Are nutrients the key driver in promoting dominance of toxic cyanobacterial blooms in a sub-tropical reservoir?

Amanda J. Posselt BSc. (Hons)

Griffith School of Environment
Science, Environment, Engineering and Technology
Griffith University

Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy

January 2009
For William and your future Brothers and Sisters
Statement of originality

The work presented in this thesis, to the best of my knowledge and belief, is original and my own work except as acknowledged in the text. This material has not been submitted either in whole or in part, for a degree at this or any other University.

Amanda J Posselt BSc. (Hons)
Abstract

Toxic cyanobacteria cause major problems, both for drinking and recreation, within water systems and bulk water storages, worldwide. Many investigations have been conducted to determine how, and why, they proliferate as well as why, and when, they produce toxins. A common assumption is that cyanobacteria grow in response to an increase in water column nutrient availability, but this is an oversimplification.

In a sub-tropical reservoir (L. Samsonvale, South East Queensland, Australia), the toxic cyanobacterium *Cylindrospermopsis raciborskii* has been dominating the phytoplankton community in the summer months for many years. The reason for this is unknown. Lake Samsonvale typically has relatively low phosphorus (P) concentrations, relatively high nitrogen (N) concentrations and *C. raciborskii* can grow without significant nutrient inputs from the catchment or point sources. The relatively high N concentrations in L. Samsonvale mean that it is unlikely to be a key nutrient in promoting dominance of *C. raciborskii*. The low phosphorus (in particular the phosphate) concentrations on the other hand may suggest a reason for *C. raciborskii* dominance in L. Samsonvale. Studies of a non-toxic strain of *C. raciborskii* originally isolated from the northern hemisphere found that a rapid phosphate uptake rate and high phosphorus storage capacity was contributing to its dominance in a phosphate-limited reservoir (Istvánovics et al. 2000).

The aim of this thesis was to characterise the relationship between phosphorus and *C. raciborskii* in L. Samsonvale. To achieve this, three levels of investigation were used: 1. Physiological studies at the species level; 2. Manipulative experiments at the phytoplankton community level; 3. Characterisation of *C. raciborskii* ecology at the whole of system level.

The relationship between *C. raciborskii* and phosphorus was studied using a multilevel approach. Knowledge gained from this allowed detailed investigation of
the relationship between the dominance of this species within the phytoplankton community of L. Samsonvale.

Using continuous culture experiments, the phosphate uptake and storage capacity of two toxic Australian strains of *C. raciborskii* was determined. One of these strains was isolated from the reservoir of interest, L. Samsonvale. P dependent growth rate and toxin production were also quantified. Both strains of *C. raciborskii* had a high maximum phosphate uptake rate (450 – 600 μmol P mg C\(^{-1}\) d\(^{-1}\)) with a relative low half saturation constant (0.64 μmol P L\(^{-1}\)). This study suggests that *C. raciborskii* is capable of taking full advantage of any available phosphate that may be introduced (such as run off) or regenerated within the phytoplankton/bacterial community. The P dependent growth rates were similar for both strains of *C. raciborskii* with a maximum growth rate at the lowest concentration of P tested (0.03 μmol P L\(^{-1}\)). Growth rates were lower overall than in other strains of *C. raciborskii*. When *C. raciborskii* cells were starved of P, they produced much more toxin than when they were grown in a nutrient sufficient environment. This indicates that toxin production may be related to a stress response.

Some phytoplankton have been shown to produce alkaline phosphatase. This enzyme cleaves phosphate from organically bound forms, targeting esters, which can be taken up and used by the cell. Since *C. raciborskii* appears to proliferate in phosphate limited systems, its potential to secrete this enzyme, and whether it was capable of growth with an organically bound source of phosphate, were investigated. Alkaline phosphatase activity was detected and *C. raciborskii* was found to be capable of multiplying in a culture media containing only an organic form of P (glucose-6-phosphate, G-6-P). However, the maximum growth rate was lower (~0.13 d\(^{-1}\)) when cells were grown in G-6-P compared to phosphate (~0.22 d\(^{-1}\)) The ability of *C. raciborskii* to use: 1. Organic P; 2. Rapidly utilise phosphate; and 3. Grow at a maximum rate at relatively low phosphate concentrations, are likely to make *C. raciborskii* a dominant competitor in phosphate-limited systems.
To determine whether *C. raciborskii* has a competitive advantage over other phytoplankton in the P-limited system of L. Samsonvale, *in situ* dialysis tube bioassays were used to test the phytoplankton response to nutrient addition. The dialysis tube bioassay is a novel approach aiming to minimise the confounding problem of artificial nutrient limitation associated with traditional closed bottle bioassays. Samples of the phytoplankton population were subjected to nutrient additions at four different times over a summer period, to test whether a change in phytoplankton species composition (with particular reference to *C. raciborskii*) could be seen after four days. In phytoplankton communities where the proportion of *C. raciborskii* was equal to, or above, 50% (biovolume), a statistically significant increase in *C. raciborskii* dominance occurred when phosphate was added as a daily spike at either of two concentrations (0.32 and 16 µM P). However, *C. raciborskii* dominance decreased when phosphate was constantly added in very high concentrations or when N and P are added together. From the bioassay experiments it can be inferred that *C. raciborskii* has a competitive advantage in L. Samsonvale due to its ability to rapidly take up phosphate. But, when the phosphate concentration is constantly high (>6.4 µmol P L\(^{-1}\)), *C. raciborskii* loses this competitive advantage. Analysis of historical data has shown that there is no correlation between periodic nutrient inputs (e.g. rainfall) and an increase in *C. raciborskii* dominance. The mechanisms by which *C. raciborskii* is accessing phosphate within L. Samsonvale were therefore examined.

One theory about how *C. raciborskii* is accessing phosphate in L. Samsonvale is that it comes from nutrient injections in the bottom waters caused by mixing the reservoir using artificial destratification. The concentration of dissolved organic phosphorus (DOP) may also provide *C. raciborskii* with available phosphate. To assess these two hypotheses, the nutrient concentration and phytoplankton cell concentrations throughout the water column were measured, both before and after artificial destratification. The DOP fraction was measured over a summer. Phosphate remained
below detection limits throughout the study, therefore the role of the destratifier in injecting phosphate into the water column was difficult to determine. A difference in phytoplankton distribution was noted with *C. raciborskii* being found at higher concentrations lower in the water column post destratification. In contrast, the other toxic species of cyanobacteria *Microcystis aeruginosa* present in substantial cell concentrations significantly decreased in cell concentrations after the destratifier was turned on. DOP was found to be a significant fraction (total mean 32%) of the total P in the water column of L. Samsonvale and may therefore provide an important source of P for *C. raciborskii* under low phosphate conditions.

This study has shown *C. raciborskii* has adapted to the low concentrations of P in L. Samsonvale to gain a competitive advantage. Reservoir management, particