in March 1998 compared to December 1997 was possibly

associated with the lower N:P ratio, associated with higher phosphorus availability (Stockner

and Shortreed 1988). Inorganic phosphorus concentrations in March 1998 were almost 10-

times limiting levels (Vollenweider 1968; Sas 1989) and the TN:TP ratio (by weight) in

March 1998 was only 3.2 compared to 6.7 in December 1997. However, the N:P ratio is not

always important for dominance of cyanobacteria (Pick and Lean 1987; Elser *et al.* 1990;

Levine and Schindler 1999) and Reynolds (1986) found in enclosure experiments that the

ratio did not govern the growth of *Anabaena*. Furthermore, the addition of phosphorus to

diffusion chambers in December 1997 did not stimulate growth, suggesting that cells already

had access to sufficient ambient concentrations.

An alternative explanation for the faster growth rates of *A. circinalis* in March 1998 is higher

light availability. In December 1997, the range of light intensities experienced by cells in the

diffusion chambers at midday ranged from 133.7 μmol m^{-2} s^{-1} at the top of the rotating

machines to 23.2 μmol m^{-2} s^{-1} at the bottom. In March 1998, the light intensities ranged from

328.8 to 30.4 μmol m^{-2} s^{-1}. Using the equation of Riley (1957), the average daily light doses received in December 1997 and March 1998 (assuming no cloud cover) corresponded to 1.9 and 3.8 mol m^{-2} day^{-1}, respectively. This difference in light dose is sufficient to significantly affect growth of *A. circinalis*, as will later be demonstrated in Chapter 5. Furthermore, the average light intensities experienced at midday in December 1997 and March 1998 corresponded to 62.7 and 125.3 μmol m^{-2} s^{-1}, respectively. McCausland *et al.* (2002) calculated a half-saturation intensity for *A. circinalis* of 45 μmol m^{-2} s^{-1}, with saturation occurring between 100 and 150 μmol m^{-2} s^{-1}. Overall, light availability for *A. circinalis* was highly favourable for growth in March 1998 and was likely to be the reason for the faster rates.

Given that the Murray River was largely influenced by turbid water from the Darling River at the time of the experiments, light availability may have played a more important role in controlling the growth of *A. circinalis* than did nutrient availability. Previous studies have shown that turbidity can alter the growth response to nutrient availability. Using water from the Murray River near Albury-Wodonga, Walker and Hillman (1982) demonstrated that phytoplankton biomass increased by a factor of 19 with phosphorus additions. At high turbidities the effect of phosphorus additions was variable but significantly less. Furthermore, Cuker (1987) showed using *in situ* limnocorrals that phosphorus fertilisation favoured the development of the nitrogen- fixers *A. circinalis* and *A. spiroides*. When turbidity was high the response was different with the assemblage dominated by flagellates, particularly *Trachelomas superba*. Overall, turbidity and light availability may strongly affect community structure in the Murray River.

Given that ambient nutrient availability in the Murray River allowed optimal growth of both *A. circinalis* and the wider phytoplankton community during the experiments, the river could

possible be considered eutrophic when under the influence of Darling River water. However, an important consideration is the way in which the diffusion chambers containing the phytoplankton were deployed. It was shown in Chapter 2 that the diffusion of nutrients into the chambers was faster than the uptake rates of the cells and therefore the internal nutrient environment realistically represented the external environment. However, in the field, the diffusion chambers remained at a static location throughout each experiment which differs from the true environment where populations are advected downstream (Baker *et al.* 2000). Assuming that the cells are contained within the same 'parcel' of water as they are advected (Reynolds *et al.* 1991), it is probable that nutrients surrounding the cells may be depleted as they are used for growth. Through suspending the diffusion chambers at a static location against the moving river flow, the amount of nutrients that the enclosed cells had access to may have been higher than would otherwise be encountered under natural conditions. To further test this theory, the diffusion cultures could be attached to a drogue system and tracked downstream. It would also be beneficial to examine eutrophication in the lower Murray River in a year when there is expected to be little influence from the Darling River.

In Chapter 2 a significant correlation was found between the F_v/F_m ratio, determined through fluorescence measurements, and growth of *A. circinalis* over 8 days. In this chapter, the F_v/F_m ratio was tested in the field for the first time. It was thought that fluorescence measurements could be used to equate growth of *A. circinalis* rather than to count tangled coils under the microscope which is time consuming and has a low accuracy. Unfortunately, in the field there was no correlation between F_v/F_m and growth, probably for logistical reasons. Sampling of *A. circinalis* in the field was completed over a few hours and by the time the samples were transported back to the field laboratory for fluorescence measurements they had been exposed to varying periods of darkness which has a direct effect on the F_v/F_m ratio. For example, the majority of non-photochemical quenching dissipates between 5 and 15 minutes in the dark

(Krause and Weis 1991; Falkowski and Kolber 1995). Due to sampling problems and the lack of correlation between predicted and measured growth rates, it is no longer considered viable to use the F_v/F_m ratio to equate growth of *A. circinalis* in the field. Furthermore, the F_v/F_m ratio provides no indication of the number of heterocysts per filament and this study has shown that this is an important measure to determine if nitrogen-fixation is occurring.
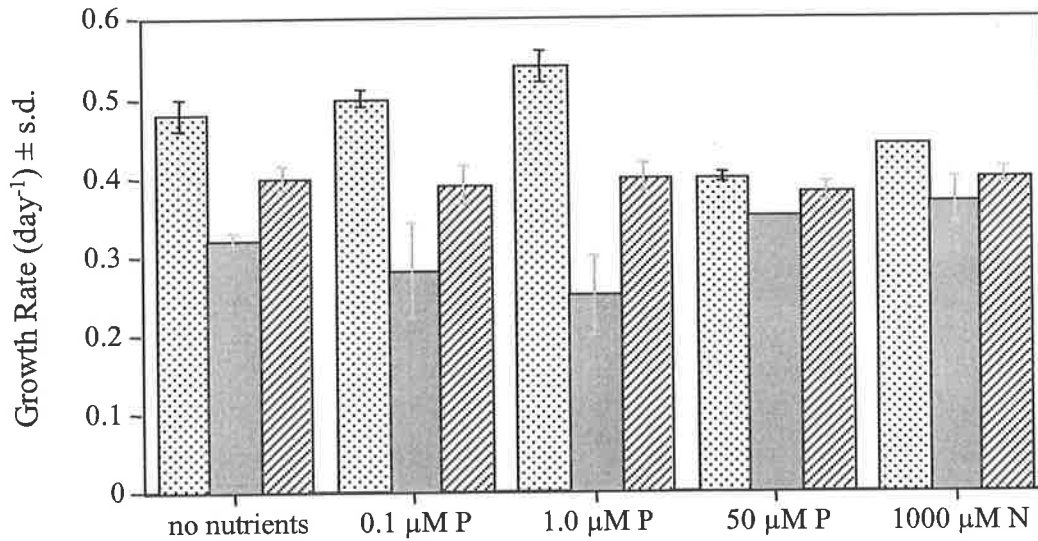
Figure 3.1: Growth of *A. circinalis* when incubated in the lower Murray River in diffusion chambers for 8 days in December 1997. Rates calculated over days 0-4 (⊡), days 4-8 (▩) and days 0-8 (☒).
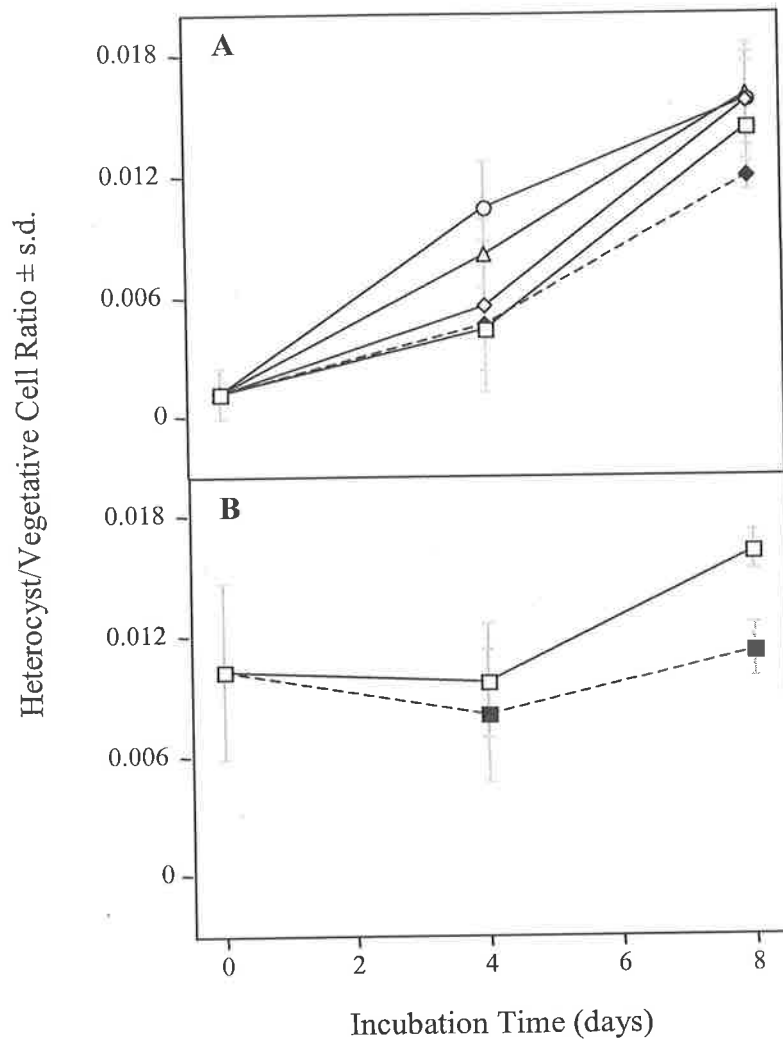
Figure 3.2: Change in the heterocyst/vegetative cell ratio of *A. circinalis* with time when incubated in the lower Murray River during a) December 1997 and b) March 1998. The nutrient treatments that *A. circinalis* was exposed to were; no nutrients (open square), 0.1 µM P (open diamond), 1 µM P (open circle), 50 µM P (open triangle), 30 µM N (solid square) and 1000 µM N (solid diamond). Treatments with nitrogen additions are indicated by a broke line.
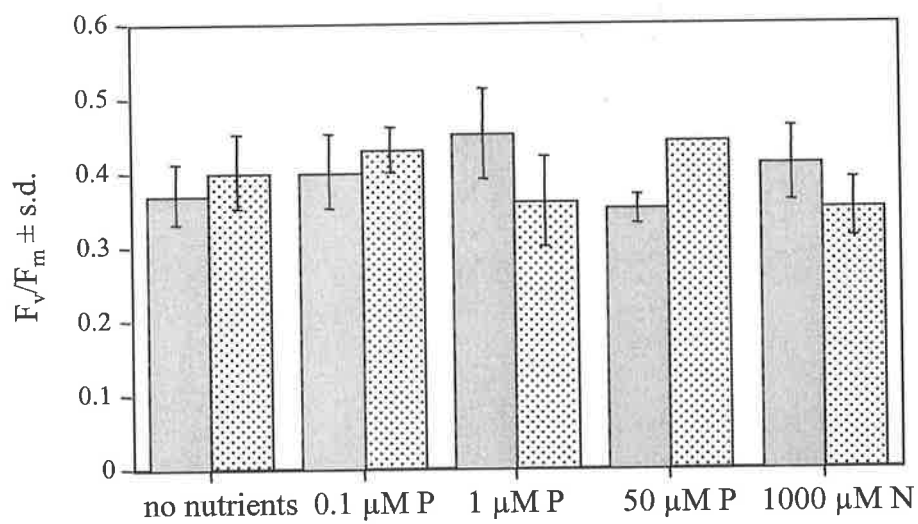
Figure 3.3: Effect of nutrient additions on the fluorescence of *A. circinalis* on days 4 (■)

and 8 (▣) after incubation in the Murray River for 8 days during December 1997.
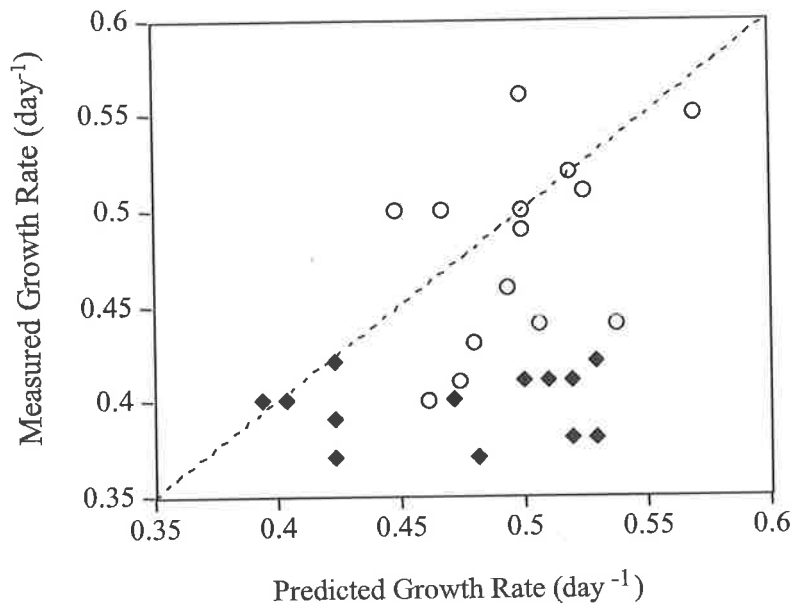
Figure 3.4: Growth rates of *A. circinalis* predicted from F_v / F_m ratios, compared to measured growth rates on day 4 (open circle, r^2 = 0.180) and day 8 (solid diamond, r^2 = 0.007) during incubation in the Murray River in December 1997. Predicted growth rates were calculated from the equations described in Chapter 2.
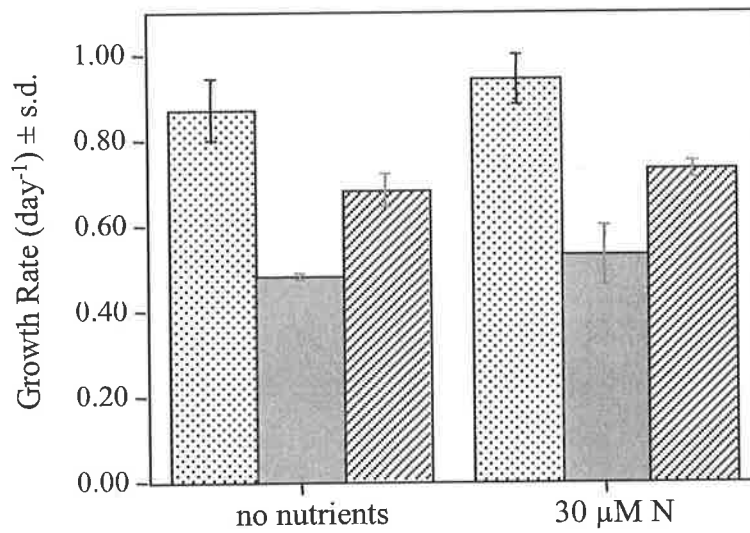
Figure 3.5: Growth of *A. circinalis* in diffusion chambers in the Murray River during March 1998. Rates calculated over days 0-4 (⊟), days 4-8 (■) and days 0-8 (▨).

Table 3.1: Nutrient and environmental data ($\pm$ standard deviation) taken at Morgan during both field experiments in the Murray River. Temperature data was taken at the depth of the rotating machines. Nutrients, $\mu g\ L^{-1}$; temperature, $^{\circ}C$; and vertical attenuation (K_d), $\ln\ m^{-1}$. TN:TP calculated by weight.

	FRP	TP	NOx	NH$_3$	TKN	TN:TP	Temp	K$_d$
Dec '97	28.7	110.3	98.7	51.0	733.3	6.7	24.6	5.0
s.d.	2.3	13.1	7.6	21.8	77.7	0.1	0.43	0.99
Mar '98	98.7	250.7	75.7	19.7	790.0	3.2	21.3	6.8
s.d.	10.7	9.0	14.2	10.8	17.3	0.1	0.75	0.57
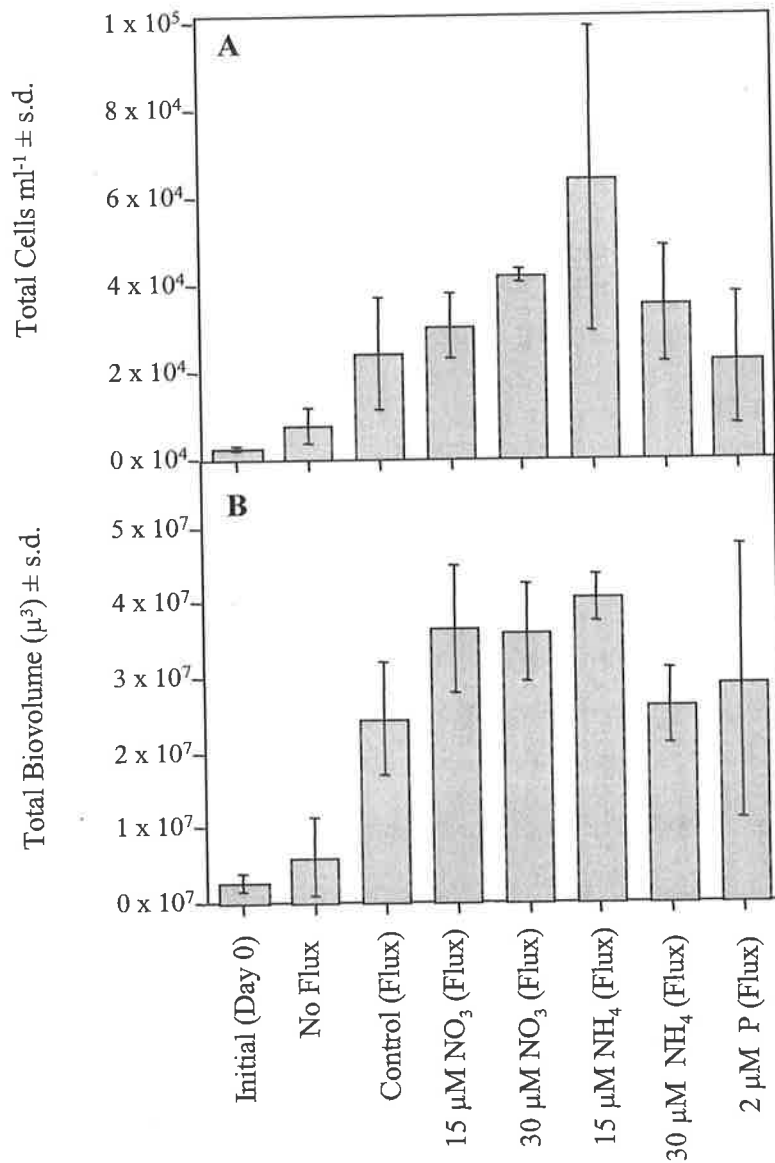
Figure 3.6: Effect of nutrient additions and nutrient flux on a) total cells ml^{-1} and b) total biovolume of the natural phytoplankton assemblage when incubated in diffusion chambers in the lower Murray River over 8 days, in March 1998.
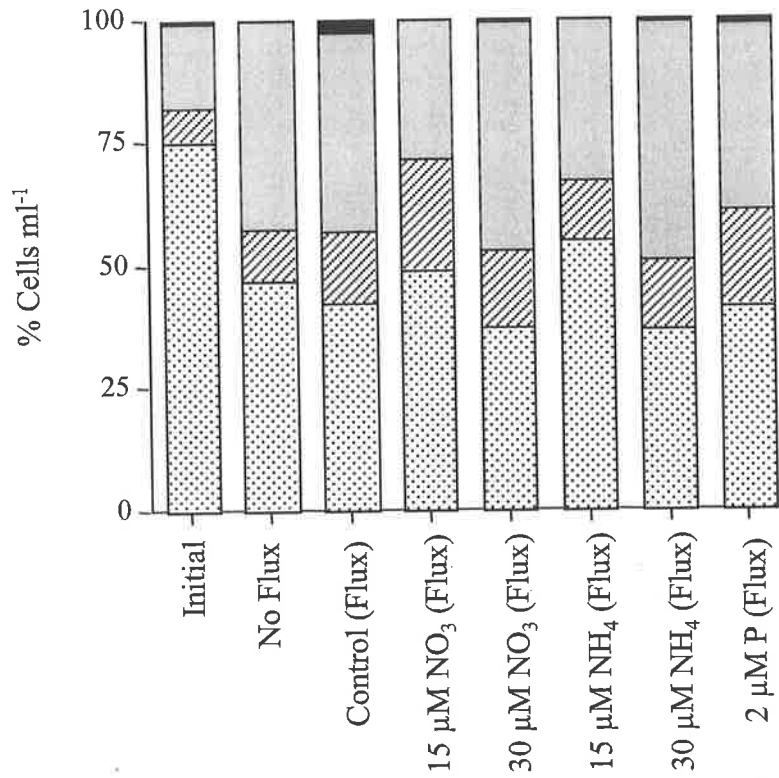
Figure 3.7: Proportions of green algae (▨), diatoms (▨), non-nitrogen fixing cyanobacteria

(▨) and nitrogen fixing cyanobacteria (■) based on cells ml⁻¹ on day 0 (initial) and after 8

days incubation in diffusion chambers in the Murray River in March 1998.
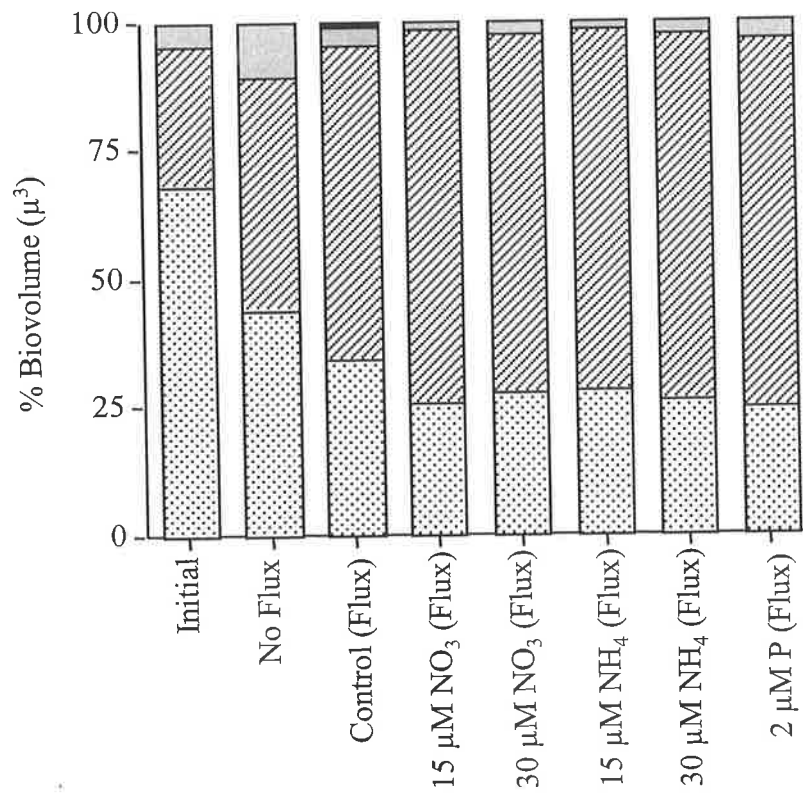
Figure 3.8: Proportion of green algae (▣), diatoms (▨), non-nitrogen-fixing

cyanobacteria (▩) and nitrogen-fixing cyanobacteria (▪) based on biovolume on day 0

(initial) and after 8 days incubation in diffusion chambers in the lower Murray River in
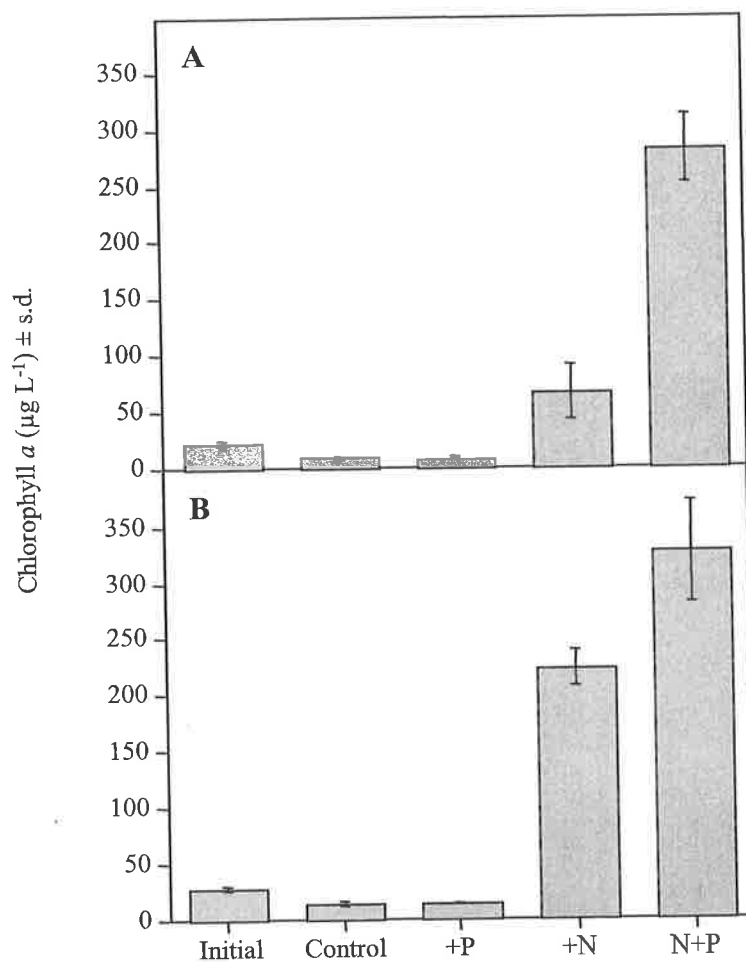
March 1998.

Figure 3.9: Results of bioassays conducted in the laboratory using water taken from the Murray River in a) December 1997 and b) March 1998. Final biomass was measured after 6 days in the December 1997 experiment and after 7 days in March 1998.

Table 3.2: Bioassay growth rates calculated from chlorophyll a measurements on day 0 and

day 6 (December 1997) and day 7 (March 1998).

	December 1997	March 1998
Control	- 0.14 ± 0.02	- 0.09 ± 0.02
+P	- 1.16 ± 0.08	- 0.11 ± 0.02
+N	+ 0.18 ± 0.04	+ 0.30 ± 0.02
N+P	+ 0.43 ± 0.01	+ 0.35 ± 0.03
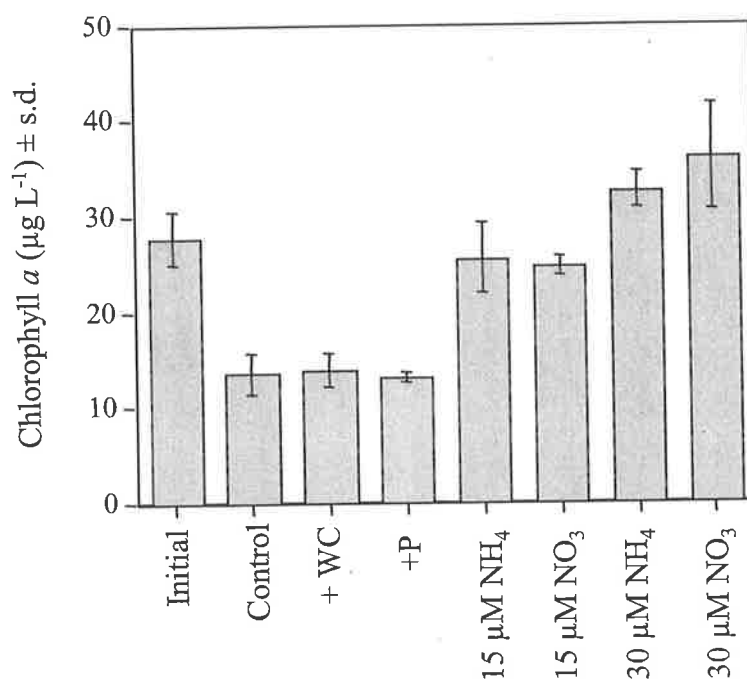
Figure 3.10: Laboratory bioassays conducted on water taken from the lower Murray River in March 1998 to determine the effect of different concentrations and forms of available nitrogen.

CHAPTER 4. Transition from *Anabaena circinalis* to *Aulacoseira granulata* in the Lower Murray River.

ABSTRACT

This chapter investigates possible reasons for the transition from *Anabaena circinalis* to *Aulacoseira granulata* when stratification breaks down and mixed conditions resume in the lower Murray River. An *in situ* experiment was conducted to examine the interaction between both species and to determine if the response was modified with nitrogen and phosphorus additions. Growth of *A. circinalis* was not affected by the presence or absence of *A. granulata*, with rates ranging from 0.28 to 0.39 day^{-1} across all nutrient treatments. A second *in situ* experiment was conducted to examine the growth response of each species when exposed to different Z_{eu}/Z_m ratios. *A. circinalis* grew faster than *A. granulata* at all Z_{eu}/Z_m ratios and the growth efficiency ($\propto$) of the former was higher when considering the response to average daily light dose. However, inorganic nitrogen availability was limiting at the time of experimentation and the ability for *A. circinalis* to fix atmospheric nitrogen may have conferred a benefit to this species. Laboratory measurements comparing the photosynthesis of *A. granulata* and *A. circinalis* when grown in nutrient replete media showed that *A. granulata* had a better efficiency under these conditions. However, the probability of nitrogen replete conditions in the lower Murray River is low throughout summer when blooms of *A. circinalis* occur and no definite reasons for the decline of *A. circinalis* during mixed conditions could be elucidated.

4.1 Introduction

Transitions between diatom and cyanobacterial dominance are commonly associated with water-column stability (Reynolds and Reynolds 1985; Talling 1986; Hötzel and Croome 1994; Harris and Baxter 1996; Sherman *et al.* 1998). In the Murray-Darling Basin, shifts in dominance between *Aulacoseira granulata* and *Anabaena* spp. are often observed and are associated with changes in river flow (Hötzel and Croome 1994; Sherman *et al.* 1998). Transitions between *Aulacoseira* spp. and *Anabaena* spp. have also been documented in studies from overseas (Izaguirre *et al.* 2001), including Lake Biwa, Japan (Miyajima *et al.* 1994), Lake Lanao, Phillipines (Lewis 1978), Crose Mere, United Kingdom (Reynolds and Reynolds 1985), and the Baía River, Brazil (Train and Rodrigues 1998). *A. granulata* is always associated with mixed conditions and dominates when river flows are high (Kilham

and Kilham 1975; Trimbee and Harris 1984; Petrova 1986; Reynolds *et al.* 1986; Hötzel and Croome 1994; Sherman *et al.* 1998). When river flows decrease and stratification develops, *Anabaena* becomes dominant and *A. granulata* declines (Sherman *et al.* 1998). *A. granulata* has a density greater than water (1077-1138 kg m^{-3} Davey 1987) and sinks at rates between 0.47 and 1.42 m day^{-1} (Reynolds *et al.* 1986; Sherman *et al.* 1998; McCausland *et al.* 2002). In contrast, *Anabaena* minimises losses through its ability to float and cells are able to maintain position within the euphotic zone where optimal light doses may be experienced. The spatial separation of *A. granulata* and *Anabaena* in the water-column under stratified conditions may also provide an additional benefit to *Anabaena* through removing the necessity to compete for nutrients. A beneficial strategy for *Aulcoseira* under stratified conditions is the ability to maintain viability through the formation of a resting stage at depth or on the sediment (Lund 1954). When mixing resumes, cells are advected back into the euphotic zone where active growth can occur (Lund 1954; Reynolds 1973; Davey 1987).

When persistent stratification breaks down and fully mixed conditions resume, the abundance of *Anabaena* decreases, whereas *A. granulata* increases and becomes dominant once more. The decline of *Anabaena* spp. during mixed conditions may be the result of one or a combination of the following reasons; a) insufficient light availability, b) competition for nutrients with *A. granulata*, c) physical disruption of the cells caused by turbulence, or d) dilution of cells caused through both the increase in flow and advection downstream, and the re-distribution of cells throughout the water-column. However, the reasons for the decline of *Anabaena* under mixed conditions are currently unknown.

As stratification is overcome and mixing resumes, the Z_{eu}/Z_m ratio decreases leading to decreased light availability. This factor may be particularly important in governing transitions between *Anabaena* and *A. granulata* in the Murray River given that the river is highly turbid.

Measurements between 1990 and 1996 averaged 51.6 ± 38.9 NTU, ranging to as high as 304 NTU (Chapter 1). *Anabaena* spp. are generally confined to systems where the Z_{eu}/Z_m ratio is within the range of 0.5 - 3.3 (Reynolds and Walsby 1975; Sherman *et al.* 1998) or in mixed conditions where cells receive > 5.8 hours of insolation per day (Reynolds and Reynolds 1985; McCausland *et al.* 2002). Furthermore, Hötzel and Croome (1994) found that cyanobacteria in the Darling River at Burtundy, New South Wales, were generally only dominant when the turbidity was less than 50 NTU. These factors suggest that *Anabaena* has a high light requirement and Sherman *et al.* (1998) calculated that this genus is only present in conditions where 10 - 13 mol m^{-2} day^{-1} are received.

The light requirements of *A. granulata* are currently unclear. Sherman *et al.* (1998) suggested that *A. granulata* is dominant at Z_{eu}/Z_m ratios of < 0.33 with a mean daily photon dose of only 4 mol m^{-2} day^{-1}. Therefore, the light requirement is less than for *A. circinalis*. However, McCausland *et al.* (2002) recently demonstrated in laboratory microcosms that the growth efficiency of *Aulacoseira* sp. was slightly lower (0.14 m^2 mol^{-1} photons) than *A. circinalis* (0.17 m^2 mol^{-1} photons). Furthermore, Coles and Jones (2000) showed that the photosynthetic efficiency of *A. granulata* var. *angutissima* was significantly lower than all cyanobacteria tested in their study *i.e. Microcystis aeruginosa*, *Oscillatoria* sp. and *Merismopedia tenuissima*. The reason for the success of *A. granulata* compared to cyanobacteria under mixed conditions may not necessarily be due to efficient growth under low light conditions. The relationship is currently unclear and requires further investigation.

The aim of this study was to examine possible reasons for the decline of *Anabaena* and the dominance of *A. granulata* under mixed conditions in the lower Murray River. Interactions between the species were investigated *in situ* and nutrient treatments administered to determine if the response was modified. *In situ* growth at various Z_{eu}/Z_m ratios was also

compared between the two species and photosynthesis compared in the laboratory. It was hypothesised that *A. circinalis* would have a faster growth rate than *A. granulata* at Z_{eu}/Z_m ratios greater than 0.5, and *vice versa*.

4.2 Methods

Field experiments were conducted at Morgan on the lower Murray River in 1998. The first field experiment commenced in February to examine the interaction between *A. circinalis* and *A. granulata*. The second field experiment commenced in December to examine the response of each species to different Z_{eu}/Z_m ratios. A laboratory experiment was also conducted to examine photosynthetic rates of both species. Laboratory cultures of *A. circinalis* (ANA271B, Chapter 2) and *A. granulata* (MEL001- personal isolate from lower Murray River) were used for all experiments. These were grown in WC media (Guillard and Lorenzen 1972) at a light intensity of 50 -60 μmol m^{-2} s^{-1} under a 14 h:10 h light/dark cycle at 25 °C.

4.2.1 Interaction Experiment

To examine the interaction between *A. circinalis* and *A. granulata* an experiment based on a de Wit (1960) replacement design was used. There were three competition treatments, each with five replicates; 100% *A. circinalis*, 100 % *A. granulata* and 50%:50% *A. circinalis* and *A. granulata* combined. The biovolume of *A. circinalis* is approximately 6-times lower than *A. granulata* which may confer a benefit if the species are competing for nutrients due to a higher surface area/volume ratio (Smith and Kalff 1982). However, it was considered that the smaller biovolume of *A. circinalis* should be included as part of the assessment of competition and therefore for the 50%:50% treatment each species was present in similar initial cell numbers.

Four nutrient treatments were overlayed on the three competition treatments to determine if the response was modified. There were two factors; nitrogen (N) and phosphorus (P), and within each factor there were two levels; + and -. The concentrations of N and P used were 30 μM and 2 μM respectively with N added in the form of $NaNO_3$ and P in the form of K_2HPO_4. Prior to experimentation, cultures of *A. circinalis* and *A. granulata* were starved of N for two days and P for four days (Chapter 2). Nutrient treatments were administered every 2 days over the course of the experiment.

All cultures were incubated *in situ* using diffusion chambers attached to rotating machines (Chapter 2). The rotating machines were suspended at a mid-axle depth of 0.475 m so that midday light availability (1800 μmol m^{-2} s^{-1}) ranged from 144.8 μmol m^{-2} sec^{-1} at the top of the cages (0.3 m) to 7.66 μmol m^{-2} s^{-1} at the bottom (0.65 m). However, on the fifth day of experimentation the cages were adjusted to 0.15 m due to high turbidity in the river. The midday light intensities at the top, middle and bottom of the cages after adjustment were 510.6, 117.4 and 26.99 μmol m^{-2} s^{-1}, respectively. The cultures were incubated for 8 days and 2 ml samples taken every 2 days and preserved in Lugol's iodine. The number of vegetative cells and heterocysts was later recorded using a Sedgwick-Rafter chamber and inverted microscope.

4.2.2 Z_{eu}/Z_m Experiment

The field experiment examining the optimal Z_{eu}/Z_m ratio for each species was conducted in December 1998. There were four Z_{eu}/Z_m treatments that were represented by varying the proportion of time that cultures were exposed to the euphotic zone and the dark. For the first treatment, $Z_{eu}/Z_m = 1$, a daylength of 12 hours was assumed and the cultures were exposed to the euphotic zone for 12 out of 12 hours (Table 4.1). For the second treatment, $Z_{eu}/Z_m = 0.5$, cultures were exposed to the euphotic zone for 6 out of 12 hours. For the final two treatments,

the Z_{eu}/Z_m ratios were 0.25 and 0.08 and corresponded to exposure times of 3 and 1 h respectively. As incoming irradiance follows a sine pattern throughout the day (assuming no cloud cover), the exposure periods were centred around midday so that cultures received sufficient light intensities. With the exception of the treatment where $Z_{eu}/Z_m = 1$, the maximum exposure period was one hour followed by darkness to ensure that photoinhibition did not occur.

During exposure periods, all cultures were suspended at the depth of the average irradiance between the surface and the euphotic depth (Z_{eu}). For darkness, the cultures were lowered below the euphotic zone down to 2 m. To calculate the average irradiance ($\bar{I}$) of the euphotic zone, the equation of Riley (1957) was used;

$$\bar{I} = \bar{I}_0' \, (1 - e^{-Kd * Zm}) / K_d * Z_m \tag{1}$$

where,

$\bar{I}_0'$ = incoming irradiance, $\mu mol \, m^{-2} \, sec^{-1}$

K_d = light attenuation, m^{-1}

Z_m = mixed depth, m

A week prior to the experiment, light measurements were taken in the river and the K_d calculated as 10 m^{-1}. Using the equation $Z_{eu} = \ln 100/K_d$ (Reynolds 1984), the euphotic depth was therefore 0.46 m. The depth of average irradiance was 0.16 m. Although the K_d slightly changed throughout the experiment, the suspension of cultures at 0.16 m during exposure periods was not altered.

In addition to the Z_{eu}/Z_m treatments, a series of cultures were incubated at static depths throughout the experiment so that *in situ* growth/irradiance curves could be constructed and

compared between the two species. The additional cultures were incubated at 0.05, 0.16, 0.35, 0.5 and 1.0 m.

Diffusion cultures were incubated in the Murray River for 6 days. Samples (2 ml) were taken from the chambers every 2 days and preserved in Lugol's for enumeration at a later date. The number of heterocysts to vegetative cells was also recorded. On the last day of the experiment the cultures were concentrated onto GF/C (Whatman 47 mm) filter papers, immersed in 10 ml 95 % ethanol and frozen until analysis of chlorophyll *a* at a later date.

It should be noted that all cultures for the December 1998 Z_{eu}/Z_m light dose experiment were incubated in diffusion chambers that were enclosed in static cages rather than tied onto rotating machines. As demonstrated in Chapter 2, the diffusion of nutrients into static chambers is 16.7 % slower than when using the rotating machines. However, as the Z_{eu}/Z_m experiment was investigating growth purely in response to light rather than nutrient availability, cells were not starved prior to *in situ* incubation. Therefore, the cells were nutrient replete at the start of the experiment and were able to use additional nutrients diffusing into the chambers over the course of the experiment.

For both field experiments, environmental data was monitored throughout the incubation periods. Details of the monitoring procedures are provided in Chapter 3. Silicate data was obtained from River Murray Water through the Australian Water Quality Centre. Bioassays were conducted by Dr Justin Brookes at the same time as the field experiments (Chapter 3). These were conducted over six days for the February 1998 interaction experiment, and over seven days for the December 1998 Z_{eu}/Z_m experiment.

4.2.3 Laboratory Examination of Photosynthesis

In addition to field work, a comparison of the photosynthetic rates of *A. circinalis* and *A. granulata* under nutrient replete conditions was conducted in the laboratory. Photosynthesis was measured using a Clarke-type Oxygen electrode with cultures (4 ml) maintained at 25 °C. The chart recorder was zeroed using sodium dithionite (Na_2SO_3) and the upper scale set using reverse osmosis water equilibrated to 25 °C which has an oxygen solubility of 8.26 mg L^{-1} or concentration of 258 µmol L^{-1}. To determine the light field experienced by the cells in the electrode chamber, the average irradiance was determined using the equation of Riley (1957) shown above (see also Dubinsky *et al.* 1987). K_d was determined by measuring intensity at the front and rear of the chamber containing the algal suspension. I_o was determined by replacing the algal suspension with water and re-measuring intensity. Finally, z is the pathlength of the chamber which was measured as 16 mm. All light intensities were varied using neutral density filters. To ensure that cells were exposed to realistic conditions in the oxygen-electrode chamber, the chamber was gently purged with nitrogen at high oxygen concentrations to avoid problems with supersaturation at high light intensities. Furthermore, since high cell densities were used to ensure a measurable response, $NaHCO_3$ was added at regular intervals to prevent carbon limitation. Photosynthesis was measured over 10 minutes for each irradiance. At the conclusion of each study, 2 ml of the algal culture was concentrated onto GF/C (Whatman 25 mm) filters for determination of chlorophyll *a*. The remainder of the culture was used to determine cell density.

4.2.4 Analysis

For both field experiments there were 5 replicates per treatment, however in many cases analysis was conducted on only 3 or 4 replicates due to the rupturing of diffusion chambers. All data was checked for normal distribution using the Shapiro-Wilk test (JMP, SAS Inc. version 3.0.2 1989-94) and skewed data was normalised using $log_{10}y$ when necessary.

For the February 1998 experiment there were 3 competition treatments *i.e.* 100% *A. circinalis*, 100% *A. granulata* and a 50%:50% combination. However, for analysis these were divided into 4 treatments; *A. circinalis* (pure), *A. granulata* (pure), *A. circinalis* (mixed) and *A. granulata* (mixed). The effects of competition, nitrogen and phosphorus on growth were analysed separately using 1-way ANOVA and a post-hoc Tukey-Kramer HSD test (JMP, SAS Inc. version 3.0.2 1989-94). As there were no significant differences caused by nitrogen or phosphorus additions for each species, the nutrient data was pooled to test the difference in growth between mixed and pure cultures for each species.

To analyse for significant increases in the heterocyst/vegetative cell ratio for both field experiments, a t-test (JMP, SAS Inc. version 3.0.2 1989-94) was used to compare data on day 0 and either day 6 (December 1998) or day 8 (February 1998). To analyse for differences in ratio according to average light dose, the ratios measured on samples taken on the last day of experimentation were analysed using 1-way ANOVA. Bioassay data and chlorophyll *a* data for both experiments was also analysed using 1-way ANOVAs (n = 3).

To determine the relationship between growth (G) and average daily light dose (I) that each species received in the river, a modified version of the hyperbolic tangent equation of Jassby and Platt (1976) was fitted to the data points. The modifications were simply a conversion from rates of photosynthesis to growth rates and the assumption that the respiration rate (Van der Bijl *et al.* 1989; Olesen and Sand-Jensen 1993) was equivalent to a loss rate:

$$G = G_{max}*Tanh(I/I_k) + R_d \qquad day^{-1} \qquad\qquad (2)$$

where,

G_{max} = Growth rate at optimal light dose, day^{-1}

I_k = Light dose where the extrapolated lines of G_{max} and the initial light limited

portion of the G-I curve intersect, mol m^{-2} day^{-1}

R_d = Constant to adjust for loss rate, day^{-1}

α = Growth efficiency, calculated from the slope of the initial linear section of

the G-I curve, day^{-1} (mol m^{-2} day^{-1})$^{-1}$

To determine the relationship between growth and average daily light dose, data from the static depth incubations and the cultures exposed to various Z_{eu}/Z_m ratios was pooled. The parameters for the equation were solved through minimisation of the sum of squares between the modelled values and the field data, and α was derived by the division of G_{max} and I_k.

The photosynthetic rates (P; mg O$_2$ mg chl *a*$^{-1}$ h^{-1}) of *A. circinalis* and *A. granulata* were modelled using the same formula of Jassby and Platt (1976) described above. The term G_{max} was replaced by P_{max} to indicate that photosynthesis and not growth was measured.

4.3 Results

4.3.1 Interaction Experiment

There was no significant effect of competition on growth of *A. circinalis* with rates ranging from 0.28 to 0.37 day^{-1} for pure cultures and 0.33 to 0.39 day^{-1} for mixed cultures (Figure 4.1). For *A. granulata*, there was high variation associated with growth and the majority of rates were negative. However, growth was significantly faster in cultures containing *A. circinalis* (-0.03 to 0.15 day^{-1}) compared to that in pure cultures (-0.19 to 0.03 day^{-1}), suggesting that *A. circinalis* stimulated *A. granulata* growth.

Nitrogen and phosphorus additions did not have a significant effect on growth of either species (Figure 4.1). Ambient filterable reactive phosphorus concentrations were extremely high and averaged 155.7 µg L^{-1} (Table 4.2). Inorganic nitrogen was slightly above limiting levels with concentrations of NOx and ammonia of 110.7 and 13.3 µg L^{-1}, respectively (Sas

1989). The TN:TP ratio (by weight) of nutrients was low due to high phosphorus availability in the river. Total silicate was abundant during experimentation and averaged 15.3 mg L^{-1} (Table 4.2).

The heterocyst/vegetative cell ratio of *A. circinalis* indicated that cells were fixing atmospheric sources of nitrogen. Therefore, although ambient inorganic nitrogen concentrations were above the limiting threshold, availability may still have been below optimal. The heterocyst/vegetative cell ratio in pure cultures increased significantly from day 0 to day 8 in the -N-P treatment with values increasing from 0.008 to 0.015 (Figure 4.2a). In mixed cultures the ratio increased significantly in both the -N-P and -N+P treatments ranging from 0.005 on day 0, to 0.017 and 0.012 on day 8, respectively (Figure 4.2b). When comparing the ratio between nutrient treatments on day 8, the ratio for the +N+P treatment was significantly lower than the treatments that received no nitrogen throughout the experiment in both the pure and mixed cultures. Therefore, in the cultures with nitrogen additions, there was sufficient nitrogen present for cells to grow without the need for significant fixation of atmospheric sources.

The laboratory bioassay on samples taken from the Murray River showed that the wider phytoplankton population was also nitrogen limited (Figure 4.3a). In the +N treatment, chlorophyll increased from 2.9 to 42.2 µg L^{-1}. This corresponds to a chlorophyll *a* based growth rate over 6 days of 0.44 day^{-1}. There was no significant difference between the Control and +P treatments, suggesting that phosphorus was not limiting to growth. However, in the +N+P treatment, growth rate over 6 days was 0.56 day^{-1}. As growth was faster than in the +N treatment, this suggests that phosphorus became limiting as growth was stimulated by the addition of nitrogen. The lack of increase in the +P treatment was caused through lack of nitrogen availability.

4.3.2 Response to Z_{eu}/Z_m Ratios

For the field experiment examining the effect of Z_{eu}/Z_m on *A. circinalis* and *A. granulata*, the average daily light doses experienced ranged from 1.8 to 35.0 mol m^{-2} day^{-1} (Table 4.3). The total light doses received over the period of experimentation ranged from 9.1 to 180.5 mol m^{-2} 6 days^{-1}. As all cultures were moved to the surface during sampling, there was a degree of light contamination for each treatment, however the extra light dose was accounted for.

The growth of *A. circinalis* and *A. granulata* was directly influenced by the average daily light dose received (Figures 4.4 a,b and 4.5 a,b). For *A. circinalis*, cultures suspended at the shallowest depth of 0.05 m grew the fastest and cell density increased from 4.4 x 10^3 to 8.0 x 10^5 cells ml^{-1} over 6 days. The slowest growth occurred in cultures suspended at 1.00 m where the density increased to 1.9 x 10^4 cells ml^{-1} (Figure 4.4 a,b).

Similar patterns in growth were measured for *A. granulata*. The highest cell density was achieved in cultures suspended at 0.05 m with an increase from 2.7 x 10^3 to 2.1 x 10^4 cells ml^{-1}. Slowest growth was measured in cultures suspended at 1.00 m and the density increased to 3.4 x 10^3 cells ml^{-1} (Figure 4.5 a,b). Cell density data for the Z_{eu}/Z_m = 0.08 treatment was unable to be included due to loss of replicates.

A strong relationship between average daily light dose and growth of both *A. circinalis* and *A. granulata* was found indicating that light limitation was significant in the lower Murray River at the time of experimentation (Figure 4.6). The data from incubations both at static depths, and from cultures that were exposed to various Z_{eu}/Z_m ratios, was pooled. The difference in the way in the which the light doses were received was not reflected in the growth rate with all points lying in the vicinity of the same curve for each species.

The maximum growth rate of *A. circinalis* was 0.80 day^{-1} which was 56.3 % higher than the maximum growth rate of 0.35 day^{-1} for *A. granulata* (Table 4.4). The I_k values were similar for each species with light becoming saturating at doses between 17 to 18 mol m^{-2} day^{-1}. The increase in growth rate with light dose at sub-saturating irradiances was faster for *A. circinalis* compared to *A. granulata* with α values of 0.04 and 0.02 mol^{-1} m^{-2} day^{-1} (day^{-1})$^{-1}$, respectively. Overall, *A. circinalis* utilised light more efficiently than *A. granulata* and growth was faster at all light doses.

At the time of experimentation inorganic phosphorus was abundant, but inorganic nitrogen concentrations were low with concentrations of oxidised nitrogen and ammonia of only 7.7 and 63.7 µg L^{-1}, respectively (Table 4.2). This suggests that nitrogen was limiting to growth (*i.e.* < 100 µg L^{-1}) and may be the reason that slow rates were found for *A. granulata*. The heterocyst/vegetative cell ratio of *A. circinalis* suggested that this species was fixing atmospheric sources of nitrogen (Figure 4.7). The ratio increased from 0.008 on day 0 to values ranging between 0.010 and 0.022 on day 8. The ratio on day 8 was related to the average daily light dose received during the experiment and increased linearly up to doses of 7.04 mol m^{-2} day^{-1} with a slight decrease at higher doses.

The total silicate concentration during December 1998 was 3.7 mg L^{-1} (Table 4.2). It was unfortunate that dissolved silicate data was unable to be obtained and it is unknown whether *A. granulata* was limited by this nutrient. However, it is thought that cells had access to sufficient silicate for a number of reasons. First, *A. granulata* was not starved of nutrients prior to experimentation as the response to light was being examined. Therefore, the cells were surrounded by nutrient-replete media when transferred into the diffusion chambers and were silicate replete when deployed in the river. Second, there was an *A. granulata* bloom in the river during the time of experimentation with a density of 1 x 10^4 cells ml^{-1} (data from the

Australian Water Quality Centre). The cells within the bloom were silicate replete, as indicated by a significant increase in chlorophyll biomass in the control treatment in the laboratory bioassay (Figure 4.3b; also see below). Furthermore, the bloom was still present in the river the following week and cell density had increased. The average amount of silicate contained in the frustules of *A. granulata* is 620 ρg cell^{-1} (Reynolds 1984). Therefore, at a cell density of 1 x 10^4 cells ml^{-1}, the silicate requirement is only 0.006 mg L^{-1} and the majority of the total silicate was likely to be available. The limiting silicate concentration for diatoms ranges from 0.1 to 0.5 mg L^{-1} (Reynolds 1984, Davey and Heaney 1989; Bormans and Webster 1999) so that uptake by *A. granulata* in the diffusion chambers was likely to have been saturated.

The laboratory bioassay showed that the natural population of phytoplankton was not nutrient limited (Figure 4.3b). The initial chlorophyll levels in the river were high with a concentration of 74.3 μg L^{-1}, due to the significant density of *A. granulata*. By day 7 the chlorophyll concentration in the control treatment had increased to 304.7 μg L^{-1} (growth rate = 0.20 day^{-1}) indicating that cells had sufficient nutrients (including nitrogen and silicate) to allow this increase, and that factors other than nutrient availability were limiting in the river (e.g. light or temperature). Given the fast growth of the control, it was not unexpected that there was no significant difference in growth between this treatment and the +P (growth 0.22 day^{-1}), +N (growth 0.16 day^{-1}) and +N+P (growth 0.22 day^{-1}) treatments.

The chlorophyll per cell measured at the end of the field experiment showed a different pattern for each species with respect to light dose (Figure 4.8). For *A. granulata* there was a decrease in chlorophyll *a* per cell at light doses greater than 7.04 mol m^{-2} day^{-1} suggesting that this species adapted to the prevailing light conditions. Chlorophyll concentration ranged from 0.3 to 2.1 pg cell^{-1}. In contrast, there was no significant difference in the chlorophyll content

of *A. circinalis* caused by light dose. The concentration ranged from 0.2 to 0.6 pg cell^{-1}, however, there was no pattern of decrease with increasing light dose and concentrations at 1.83 and 34.97 mol m^{-2} day^{-1} were 0.40 and 0.38 pg cell^{-1}, respectively. For both species the chlorophyll concentrations were close to the same range of values found in the literature (Table 4.5).

4.3.3 Photosynthesis

Since it was possible that *A. granulata* was nitrogen limited in the field leading to low growth rates, an additional laboratory experiment was conducted to examine differences in photosynthetic rate between the two species in nutrient replete conditions. The maximum photosynthetic rate was 63.6 % higher for *A. circinalis* compared to *A. granulata* with rates of 4.4 and 1.6 mg O$_2$ mg chl a^{-1} h^{-1}, respectively (Figure 4.9 a,b; Table 4.6). However, in contrast to the field experiment, α of *A. granulata* was almost double that of *A. circinalis* when comparing rates per 10^9 cells with values of 12.67 and 6.69 mg O$_2$ (10^9 cells)$^{-1}$ mol^{-1} m^2, respectively. When comparing α based on photosynthesis per mg chl *a* the values were more similar with measurements of 9.44 mg O$_2$ (mg chl *a*)$^{-1}$ mol^{-1} m^2 for *A. granulata* and 8.72 mg O$_2$ (mg chl *a*)$^{-1}$ mol^{-1} m^2 for *A. circinalis* (Figure 4.9 a,b; Table 4.6). Overall, at sub-saturating light intensities, *A. granulata* was more efficient at harvesting light in nutrient replete conditions.

The I$_k$ value for *A. granulata* was determined as 45 μmol m^{-2} s^{-1}, and for *A. circinalis*, 141 μmol m^{-2} s^{-1} (Table 4.6). The specific switch-over point where the photosynthetic rate of *A. circinalis* was faster than *A. granulata* occurred at 93.3 μmol m^{-2} s^{-1} when considering rates per 10^9 cells, and 27.2 μmol m^{-2} s^{-1} when considering rates per mg chl *a*. Respiration rates for both species were similar with measurements between -0.13 and -0.12 mg O$_2$ (mg chl *a*)$^{-1}$ h^{-1} (Table 4.6).

Photoinhibition was found in *A. circinalis* and *A. granulata* in both the field and laboratory experiments when cells were exposed to high light. At 33.97 mol m^{-2} day^{-1} growth of *A. circinalis* in the field fell below G$_{max}$ by 9.96% and growth of *A. granulata* was reduced by 7.52%. In the laboratory, photoinhibition was not detected at light intensities less than 323 µmol m^{-2} s^{-1}. However, at a light intensity of 583 µmol m^{-2} s^{-1}, photosynthesis (mg O$_2$ mg chl *a*$^{-1}$ h^{-1}) decreased by 9.98% below P$_{max}$ for *A. circinalis* and 16.9% for *A. granulata* (Table 4.5). This light intensity is equal to a dose of 25.2 mol m^{-2} day^{-1} if it is assumed that cells are constantly exposed for 12 hours daily. Therefore, there is fairly good agreement between results from the field and laboratory studies.

The photosynthetic parameters determined for each species in this study were lower than values found in the literature (Table 4.5). The largest difference was in the P$_{max}$ values. For *A. circinalis* this was measured as 4.4 mg O$_2$ (mg chl *a*)$^{-1}$ h^{-1} compared to values as high as 23 mg O$_2$ (mg chl *a*)$^{-1}$ h^{-1} in the literature (Foy and Gibson 1982). Furthermore, for *A. granulata*, the P$_{max}$ was 1.6 compared to 14.1 mg O$_2$ (mg chl *a*)$^{-1}$ h^{-1} (Coles and Jones 2000). The I$_k$ and α values were also lower for both species.

4.4 Discussion

The experiments conducted in the lower Murray River provided no clear evidence as to why *A. circinalis* decreases and *A. granulata* increases in abundance when persistent stratification breaks down and fully mixed conditions resume. There was no difference in growth of *A. circinalis* when grown as pure culture or mixed with *A. granulata*. Therefore, *A. granulata* did not have a detrimental effect on growth of *A. circinalis*. Furthermore, *A. circinalis* had a faster growth rate than *A. granulata* at all Z$_{eu}$/Z$_m$ ratios indicating that the former is able to grow successfully under mixed conditions. The hypothesis that *A. granulata* would grow better than *A. circinalis* at Z$_{eu}$/Z$_m$ ratios < 0.5 was not supported. When comparing growth of

A. circinalis and *A. granulata* over a range of average daily light doses in the field, the onset of saturation was the same for both species (I_k = 17 - 18 mol m^{-2} day^{-1}) but the maximum growth of *A. circinalis* was 56 % higher than *A. granulata* and the growth efficiency almost double. The fast G_{max} of *A. circinalis* may be the reason that it is successful in stratified waters where cells are continually exposed to higher average irradiance.

A. granulata and *A. circinalis* may have been limited by soluble inorganic nitrogen during the Z_{eu}/Z_m field experiment (December 1998). Examination of the heterocyst/vegetative cell ratio of *A. circinalis* suggested that this species was fixing atmospheric sources, whereas *A. granulata* does not have this ability. Given that nitrogen is required for the construction of photosynthetic pigments (Tilzer 1987), low availability may directly influence the light harvesting capacity of cells which may have been particularly detrimental for *A. granulata*. When photosynthesis of both species was compared in the laboratory under nutrient replete conditions, the results were the opposite of the field experiment with the efficiency of *A. granulata* at sub-saturating irradiances almost double that of *A. circinalis*. This disagrees with the study of Coles and Jones (2000) who showed that the photosynthetic efficiency of *A. granulata* was significantly lower than cyanobacteria under the same conditions.

Although the photosynthetic efficiency of *A. granulata* was higher than that of *A. circinalis* in nitrogen-replete conditions, the P_{max} of the latter species was 63.6% higher. Therefore, at high irradiance, *A. circinalis* would be expected to dominate and *vice versa*. The switch-over point where the photosynthetic rate of *A. granulata* was faster than *A. circinalis* was at irradiances lower than 93.3 µmol m^{-2} s^{-1}. If it is assumed that cells are exposed to this light intensity for 12 hours each day, the total daily light dose would be 4.0 mol m^{-2} day^{-1}. This is the exact dose that Sherman *et al.* (1998) suggested was necessary for the dominance of *A. granulata* at Maude Weir in the Murray-Darling Basin.

In the turbid lower Murray River, the light intensity experienced by cells mixed throughout the water column would often be less than 93.3 μmol m^{-2} s^{-1} or 4.0 mol m^{-2} day^{-1}. For example, assuming a K_d of 7 m^{-1} and a mixed depth of 5 m, the average irradiance experienced by mixed cells at midday (1800 μmol m^{-2} s^{-1}) is only 51.4 μmol m^{-2} s^{-1}. The average irradiance experienced by cells at other times of the day would be less. Therefore, assuming a 1:1 relationship between photosynthesis and growth, *A. granulata* would increase faster than *A. circinalis* and would be expected to dominate under nutrient replete conditions. Despite these calculations, it is rare that nitrogen is abundant in the lower Murray River. For example, between 1990 and 1996, oxidised nitrogen exceeded 100 μg L^{-1} on only 1% of sampling occasions during summer (see Chapter 1). Furthermore, blooms of *Anabaena* spp. are normally associated with low N:P ratios (Sakshaug *et al.* 1983; Tilman and Kiesling 1984; Bowling and Baker 1996). It is possible that *A. granulata* accumulates nutrients in the hypolimnion and sediments during stratified conditions (Cimbleris and Cáceres 1991), allowing cells to be nutrient replete when mixing resumes and favourable light conditions are encountered. However, following initial cell division, further nutrient supplies would be needed to ensure sustained growth.

The faster growth of *A. circinalis* compared to *A. granulata* under different Z_{eu}/Z_m ratios in the river may also have been the result of the experimental design. To examine the growth response, diffusion cultures were intermittently suspended at the depth of average irradiance between the surface and the euphotic zone (Riley 1957). Therefore, the cells were held at a constant depth and the light experienced was controlled by solar irradiance and time of day. Short-term fluctuations in light intensity, that are normally experienced through circulation, were prevented. This may have provided a benefit to *A. circinalis* as this species is known to grow better under constant compared to fluctuating light when average irradiance is low (Fogg *et al.* 1973; Litchman 1998; Nicklisch 1998; Sherman *et al.* 1998). In contrast, the

suspension of diffusion cultures at a static depth may have been detrimental to *Aulacoseira granulata* as diatoms generally favour turbulence and fluctuating light conditions (Marra 1978; Litchman 1998). Nicklisch (1998) found that when $Z_{eu}/Z_m = 1$, both cyanobacteria and diatoms grow better under continuous compared to fluctuating light when the light dose is the same. Fluctuating light resulted in a 15-20% decrease in growth for diatoms and a 35-40% decrease for cyanobacteria. The high reduction for cyanobacteria suggests that growth of *A. circinalis* under natural conditions of Z_{eu}/Z_m in the lower Murray River may be expected to be significantly less than determined using the diffusion chambers.

The experiment on the interaction between *A. circinalis* and *A. granulata* showed that growth of *A. granulata* was significantly stimulated when the species were grown together in the lower Murray River. This may have been related to nitrogen fixation by *A. circinalis* leading to an increase in availability for *A. granulata*. If so, *A. circinalis* may be an important contributor to nitrogen availability in the Murray River through fixation of atmospheric sources. The importance of cyanobacteria in contributing towards nitrogen budgets has been previously demonstrated. For example, Howarth *et al.* (1988) found that nitrogen-fixation accounted for 6 to 82% of total nitrogen inputs in eutrophic lakes with rates of 0.2 to 9.2 g N m^{-2} y^{-1}. Furthermore, in Lake Valencia, Venezuela, nitrogen fixation accounted for 23% of the total nitrogen supply and largely occurred in the surface waters where adequate light was available and where heterocyst density was the highest due to flotation of cyanobacterial filaments (Levine and Lewis 1987). The maximum fixation rates of *Anabaena* spp. in the lake ranged between 2.3 and 38.0 nmol $(10^6$ heterocysts$)^{-1}$ h^{-1} at light doses between 1.0 and 2.5 mol m^{-2} h^{-1} (Lewis and Levine 1984).

An unexpected result for the growth of *A. granulata* was that the majority of the rates were negative (February 1998), yet in the Z_{eu}/Z_m ratio experiment (December 1998), the rates were

all positive. Both nitrogen and silicate concentrations were significantly higher during February 1998 so that nutrient availability was unlikely to be the cause of the negative growth rates. It is possible that *A. granulata* did not respond well to nutrient starvation prior to experimentation in February 1998. *A. circinalis* had a positive growth rate in the experiment, however this species may be better at storing phosphorus and nitrogen so that starvation prior to experimentation did not have as marked an effect (Allen 1984; Kromkamp 1987). Alternatively, the rotation of the diffusion chambers in February 1998 may have disrupted the growth of *A. granulata*, although this is unlikely given that this species typically grows under turbulent conditions.

In the field and laboratory, inhibition of growth and photosynthesis of *A. circinalis* and *A. granulata* was found at light availabilities greater than 25 mol m^{-2} day^{-1} or 583 μmol m^{-2} s^{-1} for both species. For *A. circinalis*, there was a 9.98% decrease in photosynthesis at 583 μmol m^{-2} day^{-1}. This is similar to the finding of Foy and Gibson (1982) where there was a 10.3% decrease from P$_{max}$ for shade adapted cells (Table 4.5). The same study showed that the decrease was reduced to 4.3% if the cells were adapted to high light intensities. For *A. granulata* the depression in photosynthesis at 583 μmol m^{-2} d^{-1} was more significant with a 16.9% reduction from P$_{max}$. This differs from the finding of Coles and Jones (2000) who found no photoinhibition of shade adapted cells of *A. granulata* even at light intensities as high as 1800 μmol m^{-2} s^{-1}. The significant depression in photosynthesis of *A. granulata* at high light intensities may reflect the preference of this species for mixed conditions. However, the effect of high light on growth rather than photosynthesis of *A. granulata* was less significant with a reduction of only 9.98% from G$_{max}$ at a dose of 25 mol m^{-2} day^{-1}. McCausland *et al.* (2002) did not detect a decrease in growth of either *Aulacoseira* sp. or *A. circinalis* at high irradiance, but maximum light intensity was only 500 μmol m^{-2} s^{-1}.

The low values for I_k suggest that both *A. circinalis* and *A. granulata* are highly efficient in harvesting light for photosynthesis and can therefore maintain fast rates at low intensities (Henley 1993). However, a low I_k value may be the result of a low P_{max} rather than an indication of a high photosynthetic efficiency (Yentsch and Lee 1966; Henley 1993). In this study, the maximum photosynthetic rates of both species in nitrogen replete conditions were significantly lower than literature values. Unfortunately the reason for the reduced photosynthetic rates is unknown. As the cultures were non-axenic it may be possible that bacteria contributed significantly to the consumption of oxygen. However, the respiration rates for *A. circinalis* and *A. granulata* were within normal limits found in the literature with similar rates of -0.13 and -0.12 mg O_2 (mg chl *a*)$^{-1}$ h^{-1} respectively. For example, Foy and Gibson (1982) found respiration rates ranging between -1.78 and -0.99 for *A. circinalis* when cells were grown at 30 and 150 µmol m^{-2} s^{-1} respectively. Overall, further work examining photosynthesis is required to validate the results in this study.

A strong relationship between growth rate and average daily light dose in the field was found for both *A. circinalis* and *A. granulata*, suggesting significant light limitation in the lower Murray River. The light dose was received by cells in two different ways. Some cultures were exposed to high light intermittently, whilst others were incubated at a static depth and exposed to a continual low light intensity. Despite the different way in which the light was received, there was no difference in growth with rates conforming to the same curve for each species. Short-term studies examining the response of phytoplankton to light are based on photosynthesis. However, rates are highly sensitive to fluctuating light availability and the photosynthetic apparatus may change over a time-scale of minutes to hours (Cullen and Lewis 1988; Ferris and Christian 1991). The data from this study suggests that growth offers a more predictable response as rates are measured over a number of days and short-term physiological variations are taken into account leading to an integrated measurement. The

calculation of the average daily light dose received by cells may be a useful indicator for the

prediction of blooms.

Table 4.1: Z_{eu}/Z_m treatments for the December 1998 field experiment. Circles indicate the time at which cultures were adjusted to the depth of the average irradiance of the euphotic zone (0.16 m) between 7:00 and 19:00 h. The duration of the exposure period was 1 hour. Non-exposed cultures were suspended at 2 m in the dark.

Time (h)	7	8	9	10	11	12	13	14	15	16	17	18	19	Total
Z_{eu}/Z_m 1.00	●	●	●	●	●	●	●	●	●	●	●	●	●	12
0.50	-	●	-	●	-	●	-	●	-	●	-	●	-	6
0.25	-	-	-	-	●	-	●	-	●	-	-	-	-	3
0.08	-	-	-	-	-	-	●	-	-	-	-	-	-	1
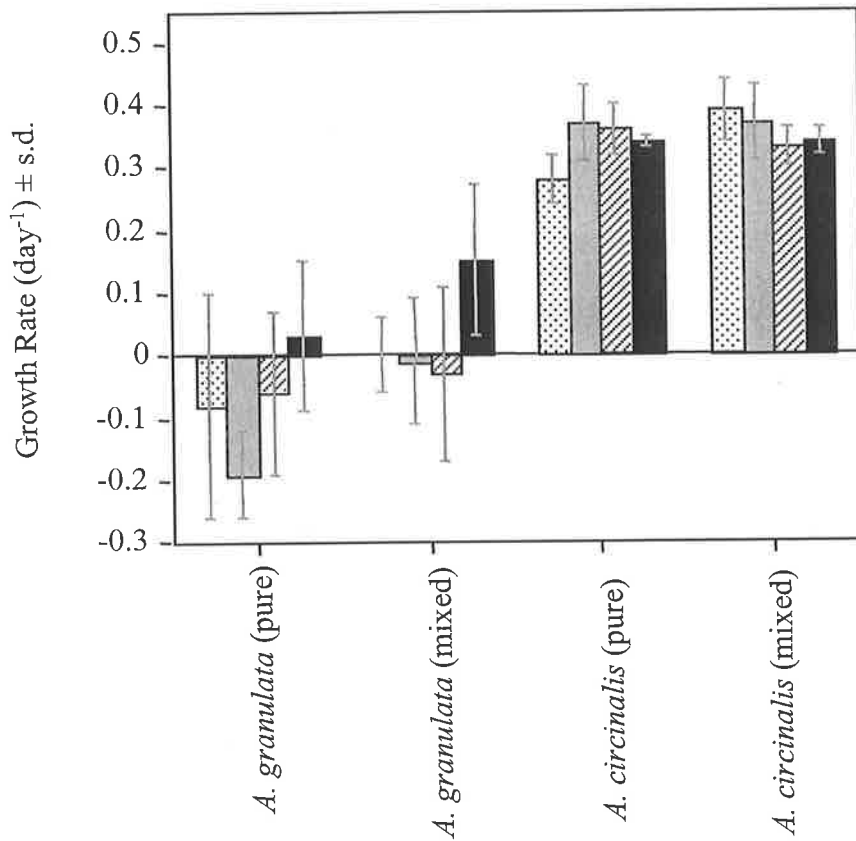
Figure 4.1: Growth of *A. granulata* and *A. circinalis* in the lower Murray River in February 1998 when grown as pure cultures or mixed together in diffusion chambers. Cultures were exposed to nitrogen (N) and phosphorus (P) treatments; -N-P (⊡), -N+P (▦), +N-P (▨) and +N+P (■). Growth rates measured between days 0 and 8.

Table 4.2: Environmental data for February 1998 and December 1998. Filterable reactive phosphorus (FRP), total phosphorus (TP), oxidised nitrogen (NOx), ammonia (NH$_4$) and total Kjeldahl nitrogen (TKN) concentrations in μg L^{-1}. Total silicate (SiO$_2$) concentrations in mg L^{-1}, TN:TP calculated by weight. Temperature (recorded at 0.5 m) and vertical attenuation (K$_d$) units in °C and m^{-1}, respectively. Standard deviations (s.d.) are shown.

	FRP	TP	NOx	NH$_4$	TKN	SiO$_2$	TN:TP	Temp	K$_d$
Feb '98	155.7	327.7	110.7	13.3	940.0	15.3	2.9	23.56	8.4
s.d.	8.4	7.1	21.5	2.3	43.6	0.58	0.1	0.47	0.50
Dec '98	108.3	394.0	7.7	63.7	1530.0	3.7	3.9	22.92	7.48
s.d.	9.5	50.3	8.6	5.7	204.2	0.58	0.03	0.36	1.1
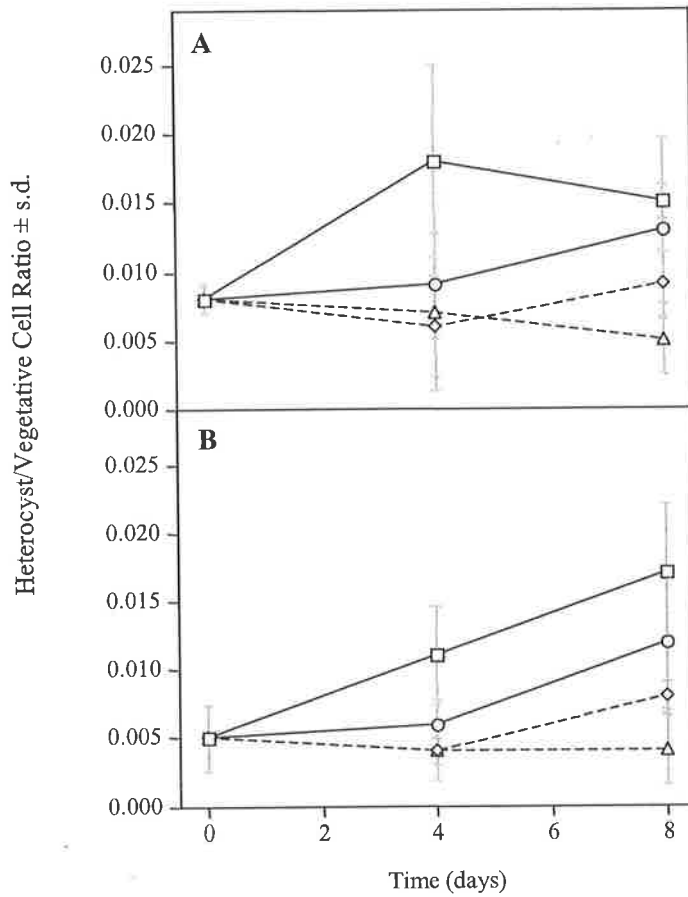
Figure 4.2: Change in the heterocyst/vegetative cell ratio with time in February 1998 in a)

pure cultures of *A. circinalis* and b) mixed cultures of *A. circinalis*, under each nutrient

treatment; -N-P (square), -N+P (circle), +N-P (diamond), and +N+P (triangle). Nitrogen

additions are depicted by a broken line.
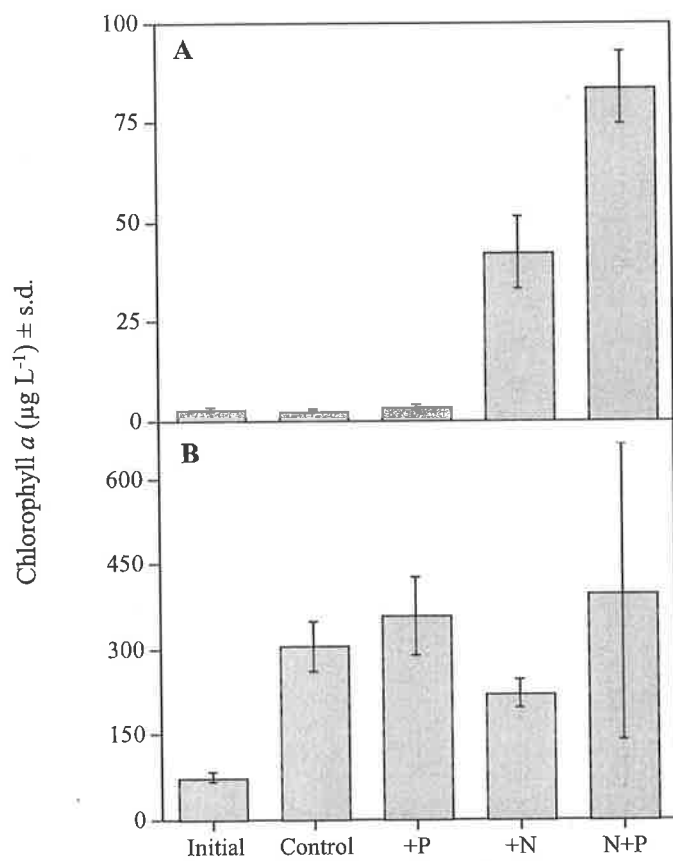
Figure 4.3: Laboratory bioassays on water samples taken from the lower Murray River in a) February 1998 and b) December 1998.

Table 4.3: Back-calculated light doses that *A. circinalis* received in experimental diffusion chambers in the lower Murray River, in December 1998.

Zeu : Zm	Hours Exposed (at 0.15 m)	Total Light mol m^{-2} 6 days^{-1}	Average Light mol m^{-2} day^{-1}
0.08	1	15.74	2.62
0.25	3	37.85	7.04
0.50	6	50.41	9.55
1.00	12	99.06	19.28
Depth (m)	Hours Exposed (at given depth)		
0.05	24	180.52	34.97
0.16	24	99.06	19.28
0.35	24	30.28	5.99
0.50	24	16.61	3.30
1.00	24	9.13	1.83
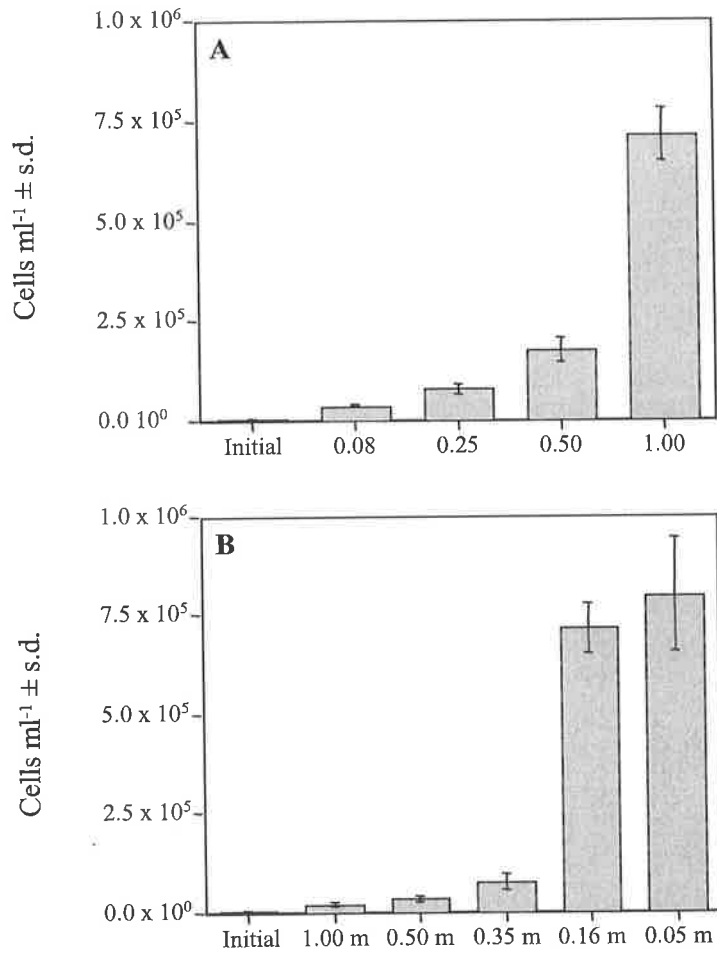
Figure 4.4: Cell density of *A. circinalis* on day 0 and day 6 for cultures incubated in diffusion chambers in the lower Murray River in December 1998 at a) various Z_{eu}/Z_m ratios and b) static depths (m).
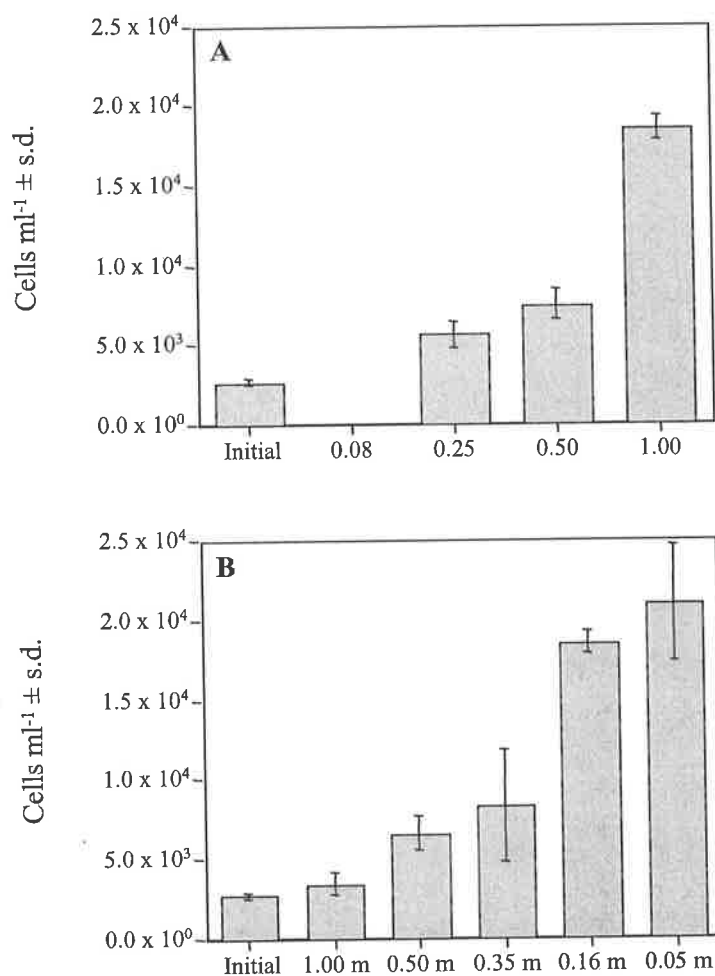
Figure 4.5: Cell density of *A. granulata* on day 0 and day 6 for cultures incubated in diffusion chambers in the lower Murray River in December 1998 at a) different Z_{eu}/Z_m ratios and b) static depths (m). N.B.; $Z_{eu}/Z_m = 0.08$ treatment not included due to loss of replicates.
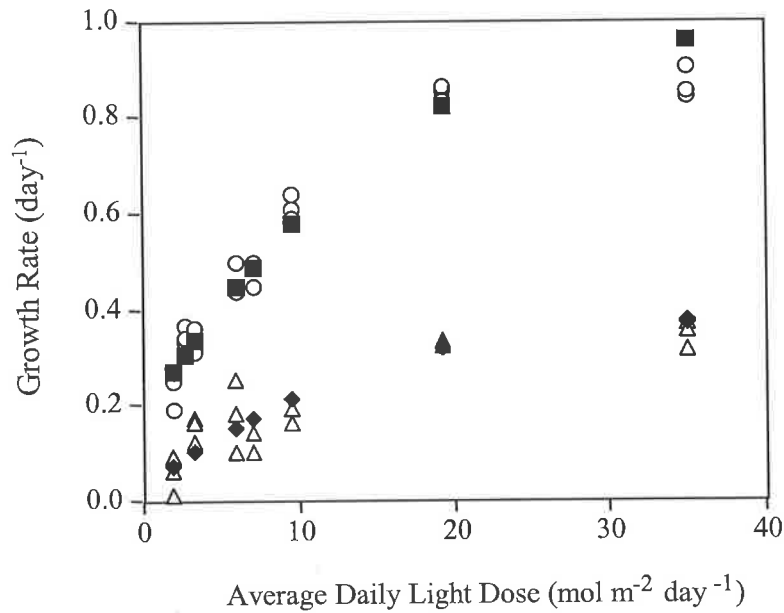
Figure 4.6: Growth rates of *A. circinalis* (circle) and *A. granulata* (triangle) at various average irradiances when grown in diffusion chamber in the lower Murray River in December 1998. Data was modelled (■ *A. circinalis*; ◆ *A. granulata*) using a modified equation from Jassby and Platt (1976).

Table 4.4: Comparison of light model parameters for *A. circinalis* and *A. granulata*. Model based on growth rates measured in diffusion chambers in the lower Murray River in December 1998. G_{max}, day^{-1}; I_k, mol m^{-2} day^{-1}; R_d, day^{-1}; alpha, day^{-1} (mol m^{-2} day^{-1})$^{-1}$.

	Anabaena circinalis	*Aulacoseira granulata*
G_{max}	0.80	0.35
I_k	18	17
R_d	0.191	0.0325
alpha	0.0444	0.0206
r-squared	0.958	0.809
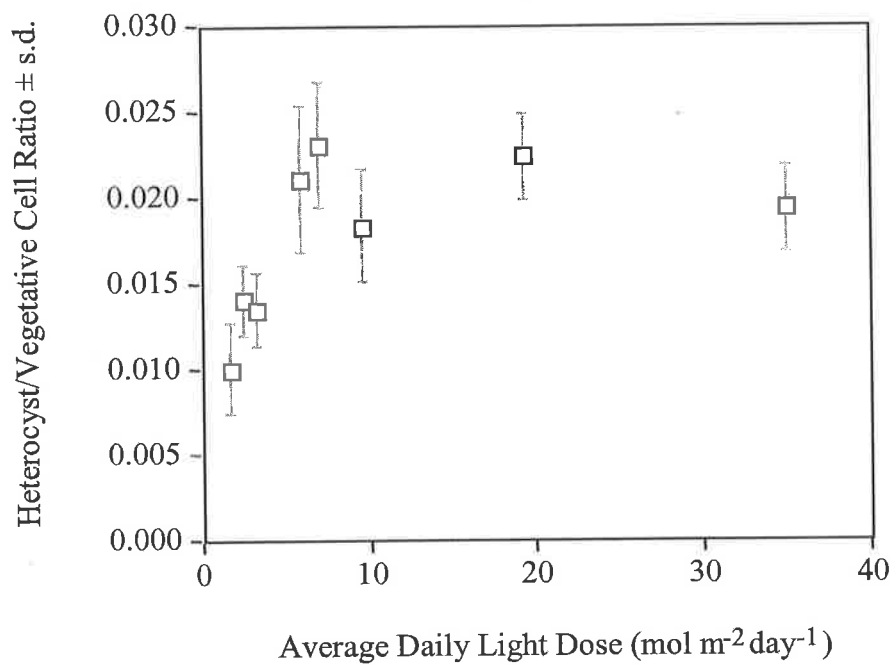
Figure 4.7: The effect of light dose on the heterocyst/vegetative cell ratio of *A. circinalis* after 6 days incubation in diffusion chambers in the lower Murray River in December 1998.
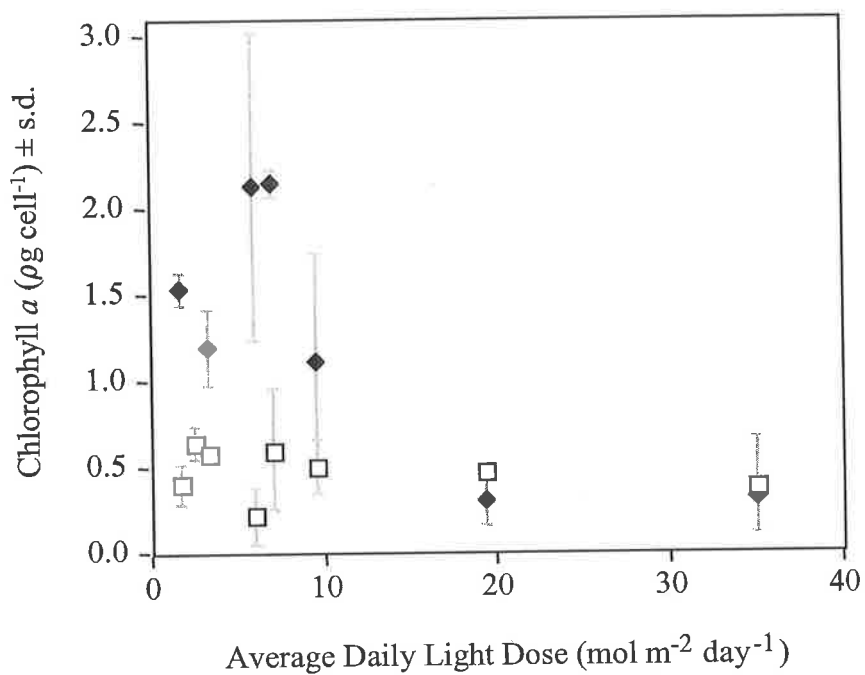
Figure 4.8: Effect of light dose on the chlorophyll content of *A. circinalis* (square) and *A. granulata* (diamond) after incubation for 6 days in diffusion chambers in the lower Murray River in December 1998.

Table 4.5: Comparison of photosynthetic parameters with values from the literature. P_{max} = mg O_2 (mg chl a)$^{-1}$ h^{-1}; I_k = μmol m^{-2} s^{-1}; α = mg O_2 (mg chl a)$^{-1}$ mol^{-1} m^2; Photoinhibition = % decrease from P_{max}; Chl a = pg chl a cell^{-1}. Data from Coles and Jones (2000) was converted assuming equimolar exchanges between CO_2 and O_2.

	P_{max}	I_k	α	Photoinhibition	Chl a
Anabaena circinalis					
This study	4.4	140	8.72	9.98%	0.2-0.6
Foy and Gibson (1982)	17.3-23.0	198-317	26.7	4.3-10.3%	-
Reynolds (1975)	12-14	-	-	-	-
Reynolds (1984)	-	-	-	-	0.6-0.9
Aulacoseira granulata					
This study	1.6	47	9.44	16.9%	0.3-2.1
Coles and Jones (2000)	14.1	138-271	14.5-18.4	none detected	0.58-1.16
Reynolds (1984)	-	-	-	-	4.0-5.0
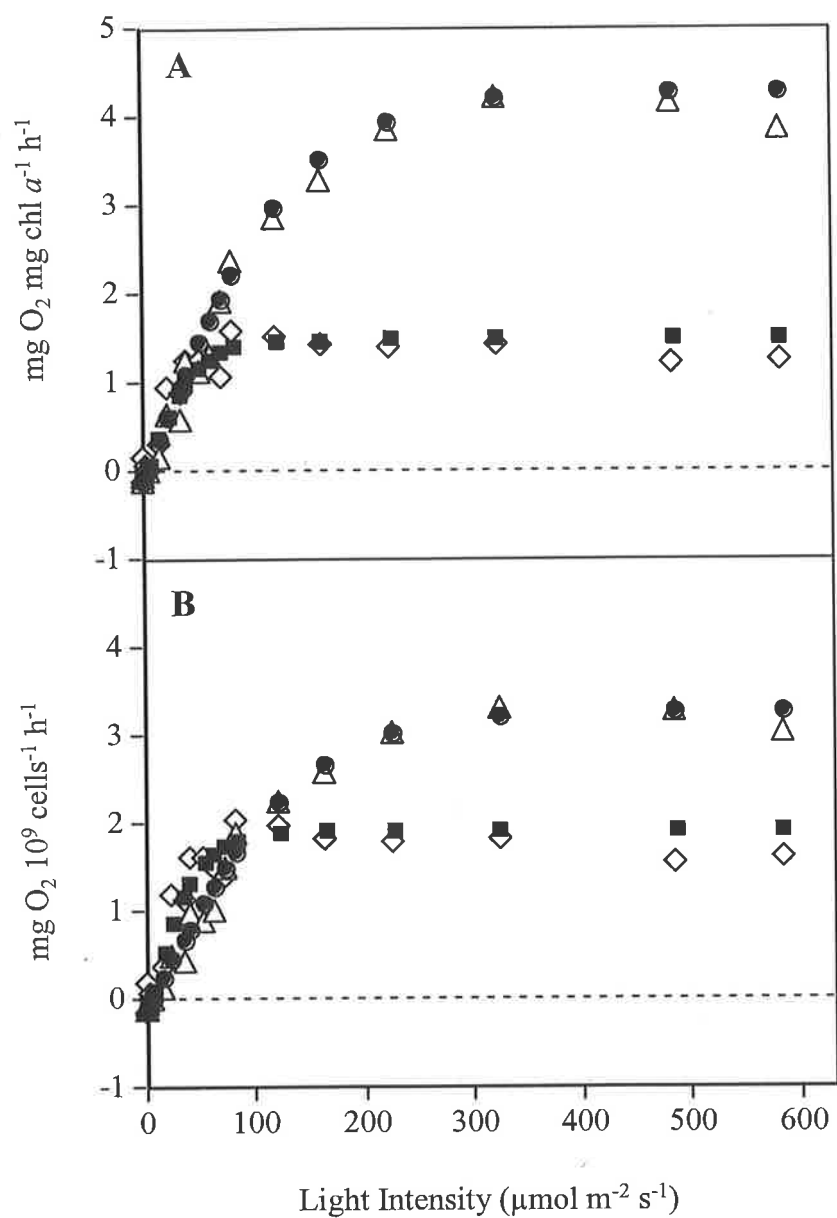
Figure 4.9: Photosynthetic rate a) per mg chlorophyll *a* and b) per 10^9 cells of *A.*

circinalis (triangle) and *A. granulata* (diamond) measured under nutrient-replete

conditions in the laboratory. Data modelled (● *A. circinalis*; ■ *A. granulata*) using

the modified formula of Jassby and Platt (1976, see text). For model parameters,

refer to Table 4.6.

Table 4.6: Comparison of photosynthesis of *A. circinalis* and *A. granulata* in nitrogen-replete conditions in the laboratory. P_{max} = mg O_2 specified units^{-1} h^{-1}; I_k = μmol m^{-2} s^{-1}; α^a = mg O_2 (specified units)$^{-1}$ h^{-1} (μmol m^{-2} s^{-1})$^{-1}$; α^b = mg O_2 (specified units)$^{-1}$ mol^{-1} m^2; R_d = mg O_2 specified units^{-1} h^{-1}

	Anabaena circinalis		*Aulacoseira granulata*	
	per mg chl *a*	**per 10^9 cells**	**per mg chl *a***	**per 10^9 cells**
P_{max}	4.40	3.40	1.60	2.05
I_k	140	141	47	45
α^a	0.0314	0.0241	0.0340	0.0456
α^b	8.72	6.69	9.44	12.67
R_d	-0.13	-0.12	-0.12	-0.13
r-squared	0.989	0.989	0.930	0.933

CHAPTER 5. Effect of Mixing Patterns and Light Dose on Growth of *Anabaena circinalis* in the Lower Murray River.

ABSTRACT.

Anabaena circinalis is common in the lower Murray River, Australia, and may compromise water quality due to the release of toxins. The water is turbid and thermal structure may significantly affect light availability. An *in situ* experiment was designed to represent complete mixing, diurnal stratification and persistent stratification and test the effect on growth of *A. circinalis*. To simulate the mixing treatments, cells were incubated in diffusion chambers that were adjusted to different positions in the water-column throughout the day. Populations exposed to persistent stratification over 6 days grew significantly faster than the other treatments at a rate of 0.65 day^{-1}. However, growth of the diurnally stratified populations was slower than (0.28 day^{-1}), or similar to (0.40 day^{-1}) the mixed population (0.40 day^{-1}). Therefore, the growth of the sub-populations exposed to the euphotic zone were insufficient to counteract the slow growth of the majority that were confined to darkness during the stratified period. A relationship between growth rate and average daily light dose was constructed and G_{max}, α and I_k solved as 0.66 day^{-1}, 0.12 day^{-1} (mol^{-1} m^{-2} day^{-1})$^{-1}$ and 5.4 mol m^{-2} day^{-1}, respectively. Using these parameters, a model was developed to predict possible differences in growth between diurnal and mixed populations under varying conditions of K_d, Z_m and incubation time.

5.1 Introduction

Blooms of the potentially toxic, nitrogen fixing cyanobacterium, *Anabaena circinalis*, are common in the Murray-Darling basin and often compromise water quality (Bowling and Baker 1996, Baker *et al.* 2000, Webster *et al.* 2000). As the Murray-Darling is a major water source for South Australia the control of blooms is an important aspect of water management (Webster *et al.* 2000). The lower Murray is a shallow, turbid river (Mackay *et al.* 1988; Blanch 1997) and the euphotic zone rarely exceeds 1m. *Anabaena* blooms tend to occur during summer when flow is low and are therefore associated with periods of thermal stratification (Burch *et al.* 1994).

Bormans *et al.* (1997) showed in the Murray River that persistent stratification occurs during low flows (< 4000 ML day^{-1}) when the daily average wind speed is < 1.2 m s^{-1}. However, low wind speeds are uncommon (Bormans *et al.* 1997; Maier *et al.* 2001) and stratification mainly

occurs on a diurnal basis with overnight cooling and average wind speeds between 1.2 and 2.9 m s^{-1} causing a significant deepening of the surface mixed layer. When the wind speed is > 3 m s^{-1} the water column is mixed on a continual basis. There is evidence to show that the break down of permanent stratification reduces both the magnitude and the probability of *A. circinalis* blooms (Reynolds *et al.* 1983; Sherman *et al.* 1998). However, there are currently no experimental data to suggest that diurnal stratification prevents bloom formation. Furthermore, there has been no comparison of growth under diurnally stratified conditions compared to continually mixed conditions. Webster *et al.* (2000) suggested that the growth of *A. circinalis* would be curtailed if the water column was mixed on at least a diurnal basis.

Diurnal stratification generally occurs for 6 hours between 11.00 and 17.00 h in the lower Murray River at Morgan (Figures 5.1 and 5.2). The water column is mixed in the early evening and overnight allowing the re-distribution of cells. As the water-column is shallow (5 m), the surface layer is mixed to the bottom and there is no parent thermocline. Therefore, the cells would be re-distributed over the depth of the water column. When diurnal stratification develops the following day, a significant proportion of a population may be trapped below the euphotic zone. In contrast, a small proportion of a population may be contained within the euphotic zone and exposed to a favourable light climate. Since the total population within the water column consists of cells that experience different daily light doses, growth needs to be examined in an integrated manner. This can be achieved by examining growth of distinct sub-populations that are manipulated to experience different light doses. The sub-populations are then combined to allow measurement of growth of the total population.

Anabaena is generally confined to systems where the Z_{eu}/Z_m ratio is 0.5 – 3.33 (Reynolds and Walsby 1975). Given that the average depth of the lower Murray River is approximately 5 m and the euphotic depth is between 1 and 0.3 m, the Z_{eu}/Z_m ratio ranges from 0.18 to 0.06. This

suggests that growth of *Anabaena* under completely mixed conditions is light limited. A laboratory study on *A. circinalis* showed that growth over four days was saturated at 100 μmol $m^{-2} s^{-1}$ on a 12 h:12 h light/dark cycle (Brookes 1997). Therefore, growth may be light limited at daily light doses of < 4.3 mol m^{-2} day^{-1}.

An *in situ* experiment was designed to investigate the light limited growth of *A. circinalis* populations subjected to diurnal stratification. This was compared with the growth of populations that were either fully mixed or persistently stratified. Given that turbidity is highly variable in the Murray River (Sullivan *et al.* 1988; Mackay *et al.* 1988), a simple model was developed to compare the difference in growth between diurnally stratified and mixed populations when the vertical attenuation changes. Responses to changes in the maximum mixed depth and the duration of favourable light conditions were also explored. The model did not include loss factors and therefore estimated the upper limits of *A. circinalis* growth.

5.2 Methods

5.2.1 Site Description

Morgan (34°01 S/139°41 E) is situated on the Murray River, 315km from the mouth and equidistant between locks 1 and 2. The river has a mean depth of 5 m and a bed slope less than 4.5 cm km^{-1} (Sullivan *et al.* 1988). Turbidity at Morgan is highly variable and is largely influenced by discharges from the clay-laden Darling River upstream (Sullivan *et al.* 1988; Mackay *et al.* 1988). Over the past ten years, turbidity has ranged from 4 to 304 NTU with an average of 51.6 ± 38.9 NTU. Salinity at Morgan is 556 ± 184 EC units and flow measured at lock 2 is 9852 ± 8022 ML day^{-1} (data from the Murray-Darling Basin Commission).

5.2.2 Sample Preparation

A. circinalis (ANA271B, Chapter 2) was cultured in WC media (Guillard and Lorenzen 1972) at a light intensity of 50 - 60 μmol $m^{-2} s^{-1}$ under a 14 h:10 h light/dark cycle at 25 °C. Log

phase cultures were transported to Morgan and transferred into 50, 140 ml diffusion chambers (Köhler 1997). These were constructed from 9.5 cm lengths of UV-transmitting polycarbonate tubing and the ends were sealed with 47 mm polysulphone filters with a pore size of 0.45 μm.

5.2.3 Incubation Protocol

To represent populations of *A. circinalis* under persistently stratified or fully mixed conditions, cultures were suspended at depths equivalent to the average column irradiance ($\bar{I}$) calculated for each mixing regime (Figure 5.3 a,b). Depths were calculated from Riley's (1957) equation:

$$\bar{I} = \bar{I}_0' \, (1 - e^{-K_d * Z_m}) / K_d * Z_m \tag{1}$$

where,

$\bar{I}_0'$ = incoming irradiance, $\mu mol \; m^{-2} \; sec^{-1}$

K_d = light attenuation, m^{-1}

Z_m = mixed depth, m

The vertical attenuation coefficient measured in the river a week prior to experimentation was 6.3 m^{-1}. The depths of average irradiance corresponding to the two mixing regimes were 0.25 m and 0.55 m. The first represented a population permanently circulating within the euphotic zone (0.73 m), the second, a population that was mixing through a 5 m water column (the mean depth of the River Murray at Morgan, $Z_{eu}/Z_m = 0.15$).

To represent a diurnally stratified water column, a population of *A. circinalis* was divided into five sub-populations. Each sub-population represented 1 m^3 assuming that cells were evenly distributed throughout the 5 m water-column. During isothermal mixing, all sub-populations were suspended at 0.55 m (Figure 5.3c). During the period of thermal stratification (11.00 to 17.00 h), four of the sub-populations were lowered into the dark to represent the proportion of

cells trapped below the euphotic zone. The remaining sub-population was suspended at 0.25 m to represent the proportion of the population still exposed to the euphotic zone during the stratified period. At 17.00 h all cultures were re-positioned to 0.55 m where they remained until 11.00 the following day.

On a daily basis random numbers were used to select which of the five sub-populations would be exposed to the average irradiance of the euphotic zone during the stratified period (the diurnal random treatment). However, to determine the influence of this randomised procedure an additional treatment, diurnal non-random, was included (Figure 5.3d). This treatment consisted of two sub-populations. One was always placed in the euphotic zone and the other in the dark during the period of thermal stratification.

There were five replicates for each sub-population within each treatment described above. An additional dark treatment was included to determine growth rate in the dark (not illustrated). For the dark treatment diffusion chambers were positioned below the euphotic zone throughout the entire incubation period.

5.2.4 Sampling

Diffusion cultures were incubated in the Murray River for six days from Feb 20 to 26 1999. Every two days between 8:00 and 11:00 h, 2 ml samples were removed from all chambers. Prior to sampling the chambers were gently agitated to ensure that cells were evenly distributed. In addition, for the diurnal random treatment, 1 ml was removed from each chamber representing each sub-population and mixed to give a 5 ml integrated sample of the total population. For the diurnal non-random treatment, 1 ml was taken from the chamber exposed to the euphotic zone and 4 ml taken from the chamber lowered into the dark during thermal stratification and mixed to give a 5 ml integrated sample. All samples were preserved

in Lugol's iodine solution. Cell numbers were determined using a Sedgwick-Rafter chamber and inverted microscope. The ratio of heterocysts to vegetative cells was also recorded.

Incident irradiance was continually recorded using a data logger and quantum sensor (LICOR 1000). Underwater light penetration was measured daily with a LICOR underwater quantum sensor. The vertical attenuation coefficient was calculated by regression analysis of underwater light measurements against depth. Water temperature was logged at 1 m using an optic stowaway (Hastings Data Loggers STEB8).

To determine nutrient concentrations, three integrated water samples (0 to 1.5 m) were collected every two days from the river. For analysis of filterable reactive phosphorus (FRP), nitrate + nitrite (NO_x) and ammonium (NH_4), an aliquot of each sample was filtered through a Whatman GF/C filter (pre-filter) and then a 0.2 μm polycarbonate filter (Gelman). Filtered and raw water samples were frozen until analysis. The analysis for FRP, NO_x, NH_4, total phosphorus, and total Kjeldahl nitrogen was conducted by the Australian Water Quality Laboratory according to standard procedures (Anon. 1995).

5.2.5 Analysis

Growth rates were calculated from the equation $\mu = (1/t) \times \ln(N_t/N_0)$ where t is days, and N_t and N_0 represent the final and initial cell concentrations. The light dose received by individual chambers was calculated by summing integrated light intensities experienced at depth over each hour. This was based upon knowledge of the vertical attenuation coefficient, the daily incoming irradiance and the depth at which the cultures were exposed. Growth rates were analysed using 1-way ANOVA and a post-hoc Tukey's HSD test (JMP, SAS Inc. version 3.0.2 1989-94) after testing for normality (Shapiro-Wilk test). Although five replicates were used for all treatments, analysis was only conducted on three replicates due to the rupturing of

some chambers in the field. Heterocyst data were analysed using both 1-way ANOVAs and t-tests (n = 3).

A growth/irradiance curve for *A. circinalis* was plotted and a modified version of the hyperbolic tangent equation of Jassby and Platt (1976) fitted to the data points. The modifications were that growth rates (G) replaced photosynthetic rates, and the respiration rate (Van der Bijl *et al.* 1989; Olesen and Sand-Jensen 1993) was assumed to be equivalent to a loss rate:

$$G = G_{max} * Tanh(I/I_k) + R_d \quad day^{-1} \tag{2}$$

where,

G_{max} = Growth rate at optimal light dose, day^{-1}

I_k = Light dose where the extrapolated lines of G_{max} and the initial light limited portion of the G-I curve intersect, mol m^{-2} day^{-1}

R_d = Constant to adjust for loss rate, day^{-1}

α = Slope of the initial linear section of the G-I curve, day^{-1} (mol m^{-2} day^{-1})$^{-1}$

The parameters for the equation were solved through minimisation of the sum of squares between the modelled values and the field data and α was derived by the division of G_{max} and I_k.

5.2.6 Model Construction

A model was constructed, based upon the field results, to examine differences in the growth of *A. circinalis* when cells were exposed to either continually mixed or diurnally stratified conditions. The model was divided into four parts; a) incoming irradiance, b) depth profiles of irradiance c) the response of cells to light dose and d) the daily integration of sub-populations representing diurnal populations. The effects of vertical light attenuation (K_d, m^{-1}), maximum

mixed depth (Z_m, m) and incubation period (days) were examined. The incubation period represented the number of cloudless days. Incoming irradiance (I_o) was calculated according to the equation of Kromkamp and Walsby (1990) which describes the sine pattern of the rise and fall of the sun:

$$I_o = I_m * \sin(\pi * t / D_L) \hspace{4cm} (3)$$

where,

I_m = maximum incoming irradiance, μmol m^{-2} s^{-1}

I_o = surface irradiance, μmol m^{-2} s^{-1}

t = time of the surface irradiance after dawn, mins

D_L = length of the light period from dawn until dusk, h

Light intensity experienced by *A. circinalis* was determined using the Beer-Lambert Law which required values for I_o, the depths where the cultures were suspended and K_d. The daily light dose that individual cultures received was calculated by summing the light at hourly intervals. The depths at which cultures were suspended were based on the design described for the mixed and diurnal non-random treatments in the field experiment. The model calculated the appropriate depths of average irradiance using Riley's (1957) equation and the Beer-Lambert Law, from Z_m and K_d.

To determine the response of the cultures to light dose the modified equation of Jassby and Platt (1976) was used (see above). G_{max}, I_k and R_d were supplied as inputs and the expected growth rates calculated based upon average daily light dose. The expected number of cells for a given light dose was calculated from the growth rate and from the values for cell density calculated the previous day.

For the diurnally stratified treatment, the cell numbers of the sub-populations were integrated at the end of each day to represent the re-distribution of cells throughout the water column when stratification breaks down and mixing resumes. The proportion of cells exposed to the euphotic zone during the stratified period was calculated from Z_{eu}/Z_m. The proportion of cells in the dark was calculated from $1 - (Z_{eu}/Z_m)$. The cell density of each sub-population was multiplied by the proportion represented and summed to give the final cell density of the total integrated population.

The ratio of cell numbers (diurnal/mixed) was used to compare diurnal with mixed treatments. Ratios of <1 indicate that the mixed treatment was more productive than the diurnal treatment. In addition, Z_{eu}/Z_m ratios were used rather than K_d and Z_m. However, it should be noted that Z_{eu}/Z_m is only directly applicable to the mixed populations. For populations exposed to diurnal stratification the Z_{eu}/Z_m increases during the stratified period.

5.3 Results

5.3.1 Light Dose

The weather was generally cloudless and the average daily light doses received by individual cultures ranged from 0 to 8.9 mol m^{-2} day^{-1} (Figure 5.4). The highest dose was received by cultures permanently exposed in the euphotic zone (8.9 mol m^{-2} day^{-1}). Cultures that were exposed to the average column irradiance received 1.5 mol m^{-2} day^{-1}, whereas cultures that were re-positioned non-randomly either into the euphotic zone or into darkness during diurnal stratification received 7.0 and 0.4 mol m^{-2} day^{-1}, respectively.

The light received by the cultures whose exposure depth during the period of diurnal stratification (11.00 to 17.00 h) was determined randomly experienced a range of doses (Figure 5.4). The highest dose was 3.3 mol m^{-2} day^{-1} for cultures located in the euphotic zone on days 1, 3, and 6. Two days exposure in the euphotic zone (days 2 and 5) gave a light dose

of 2.7 mol m^{-2} day^{-1} and a single day (day 4) 1.8 mol m^{-2} day^{-1}. The two sets of diffusion chambers that were never re-positioned into the euphotic zone during the stratified period only received 0.4 mol m^{-2} day^{-1}.

5.3.2 Cell Numbers

The initial *A. circinalis* cell density was 4.7 x 10^3 cells ml^{-1}. The final cell densities ranged from 6.7 x 10^3 to 2.3 x10^5 cells ml^{-1} (Figure 5.4). Cultures permanently located in the euphotic zone increased cell numbers by a factor of 49, whereas those permanently exposed to the average column irradiance showed a 10-fold increase. Cultures that were located at the average column irradiance during periods of isothermy and repositioned into the euphotic zone during stratification increased by a factor of 40. Cultures positioned in the dark during the stratified period showed no significant increase in cell numbers above the initial or cultures permanently located in the dark.

Cultures randomly distributed into the euphotic zone had cell numbers that ranged from 5.6 x 10^4 cells ml^{-1} in the cultures that had been re-positioned into the euphotic zone for three of the six days, to concentrations that were not significantly different from the initial concentrations (Figure 5.4).

5.3.3 Growth Rates v Light Dose

Growth rates of the sub-populations randomly exposed to the euphotic zone during diurnal stratification ranged from 0.22 to 0.41 day^{-1}. The growth rates for the non-random sub-populations were 0.23 and 0.62 day^{-1}. These non-random growth rates represent the lower and upper limits for the growth of sub-populations randomly exposed to the euphotic zone each day.

The relationship between individual growth rates and light (Figure 5.5) showed that rates increased rapidly as the average daily dose increased from 0 to 3.3 mol m^{-2} day^{-1}. At higher light doses the growth rate tended towards a maximum (G_{max}) of 0.66 day^{-1}. The parameters I_k, R_d and α were determined as 5.4 mol m^{-2} day^{-1}, 0.056 day^{-1} and 0.12 mol m^{-2} day^{-1} (day^1)$^{-1}$, respectively ($r^2 = 0.884$).

5.3.4 Integrated Population Growth

The mean growth rate of populations permanently located within the euphotic zone was 0.65 ± 0.008 day^{-1}. In contrast, cultures located at the depth of the average column irradiance grew at a significantly slower rate of 0.40 ± 0.005 day^{-1}. There was no difference in the growth rates of cultures exposed to the average column irradiance and diurnal populations that were non-randomly exposed during thermal stratification (Figure 5.4, 0.40 ± 0.02 day^{-1}). The slowest growth rate (0.28 ± 0.02 day^{-1}) was recorded for the diurnal treatment that consisted of 5 sub-populations which were randomly exposed to the euphotic zone during the stratified period each day. Overall, populations exposed to diurnal stratification did not grow faster than the mixed population. Therefore, the fast growth of sub-populations exposed to the euphotic zone during the stratified period was insufficient to counteract the slow growth of the sub-populations that remained in the dark.

5.3.5 Nutrients

The average filterable reactive phosphorus concentration in the river water throughout the experiment was 51.8 ± 9.8 µg L^{-1}, a concentration that is unlikely to limit phytoplankton growth (Vollenweider 1968; Sas 1989). However, oxidised nitrogen was undetectable on 50% of the sampling occasions but averaged 3.3 ± 3.3 µg L^{-1}, and ammonia concentrations averaged 46.8 ± 8.9 µg L^{-1}. These nitrogen levels may restrict algal growth and a TN:TP ratio (by weight) of 5.8 also suggests that nitrogen was limiting. To investigate this possibility the heterocyst/vegetative cell ratio of *A. circinalis* was measured. The ratio increased significantly

from an initial value of 0.013 ± 0.005 for light doses > 0.4 mol m^{-2} d^{-1}. The final ratios (day 6) were significantly related to light dose with cultures receiving 8.9 mol m^{-2} day^{-1} having approximately triple the ratio of cultures which received an average dose of 0.4 mol m^{-2} day^{-1} (P = < 0.05, Figure 5.6).

5.3.6 Modelling the Growth of A. circinalis

To assess the influence of K_d, Z_m and incubation time on growth rate, cell numbers and the diurnal/mixed ratio the following constants were used: Midday irradiance = 1500 μmol m^{-2} s^{-1} (= 45.5 mol m^{-2} day^{-1}); Daylength = 14 h; Initial cell density = 800 cells ml^{-1}. The parameters used to predict growth of *A. circinalis* according to light dose were derived from Figure 5.5. The exception was the value for R_d which was set to 0. Buoyancy of *A. circinalis* was assumed to be neutral at all times.

Application of the model (Figures 5.7 and 5.8) shows how these factors interact. The daily growth of both mixed and diurnally stratified populations increases with increasing Z_{eu}/Z_m (Figure 5.7). The increase in growth rate follows the trend of a curve that has a different shape for each treatment. Growth of mixed populations is faster than diurnal populations between Z_{eu}/Z_m values of approximately 0.2 and 0.9. However, when Z_{eu}/Z_m is < 0.2 or between 0.9 and 1.0, growth of mixed and diurnally stratified populations is similar (Table 5.1). Diurnal populations do not grow faster than mixed populations at any Z_{eu}/Z_m values.

The difference in growth rate between mixed and diurnal populations leads to differences in the cell density when comparing the two treatments. These differences are illustrated through use of the diurnal/mixed ratio. As suggested from Figure 5.7, the largest difference in growth between mixed and diurnal populations occurs at Z_{eu}/Z_m values between 0.2 and 0.9. This is supported by the diurnal/mixed ratio with the lowest values occurring within this range (Figure 5.8). With increasing incubation time the diurnal/mixed ratio decreases as the

differences in cell density between the two treatments become larger. The diurnal/mixed ratio ranges from 0.85 to 1.0 after 3 days incubation, from 0.5 to 1.0 after 12 days incubation, and from 0.3 to 1.0 after 21 days incubation. In all cases the lowest ratios occur at $Z_{eu}/Z_m = 0.5$. Low ratios represent large differences in cell density. For example, when $K_d = 8$ m^{-1} and the maximum mixed depth = 1 m ($Z_{eu}/Z_m = 0.576$), the predicted cell density of diurnal populations after 9 days is 5.3×10^4. For mixed populations the predicted density is 8.6×10^4 (Table 5.1).

If Z_m is equal to or less than Z_{eu} the mixing pattern does not affect growth since the cells are always circulating within the euphotic zone and the light dose received is analogous to persistently stratified conditions. The growth rate under conditions where $Z_{eu}/Z_m = 1.0$ is 0.63 day^{-1} (Table 5.1). The cell densities for persistently stratified populations of *A. circinalis* after 3, 6, 9, 15 and 21 days growth are 5.2×10^3, 3.4×10^4, 2.3×10^5, 9.7×10^6 and 4.5×10^8 cells ml^{-1}, respectively. These values are 4.3, 20.0, 95.8, 1 865 and 40 909 times higher than cell densities achieved by diurnally stratified populations and 4.3, 18.8, 82.1, 1 516 and 30 000 times higher than mixed populations when $K_d = 8$ m^{-1} and $Z_m = 5$ m (Table 5.1).

5.4 Discussion

Populations of *A. circinalis* randomly and non-randomly exposed to diurnally stratified conditions did not grow faster than mixed populations in the lower Murray River. Although 1/5 of each population was exposed to the euphotic zone for 6 hours during the stratified period, the growth of cells in the euphotic zone was insufficient to counteract the slower growth of the majority of the population trapped in darkness. These results imply that artificial mixing of the lower Murray River to prevent diurnal stratification would be of no benefit. In fact, the diurnal random treatment suggested that artificial mixing may actually enhance growth of *A. circinalis* rather than restrict it.

Growth of the diurnal non-random population was significantly faster than the diurnal random population, and similar to the mixed population. This was due to the higher average daily light dose experienced by the sub-population within this treatment that had repeated access to the euphotic zone each day. Growth of the sub-population was rapid (0.62 day^{-1}) and contributed significantly to the total integrated population. The maximum growth of the sub-populations from the diurnal random treatment was only 0.22 day^{-1}.

The population subjected to persistent stratification ($Z_{eu}/Z_m = 1$) had the fastest growth rate (0.66 day^{-1}) which can be attributed to its prolonged exposure to the euphotic zone throughout the incubation period. Since growth of the persistently stratified population was significantly higher than both mixed and diurnally stratified populations, artificial destratification would be a beneficial tool if environmental factors leading to this condition were to occur (Sherman *et al.* 1998; Reynolds *et al.* 1983). However, if the euphotic depth is similar to the bottom depth, destratification may prove ineffective (Nakano *et al.* 2001). This was demonstrated by the model which shows that *A. circinalis* grows at 0.63 day^{-1} when $Z_{eu}/Z_m = 1$, regardless of the mixing pattern.

The onset of light saturated growth was 5.4 mol m^2 day^{-1} compared with the dose of 4.3 mol m^2 day^{-1} calculated from the data of Brookes (1997). This may be due to the light required to satisfy both photosynthesis and nitrogen fixation given that combined nitrogen concentrations were < 300 μg L^{-1} (Horne and Goldman 1972) and heterocyst formation was stimulated (Golden *et al.* 1985). Oxidised nitrogen concentrations in the lower Murray River were mostly undetectable in this and previous studies (Brookes *et al.* 1997; Baker *et al.* 2000). The previous studies also demonstrated nitrogen limitation of phytoplankton in the lower Murray River via bioassays and fluorescein diacetate-based metabolic assays. Nitrogen fixation competes for energy and reductant with photosynthesis (Lex *et al.* 1972; Paerl and Kellar

1979; Tilzer 1987) and the light requirements of nitrogen-fixing cyanobacteria are therefore higher. Since increases in the heterocyst/vegetative cell ratio were correlated with increases in light availability (Lewis and Levine 1984), the optimal growth of *A. circinalis* at doses > 5.4 mol m^{-2} day^{-1} may be related to both light and nitrogen availability. Interestingly, blooms of *A. circinalis* in the Murray River are mainly associated with shallow lagoons that adjoin the main river channel (Baker 1999). The depths of the lagoons range from approximately 0.5 to 1 m and the high density of cells may be associated with the high light doses experienced.

The model supported the data from the field experiment and provided additional information about the response of *A. circinalis* to mixing patterns with changes in vertical light attenuation, maximum mixed depth and incubation time. It demonstrated that differences in daily growth between diurnally stratified and mixed populations are dependent on Z_{eu}/Z_m. At ratios between approximately 0.2 and 0.9 the growth of diurnally stratified populations was predicted to be significantly lower than mixed populations. At Z_{eu}/Z_m values outside this range (but less than 1 due to restrictions of the model) the growth of diurnally stratified populations was predicted to be similar to, but never faster than, mixed populations. When considering cell density, any differences in growth between diurnally stratified and mixed populations were accentuated with incubation time. Overall, the model predicts that changes in turbidity and the maximum mixed depth in the Murray River would not lead to the situation where diurnally stratified populations would grow faster than mixed populations.

The predictions of the model are based on a daily time-scale and should therefore lead to conservative outcomes. Short-term fluctuations (*i.e.* hours) in light, nutrient availability and temperature are unlikely to significantly affect overall growth of *A. circinalis*. Harris (1983) suggests a lag phase of 1 to 2 generations before cellular responses are reflected by growth responses. Furthermore, cyanobacteria do not respond rapidly to changing light availability

and require up to 48 hours to adapt (Ward and Wetzel 1980). The use of average daily light dose and growth rate means that short-term physiological variations are taken into account. However, extended changes in environmental conditions (days - weeks) may alter the growth response curve of *A. circinalis*, thus changing the predictions.

The model differs from the detailed models of Elliot *et al.* (2001) and Walsby (1997). It predicts the upper limits to *A. circinalis* growth and does not take into account loss factors or competition with other algal species. Enclosure of cells in diffusion chambers may cause an increase in biovolume (13%) compared with river populations (Köhler 1997). Furthermore, self-shading may cause a significant reduction in growth rate. *Anabaena* has a specific attenuation coefficient of 0.009 m^2 mg^{-1} chl *a* (Oliver 1990) and a chlorophyll content of 0.72 pg $cell^{-1}$ (Reynolds 1984), and at cell densities of > 10^6 cells ml^{-1} would be the major component of light attenuation. The examples of *A. circinalis* growth provided in this study were less than 10^6 cells ml^{-1} (with the exception of examples where Z_{eu}/Z_m = 1) so that self-shading should not be a major factor.

Baker *et al.* (2000) have previously tracked a population of *A. circinalis* down the Murray River and found that cells were advected at a rate of 6.6 km day^{-1}, a rate similar to the river flow. Given that Morgan is 315 km from the mouth of the Murray River it would therefore take 47.7 days for cells to travel there. Using the model under typical Murray River conditions (K_d = 8 m^{-1}, Z_m = 5 m, Z_{eu}/Z_m = 0.115), the predicted cell density after 48 days for diurnal and mixed populations is 3.1 x 10^5 and 6.1 x 10^5 cells ml^{-1}, respectively. Cell densities are rarely this high which supports the suggestion that loss factors are important for the overall balance of the population. However, the average *in situ* growth rate of *A. circinalis* calculated by Baker *et al.* (2000) in the Murray River was 0.18 day^{-1}. In Maude weir, *A. circinalis* grew at

0.37 day^{-1} (Sherman *et al.* 1998). These rates are not markedly different from the rate of 0.28

day^{-1} measured for the diurnal random population in this study.
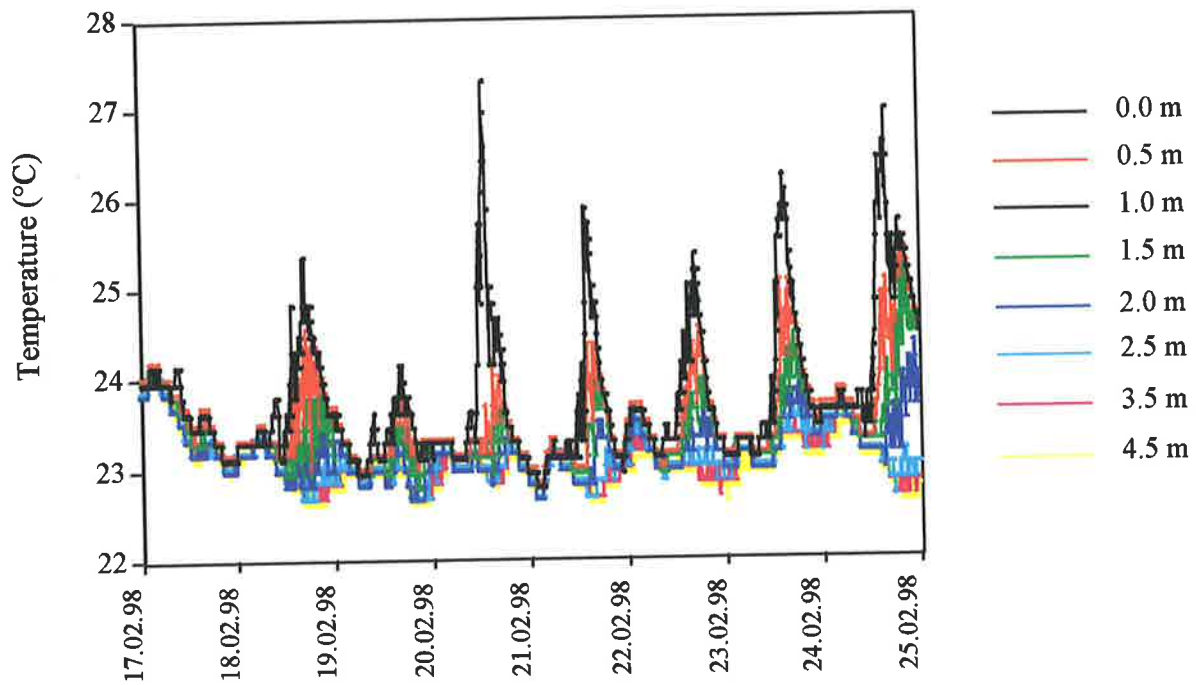
Figure 5.1: Temperature depth profiles taken in the lower Murray River at

Morgan between 17 and 25 February, 1998. Temperature recorded at 0.1, 0.5,

1.0, 1.5, 2.5, 3.5 and 4.5 m.
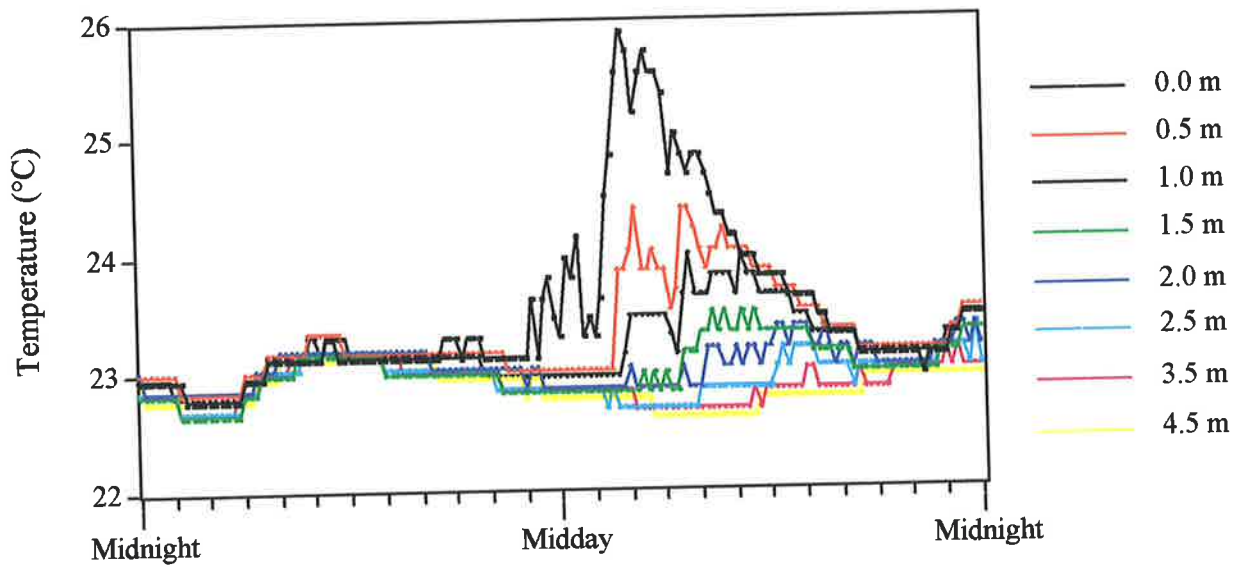
Figure 5.2: High resolution of the pattern of diurnal stratification that occurs in the lower Murray River at Morgan, recorded on February 21st 1998. The onset of stratification occurs at approximately 11:00 h and begins to break down at 17:00 h.
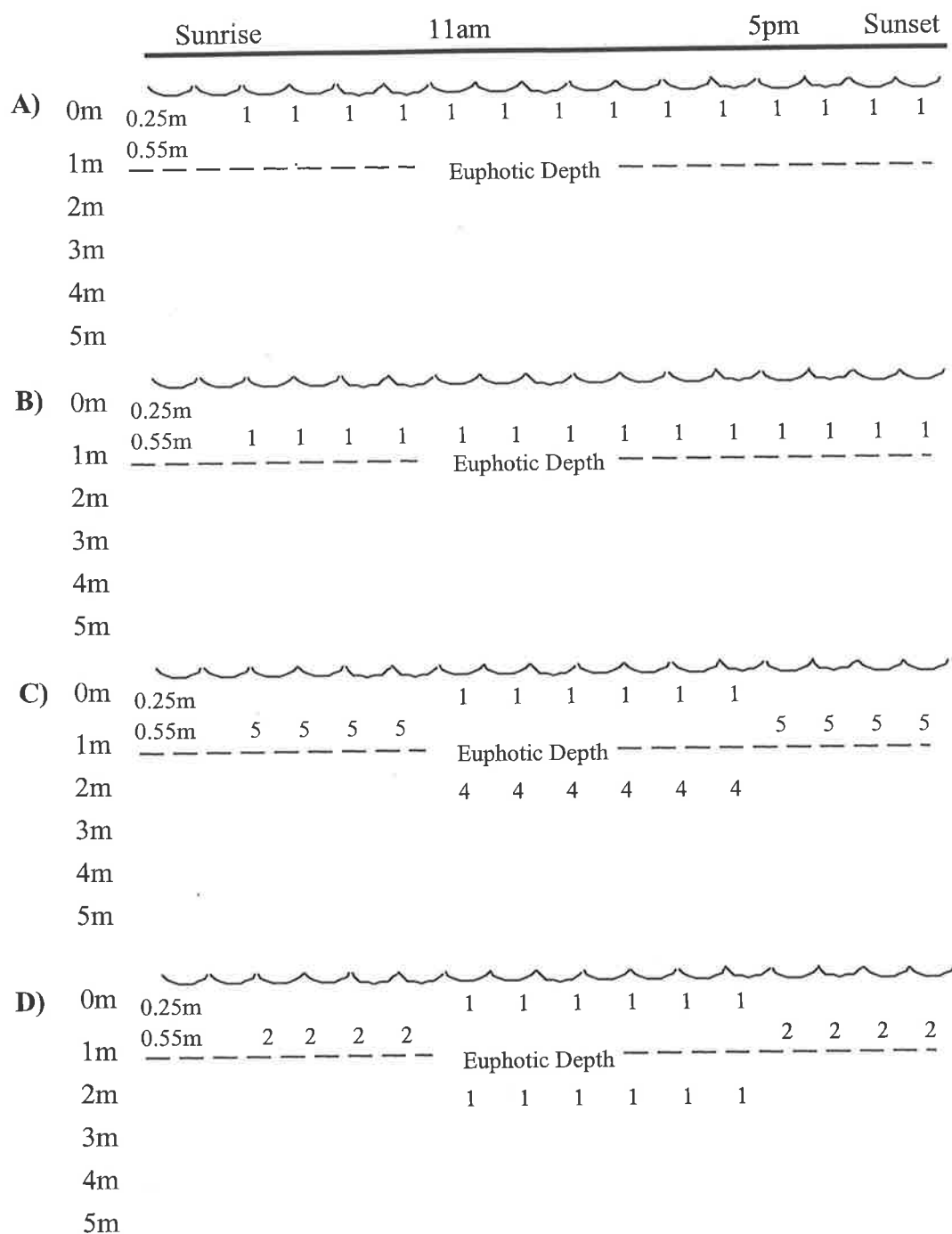
Figure 5.3: Position of diffusion chambers representing sub-populations of *A. circinalis* in a 5 m water-column in the lower Murray River;   a) persistently stratified, b) mixed, c) diurnal random and d) diurnal non-random. See text for details.
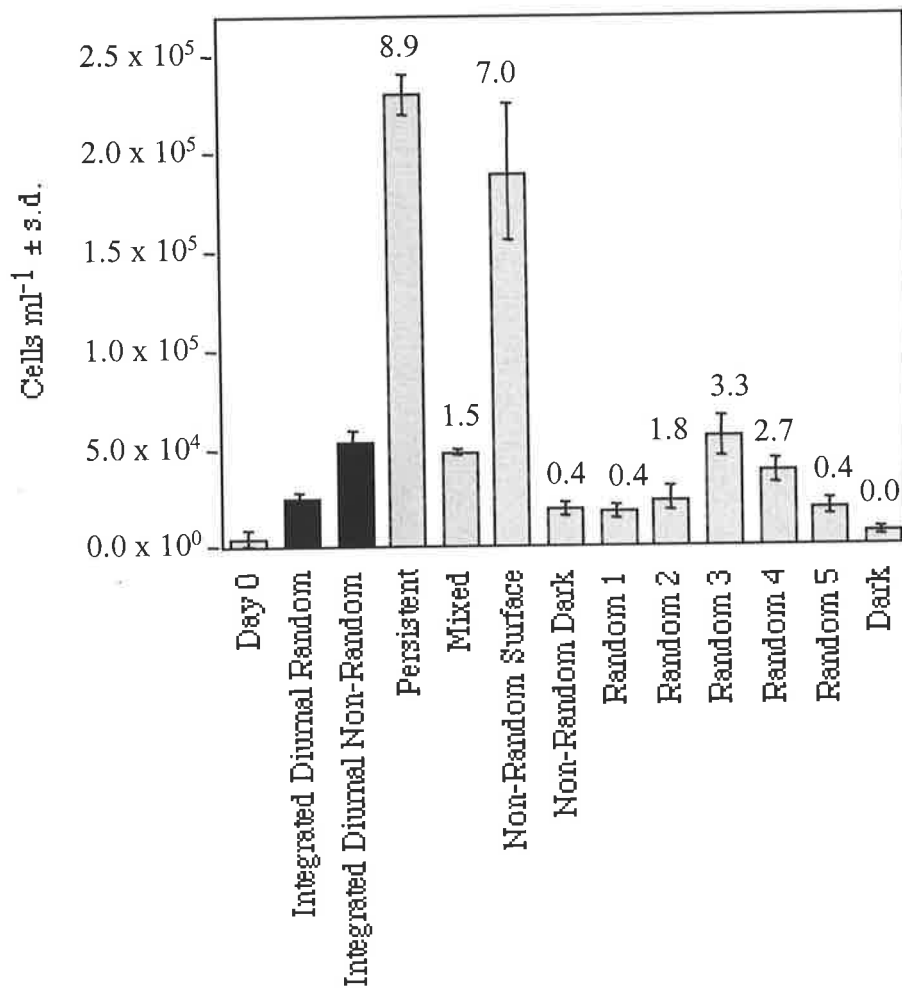
Figure 5.4: Cell densities of *A. circinalis* on day 0 and after 6 days growth in diffusion chambers suspended in the lower Murray River at Morgan. The light dose received by sub-populations is indicated (mol m^{-2} day^{-1}). Persistent = chambers permanently located in the euphotic zone; Mixed = chambers exposed to the average column irradiance; Random and Non-Random = sub-populations either randomly or non-randomly exposed to the euphotic zone during diurnal stratification. Black bars indicate the cell numbers when sub-populations were integrated to represent the total population for the diurnal random and diurnal non-random treatments.
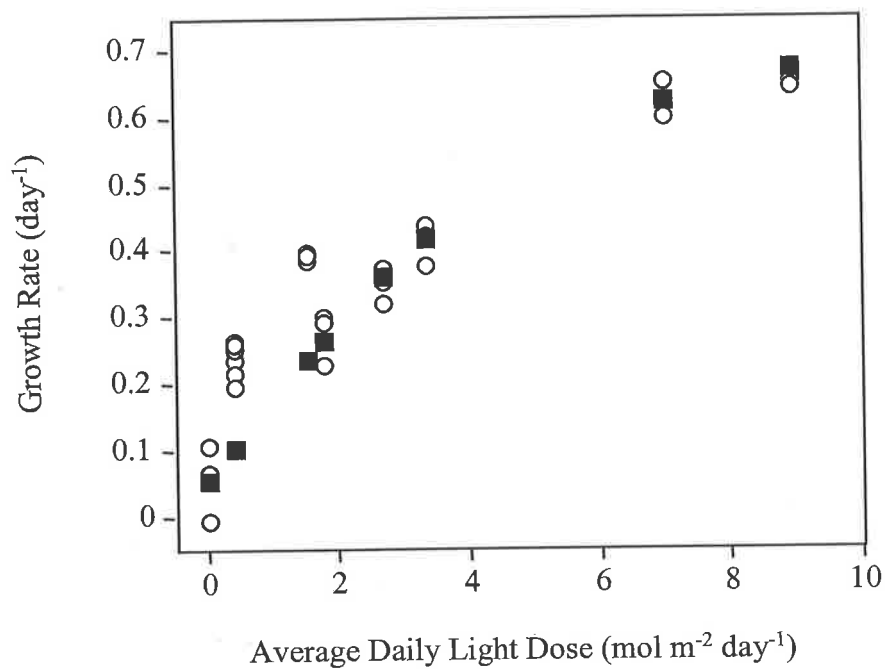
Figure 5.5: Field data showing the relationship between the growth rate of *A. circinalis* (days 0-6) and light dose. Cultures were incubated in diffusion chambers at Morgan on the lower Murray River. Solid symbols represent the model of Jassby and Platt (1976).
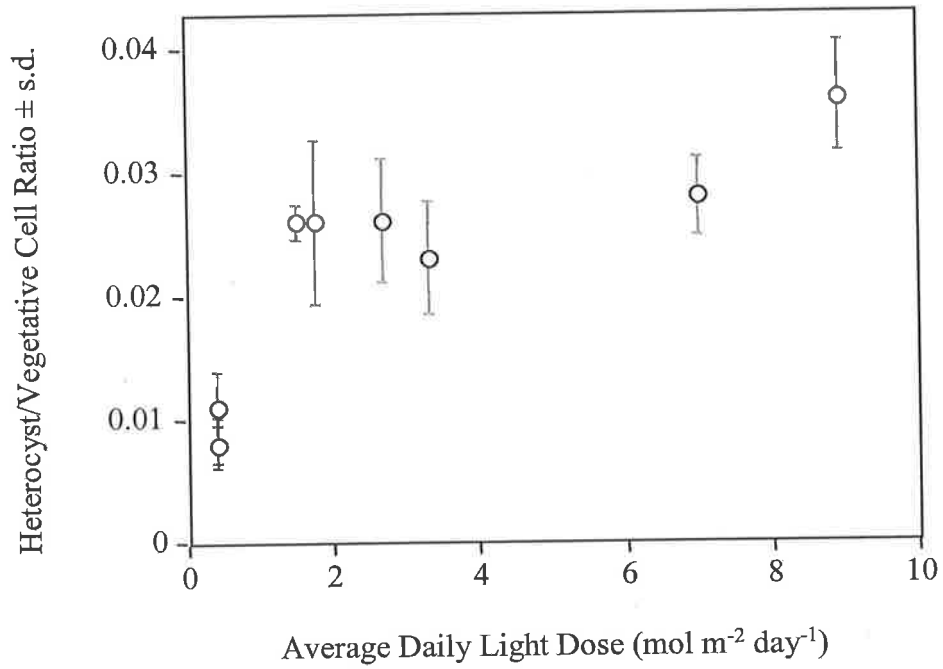
Figure 5.6: Effect of light dose on the heterocyst/vegetative cell ratio of *A. circinalis* after 6 days growth diffusion chambers in the lower Murray River at Morgan.
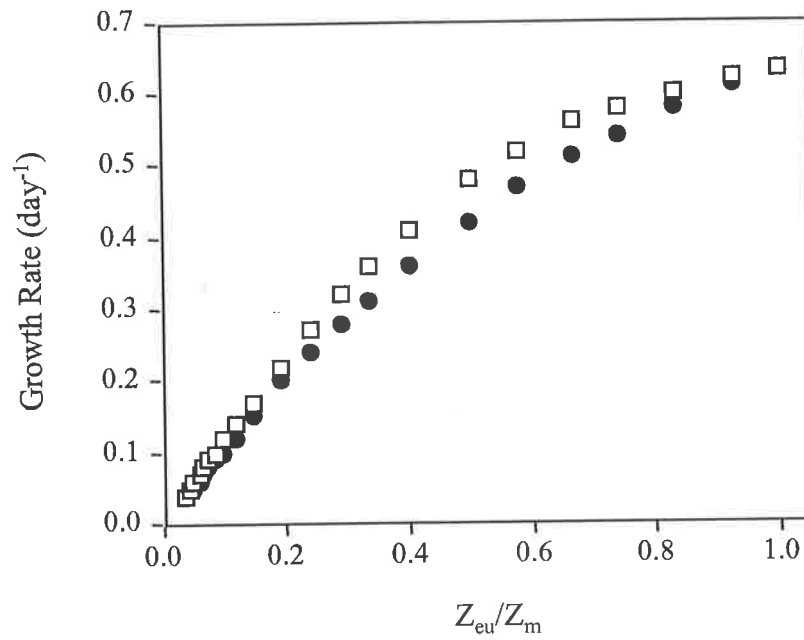
Figure 5.7: Effect of Z_{eu}/Z_m on the daily growth rate of populations of *A. circinalis* exposed to mixed (square) and diurnally stratified (circle) conditions.
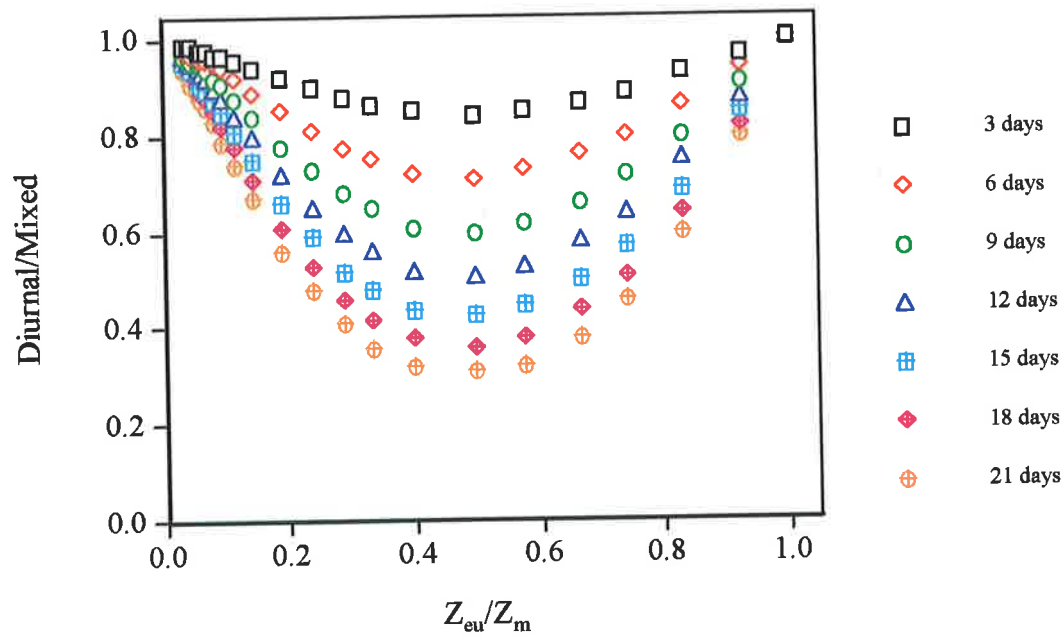
Figure 5.8: Effect of Z_{eu}/Z_m on the predicted ratio of cell densities (diurnal/mixed) for populations of *A. circinalis* exposed to diurnally stratified and mixed conditions over the designated number of days.

Table 5.1: Model parameters; vertical attenuation coefficient (K_d, m^{-1}), mixed depth (Z_m, m), and growth period (days), used to predict cell densities (cells ml^{-1}) and daily growth rates (day^{-1}) for populations of *A. circinalis* exposed to mixed and diurnally stratified conditions. The predicted ratios of cell densities, diurnal to mixed, are given.

K_d	Z_m	Days	Zeu:Zm	Mixed cells ml^{-1}	day^{-1}	Diurnal cells ml^{-1}	day^{-1}	Ratio
4.61	1	3	1.000	5.2×10^3	0.63	5.2×10^3	0.63	1.00
4.61	1	6	1.000	3.4×10^4	0.63	3.4×10^4	0.63	1.00
4.61	1	9	1.000	2.3×10^5	0.63	2.3×10^5	0.63	1.00
5	0.922	15	1.000	9.7×10^6	0.63	9.7×10^6	0.63	1.00
5	0.922	21	1.000	4.5×10^8	0.63	4.5×10^8	0.63	1.00
5	1	6	0.922	3.2×10^4	0.62	3.0×10^4	0.60	0.93
5	2	6	0.461	1.2×10^4	0.46	8.8×10^3	0.40	0.71
5	5	6	0.184	2.9×10^3	0.22	2.5×10^3	0.19	0.85
5	8	6	0.115	1.8×10^3	0.14	1.7×10^3	0.12	0.92
5	10	6	0.092	1.6×10^3	0.11	1.5×10^3	0.10	0.94
5	14	6	0.066	1.3×10^3	0.08	1.2×10^3	0.07	0.96
8	1	3	0.576	3.8×10^3	0.52	3.2×10^3	0.47	0.85
8	3	3	0.192	1.6×10^3	0.22	1.4×10^3	0.20	0.92
8	5	3	0.115	1.2×10^3	0.14	1.2×10^3	0.12	0.96
8	5	6	0.115	1.8×10^3	0.14	1.7×10^3	0.12	0.92
8	5	9	0.115	2.8×10^3	0.14	2.4×10^3	0.12	0.88
8	5	12	0.115	4.2×10^3	0.14	3.6×10^3	0.12	0.84
8	5	15	0.115	6.4×10^3	0.14	5.2×10^3	0.12	0.81
8	5	18	0.115	9.6×10^3	0.14	7.5×10^3	0.12	0.78
8	5	21	0.115	1.5×10^4	0.14	1.1×10^4	0.12	0.74
8	1	9	0.576	8.6×10^4	0.52	5.3×10^4	0.47	0.62
8	2	9	0.288	1.4×10^4	0.32	9.8×10^3	0.28	0.68
8	5	9	0.115	2.8×10^3	0.14	2.4×10^3	0.12	0.88
8	8	9	0.072	1.8×10^3	0.09	1.6×10^3	0.08	0.94
8	10	9	0.058	1.5×10^3	0.07	1.4×10^3	0.06	0.95
8	12	9	0.048	1.4×10^3	0.06	1.3×10^3	0.05	0.96
8	14	9	0.041	1.3×10^3	0.05	1.2×10^3	0.05	0.97
8	16	9	0.036	1.2×10^3	0.04	1.2×10^3	0.04	0.97
10	1	6	0.461	1.2×10^4	0.46	8.8×10^3	0.40	0.71
10	2	6	0.231	3.9×10^3	0.26	3.2×10^3	0.23	0.81
10	5	6	0.092	1.6×10^3	0.11	1.5×10^3	0.10	0.94
10	8	6	0.058	1.2×10^3	0.07	1.2×10^3	0.06	0.97
10	10	6	0.046	1.1×10^3	0.06	1.1×10^3	0.05	0.98
10	14	6	0.033	1.0×10^3	0.04	1.0×10^3	0.04	0.98

CHAPTER 6. Effect of Cell Flotation on Growth of *Anabaena circinalis* under Diurnally Stratified Conditions.

ABSTRACT

This chapter tested the hypothesis that the growth rate of *Anabaena circinalis*, under diurnally stratified conditions, would increase as flotation velocity increased. An *in situ* experiment compared the growth of populations with flotation velocities of 0.5 and 1.0 m h^{-1}, with neutrally buoyant populations that were exposed to either mixed or persistently stratified conditions. To represent the mixing patterns, *A. circinalis* was contained in diffusion chambers that were moved to different positions in the water-column throughout the day. Diurnal populations with flotation velocities of 1.0 and 0.5 m h^{-1} grew at 0.23 ± 0.01 and 0.15 ± 0.01 day^{-1}, respectively. Mixed populations grew at 0.19 ± 0.01 day^{-1}, whereas, persistently stratified populations grew at 0.43 ± 0.01 day^{-1}. Results were used to extend a model that predicts growth of *A. circinalis* when exposed to the different mixing patterns. The model showed that blooms are unlikely to be formed when the period of diurnal stratification is less than one week, regardless of flotation velocity. When the diurnally stratified period is greater than one week, flotation velocity is important and a bloom may form depending on values assigned to the growth period and maximum mixed depth (Z_m).

6.1 Introduction

At cell densities >15,000 cells ml^{-1}, the cyanobacterium *Anabaena circinalis* poses a potential health hazard in lakes, reservoirs and rivers that are used as a water supply (Jones 1993). In lakes and reservoirs this problem may be addressed by the introduction of artificial mixers that overcome thermal stratification and reduce the benefits of buoyancy. However, there have been varying degrees of success (Reynolds *et al.* 1983; Visser *et al.* 1996a; Visser *et al.* 1996b; Nakano *et al.* 2001). Artificial destratification has also been considered for regulated rivers and may be achieved by increasing river flow. A detailed understanding of the potential growth of *A. circinalis* under different mixing patterns is required to determine the outcome of artificial destratification in rivers, as this may be costly in terms of both finances and wasted resource.

Sherman *et al.* (1998) demonstrated in Maude Weir, Australia, that *A. circinalis* dominated during low flows when the vertical extent of the surface mixed layer was similar to the euphotic zone (Z_{eu}/Z_m = 1). However, *A. circinalis* would be replaced by the diatom *Aulacoseira granulata* if flow rates were increased to deepen the surface mixed layer to the point where Z_{eu}/Z_m was < 0.3. Their study provided strong evidence that persistent stratification leads to the formation of *A. circinalis* blooms. However, some studies have since assumed that this is an absolute requirement (Bormans *et al.* 1997; Webster *et al.* 2000; Maier *et al.* 2001; McCausland *et al.* 2002). It may be argued that stratification on a diurnal basis may also provide *A. circinalis* with sufficient opportunity to float into the euphotic zone to receive adequate light and grow rapidly.

A. circinalis blooms are common in the Murray-Darling Basin, Australia (Baker *et al.* 1993; Hötzel and Croome 1994; Bowling and Baker 1996). Using average wind speeds and entitlement flows (7000 ML day^{-1}), Maier *et al.* (2001) calculated that the probability of persistent stratification in the lower Murray River over 7 days is only 0.65% in January (the main growth period). Bormans *et al.* (1997) measured temperature-depth profiles in the lower Murray from 26 January to 13 February 1996. Over this period they showed that persistent stratification occurred for 2 days, diurnal stratification for 13 days, and isothermy for 4 days. Overall, the probability of persistent stratification is low in the lower Murray River, and diurnal stratification is the predominant mixing pattern. Under diurnally stratified conditions, the proportion of a population that would float into the euphotic zone during stratification would be dependent on the flotation velocity of the cells, the initial position of the cells in the water-column, and the length of the stratified period.

Chapter 5 studied the growth of neutrally buoyant populations of *A. circinalis* in the lower Murray River. Growth of diurnally stratified populations was slower than continually mixed

populations and therefore artificial destratification would enhance, rather than restrict growth of *A. circinalis*. However, reported flotation velocities of *A. circinalis* typically range from 0.01 to 0.40 m h^{-1} (Reynolds *et al.* 1987; Brookes *et al.* 1999; Mitrovic *et al.* 2001). Velocities as fast as 2.0 m h^{-1} can be achieved in populations where large filament aggregations are formed under light limited conditions (Brookes *et al.* 1999). Mitrovic *et al.* (2001) used the model of Walsby (1997) to show that the daily integral of photosynthesis of *A. circinalis* in the Darling River, Australia, was five times faster in buoyant populations compared to populations that were evenly distributed throughout the water column. However, this benefit may not have a direct influence on growth rate. For example, Bright and Walsby (2000) demonstrated that at light-saturating irradiance, the efficiency of carbon assimilation for growth of *Planktothrix rubescens* was only 53%. The difference was partly explained by the use of energy to produce extracellular products, and by photoinhibition of cells when exposed to high irradiance for extended periods (a decrease of 1% per minute). Growth rates are also expected to be lower than photosynthetic rates, as net growth is the net result of both photosynthesis and respiration (Falkowski *et al.* 1985). Furthermore, under nitrogen limited conditions, a significant amount of energy may be diverted for nitrogen-fixation (Paerl and Kellar 1979).

The aim of this study was to determine the *in situ* effect of flotation on growth of populations of *A. circinalis* exposed to diurnally stratified conditions in the lower Murray River. The response was compared with that of neutrally buoyant populations exposed to mixed and persistently stratified conditions. Results from the field experiment were used to improve the predictions of the model described in Chapter 5. The model extends the field results and details the growth response under varying conditions of flotation velocity, Z_m, K_d and growth period.

6.2 Methods

6.2.1 Site Description

Morgan (34°01 S/139°41 E) is situated on the Murray River, 315km from the mouth and equidistant between locks 1 and 2. The river has a mean depth of 5 m and a bed slope less than 4.5 cm km^{-1} (Sullivan *et al.* 1988). Turbidity at Morgan is highly variable and is largely influenced by discharges from the clay-laden Darling River upstream (Sullivan *et al.* 1988; Mackay *et al.* 1988). Over the past ten years, turbidity has ranged from 4 to 304 NTU with an average of 51.6 ± 38.9 NTU. Salinity at Morgan is 556 ± 184 EC units and flow measured at lock 2 is 9852 ± 8022 ML day^{-1} (data from the Murray-Darling Basin Commission).

6.2.2 Sample Preparation

A. circinalis (ANA271B, Chapter 2) was cultured in WC media (Guillard and Lorenzen 1972) at a light intensity of 50 - 60 µmol m^{-2} s^{-1} under a 14 h:10 h light/dark cycle at 25 °C. Log phase cultures were transported to Morgan and transferred into 50, 140 ml diffusion chambers (Köhler 1997). These were constructed from 9.5 cm lengths of UV-transmitting polycarbonate tubing and the ends were sealed with 47 mm polysulphone filters with a pore size of 0.45 µm.

6.2.3 Incubation Protocol

To represent populations of *A. circinalis* under persistently stratified or fully mixed conditions, cultures were suspended at depths equivalent to the average column irradiance ($\bar{I}$) calculated for each mixing regime (Figure 6.1 a,b). Depths were calculated from Riley's (1957) equation:

$$\bar{I} = \bar{I}_0{}' (1 - e^{-K_d * Z_m}) / K_d * Z_m \qquad \text{µmol m}^{-2} \text{ sec}^{-1} \qquad (1)$$

where,

$\bar{I}_0{}'$ = incoming irradiance, µmol m^{-2} sec^{-1}

K_d = light attenuation, m^{-1}

Z_m = mixed depth,

The vertical attenuation coefficient measured in the river a week prior to experimentation was 3.6 m^{-1}. The depths of average irradiance that corresponded to the two mixing regimes were 0.43 m and 0.80 m. The first represented a population permanently circulating within the euphotic zone (1.28 m), the second, a population that was mixing through a 5 m water column (the mean depth of the River Murray at Morgan, $Z_{eu}/Z_m = 0.26$).

To represent a diurnally stratified water column, a population of *A. circinalis* was divided into five sub-populations. Each sub-population represented 1 m^3 assuming that cells were evenly distributed at pre-dawn throughout the 5 m water-column. During isothermal conditions, all sub-populations were suspended at 0.80 m (Figure 6.1 c,d). At the onset of thermal stratification (11:00 h), four of the sub-populations were lowered into the dark to represent the proportion of cells trapped below the euphotic zone. The remaining sub-population was suspended at 0.43 m to represent the proportion of the population still exposed to the euphotic zone. Throughout the stratified period (11:00 to 17:00 h), additional sub-populations were moved from the darkness to 0.43 m to simulate the flotation of cells into the euphotic zone (see below). At 17:00 h all cultures were re-positioned to 0.80 m where they remained until 11:00 the following day.

Two flotation velocities were tested for populations exposed to diurnally stratified conditions; 0.5 and 1.0 m h^{-1}. Since each sub-population represented 1 m^3 of the water column, an additional culture was introduced every 2 hours into the euphotic zone throughout the stratified period for the 0.5 m h^{-1} diurnal treatment and every hour for the 1.0 m h^{-1} diurnal treatment (Figure 6.1 c,d). The cultures exposed were chosen randomly. For the 0.5 m h^{-1} diurnal treatment, 2/5 of the population was still in darkness at the resumption of isothermal

mixing at 17:00 h. In contrast, the entire population was in the euphotic zone by 15:00 h for the 1.0 m h^{-1} diurnal treatment.

There were 5 replicates for each sub-population within each treatment described above. To extend an examination of the response of *A. circinalis* to irradiance, a surface population was also included. Diffusion chambers (5 replicates) were suspended at 0.2 m throughout the incubation period.

6.2.4 Sampling

Diffusion cultures were incubated in the Murray River for eight days from Feb 20 to 28, 2000. Every two days between 8:00 and 11:00 h, 2 ml samples were removed from all chambers. Prior to sampling the chambers were gently agitated to ensure that cells were evenly distributed. In addition, for both diurnal treatments (0.5 and 1.0 m h^{-1}), 1 ml was removed from each chamber representing each sub-population and mixed to give a 5 ml integrated sample of the total population. All samples were preserved in Lugol's iodine solution. Cell numbers were determined using a Sedgwick-Rafter chamber and inverted microscope. The ratio of heterocysts to vegetative cells was also recorded.

To determine the proportion of *A. circinalis* filaments that were buoyant, 1 ml samples were removed from diffusion chambers between 8:00 and 11:00 h on the last day of experimentation. The samples were placed in a Sedgwick-Rafter chamber and the floating and sinking filaments enumerated by focussing on different planes using an inverted microscope (Walsby and Booker 1980).

Incident irradiance was continually recorded using a data logger and quantum sensor (LICOR 1000). Underwater light penetration was measured daily with a LICOR underwater quantum sensor. The vertical attenuation coefficient was calculated by regression analysis of

underwater light measurements against depth. Water temperature was logged at 1 m using an optic stowaway (Hastings Data Loggers STEB8).

To determine nutrient concentrations, three integrated water samples (0 to 1.5 m) were collected every two days from the river. For analysis of filterable reactive phosphorus (FRP), nitrate + nitrite (NO_x) and ammonium (NH_4), an aliquot of each sample was filtered through a Whatman GF/C filter (pre-filter) and then a 0.2 μm polycarbonate filter (Gelman). Filtered and raw water samples were frozen until analysis. The analysis for FRP, NO_x, NH_4, total phosphorus, and total Kjeldahl nitrogen was conducted by the Australian Water Quality Laboratory according to standard procedures (Anon. 1995).

6.2.5 Analysis

Growth rates were calculated from the equation $\mu = (1/t) \times \ln(N_t/N_0)$ where t is days, and N_t and N_0 represent the final and initial cell concentrations. The light dose received by individual chambers was calculated by summing integrated light intensities experienced at depth over each hour. This was based upon knowledge of the vertical attenuation coefficient, the daily incoming irradiance and the depth at which the cultures were exposed. Growth rates were analysed using 1-way ANOVA and a post-hoc Tukey's HSD test (JMP, SAS Inc. version 3.0.2 1989-94) after testing for normality (Shapiro-Wilk test). Although 5 replicates were used for all treatments, analysis was only conducted on 3 replicates due to the rupturing of some chambers in the field. Heterocyst data were analysed using both 1-way ANOVAs and t-tests (n = 3).

Field data on the growth rate (G) of individual cultures of *A. circinalis* according to average daily light dose was plotted against values predicted using the modified model of Jassby and Platt (1976), described in Chapter 5;

$$G = G_{max} * Tanh(I/I_k) + R_d \qquad day^{-1} \qquad\qquad (2)$$

where,

G_{max} = Growth rate at optimal light dose, day^{-1}

I_k = Light dose where the extrapolated lines of G_{max} and the initial light limited

portion of the G-I curve intersect, $mol\ m^{-2}\ day^{-1}$

R_d = Constant to adjust for loss rate, day^{-1}

α = Slope of the initial linear section of the G-I curve, $day^{-1}\ (mol\ m^{-2}\ day^{-1})^{-1}$

The parameters used in the model were those derived in Chapter 5 a year earlier *i.e.* G_{max}, α, I_k and R_d were 0.66 day^{-1}, 0.12 $day^{-1}\ (mol\ m^{-2}\ day^{-1})^{-1}$, 5.4 $mol\ m^{-2}\ day^{-1}$ and 0.056 day^{-1}, respectively.

6.2.6 Model Construction

The model described in Chapter 5 was extended in this study to include a consideration of flotation velocity and its effect on the growth of *A. circinalis*. The model examines differences in growth when cells are exposed to either continually mixed or diurnally stratified conditions, and is divided into four parts; a) incoming irradiance, b) depth profiles of irradiance c) the response of cells to light dose and d) the daily integration of sub-populations representing diurnal populations. The effects of vertical light attenuation (K_d, m^{-1}), maximum mixed depth (Z_m, m) and incubation period (days) were examined. The incubation period represented the number of cloudless days.

Incoming irradiance (I_o) was calculated according to the equation of Kromkamp and Walsby (1990) that describes the sine pattern of the rise and fall of the sun:

$$I_o = I_m * sin(\pi * t / D_L) \qquad \mu mol\ m^{-2}\ s^{-1} \qquad\qquad (3)$$

where,

I_m = maximum incoming irradiance, $\mu mol \ m^{-2} \ s^{-1}$

I_o = surface irradiance, $\mu mol \ m^{-2} \ s^{-1}$

t = time of the surface irradiance after dawn (min)

D_L = length of the light period from dawn until dusk (h)

Light intensity experienced by *A. circinalis* was determined using the Beer-Lambert Law which required values for I_o, the depths where the cultures were suspended and K_d. The daily light dose that individual cultures received was calculated by summing the light at hourly intervals. The depths at which cultures were suspended were based on the design described for the mixed and diurnal treatments in the field experiment (see below). The model calculated the appropriate depths of average irradiance using Riley's (1957) equation and the Beer-Lambert Law, from Z_m and K_d.

For the diurnally stratified populations, the depths where sub-populations were suspended changed with time to account for the flotation of cells into the euphotic zone during the stratified period (Table 6.1). Four flotation velocities were modelled; 0.0, 0.2, 0.5, 1.0 and 2.0 m h⁻¹. Culture 'A' was exposed to the euphotic zone from 11:00 h onwards, with additional sub-populations exposed in a serial manner with time *i.e.* B,C,D ... J, K. At 17:00 h, all sub-populations were re-positioned to the depth of the average irradiance between the surface and Z_m.

To determine the response of the cultures to light dose the modified equation of Jassby and Platt (1976) was used (see above). G_{max}, I_k and R_d were supplied as inputs and the expected growth rates calculated based upon average daily light dose. The expected number of cells for a given light dose was calculated from the growth rate and from the values for cell density calculated the previous day.

For the diurnally stratified treatment, the cell numbers of the sub-populations were integrated at the end of each day to represent the re-distribution of cells throughout the water column when stratification breaks down and mixing resumes. The proportion of the total population that Culture 'A' represented was Z_{eu}/Z_m. Additional sub-populations that floated into the euphotic zone during the stratified period represented the proportion $1/Z_m$ as each represented $1m^3$ of the water-column. Sub-populations that remained in the dark during stratification represented the proportion $1 - (Z_{eu}/Z_m + (1/Z_m * n))$, where n = the number of additional sub-populations that floated into the euphotic zone during the stratified period. In some cases the whole population had the opportunity to enter the euphotic zone before the end of the stratified period. The final proportion of the total population, indicated by the term 'Remainder' in Table 6.1, was also represented by the equation $1 - (Z_{eu}/Z_m + (1/Z_m * n))$. In these instances there were no sub-populations continually exposed to the dark. The cell density of each sub-population was multiplied by the appropriate proportion and summed to give the final cell density of the total integrated population.

The model was run assuming 3 water column depths; 5, 10, and 15 m. For mixed populations, it was assumed in the model that flotation velocity did not affect growth as cells are fully entrained within the water column. Comparisons of cell densities were made between diurnally stratified populations and mixed populations through use of the diurnal/mixed ratio. Ratios > 1 indicated that diurnally stratified populations were more productive than mixed and that artificial destratification may be beneficial.

6.3 Results

6.3.1 Light Dose

Sub-populations of *A. circinalis* exposed to diurnal stratification with a flotation velocity of 0.5 m h^{-1} received light doses ranging from 0.53 to 1.45 mol m^{-2} day^{-1} (Table 6.2). The lowest

dose was experienced by sub-populations exposed to the euphotic zone for only 3 out of 8 days. The highest dose was experienced by sub-populations exposed to the euphotic zone each day during the stratified period. For sub-populations with a flotation velocity of 1.0 m h^{-1}, the light doses experienced ranged from 1.45 to 2.28 mol m^{-2} day^{-1}. All sub-populations were exposed to the euphotic zone each day, however the light dose differed due to the time of day that cultures were first introduced into the euphotic zone.

Cultures exposed to the average irradiance of the water column received an average daily light dose of 0.67 mol m^{-2} day^{-1} over 8 days. Surface cultures experienced 17.43 mol m^{-2} day^{-1} and cultures exposed to the average irradiance of the euphotic depth received 3.46 mol m^{-2} day^{-1}. Surface cultures experienced light intensities as high as 550 μmol m^{-2} s^{-1} at midday during sunny conditions.

6.3.2 Cell Numbers

Initial cell density of *A. circinalis* was 1.7 x 10^4 cells ml^{-1}. On day 8, density had increased to as high as 1.6 x 10^6 cells ml^{-1} for the surface treatment (Figure 6.2). Cultures exposed to persistently stratified conditions and mixed conditions increased to 5.2 x 10^5 and 7.9 x 10^4 cells ml^{-1}, respectively. Cell numbers for sub-populations exposed to diurnally stratified conditions with a flotation velocity of 0.5 m h^{-1} ranged from 4.5 x 10^4 to 8.3 x 10^4 cells ml^{-1}. Sub-populations with a flotation velocity of 1.0 m h^{-1} grew to cell densities ranging from 7.4 x 10^4 to 1.4 x 10^5 cells ml^{-1}.

6.3.3 Growth Rates v Light Dose

Light dose had a strong influence on growth rate of *A. circinalis* and the fit of the field data to the model described in Chapter 5 was significant (Figure 6.3; r^2= 0.91). However, the model

overestimated the growth of surface populations that received a light dose of 17.43 mol m^{-2} day^{-1} by 20% (predicted = 0.72 day^{-1}, measured = 0.57 day^{-1}).

6.3.4 Nutrients

Filterable reactive and total phosphorus concentrations in the river were 50.7 ± 4.8 and 140.7 ± 12.4 µg L^{-1}, respectively. Oxidised nitrogen was undetectable on 2 out of 3 sampling occasions with an average of 6.3 ± 9.0 µg L^{-1}. Ammonium and total Kjeldahl nitrogen concentrations were 36.0 ± 5.4 and 693.3 ± 54.4 µg L^{-1}, respectively. Average water temperature was 24.3 ± 1.1 °C and K_d was 4.52 ± 0.36 m^{-1} over 8 days. Inorganic nitrogen concentrations and a TN:TP ratio (by weight) of 4.9 suggest that nitrogen was limiting.

The heterocyst/vegetative cell ratio in filaments of *A. circinalis* changed from an initial of 0.0057 ± 0.003 to values between 0.011 and 0.045 on day 8. The ratio on day 8 was strongly correlated to light dose (Figure 6.4) and increased linearly up to a light dose of 3.46 mol m^{-2} day^{-1} (y = 0.01x + 0.011; r^2 = 0.816). At a light dose of 17.4 mol m^{-2} day^{-1} the ratio decreased to 0.035.

6.3.5 Buoyancy

For sub-populations exposed to diurnal mixing the percentage of filaments with positive buoyancy on day 8 ranged between 90.8 to 100 %. For populations exposed to mixed or persistently stratified conditions, the proportion of floating filaments was 100 and 74.8 % respectively. For the surface cultures only 29.2 % of filaments were floating. A significant negative correlation was detected between the percentage of floating filaments and the average daily light dose experienced by cultures (y = -3.6 x + 103.1; r^2 = 0.82).

6.3.6 Integrated Population Growth

Integrated growth rates for each mixing treatment were all significantly different from each other ($P < 0.05$; n = 3). Growth of the persistently stratified population of *A. circinalis* was 0.43 ± 0.01 day^{-1} and the population exposed to mixed conditions grew at 0.19 ± 0.01 day^{-1}. The population exposed to diurnal mixing with a flotation velocity of 0.5 m h^{-1} grew at a rate of 0.15 ± 0.01 day^{-1} (integrated population density 5.4 x 10^4 cells ml^{-1}, Figure 6.2). The diurnally stratified population with a flotation velocity of 1.0 m h^{-1} grew at a rate of 0.23 ± 0.01 day^{-1} (integrated population density 8.5 x 10^4 cells ml^{-1}, Figure 6.2). The ratio of the cell density of the persistently stratified population to the mixed population was 6.82. Ratios of the 1.0 m h^{-1} diurnal and 0.5 m h^{-1} diurnal populations to the mixed population corresponded to 1.42 and 0.72, respectively.

6.3.7 Model

To assess the influence of flotation rate, K_d, Z_m and incubation time on growth rate, cell numbers and the diurnal/mixed ratio, the following constants were used: Midday irradiance = 1500 µmol m^{-2} s^{-1} (= 45.5 mol m^{-2} day^{-1}); Daylength = 14 h; Initial cell density = 800 cells ml^{-1}. The parameters used to predict growth of *A. circinalis* according to light dose were derived from Chapter 5, *i.e.* G_{max}, α and I_k were 0.66 day^{-1}, 0.12 day^{-1} (mol m^{-2} day^{-1})$^{-1}$ and 5.4 mol m^{-2} day^{-1}, respectively. The exception was the value for R_d which was set to 0. The five flotation velocities tested were 0.0, 0.2, 0.5, 1.0 and 2.0 m h^{-1}. The term 'bloom' applies to the situation where cell density is ≥ 1 x 10^4 cells ml^{-1}.

6.3.8 Daily Growth Rates

The predicted growth of *A. circinalis* subjected to diurnally stratified conditions shows that rates significantly increase with increasing flotation velocity (Figures 6.5 to 6.7). When comparing daily growth at flotation velocities of 0.0 and 2.0 m h^{-1}, the difference in rates can

be as high as 0.37 day^{-1}. Therefore, flotation provides a clear benefit to *A. circinalis* in diurnally stratified systems. There is little difference in growth when comparing diurnally stratified populations with flotation velocities of 0.0 and 0.2 m h^{-1}, and mixed populations. However, when comparing these treatments with diurnal populations with flotation velocities of 0.5 or 1.0 m h^{-1} (the same as the field experiment), the maximum differences in rates correspond to 0.13 and 0.29 day^{-1}, respectively. The maximum daily growth rates of diurnally stratified populations when flotation rates are 0.0, 0.2, 0.5, 1.0 and 2.0 are 0.19, 0.21, 0.31, 0.45 and 0.51 day^{-1}, respectively. These values correspond to a Z_m of 5 m and a K_d of 5 m^{-1}.

The degree of increase in growth rate when flotation velocity is increased from 0.0 to 2.0 m h^{-1} is dependent on Z_m (Figure 6.5), and to a small extent K_d (Figure 6.6). The shallower the maximum mixed depth (Z_m), the greater the increase in growth when flotation velocity is increased. At shallow Z_m's a larger proportion of the total population is able to float into the euphotic zone during the stratified period.

6.3.9 Diurnal/Mixed Ratio

Differences in cell density between diurnally stratified and mixed populations are indicated by the diurnal/mixed ratio. With increasing flotation velocity the diurnal/mixed ratio increases (Figures 6.5 to 6.7). A comparison of the ratio when flotation is 0.0 m h^{-1} compared to 2.0 m h^{-1} shows that the ratio may increase by as much as 23 over 9 days (Figure 6.7). The strongest influence on the diurnal/mixed ratio is incubation time (Figure 6.7). The differences in cell density between the two treatments, caused by the differing daily growth rates, become larger with increasing number of days. As with growth rates, Z_m also has a significant influence on the diurnal/mixed ratio (Figure 6.5), however K_d has little effect (Figure 6.6). The diurnal/mixed ratio decreases with increasing Z_m. When flotation velocity is 0.0 m h^{-1}, growth

of diurnally stratified populations is slower than mixed and the diurnal/mixed ratio is < 1 (Figures 6.5 to 6.7). This was also demonstrated in Chapter 5.

6.3.10 Cell Numbers

When the period of diurnal stratification is ≤ 1 week the probability that *A. circinalis* will form a bloom is low, regardless of the flotation rate. Over 3 days of diurnal stratification, with a flotation velocity as high as 2.0 m h^{-1}, the expected density is only 3.4 x 10^3 cells ml^{-1} when Z_m is 5 m (Table 6.3). With slower flotation velocities the predicted cell densities are even lower. When diurnal stratification occurs over 5 days the predictions are similar (data not shown). The first indication that a bloom will occur is when diurnal stratification persists for a period of 7 days and flotation velocity is 2.0 m h^{-1}. Cell density is predicted to reach 2.3 x 10^4 cells ml^{-1}.

When cells are exposed to diurnal stratification for extended periods (> 1 week) the probability of a bloom is dependent on the interaction between flotation velocity, incubation time and Z_m. When flotation velocity is rapid (1.0 or 2.0 m h^{-1}), the probability of a bloom occurring is high, particularly when the water column depth is shallow (Table 6.3). For example, when cells with a flotation rate of 1.0 m h^{-1} are exposed to diurnal stratification for 1 week and Z_m is 5 m, the predicted cell density is 1.3 x 10^4 cells ml^{-1} (Table 6.3). If Z_m is deep (e.g. 15 m), a minimum of 2 weeks of diurnal stratification is required for bloom formation at these flotation velocities.

When flotation velocity is mid-range (0.5 m h^{-1}), the probability of a bloom is less, but still possible. For example, if cells have a flotation velocity of 0.5 m h^{-1} and are exposed to diurnal mixing for 1 week in a 5 m water column, the predicted cell density is only 4.6 x 10^3 cells ml^{-1} (Table 6.3). If the length of the diurnal period is extended to 2 weeks the predicted density is

2.7×10^4 cells ml^{-1} which is considered a bloom. However, if the diurnal period is 2 weeks but the water column depth is increased to

15 m, cells with a flotation velocity of 0.5 m h^{-1} will only achieve a cell concentration of 2.9 x 10^3 cells ml^{-1}. Cells with a flotation velocity of 0.5 m h^{-1} will not form blooms when Z_m is deep, regardless of incubation time.

With a slow flotation velocity (0.0 or 0.2 m h^{-1}), the probability of a bloom occurring under diurnally stratified conditions is low, regardless of Z_m and incubation time. The predicted cell densities of diurnal populations are not significantly different from populations exposed to continual mixing under the same conditions. For example, after 2 weeks growth in a 5 m water column, cells with a flotation velocity of 0.2 m h^{-1} are predicted to grow to only 5.9 x 10^3 cells ml^{-1} under diurnally stratified conditions and 5.5 x 10^3 cells ml^{-1} under continually mixed conditions.

6.4 Discussion

The growth of *A. circinalis* under diurnally stratified conditions increases as flotation velocity increases. In the lower Murray River, populations with a flotation rate of 0.5 m hr^{-1} grew at 0.15 day^{-1}, significantly slower than 0.19 day^{-1} for the mixed population. However, cells with a flotation rate of 1.0 m h^{-1} grew significantly faster than both treatments at a rate of 0.23 day^{-1}. The fastest growth was for the population exposed to persistently stratified conditions, 0.43 day^{-1}. Mitrovic *et al.* (2001) reported a five-fold increase in the daily integral of photosynthesis of *A. circinalis* when populations were buoyant. However, in this study growth of the diurnally stratified population (flotation 1.0 m h^{-1}) and the persistently stratified population, compared to the mixed population, was only 1.2 and 2.3 times faster, respectively.

The model demonstrates that persistent stratification is not an essential requirement for the development of *A. circinalis* blooms. The daily growth rate of diurnally stratified populations

of *A. circinalis* may increase by as much as 0.37 day^{-1} when comparing flotation velocities of 0.0 and 2.0 m h^{-1}. In terms of cell numbers, it is unlikely that a bloom will form when the period of diurnal stratification is less than one week. Diurnal populations are expected to grow faster than mixed populations on a daily basis, but the growth period is not sufficient for cells to reach a high density. However, when the diurnally stratified period is longer than one week, the probability of bloom formation depends on the flotation velocity, incubation time and maximum mixed depth (Z_m). The vertical attenuation (K_d) has only a small effect so that changes in turbidity in the lower Murray River (e.g. from Darling River water) will not significantly alter the model outcomes.

When diurnal stratification is longer than one week, the conditions most conducive to bloom formation are a rapid flotation velocity and a shallow maximum mixed depth (Z_m). With a rapid flotation velocity (2.0 or 1.0 m h^{-1}), blooms of *A. circinalis* will develop when diurnal stratification lasts between 1 and 2 weeks provided that Z_m is shallow. If the diurnally stratified period is longer than 2 weeks, a bloom will form regardless of Z_m. If the flotation velocity is mid-range (0.5 m h^{-1}), blooms of *A. circinalis* are unlikely if diurnal stratification lasts between 1 and 2 weeks. Blooms will only form when diurnal stratification is longer than 2 weeks and Z_m is shallow. If the flotation velocity is slow (≤ 0.2 m h^{-1}), growth of *A. circinalis* under diurnally stratified conditions is not significantly different from populations exposed to continual mixing, regardless of the growth period and Z_m. Populations with slow flotation velocities will not form a bloom under diurnally stratified conditions.

In cases where the probability of a bloom of *A. circinalis* is high, consideration of the diurnal/mixed ratio indicates whether artificial destratification would be effective in preventing growth. The model shows that the largest influence on the diurnal/mixed ratio is the growth period, and the ratio increases with time. Long growth periods accentuate small

differences in the daily growth rate between diurnally stratified and mixed populations as the differences in cell densities become larger with time. Since blooms of *A. circinalis* under diurnally stratified conditions are not expected to develop unless the growth period is longer than one week, the diurnal/mixed ratio is high enough to suggest that in all cases artificial destratification would be beneficial.

McCausland *et al.* (2002) used laboratory microcosms to test the effect of mixing on growth of *A. circinalis*. They concluded that there was little difference in growth rates of populations subjected to calm conditions (0.45 day^{-1}; mixed every 48 hr) versus mixed conditions (0.47 day^{-1}; mixed every 10 min). This differs from our conclusions which show that both persistent and diurnal stratification allow *A. circinalis* to grow faster than would be expected under mixed conditions. In McCausland *et al.* (2002) the microcosms had a maximum surface irradiance of 225 and a minimum bottom irradiance of 18 µmol m^{-2} s^{-1} and therefore Z_{eu}/Z_{m} was > 1. Our experiments were conducted *in situ* and allowed a Z_{eu}/Z_{m} ratio of 0.26. Therefore, growth of mixed populations was slower due to less light availability. Interestingly, the maximum growth rates of *A. circinalis* at saturating irradiance were similar in both studies (G_{max} = 0.66 day^{-1}) and there was a direct similarity between our population growth rate under persistently stratified conditions (Z_{eu}/Z_{m} = 1), and theirs (0.46 v. 0.45 day^{-1}). Another important reason for the differing conclusions regarding *A. circinalis* growth and mixing patterns is the difference in flotation rate. In McCausland *et al.* (2002) the flotation rate was measured as only 0.2 cm hr^{-1} (0.04 m day^{-1}), whereas we used rates between 0 and 2.0 m hr^{-1}. The results of our model show that at 0.2 cm hr^{-1} there would be little difference in growth between mixed and diurnally stratified populations.

The growth response of *A. circinalis* to average daily light dose in this study is remarkably similar to that described in Chapter 5, even though the experiment in this study was

conducted a year later. The fit of the data to the growth/light curve constructed from the previous year is significant showing that the measurement of growth according to average daily light dose is conservative and leads to robust predictions. Nutrient and temperature conditions in the Murray River were similar in both years. Furthermore, inorganic nitrogen concentrations and the heterocyst/vegetative cell ratios suggested that *A. circinalis* was fixing nitrogen gas. As in the previous study, the heterocyst/vegetative cell ratio was strongly correlated with light availability and rapid growth at high light doses may have been related to increased carbon and nitrogen availability.

The maximum light dose that *A. circinalis* was exposed to in Chapter 5 was only 8.9 mol m^{-2} day^{-1}. In this study, *in situ* growth was tested at a light dose of 17.4 mol m^{-2} day^{-1} (surface cultures) and the rate was 20% lower than predicted from the model. This suggests that *A. circinalis* was photoinhibited. However, the maximum light intensity experienced by *A. circinalis* in this study was 550 μmol m^{-2} s^{-1} at midday, and at other times of the day the intensity would have been less. Foy and Gibson (1982) found only slight photoinhibition of *A. circinalis* at irradiances > 700 μmol m^{-2} s^{-1}, and in Chapter 4 photoinhibition only occurred at intensities greater than 583 μmol m^{-2} day^{-1} (25.2 mol m^{-2} day^{-1}). Furthermore, *Anabaena* spp. can tolerate high light and oxygen toxicity through increasing carotenoid content, and through reducing carbon fixation whilst increasing nitrogen fixation (Paerl and Kellar 1979). These studies suggest that *Anabaena* uses light very efficiently and the decrease in growth rate in this study is not necessarily the consequence of photoinhibition. Rather, *A. circinalis* grew rapidly and the high cell densities in the diffusion chambers (1.6 x 10^6 cells ml^{-1}) may have caused self-shading and nutrient limitation. It is currently unclear whether photoinhibition should be included as a response in the model for the above reasons, and because the Murray River is turbid with high light attenuation. In turbid systems, it is unlikely that cells would be exposed to unfavourable light intensities for sufficient time for photoinhibition to occur. For

example, in turbid Lake Biwa, Japan, MacIntyre (1996) showed that an algal cell can circulate from 90% to 5% surface irradiance within 3 to 4 minutes. Furthermore, only a small proportion of the total population would be exposed to unfavourable light conditions at any given time.

The formation of scum at the water surface may have an influence on overall growth of buoyant populations. Sherman and Webster (1994) modelled the relationship between buoyancy and light limited growth of cyanobacteria in a turbid lagoon and included the development and dispersal of scum at the water surface as a function of wind speed. The model assumed a maximum growth and flotation rate of 1.0 day^{-1} and 3.6 m h^{-1}, respectively. Light saturation was assumed to be at 11.9 μmol m^2 s^{-1} (= 1.03 mol m^{-2} day^{-1}). The potential growth rate when comparing the diurnal mixed surface layer, with a fully mixed water column, was found to be two times higher when flotation velocity was 0.36 m h^{-1}, increasing to a maximum of two and a half-times higher at a flotation velocity of 1.08 m h^{-1}. However, at flotation velocities > 1.08 m h^{-1} the benefit of buoyancy started decreased due to the formation of the scum. A scum may form at the water surface, even under mixed conditions, at a rate partly proportional to the flotation velocity of the cells. Therefore, with fast flotation velocity, a relatively greater proportion of the population moves into the scum under mixed conditions. Consequently, a greater proportion of cells are maintained in a favourable light climate under mixed conditions, thus reducing the difference in growth between mixed and diurnally stratified populations. From the work of Sherman and Webster (1994) the growth predictions from the model when flotation rate is 2.0 m h^{-1}, may be less due to scum formation. Webster and Huchinson (1994) suggest that scum is entrained when wind speed is > 3 m s^{-1}. Given that one of the conditions for diurnal stratification in the lower Murray River is when flow is < 4000 ML day^{-1} and wind speed is 1.2-2.9 m s^{-1}, the formation of scum is likely.

Loss of buoyancy is another factor that was not considered in the model. This may lead to a redistribution of cells (Patterson and Wilson 1995) and a consequent change in the average daily light dose experienced by *A. circinalis*. Buoyancy loss is associated with carbohydrate accumulation and the collapse of gas vesicles by changes in turgor pressure, and in *A. circinalis* occurs at photosynthetic rates as low as 1.8 mg O_2 (mg chlorophyll *a*)$^{-1}$ h^{-1} over 2 to 4 hours (Reynolds 1975). In the field experiment a negative linear correlation was found between light dose and the percentage of floating filaments, as found in other studies (Ibelings *et al.* 1991a,b ; Visser *et al.* 1996b). However, for the sub-populations that constituted the populations exposed to diurnal stratification, the maximum average daily light dose experienced was only 2.3 mol m^{-2} day^{-1} and at least 90.8% of filaments were floating. Furthermore, Brookes *et al.* (1999) observed persistent buoyancy in colonies exposed to light doses < 6 mol m^{-2} day^{-1}. Under diurnally stratified conditions, the population is unlikely to be subject to buoyancy loss and the model predictions are appropriate.

The scope of the model only included examples where the minimum Z_m was 5 m and the minimum K_d was 5 m^{-1}, as these values are relevant to the lower Murray River. Therefore, the maximum Z_{eu}/Z_m considered by the model was 0.18, and for an *A. circinalis* bloom to develop under diurnally stratified conditions, the minimum flotation velocity of the cells has to be > 0.5 m h^{-1}. However, in other aquatic systems, and some parts of the Murray River, Z_{eu}/Z_m may be > 0.18. As Z_{eu}/Z_m increases, the importance of flotation velocity decreases as a higher proportion of the water column provides favourable light for growth, regardless of where the cells are positioned (Humphries and Lynne 1988). Therefore, under these circumstances, a bloom may develop when flotation velocity of *A. circinalis* is < 0.5 m h^{-1}. A good example is from the study of Mitrovic *et al.* (2001) who examined the migration of *A. circinalis* in the Darling River. The diurnal pattern of stratification was similar to the lower

Murray River with stratification for eight hours each day and complete mixing to the bottom overnight. The maximum depth ranged from 2.0-2.5 m and Z_{eu}/Z_m was 0.4 to 0.5. *A. circinalis* was present in bloom proportions but the measured flotation velocity was only 0.15 m h^{-1}. The model in Chapter 5 predicted that growth of neutrally buoyant populations of *A. circinalis* when Z_{eu}/Z_m = 0.5 would be 0.42 day^{-1} (Figure 5.7). With a flotation velocity of 0.15 m h^{-1}, growth would be slightly faster. Given the fast predicted growth rate, a bloom of *A. circinalis* may well be expected to develop under the conditions in the Darling River. Therefore, the model agrees with the work of Mitrovic *et al.* (2001). From previous knowledge of the light requirements of *A. circinalis* (Reynolds and Walsby 1975), and the predictions of the model for neutrally buoyant cells in Chapter 5 (Figure 5.7), *Anabaena* blooms may only occur when flotation velocity is < 0.5 m h^{-1} if Z_{eu}/Z_m is > 0.5, as the daily growth of neutrally buoyant populations will be > 0.42 day^{-1} under diurnally stratified conditions.
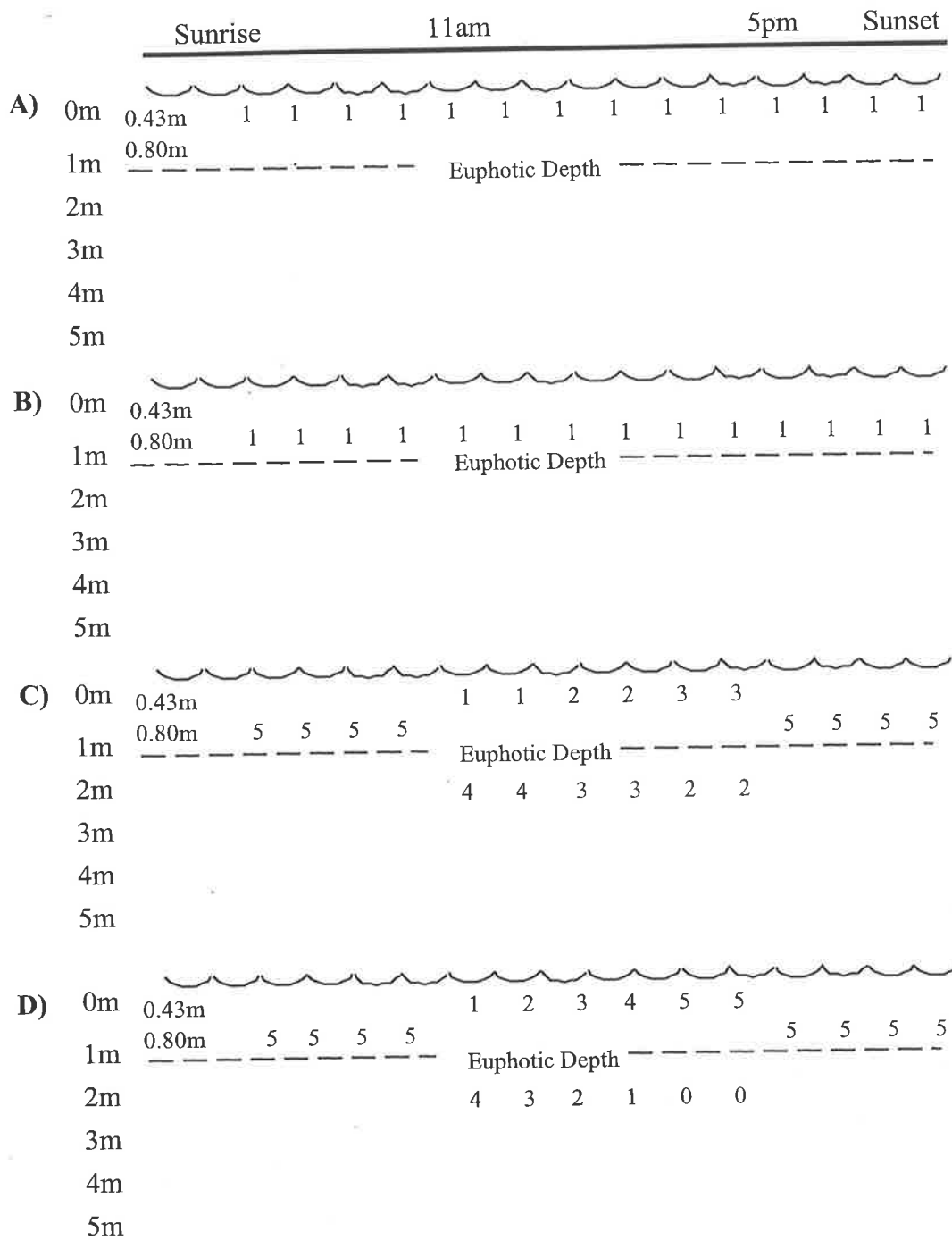
Figure 6.1: Position of diffusion chambers representing sub-populations of *A. circinalis* in a 5 m water-column in the lower Murray River; a) persistently stratified, b) mixed, c) diurnal 0.5 m h^{-1} flotation and d) diurnal 1.0 m h^{-1} flotation. See text for details.

Table 6.1: Time at which sub-populations (A-K) within populations of *A. circinalis* exposed to diurnally stratified conditions were introduced into the euphotic zone during the stratified period (11:00 - 17:00 h). Flotation velocity ranged from 0.0 to 2.0 m h^{-1}. Three maximum mixed depths (Z_m) were tested; a) Z_m = 5, b) Z_m = 10, and c) Z_m = 15 m. Isothermal mixing resumed at 17:00 and continued overnight until the onset of stratification the next day at 11:00 h. Total (n) = number of additional sub-populations that floated into the euphotic zone during the stratified period, each representing 1m^3 of the water-column. Entries labelled 'Remainder' indicate the final proportion of the total population that enters the euphotic zone.

Flotation	11:00 h	12:00 h	13:00 h	14:00 h	15:00 h	16:00 h	Total (n)
A)							
0.0 m h^{-1}	A	-	-	-	-	-	0
0.2 m h^{-1}	A	-	-	-	-	B	1
0.5 m h^{-1}	A	-	B	-	C	-	2
1.0 m h^{-1}	A	B	C	D	E	Remainder	4
2.0 m h^{-1}	A	B,C	D,E	Remainder	-	-	4
B)							
0.0 m h^{-1}	A	-	-	-	-	-	0
0.2 m h^{-1}	A	-	-	-	-	B	1
0.5 m h^{-1}	A	-	B	-	C	-	2
1.0 m h^{-1}	A	B	C	D	E	F	5
2.0 m h^{-1}	A	B,C	D,E	F,G	H,I	J, Remainder	9
C)							
0.0 m h^{-1}	A	-	-	-	-	-	0
0.2 m h^{-1}	A	-	-	-	-	B	1
0.5 m h^{-1}	A	-	B	-	C	-	2
1.0 m h^{-1}	A	B	C	D	E	F	5
2.0 m h^{-1}	A	B,C	D,E	F,G	H,I	J,K	10

Table 6.2: Time of day (between 11:00 and 15:00 h) and day of experimentation, that sub-populations within populations of *A. circinalis* exposed to diurnally stratified conditions were introduced into the euphotic zone during the stratified period in the Murray River. Cultures remained there until 17:00 h. The average daily light dose that cultures received over 8 days of experimentation is indicated in the last column (mol m^{-2} day^{-1}). Sub-populations had a flotation velocity of either 0.5 or 1.0 m h^{-1}.

	11:00	12:00	13:00	14:00	15:00	Light Dose
Flotation 1.0 m h^{-1}						
a	1,3,4	7	5	2,8	6	2.28
b	2	1,4,5	-	6	3,7,8	1.71
c	5,6	2,8	1,4	3,7	-	1.96
d	7,8	3,6	-	1,4,5	2	1.90
e	-	-	2,3,6,7,8	-	1,4,5	1.45
Flotation 0.5 m h^{-1}						
a	1,4	-	5	-	3,6	1.24
b	5,6	-	1	-	2,7	1.39
c	-	-	2,3,6,7,8	-	1,4,5	1.45
d	2	-	4	-	8	0.53
e	3,7,8	-	-	-	-	1.17
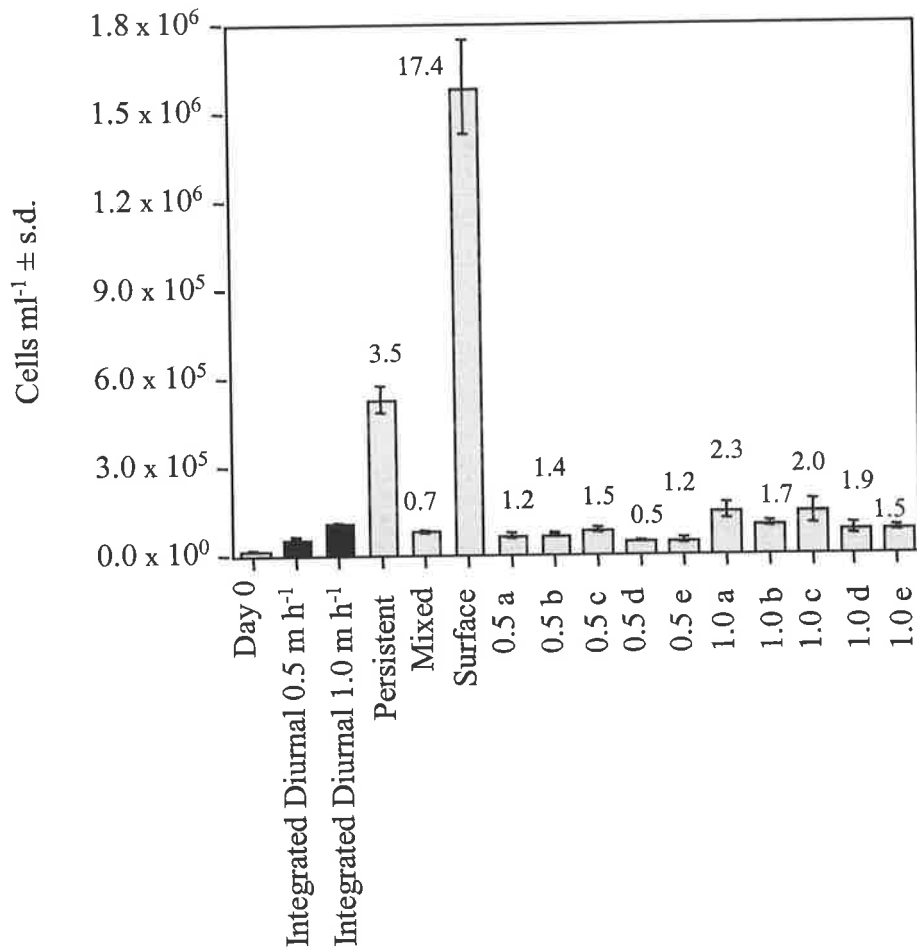
Figure 6.2: *A. circinalis* cell density on day 0 and after 8 days growth in the lower Murray River. The average daily light dose that sub-populations received is indicated (mol m^{-2} day^{-1}). Black bars indicate the cell density of integrated populations that were exposed to diurnal stratification.
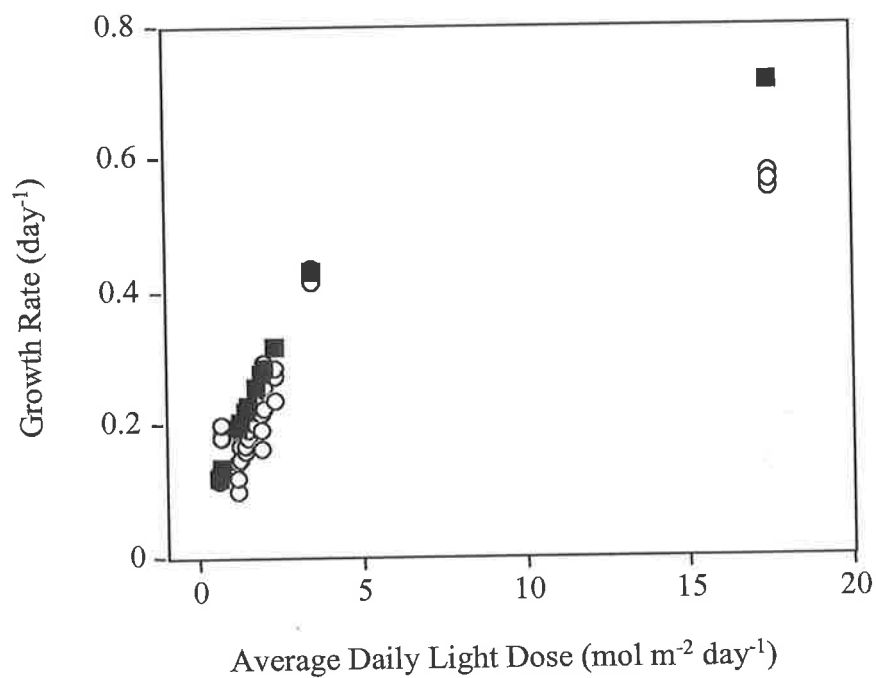
Figure 6.3: Comparison of field data (circles) with values predicted by the model (squares) of the growth of *A. circinalis* in the lower Murray River, from Chapter 5. Model adapted from Jassby and Platt (1976).
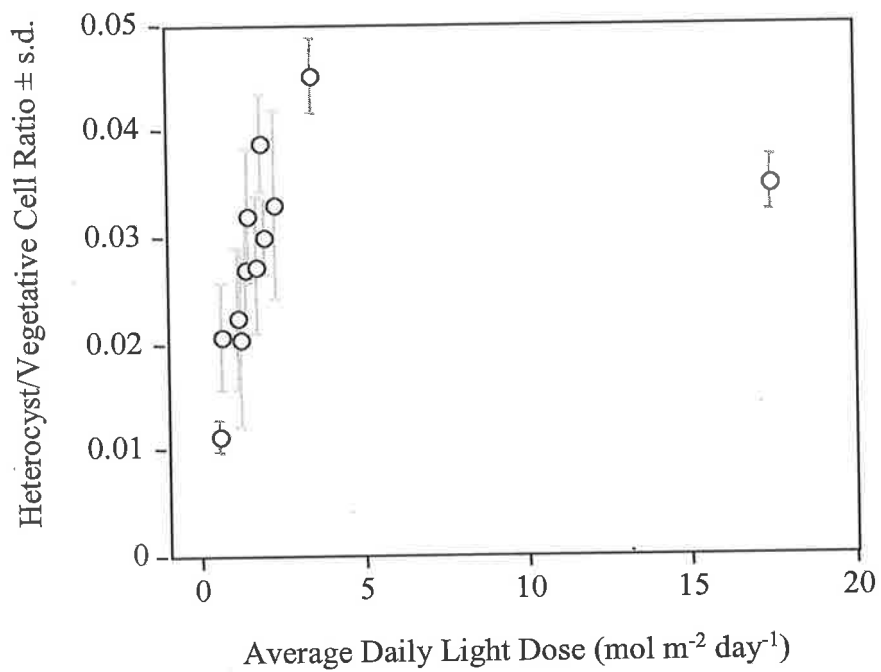
Figure 6.4: Effect of light dose on the heterocyst/vegetative cell ratio after incubation of *A. circinalis* in the lower Murray River for 8 days.
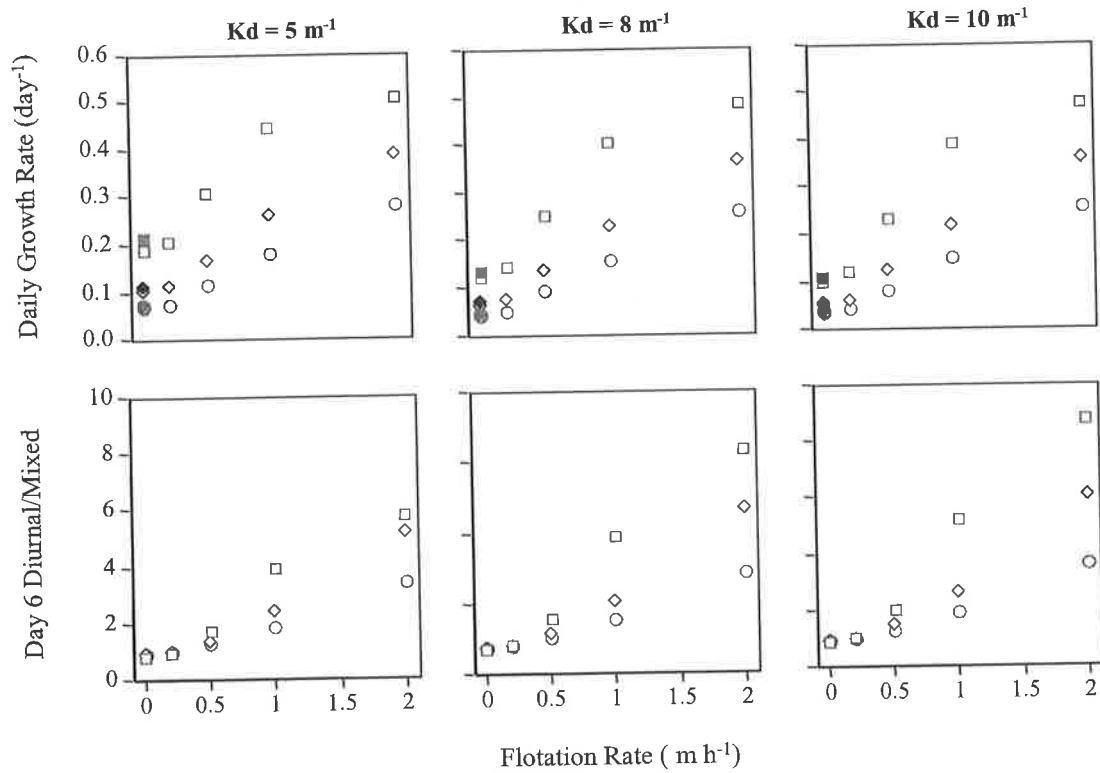
Figure 6.5: Effect of flotation rate and maximum mixed depth (Z_m) on the daily growth of *A. circinalis* and on the ratio of cell density, diurnal to mixed, after 6 days growth at K_d = 5, 8, and 10 m^{-1}. Z_m = 5 (square), 10 (diamond) and 15 (circle) m. In the graph showing daily growth rate, solid symbols represent the growth of mixed populations (neutrally buoyant) and open symbols represent diurnally stratified populations.
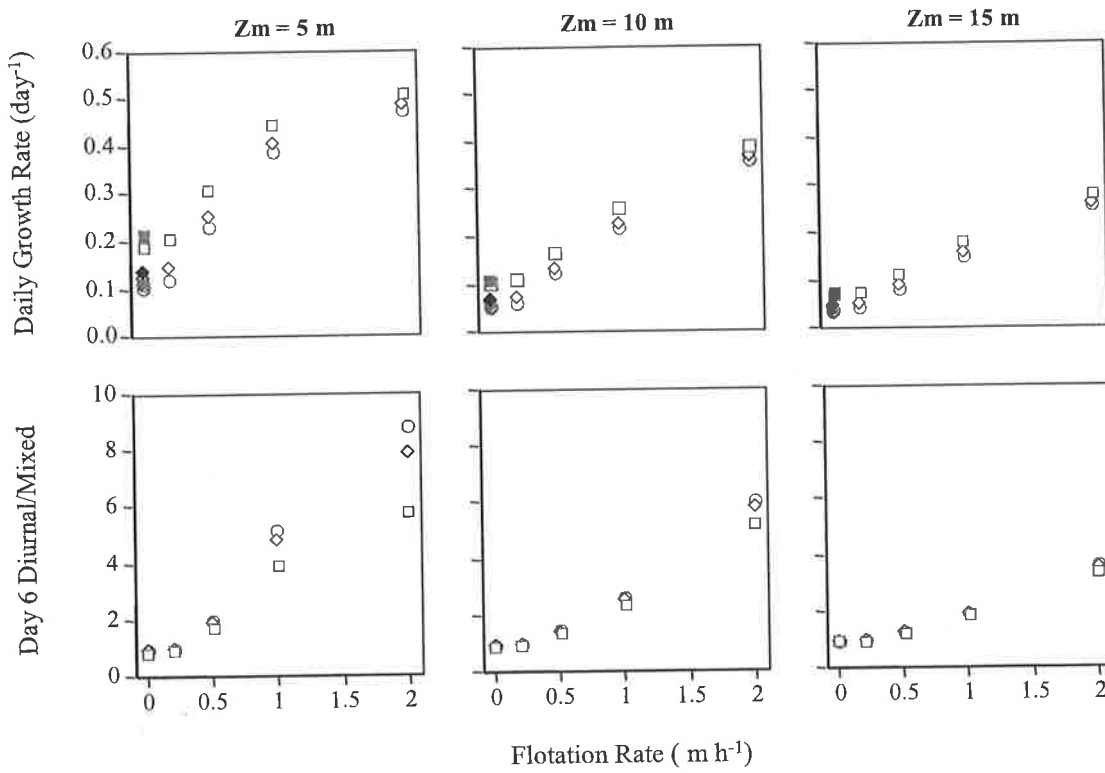
Figure 6.6: Effect of flotation rate and vertical light attenuation (K_d) on the daily

growth of *A. circinalis* and on the ratio of cell density, diurnal to mixed, after 6

days growth at Z_m = 5, 10, and 15 m. K_d = 5 (square), 8 (diamond) and 10 (circle)

m^{-1}. In the graph showing daily growth rate, solid symbols represent the growth

of mixed populations (neutrally buoyant) and open symbols represent diurnally

stratified populations.
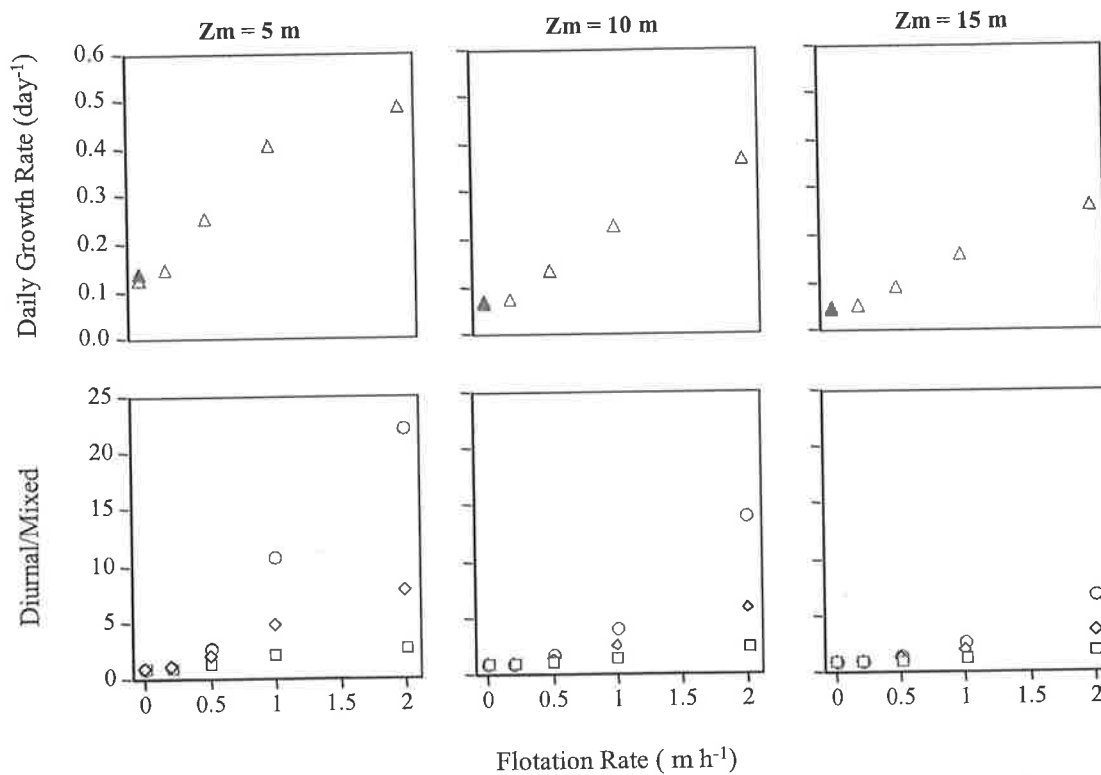
Figure 6.7: Effect of flotation rate and incubation period (days) on the daily growth of *A. circinalis* and on the ratio of cell density, diurnal to mixed, when $K_d = 8$ m^{-1}, at maximum mixed depths (Z_m) of 5, 10 and 15 m. Incubation times are 3 (square), 6, (diamond) and 9 (circle) days. Growth rates are represented by triangles for neutrally buoyant mixed populations (solid) and diurnally stratified populations with neutral to positive buoyancy (open). Daily growth rates do not change with incubation time.

Table 6.3: Effect of flotation velocity (m h^{-1}), maximum mixed depth (Z_m, m) and incubation time (days) on cell density and growth rate of populations of *A. circinalis* exposed to diurnally stratified and mixed conditions, and on the ratio of the cell density, diurnal to mixed.

Treatment	K_d	Days	Z_m	Cells ml^{-1}	Day^{-1}	Diurnal:Mixed
Mixed	8	3	5	1.2×10^3	0.138	-
0.0 m h^{-1}	8	3	5	1.2×10^3	0.124	0.959
0.2 m h^{-1}	8	3	5	1.2×10^3	0.143	1.015
0.5 m h^{-1}	8	3	5	1.7×10^3	0.250	1.398
1.0 m h^{-1}	8	3	5	2.7×10^3	0.407	2.237
2.0 m h^{-1}	8	3	5	3.4×10^3	0.485	2.828
Mixed	8	7	5	2.1×10^3	0.138	-
0.0 m h^{-1}	8	7	5	1.9×10^3	0.124	0.906
0.2 m h^{-1}	8	7	5	2.2×10^3	0.143	1.035
0.5 m h^{-1}	8	7	5	4.6×10^3	0.250	2.186
1.0 m h^{-1}	8	7	5	1.3×10^4	0.402	6.327
2.0 m h^{-1}	8	7	5	2.3×10^4	0.482	11.113
Mixed	8	14	5	5.5×10^3	0.138	-
0.0 m h^{-1}	8	14	5	4.6×10^3	0.124	0.821
0.2 m h^{-1}	8	14	5	5.9×10^3	0.143	1.072
0.5 m h^{-1}	8	14	5	2.7×10^4	0.250	4.780
1.0 m h^{-1}	8	14	5	2.2×10^5	0.402	40.036
2.0 m h^{-1}	8	14	5	6.8×10^5	0.482	123.505
Mixed	8	7	15	1.1×10^3	0.047	-
0.0 m h^{-1}	8	7	15	1.1×10^3	0.043	0.978
0.2 m h^{-1}	8	7	15	1.1×10^3	0.050	1.025
0.5 m h^{-1}	8	7	15	1.5×10^3	0.091	1.364
1.0 m h^{-1}	8	7	15	2.4×10^3	0.157	2.169
2.0 m h^{-1}	8	7	15	4.9×10^3	0.259	4.433
Mixed	8	14	15	1.5×10^3	0.047	-
0.0 m h^{-1}	8	14	15	1.5×10^3	0.043	0.956
0.2 m h^{-1}	8	14	15	1.6×10^3	0.050	1.052
0.5 m h^{-1}	8	14	15	2.9×10^3	0.091	1.860
1.0 m h^{-1}	8	14	15	7.2×10^3	0.157	4.703
2.0 m h^{-1}	8	14	15	3.0×10^4	0.259	19.648
Mixed	8	21	15	2.1×10^3	0.047	-
0.0 m h^{-1}	8	21	15	2.0×10^3	0.043	0.935
0.2 m h^{-1}	8	21	15	2.3×10^3	0.050	1.078
0.5 m h^{-1}	8	21	15	5.4×10^3	0.091	2.537
1.0 m h^{-1}	8	21	15	2.2×10^4	0.157	10.198
2.0 m h^{-1}	8	21	15	1.9×10^5	0.259	87.093

CHAPTER 7. General Discussion

This thesis has provided a field evaluation of the factors that control the growth of *A. circinalis* in the lower Murray River. To enable the measurement of realistic growth responses, the technique of *in situ* diffusion culturing was utilised to allow both the chemical and physical environment to affect the outcome. Of the various hypotheses that have emerged to explain cyanobacterial dominance (Shapiro 1990; Blomqvist *et al.* 1994), the success of *A. circinalis* in the lower Murray River was initially thought to be related to phosphorus storage, nitrogen-fixation and buoyancy regulation (Chapter 1). The importance of phosphorus storage for *A. circinalis* was unable to be tested as significant volumes of water were released from the Darling River during every field experiment, causing phosphorus concentrations to be elevated (Mackay *et al.* 1988; Sullivan *et al.* 1988). It was unfortunate that a year with low input from the Darling River, as in 1994/1995 (Chapter 1), was not encountered over the course of study to test the effect on growth of *A. circinalis*. The variable nature of the Murray River demonstrates that results from one year are not necessarily representative of another. Nevertheless, a comparison of the data presented in Chapters 3 to 6 (see below) may provide some insight into the importance of nitrogen-fixation and buoyancy regulation in the control of *A. circinalis* growth.

7.1 Nitrogen Fixation

Laboratory bioassays and nutrient data from the lower Murray River indicated that nitrogen was the main limiting nutrient for growth during the project. In bioassays, nitrogen additions generally caused a significant increase in chlorophyll concentration, whereas phosphorus additions failed to elicit a response in any experiment. The TN:TP ratio (by weight) in the river was always < 7.0 and oxidised nitrogen was often below the limit of detection. Nitrogen limitation has been demonstrated previously in the lower Murray River by Brookes (1997)

who examined the response of the natural phytoplankton community to nutrient enrichment using fluorescein-diacetate to measure metabolic activity.

An increase in the heterocyst/vegetative cell ratio of *A. circinalis* in the lower Murray River indicated that nitrogen was obtained through atmospheric sources. A comparison of field experiments between years shows that the heterocyst/vegetative cell ratio was affected by both light dose and inorganic nitrogen availability (Figure 7.1). The ratio increased with average daily light dose to a maximum, and in some cases decreased at higher light doses suggesting inhibition - a similar pattern was found by Lee and Rhee (1999) in laboratory studies of *Anabaena flos-aquae*. The increase in heterocyst/vegetative cell ratio with light dose varied between years, as did the maximum (Figure 7.1). The lowest ratios were in December 1998, ranging to the highest in February 2000. Inorganic nitrogen concentrations in the river during each experiment were 71.4 μg L^{-1} (December 1998), 54.6 μg L^{-1} (February 1999) and 42.3 μg L^{-1} (February 2000), respectively. This suggests that *A. circinalis* produced heterocysts according to the necessity to compensate for low ambient nitrogen concentrations.

In natural populations of *A. circinalis*, the heterocyst/vegetative cell ratio may increase from 0.0001 to as high as 0.10 under nitrogen limited conditions (Horne *et al.* 1972; Reynolds 1986). However, in the lower Murray River the maximum ratio was 0.048 (Figure 7.1). The intermediate values for the heterocyst/vegetative cell ratio suggest that nitrogen-limitation was not severe. The incubation of *A. circinalis* using *in situ* diffusion chambers meant that cells had access to fluxes of nutrients from the river. In contrast, the natural phytoplankton community in laboratory bioassays had access to finite nutrients, causing nitrogen limitation to be amplified. In nature, the degree of nutrient limitation may fall somewhere between the results of the field and laboratory incubations. As cells are advected down the river, they may utilise surrounding nutrients within the parcel of water that is occupied. However, diffusion chambers were suspended at a static location against the river flow so that nutrient availability

may have been elevated. This may explain the lack of growth response of either *A. circinalis* or the natural phytoplankton community to nitrogen additions over the initial years of the project. Although, in these years inorganic nitrogen was similar to, or above the limiting threshold of 100 μg L^{-1} (Chapter 1) with concentrations of 149.7 μg L^{-1} (December 1997), 124.0 μg L^{-1} (February 1998) and 95.4 μg L^{-1} (March 1998), respectively. It was unfortunate that light, rather than nutrient availability, was investigated on the occasions when inorganic nitrogen fell below the limiting threshold (December 1998 - February 2000, see above). It is possible that a response to nitrogen additions may have been measured under these conditions. Nevertheless, the highest growth rate of *A. circinalis* was observed during a year of low inorganic nitrogen availability. This was in December 1998 (maximum growth 0.88 day^{-1}) when oxidised nitrogen was 7.7 μg L^{-1} and total inorganic nitrogen was 71.4 μg L^{-1}.

7.2 Light and the Physical Environment

Steinberg and Hartmann (1988) suggest that the physical environment becomes important for cyanobacterial growth when phosphorus availability exceeds limiting thresholds. In the lower Murray River, phosphorus was significantly higher than the limiting concentration of 10 μg L^{-1} (Sas 1989) during all field experiments, ranging from 28.7 to 155.7 μg L^{-1}. The importance of the physical environment under these conditions was demonstrated in Chapters 5 and 6 where examination of the interaction between mixing patterns and buoyancy of *A. circinalis* gave rise to predictable growth responses. As the lower Murray River is turbid, K$_d$ ranged between 3.6 and 8.4 m^{-1} across all field experiments and light limitation was significant. Changes in the physical environment directly modified the growth of *A. circinalis* through changing the average daily light dose that populations received.

The growth of *A. circinalis* under light limited conditions was similar across the three field experiments that specifically examined this response (Figure 7.2). Limitation occurred at light doses ranging from 0 to 10 mol m^{-2} day^{-1}, with growth increasing from 0.00 to 0.63 day^{-1}.

Nitrogen limitation may affect both the light-limited and light-saturated portions of the growth/light curve through influencing the production of pigments for light harvesting, the production of new cellular material for growth, and the necessity to divert energy for nitrogen-fixation processes (Tilzer 1987; Paerl and Kellar 1979). However, there was no effect of inorganic nitrogen variability on growth of *A. circinalis* under light limited conditions (Figure 7.2). This was unexpected, given that the heterocyst/vegetative cell ratio varied according to nitrogen availability and that energy is required for heterocyst production and nitrogen-fixation (Turpin 1991). However, as suggested above, nitrogen-limitation of *A. circinalis* may not have been severe in the diffusion chambers. Furthermore, phytoplankton require only small quantities of nitrogen relative to carbon for active growth (16 N : 106 C by atoms, 224 N : 1272 C by weight, Redfield 1958). The amount of energy diverted for fixation processes may have been too small to be reflected in the growth rate. Over longer-term experiments (> 8 days) and lower inorganic nitrogen availability, small changes in growth rate related to nitrogen-fixation may have emerged. Regardless, it appears as though the ability to fix molecular nitrogen compensates for low concentrations of inorganic nitrogen with the result that growth rates under light limited conditions are unaffected. This provides a definite ecological advantage for *A. circinalis* in the lower Murray River (Figure 7.3).

At light doses > 10 mol m^{-2} day^{-1}, growth of *A. circinalis* was saturated (Figure 7.2). Saturated growth in February 2000 was significantly lower than in December 1998. This may have been due to higher cell density inside the diffusion chambers (1.6 x 10^6 cells ml^{-1} in February 2000 v 8.0 x 10^5 cells ml^{-1} in December 1998) which may have induced nutrient limitation or self-shading (Chapter 6). Alternatively, slower growth was caused through lower external nitrogen availability (see concentrations above). If so, nutrient availability may have an important effect on the maximum growth rate. Water temperature had no influence on saturated growth with average measurements in December 1998, February 1999 and February 2000 corresponding to 22.9, 23.1 and 24.3 °C, respectively. The fastest growth of *A. circinalis* was

in December 1998 when the water temperature was lowest. Although water temperature may have an effect on maximum photosynthetic rates, respiration rates, and overall growth rates of phytoplankton populations (Falkowski *et al.* 1985; Robarts and Zohary 1987), the association of cyanobacteria with warm temperatures may be related more to the development of stratification, than to changes in cell physiology (Reynolds and Walsby 1975).

From Chapters 5 and 6, light saturated growth of *A. circinalis* may only occur under persistently stratified conditions in the lower Murray River, as this is the only mixing pattern that allows an average daily light dose > 10 mol m^{-2} day^{-1}. Therefore, the maximum growth of *A. circinalis* can only occur when river flow is < 4000 ML day^{-1} and average daily wind speed is < 1.2 m s^{-1} (Bormans *et al.* 1997), or in shallow parts of the river where Z_{eu}/Z_m is close to 1. The dominance of *Anabaena* under low flows and persistently stratified conditions was demonstrated in Maude Weir, where populations received a light dose of 10 to 13 mol m^{-2} day^{-1} (Sherman *et al.* 1998). However, the predominant mixing pattern in the lower Murray River is diurnal stratification that occurs when flow is < 4000 ML day^{-1} and average daily wind speed is between 1.2 and 2.9 m s^{-1}, or at flows between 4000 and 10 000 ML day^{-1} (Bormans *et al.* 1997). Under diurnal stratification, the extremes in light dose that sub-populations are exposed to ranges from 0.14 mol m^{-2} day^{-1} (sub-population trapped in darkness during stratification) to 6.7 mol m^{-2} day^{-1} (sub-population trapped within the euphotic zone at the onset of stratification). Therefore, growth of *A. circinalis* under diurnally stratified conditions is always restricted to below the maximum. The flotation of sub-populations into the euphotic zone during the stratified period directly increases growth rate through increasing light availability (Chapter 6). This may cause an increase in growth of the total integrated population, provided that a sufficient proportion of the population floats into the euphotic zone and that cell density increases by a large enough degree to affect the overall population.

7.3 Buoyancy

Under typical conditions in the lower Murray River, K_d > 5 m^{-1} and average Z_m (bottom depth) is 5 m so that Z_{eu}/Z_m < 0.18. The model developed in Chapters 5 and 6 demonstrated that for a bloom of *A. circinalis* to occur under diurnally stratified conditions when Z_{eu}/Z_m < 0.18, the flotation velocity of cells must be > 0.5 m h^{-1} and the time for bloom development is > 1 week. If flotation velocity is < 0.5 m h^{-1}, the average daily light dose received by sub-populations is not significantly different from that experienced under completely mixed conditions and growth rates are similar between the two mixing patterns (Figure 5.7). In cases where blooms of *A. circinalis* are predicted to develop under diurnally stratified conditions, the diurnal/mixed ratio indicated that it would be beneficial to artificially mix the water-column to reduce cell density.

In other aquatic systems, and parts of the Murray River, Z_{eu}/Z_m may be > 0.18. As Z_{eu}/Z_m increases, the importance of flotation velocity decreases as a higher proportion of the water column provides favourable light for growth, regardless of where the cells are positioned (Humphries and Lynne 1988). Under diurnally stratified conditions, a bloom of *A. circinalis* may develop when Z_{eu}/Z_m is > 0.5 (Reynolds and Walsby 1975), regardless of the flotation velocity of the cells, as the daily growth of neutrally buoyant populations is > 0.42 day^{-1} (Chapter 5, Figure 5.7). As Z_{eu}/Z_m approaches 1.0, the light dose received by populations of *A. circinalis* is analogous to persistently stratified conditions regardless of the mixing pattern and growth rate approaches the maximum of 0.63 day^{-1}. Under persistently stratified conditions, the use of artificial destratification to decrease Z_{eu}/Z_m to < 1.0 is beneficial as growth would be restricted to below the maximum. However, if the euphotic depth reaches the bottom, artificial destratification would be of little benefit given that light dose would not change significantly. This was demonstrated by Visser *et al.* (1996b) who found that *Microcystis* was unable to be completely eliminated in the De Gijster reservoir because Z_{eu}/Z_m in shallow areas of the basin ranged from 0.98 to 2.40.

An important consideration for growth of *A. circinalis* under diurnally stratified conditions in the lower Murray River is the effect of nitrogen limitation on cell flotation. The flotation velocity of cells is partly dependent on the production of gas vesicles that are filled with air and provide lift (Oliver 1994). However, gas vesicles are constructed from proteins and nitrogen limitation may restrict production (Klemer *et al.* 1982; Spencer and King 1989; Oliver and Ganf 2000). As the population divides, the existing gas vesicles are diluted, as demonstrated in *Microcystis aeruginosa* by Brookes and Ganf (2001). Nitrogen limitation may also hinder the metabolism of carbohydrate, causing heavy molecules to accumulate (Turpin 1991). The overall implication of nitrogen limitation is that flotation velocities are likely to be slow (*i.e.* < 0.5 m h^{-1}), or that cells will lose buoyancy and sink. However, *A. circinalis*, unlike *Microcystis aeruginosa*, is able to fix nitrogen so that the production of gas vesicles may be sustained. If so, nitrogen-fixation provides an ecological advantage to *A. circinalis* in two ways; both for the production of new cellular material for growth, and the production of gas vesicles for buoyancy regulation (Figure 7.3). An important consideration for *A. circinalis* and other nitrogen-fixing cyanobacteria, under diurnally stratified and nitrogen limited conditions, is that the ability to fix nitrogen may essentially create a positive feed-back to light availability (Figure 7.3). The sustained production of gas vesicles means that cells may float into the euphotic zone to obtain more light. In turn, increased light promotes further heterocyst production (Figure 7.1) and nitrogen-fixation so that further gas vesicles can be produced (Figure 7.3). This positive feed-back provides a possible mechanism that enables *A. circinalis* to dominate in the lower Murray River. Under persistent stratification, nitrogen-fixation may simply benefit cyanobacteria through directly increasing growth rate, rather than through stimulation of gas vesicle production. Interestingly, Brookes (1997) found that gas vesicle production in *Anabaena* could not match growth under light-saturating conditions, but under light limitation the number of gas-vesicles per cell was maintained (in nitrogen-replete media).

The ability for *A. circinalis* to maintain positive buoyancy under nitrogen limited conditions was demonstrated in Chapter 6, with > 90% of filaments floating after exposure to diurnally stratified conditions when inorganic nitrogen was 42.3 μg L^{-1} and TN:TP (by weight) was 4.9. There are also a number of examples from the literature. Mitrovic *et al.* (2001) examined the migration of a bloom of *A. circinalis* in the Darling River. The TN:TP ratio (by weight) was 4.0, suggesting strong nitrogen limitation and the measured flotation velocity of *A. circinalis* was 0.15 m h^{-1}. Furthermore, in Lake Cargelligo on the Lachlan River, New South Wales, a toxic bloom of *Anabaena circinalis* occurred in 1990 (Bowling 1994) and the TN:TP ratio during the bloom was 4.0. Flotation rates were not measured, as the majority of data were extracted in retrospect to the bloom occurring. However, the water-column depth was 5 m and thought to be mixed intermittently. *A. circinalis* would need to have had a reasonably fast flotation velocity to dominate, despite nitrogen limited conditions. Interestingly, Bowling (1994) suggested that an increase in the TN:TP ratio to > 30 caused a decline in the bloom, but a subsequent decrease of TN:TP to < 12 two months later caused re-emergence. The buoyancy-regulating, but non-nitrogen fixing cyanobacterium *Microcystis* only became dominant when nitrogen became abundant, after the initial decline of the *A. circinalis* bloom.

7.4 Why *Anabaena circinalis* in the Lower Murray River?

The underlying necessity to fix atmospheric nitrogen to compensate for low dissolved inorganic availability in the lower Murray River is likely to preclude other buoyant, but non-nitrogen-fixing cyanobacteria from dominating. For example, *Microcystis aeruginosa* can achieve fast flotation rates (> 3.83 m day^{-1}; Ganf 1974) and is associated with stratified conditions, but generally only dominates when N:P > 10, ranging to > 80 (Harris 1986; Zohary and Robarts 1989). However, the question arises as to why a range of other cyanobacterial species that are able to fix atmospheric nitrogen and regulate buoyancy are not as prominent as *A. circinalis* in the lower Murray River. Alternative nitrogen-fixing species

that are known to bloom under stratified conditions are the larger morphological forms, including *Anabaena flos-aquae*, *Anabaenopsis* spp., *Aphanizomenon flos-aquae* and *Gleotrichia* spp. (Viner and Smith 1973; Reynolds and Walsby 1975; Foy *et al.* 1976; Rhee and Lederman 1983; Spencer and King 1989; Istvánovics *et al.* 1993; Walsby *et al.* 1987; Oliver and Ganf 2000). In the Murray River, blooms of *Anabaena aphanizomenoides*, *Anabaena flos-aquae*, *Anabaena spiroides*, *Anabaena solitaria*, *Anabaenopsis elenkinii*, *Aphanizomenon issatschenkoi* and *Cylidrospermopsis raciborskii* have been recorded, but usually as a single event and with significantly less prevalence than *A. circinalis* blooms (Baker *et al.* 1993; Velzeboer *et al.* 2000).

One possibility for the dominance of *A. circinalis*, rather than alternative species, is that flotation velocities may be faster, thus allowing a higher proportion of a population to reach the euphotic zone that is generally shallow due to high turbidity. From the literature, measured flotation velocities of cyanobacteria are variable. For example, in the study of Brookes *et al.* (1999) the flotation rate of *A. circinalis* ranged from 0.01 to 2.0 m h^{-1}, depending on light and nutrient availability. Reynolds (1975) compared the flotation of *A. circinalis*, *Aphanizomenon flos-aquae* and *Anabaena spiroides* and showed maximum rates corresponding to 0.21, 0.06 and 0.11 m h^{-1}, respectively. Walsby *et al.* (1997) found that *Aphanizomenon flos-aquae* could achieve flotation velocities between 0.2 to 1.5 m h^{-1} in the Baltic Sea, depending on the size and density of colonies. Furthermore, flotation rates of *Anabaena flos-aquae* range from 0.036 to 0.36 m h^{-1} (Oliver and Ganf 2000). Although *A. circinalis* and *Aphanizomenon flos-aquae* may float faster than the other species, this can only be achieved when large colony aggregations are formed through high cell densities (Oliver 1994; Walsby *et al.* 1997; Brookes *et al.* 1999). However, when considering the initiation of a bloom, flotation velocities are more likely to be at the lower end of the range as cell density would be too low for colony aggregation. The importance of flotation velocity for dominance of *A. circinalis* rather than other species in the Murray River is currently unclear.

Another possibility for dominance of *A. circinalis* is that it has a faster growth rate than the other species and survives better in rivers where populations may be subjected to high losses from dilution and advection downstream. Foy and Gibson (1982) studied the photosynthetic characteristics of a range of nitrogen fixing cyanobacteria, including *A. circinalis*, *Anabaena flos-aquae*, *Anabaena spiroides* and *Anabaena solitaria*. The maximum photosynthetic rate was significantly higher for *A. circinalis* (P_{max} = 17.3 mg O_2 mg chl a^{-1} h^{-1} low light-adapted; 23 mg O_2 mg chl a^{-1} h^{-1} high light-adapted), compared to the other species (next highest P_{max} = 13.3 mg O_2 mg chl a^{-1} h^{-1} low light-adapted; 12.3 mg O_2 mg chl a^{-1} h^{-1} high light-adapted, *Aphanizomenon flos-aquae*). Furthermore, low light-adapted cells of *A. circinalis* had a higher α than the other species with a value of 26.7 mg O_2 mg Chl a^{-1} E^{-1} m^2 compared to the range 15.8 to 23.7 mg O_2 mg Chl a^{-1} E^{-1} m^2. Assuming that photosynthetic rates convert directly to growth, *A. circinalis* may have a better ability to harvest light under the turbid conditions that characterise the Murray-Darling Basin and grow better under diurnal and persistently stratified conditions.

In contrast to the study of Foy and Gibson (1982), studies on growth (rather than photosynthesis) of *A. circinalis* under optimal conditions of light and nutrient availability suggest that rates are similar to the alternative species (McCausland *et al.* 2002; Winder and Cheng 1995). McCausland *et al.* (2002) determined a growth rate for *A. circinalis* of 0.65 day^{-1} in laboratory microcosms, and in this project the maximum growth rate in the lower Murray River in diffusion chambers was 0.88 day^{-1}. Maximum growth rates for *Anabaena flos-aquae* range from 0.67 to 1.25 day^{-1} (Gorham *et al.* 1964; Healey *et al.* 1973; Foy *et al.* 1976). Rates determined for *Aphanizomenon flos-aquae* range from 0.55 to 0.99 day^{-1} (Foy *et al.* 1976; Rother and Fay 1979; Lee and Rhee 1999). In addition, *Anabaena solitaria* can divide at 0.50 day^{-1} and *Anabana spiroides* at 0.62 day^{-1} (Foy 1980). Growth under optimal light and nutrient conditions is generally positively correlated with the surface area/volume ratio (Fogg 1975; Foy 1980).

Under sub-optimal nutrient conditions, growth of *A. circinalis* may be faster than the other species. De Nobel *et al.* (1998), compared the light limited growth of *Anabaena* sp. and *Aphanizomenon flos-aquae* in the laboratory under nitrogen limitation. Growth of low light-adapted cells of *A. flos-aquae* was faster than *Anabaena* at light intensities < 17.7 μmol m^{-2} s^{-1} (0.76 mol m^{-2} 12 h^{-1}), but at higher light intensities, *Anabaena* grew faster. The maximum growth rate of *Anabaena* was also faster than *A. flos-aquae*; 0.046 h^{-1} compared to 0.026 h^{-1}, respectively. When both species were grown together under light-limited and nitrogen-limited conditions, *Anabaena* excluded *A. flos-aquae* from the experiment. Under diurnally stratified conditions in the lower Murray River, the light doses that sub-populations are exposed to is always > 0.76 mol m^{-2} day^{-1}, with the exception of sub-populations that remain trapped in darkness throughout the stratified period (0.14 mol m^{-2} day^{-1}, see above). It is possible that *A. circinalis* has a better ability to cope with nitrogen-limitation than the other species under diurnally stratified conditions.

7.5 Transitions Between *Anabaena* and *Aulacoseira.*

The rise of *Anabaena* and decline of *Aulacoseira* when stratification develops in the lower Murray River can be attributed to differences in cell density (Sherman *et al.* 1998). *Aulacoseria* is denser than water (Davey 1987) and sinks rapidly out of the euphotic zone, whereas *Anabaena* is able to float and have continued access to light for active growth (Sherman *et al.* 1998). Less is known about the decline of *Anabaena* and increase in *Aulacoseira* when mixed conditions resume and Chapter 4 examined possible reasons for this transition. It was expected that growth of *A. granulata* would be faster than *A. circinalis* with decreasing Z_{eu}/Z_m, but this was not the case. Rather, growth of *A. circinalis* was faster than *A. granulata* at all Z_{eu}/Z_m ratios (0.08 to 1.00). At the lowest ratio ($Z_{eu}/Z_m = 0.08$), growth of *A. circinalis* was 0.2 day^{-1}, compared to 0.05 day^{-1} for *A. granulata* (Figure 4.6). Growth of *A. circinalis* was rapid under a wide range of light doses, suggesting that the decline of this

species under mixed conditions is not necessarily caused through a decrease in light availability. Diffusion chambers were suspended at static depths in the Murray River during exposure, thus removing short-term fluctuations in light availability which may have caused an increase in growth rate of *A. circinalis* (Nicklisch 1998; Chapter 4). Nevertheless, instantaneous measurements of photosynthesis in the laboratory provided further evidence that *A. circinalis* may achieve fast photosynthetic rates and that the rate is higher than *A. granulata* when intensity is > 93.3 μmol m^{-2} s^{-1}, or 4.0 mol m^{-2} day^{-1} (Figure 4.9). Other laboratory studies have also demonstrated that growth and photosynthetic efficiencies of cyanobacteria are higher than *Aulacoseira* sp. (Coles and Jones 2000; McCausland *et al.* 2002). In all, the evidence that light availability plays an important role in the transition from *Anabaena* to *Aulacoseira* under mixed conditions is not convincing.

An alternative reason for the decline of *Anabaena* under mixed conditions is that cells may be affected by turbulence, either directly or indirectly. An increase in turbulence may override the benefits provided to *A. circinalis* through buoyancy regulation as cells are unable to disentrain from the flow, assuming that flow velocity is an order of magnitude greater than the flotation velocity of the cells (MacIntyre 1993). In contrast, *Aulacoseira* may benefit from turbulent conditions as long filaments are easily entrained within the flow and cells may be advected back into the euphotic zone.

Turbulence may also be detrimental to cyanobacteria through the generation of shear stress that may have a negative effect on nitrogen-fixation rates and growth rates (Fogg 1969; Paerl 2000). For example, in a study by Kucera and Paerl (unpublished, in Paerl 2000), the nitrogen-fixation rate for *A. circinalis* under high shear stress (4 to 6 hours) was 4.92 nmol C_2H_4 mg chl^{-1} h^{-1} compared to 8.52 nmol C_2H_4 mg chl^{-1} h^{-1} in the control. Carbon fixation rates were only slightly affected with a rate of 1.32 mg C mg chl^{-1} h^{-1} under high sheer stress compared to 1.75 mg C mg chl^{-1} h^{-1} in the control. Paerl (2000) suggests that reductions in

nitrogen and carbon fixation rates from turbulence may be caused through filament breakage (Lang and Fay 1971) or the deactivation of nitrogenase through increased oxygen availability (Fogg 1969). Reduced nitrogen-fixation would be particularly detrimental for cyanobacteria in the lower Murray River where nitrogen is limiting. Filament breakage may also lead to slower flotation of *A. circinalis*, as fast velocities are associated with filament aggregation and high cell densities (Brookes *et al.* 1999). If cells are provided with the opportunity to disentrain from the river flow for short periods, the flotation velocity of cells may be insufficient for a significant proportion of the population to reach the euphotic zone to gain adequate light for growth.

Another possibility for a decrease in *Anabaena* during mixed conditions is that it is an apparent rather than a realistic observation. During stratified conditions, *Anabaena* may become concentrated towards the surface due to the flotation of colonies. With the onset of mixing, the population is re-distributed throughout the entire water-column, thus reducing the cell density in surface waters where samples are taken, but maintaining the total cell number per unit volume of the total water column. Sherman *et al.* (1998) also suggest that it is difficult to measure the growth of a population of *Anabaena* once mixed conditions resume, as the population doubling time may be slower than retention time due to increased flow. To correctly determine growth, the population would need to be tracked downstream (Baker *et al.* 2000). However, this suggestion applies equally to *A. granulata*, yet cell density increases under mixed conditions. It is interesting that high cell numbers of *A. granulata* are able to be sustained in rivers where populations are continually subjected to advection downstream. This implies either that growth rates of *A. granulata* are rapid upstream, and/or that a seeding population may contribute a significant number of cells to the channel population (Reynolds *et al.* 1991). Success of *A. granulata* may be related to the ability to form a resting stage on the sediment which ensures that a seeding population is available when conditions become more favourable for growth (Kilham and Kilham 1975; Reynolds 1984; Davey 1987).

Although, *A. circinalis* can also form cysts so that the suggestion of a seeding population does not preclude *A. circinalis* from dominating under mixed conditions.

A. granulata is unable to fix atmospheric nitrogen so that growth is dependent upon ambient inorganic nitrogen sources. As the lower Murray River is nitrogen-limited, this species may be more tolerant of low inorganic nitrogen availability compared to other species. Although, some studies have shown that *Aulacoseria* has a high sensitivity to nitrogen availability, but a low sensitivity to light availability (Lewis 1978; Sherman *et al.* 1998). It is possible that *A. granulata* accumulates nitrogen in the sediments during stratified conditions (Cimbleris and Cáceres 1991), allowing rapid growth when filaments are advected back into the euphotic zone with the onset of turbulence. It was also interesting that in Chapter 4, growth of *A. granulata* was stimulated when grown in the same diffusion chambers as *A. circinalis*, suggesting that nitrogen limitation may have been alleviated. If so, *A. circinalis* may be an important contributor to nitrogen availability in the lower Murray River through fixation of atmospheric sources. In Maude Weir, the TN:TP ratio prior to an *Anabaena* bloom ranged from 2.7 to 4.8 (Sherman *et al.* 1998). The TN:TP ratio increased to 9.5 during the bloom and this was attributed to nitrogen-fixation by the *Anabaena* population. Lewis and Levine (1984) measured nitrogen-fixation of *Anabaena* spp. in Lake Valencia, Venezuela and found maximum rates ranging between 2.3 and 38.0 nmol $(10^6 \text{ heterocysts})^{-1}$ h^{-1}, at light doses between 1.0 and 2.5 mol m^{-2} h^{-1}. Nitrogen fixation was modelled using the photosynthetic curve of Platt *et al.* (1980) and fastest fixation and highest α occurred in high light-adapted cells that were sampled from the lake surface. Assuming an intermediate nitrogen-fixation rate of 14 nmol $(10^6 \text{ heterocysts})^{-1}$ h^{-1}, a cell density of 1 x 10^4 cells ml^{-1} and a heterocyst/vegetative cell ratio of 0.10 (= 1000 heterocysts ml^{-1}), the amount of nitrogen that *A. circinalis* could fix in 12 hours is 2.4 μg L^{-1}. In a nitrogen-limited environment, this may be a significant contribution given that a single cell of *A. granulata* is composed of 2.1 x 10^{-5} μg of nitrogen (calculated from Reynolds 1984). However, nitrogen that is fixed by *A.*

circinalis would not be immediately available for growth so that nitrogen cycling rates are also important.

7.6 Future Research

Further research into possible reasons for the decline of *Anabaena* and increase in *Aulacoseira* under mixed conditions is required. The evidence from Chapter 4 and other studies (Coles and Jones 2000; McCausland *et al.* 2002) suggests that light availability may not play an important role in the transition. To confirm the results of Chapter 4, additional field experiments could be conducted to examine growth under various Z_{eu}/Z_m ratios using diffusion chambers that are circulated through a fluctuating light environment. The effect of turbulence on nitrogen-fixation of *A. circinalis* and growth of both *A. circinalis* and *A. granulata* also requires examination. Assuming that shear stress has an influence, threshold values for turbulent velocity are required. Mitrovic *et al.* (2003) recently examined the development of persistent stratification at several sites within the Murray-Darling Basin and determined that a critical flow velocity of 0.05 m s^{-1} was sufficient to suppress stratification and reduce *A. circinalis* growth at all sites. The turbulent velocity (μ^*) under weak mixing varied between 2.66 x 10^{-3} and 2.91 x 10^{-3} m s^{-1}.

To gain a better understanding of nutrient limitation in the lower Murray River, it would also be useful to incubate diffusion chambers in a year when the contribution of water from the Darling River is minimal. Validation of the slope of the growth/light dose curve under more severe nutrient limited conditions is required to ensure that the model predictions for growth of *A. circinalis* under diurnally stratified conditions are reasonable. For example, phosphorus availability was above the limiting threshold throughout the project, but nitrogen-fixation is known to be stimulated by phosphorus availability due to the production of ATP that provides energy for fixation (Stewart and Alexander 1971). Under reduced phosphorus and nitrogen availability, growth of *A. circinalis* may decrease significantly.

Although the importance of phosphorus storage was unable to be tested in the lower Murray River, the laboratory experiments in Chapter 2 suggested that *A. circinalis* has a large capacity for phosphorus storage. This was indicated by the high half-saturation for phosphorus uptake (10 μM), compared to growth (0.1 μM). Experiments to determine the length of time that stored phosphorus can sustain growth and buoyancy of *A. circinalis* under phosphorus and nitrogen-limited conditions would be useful for interpretation of field results. To more accurately test nutrient availability in the lower Murray River, it would be better to attach the diffusion chambers to a drogue system that can be tracked downstream. This would overcome the possible problem of cells encountering higher nutrient availability through static placement against a moving river flow. In nature, cells may deplete surrounding nutrients as they are advected downstream.

The buoyancy of *A. circinalis* under nitrogen-limited conditions also requires examination. It is necessary to determine if *A. circinalis* can maintain the production of gas vesicles and positive flotation under nitrogen-fixing conditions, and to determine the flotation velocities that can be achieved. Brookes (1997) attempted to examine growth and relative gas vesicle volume of nitrogen-deficient cultures of *A. circinalis* that were flushed with argon gas to prevent atmospheric nitrogen from influencing nitrogen limitation. This was compared with cultures that were nitrogen-deficient but had access to atmospheric nitrogen. However, there were no significant differences in growth rate or relative gas vesicle volume and Brookes (1997) concluded that atmospheric nitrogen had entered the cultures with argon gas due to a high concentration gradient with the external environment. Further work is required in this area.

7.7 Summary

Through examining long-term growth, rather than instantaneous measurements of fluorescence and photosynthesis of *A. circinalis* in the lower Murray River, this thesis has provided information that may be easily interpreted by water managers. The measurement of growth means that short-term variability in physiological responses of *A. circinalis* are taken into account, thus allowing conservative predictions from model outcomes. Growth has been related to measurements that are easily determined in monitoring studies, *i.e.* nutrient and light availability.

The over-riding factor that is likely to lead to dominance of *A. circinalis* in the lower Murray River is nitrogen limitation. *A. circinalis* is able to fix atmospheric nitrogen sources when required, thus gaining a distinct ecological advantage through the ability to maintain production of gas vesicles for buoyancy regulation and cellular material for growth. Both heterocyst production and growth are strongly influenced by light dose. Given the turbid nature of the lower Murray River, the light dose received by populations is directly influenced by river flow, mixing patterns and the flotation velocity of the cells. The provision of adequate light for rapid growth of *A. circinalis* and the development of blooms in the lower Murray River may occur under one of three conditions;

a) when flow is < 4000 ML day^{-1} and average daily wind speed is < 1.2 m s^{-1}. Under these conditions persistent stratification develops allowing a growth rate close to the maximum and possible bloom development within one week.

b) when flow is < 4000 ML day^{-1}, average daily wind speed is 1.2 to 2.9 m s^{-1} and flotation velocity of *A. circinalis* is > 0.5 m h^{-1}. Under these conditions, diurnal stratification occurs and a bloom could develop over 1 to 2 weeks, depending on the flotation velocity of the cells, the maximum mixed depth and the length of the diurnally stratified period. As growth is light

limited, nutrient availability may have a small influence on growth but may set the upper limit to cell density.

c) when flow is 4000 - 10 000 ML day^{-1} and flotation velocity of *A. circinalis* is > 0.5 m h^{-1}. Again, diurnal stratification is the predominant mixing pattern and a bloom may develop over 1 to 2 weeks.

Under other conditions of flow, wind speed and flotation velocity of *A. circinalis* in the lower Murray River, a bloom is unlikely to develop unless the water column or maximum mixed depth is shallow (Z_{eu}/Z_m > 0.5). As Z_{eu}/Z_m approaches 1.0, the light conditions become analogous to persistently stratified conditions and growth rate approaches the maximum regardless of the flow rate, mixing pattern or the buoyancy status of the population. Under circumstances where a bloom is expected to develop, the diurnal/mixed ratio indicated that it would be beneficial to increase river flow and artificially destratify the water-column to reduce cell growth. However, when the bottom depth is similar to the euphotic depth (Z_{eu}/Z_m = 1), artificial destratification would be ineffective given that light dose would not change significantly.
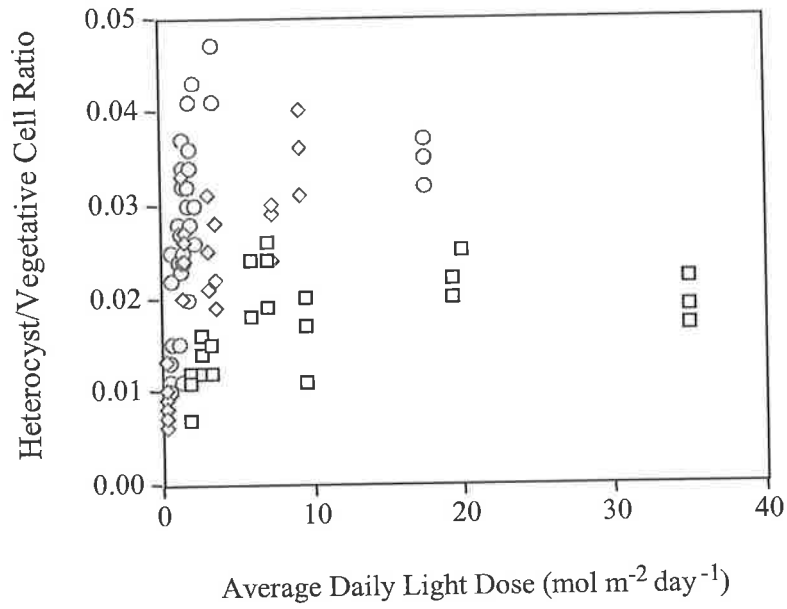
Figure 7.1: Effect of light dose on the heterocyst/vegetative cell ratio of *A. circinalis* after incubation in the lower Murray River in diffusion chambers in December 1998 (squares), February 1999 (diamonds) and February 2000 (circles).

Figure 7.2: Effect of light dose on growth of *A. circinalis* when incubated in diffusion chambers in the lower Murray River in December 1998 (square), February 1999 (diamond) and February 2000 (circle).

↑ Photosynthesis ⟶ ↑ C ⟶ ↑ Growth

↑ Light

↓ Inorganic N

↑ Heterocyst Production ⟶ ↑ N

↑ Flotation ⟵ ↑ Gas Vesicles

Figure 7.3: Model showing the interactive effects of light, inorganic nitrogen availability, heterocyst production, nitrogen-fixation, cell flotation, and photosynthesis on growth of *A. circinalis*. The interaction may promote dominance of *A. circinalis* under diurnally stratified conditions in the lower Murray River.

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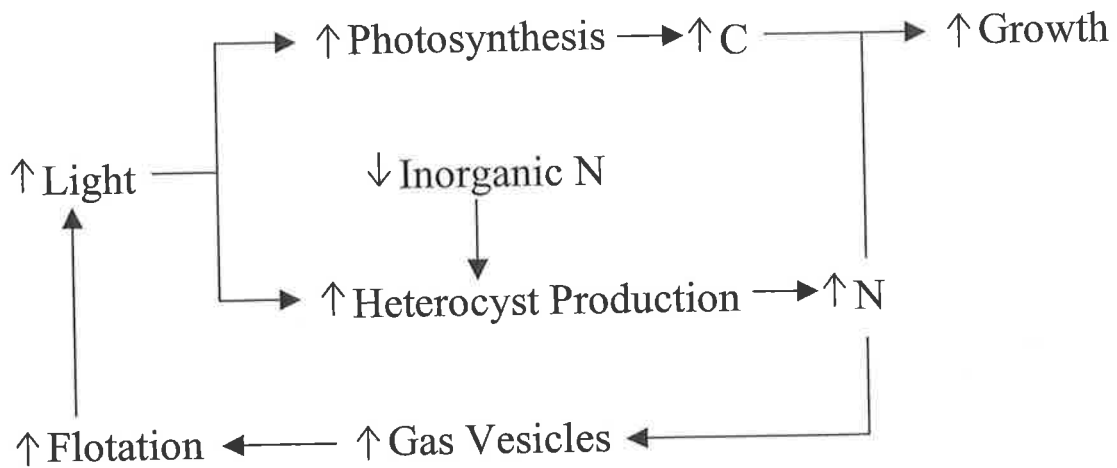
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AMENDMENTS TO THESIS

CHAPTER 1, Page 6, 2nd Paragraph

Add: "A difficulty with using the N:P ratio is that it does not provide a true indication of bioavailability. In general, the ratio is considered in terms of total amounts of nitrogen and phosphorus (TN:TP). This may be appropriate in clearwater studies where a significant amount of nutrient can be present in a useable form. However, in many systems, a significant proportion of the total nutrient pool may be biologically unavailable. For example, in northern Minnesota lakes, the TN:TP ratio (20:1) indicated phosphorus rather than nitrogen limitation, yet bioassays indicated that nitrogen was the main limiting nutrient (Axler *et al.* 1994). It was suggested that a significant amount of the TN pool in the lakes was comprised of refractory dissolved organic nitrogen, and thus unavailable for growth. A better relationship between phytoplankton biomass and nutrient availability was found in the lakes when using dissolved inorganic nitrogen concentrations. DIN:TP ratios > 4.0 indicated phosphorus limitation and ratios < 1.3 nitrogen limitation. At ratios between 1.3 and 4.0, phosphorus and nitrogen were co-limiting. Varis (1990) also analysed data (1971-1988) from Lake Tuusulanjärvi in Finland. Canonical correlation indicated that cyanobacterial abundance was strongly negatively correlated with dissolved inorganic N:P ratios and nitrate concentrations.

The measurement of dissolved inorganic phosphorus concentrations may further complicate the use of the N:P ratio to predict phytoplankton biomass. In standard analysis, raw water samples are filtered through a 0.45 μm membrane and the filterable reactive phosphorus (FRP) determined (Anon. 1995). However, recent studies have shown that a significant proportion of the FRP pool may be unavailable for growth, comprising only small amounts of orthophosphate (Hudson *et al.* 2000). Furthermore, the behaviour of phosphorus in turbid Australian freshwaters is different than would be expected from studies overseas (Oliver *et al.* 1993). Normally, suspended particulate matter has a high binding capacity and rapidly takes up orthophosphate (Fox 1993). However, in Australian waters, the concentration of phosphorus in the dissolved fraction is high and it is possible that natural organic material associated with the particulate matter causes a low sorption capacity (Hart *et al.* 1995). Furthermore, problems have been encountered through using the standard 0.45 μm membrane for filtering. In the Darling River, the concentration of "dissolved" FRP using a 0.45 μm membrane was up to 40 μg L^{-1} higher than the concentration in the < 0.003 μm fraction (Hart *et al.* 1995). Turbid systems also complicate the predictive power of N:P models as light limitation prevents the nutrient pool from being utilised to its maximum capacity (Smith

1990). Studies by Walker and Hillman (1982) and Cuker (1987) suggest that the presence of suspended clays can supersede phosphorus as the main factor organising community structure."

Axler, R.P., Rose, C., Tikkanen, C.A. 1994. Phytoplankton nutrient deficiency as related to atmospheric deposition in northern Minnesota acid-sensitive lakes. *Canadian Journal of Fisheries and Aquatic Science* **51**: 1281-1296.

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Smith, V.H. 1990. Nitrogen, phosphorus, and nitrogen fixation in lacustrine and estuarine ecosystems. *Limnology and Oceanography* **35**: 1852-1859.

Varis, O. 1990. Development of phytoplankton community in a restored eutrophic lake - a multivariate analysis. *Verhandlungen Internationale Vereinigung für Limnologie* **24**: 693-697.

CHAPTER 2, Page 34, 2nd Line

An alternative equation that may have been used to determine the appropriate incubation time for Fe-strips is the Langmuir isotherm and related kinetic expression:

$$d[\equiv XC]/dt = k \{([\equiv X][C]) - ([\equiv XC]/K_{ads})\}$$

where;

$\equiv X$ = concentration of the ferrihydrite surface on the Fe-strip

$[C]$ = unbound phosphate

$[\equiv XC]$ = concentration of the bound phosphate complex

K_{ads} = equilibrium constant for the sorption

k = rate constant

This equation assumes that there is a monolayer coverage of the ferrihydrite surface by the phosphate, that electrostatic interactions between the surface and phosphate are constant at all

phosphate loadings, and that all binding sites are equal in energy (Dr Mike Grace, personal communication, Monash University, Australia).

CHAPTER 3, Page 79, Line 8

Add: "The difference in the outcome of bioassays between the *in situ* diffusion chamber technique and the laboratory bioassay technique was likely to be a consequence of the amount of nutrients that the phytoplankton community had access to. With the *in situ* diffusion chamber bioassay, cells had access to a continual flux of nutrients from the river, whereas no fluxes were available in the laboratory bioassay technique. The difference in results highlights a potential problem with laboratory bioassays in that they may simply reflect a nutrient limitation that has been induced through experimentation rather than a real result. The diffusion chamber bioassay may provide a more accurate representation of nutrient availability in a natural system. This is further discussed in Chapter 7."

CHAPTER 4, 2nd Paragraph

Change to: "When persistent stratification breaks and fully mixed conditions resume, the abundance of *Anabaena* decreases, whereas *A. granulata* increases and becomes dominant once more. However, the reasons for the decline of *Anabaena* under mixed conditions are currently unknown. Possible reasons include; a) insufficient light availability, b) competition for nutrients with *A. granulata*, c) physical disruption of cells caused by turbulence, and d) dilution of cells caused through an increase in river flow and advection downstream, and the re-distribution of cells throughout the water column. Sherman *et al.* (1998) suggested that (d) is likely to be an important reason for the decline of *Anabaena* under mixed conditions. This chapter investigates the importance of (a) and (b)."

CHAPTER 4, Page 98

Replace Line 7 with: "These factors suggest that *Anabaena* has a high light requirement and Sherman *et al.* (1998) calculated that this genus is only present in conditions where cells receive > 10-13 mol m^{-2} day^{-1}."

CHAPTER 4, Page 98

Replace Line 12 with: "However, McCausland *et al.* (2002) recently demonstrated in laboratory microcosms that the growth efficiency (initial slope of the growth/irradiance curve) of *Aulacoseira* sp. was slightly lower (0.14 m^2 mol^{-1} photons) than *A. circinalis* (0.17 m^2 mol^{-1} photons)."

Typographic Errors

P07 ln 03 : replace "cyanophyin" with "cyanophycin"

P09 ln 23 : Z_{eu} represents the euphotic depth and is defined as the depth at which 1% of surface irradiance remains. Z_m represents the mixed depth and is defined as the depth of the surface mixed layer. The mixed depth is directly influenced by wind-generated tubulence and surface heat-fluxes (discussed later).

P19 ln 22 : replace "inorganic phosphorus" with "filterable reactive phosphorus"

P21 ln 17 : replace "μM" with "μm"

P22 ln 04 : diurnal stratification also occurs at flows between 4000 and 10 000 ML day^{-1} when wind speed is < 1.2 m s^{-1}

P30 ln 07 : replace "inorganic phosphorus" with "filtrable reactive phosphorus"

P32 ln 15 : replace with "As total phosphorus entered the diffusion chambers via a 0.45 μm membrane, this measurement was termed TP<0.45 μm"

P34 ln 16 : For *in situ* "flux" treatments, Fe-strips were incubated in 450 ml canisters with fourteen 0.7 cm diameter holes drilled at each end. The Fe-strips were therefore in direct contact with the river water.

P37 eqn 2 : replace equation (2) with "$G = (G_{max} \times [P]) / (K_s + [P])$"

P37 ln 15 : replace "FRP+DOP" with "TP<0.45 μm" throughout paragraph

P37 ln 22 : remove "or dissolved organic molecules that were too large to cross the membrane"

P38 ln 02 : replace "inorganic phosphorus" with "filtrable reactive phosphorus"

P39 ln 09 : remove "due to the theoretically faster diffusion of molecules towards the binding sites" and replace with "due to the higher number of phosphorus molecules that came into contact"

P39 ln 13 : replace "v" with "versus"

P41 ln 15 : remove "arbitrary units" throughout paragraph

P42 ln 07 : replace "FRP+DOP" with "TP<0.45 μm"

P42 ln 08 : replace "FRP+DOP" with "TP<0.45 μm"

P42 ln 15 : replace "μS h^{-1}" with "h^{-1}"

P42 ln 16 : replace "μS h^{-1}" with "h^{-1}"

P81 ln 17 : replace "but significantly less" with "but the increase in phytoplankton biomass was significantly less"

P105 ln 11: add "Respiration rates were determined from oxygen electrode measurements taken in the dark"

P107 ln 04: add "(cultures only exposed to light for half a day on the first day of experimentation)"

P138 ln 03: replace with "Incident irradiance was continually recorded using a data logger and PAR

    quantum sensor"

P140 ln 02: replace "The incubation period represented the number of cloudless days" with "It was

    assumed that the modelled incubation period was cloudless"

P163 ln 02: replace with "Z_m = mixed depth, m"

P163 ln 03: add "The vertical attenuation coefficient measured in the river a week prior to

    experimentation (February 2000) was 3.6 m^{-1}"

P163 ln 11: move "(Figure 6.1 c,d)" to end of sentence on line 13

P164 ln 21: replace with "Incident irradiance was continually recorded using a data logger and PAR

    quantum sensor"

P166 ln 19: replace "The incubation period represented the number of cloudless days" with "It was

    assumed that the modelled incubation period was cloudless"

P171 ln 18: add "R_d was set to 0 so that the maximum potential for bloom development could be

    assessed."

P174 ln 05: replace "deep" with "15 m"

P178 ln 08: replace with "The model of Sherman and Webster (1994) assumed a maximum growth

    and flotation rate of 1.0 day^{-1} and 3.6 m h^{-1}, respectively"

P192 ln 04: replace with "An increase in the heterocyst/vegetative cell ratio of *A. circinalis* in the

    lower Murray River indicated that dissolved nitrogen gas was being utilised"

P200 ln 10: replace "mg O_2 mg Chl a^{-1} E^{-1} m^2" with "mg O_2 mg Chl a^{-1} Es^{-1} m^2"

P200 ln 11: replace "mg O_2 mg Chl a^{-1} E^{-1} m^2" with "mg O_2 mg Chl a^{-1} Es^{-1} m^2"

P204 ln 01: replace "Although" with "However"

P204 ln 05: replace "Although" with "However"

P204 ln 18: replace "nmol $(10^6$ heterocysts$)^{-1}$ h^{-1}" with "fmol $heterocyst^{-1}$ h^{-1}"

P204 ln 22: replace "nmol $(10^6$ heterocysts$)^{-1}$ h^{-1}" with "fmol $heterocyst^{-1}$ h^{-1}"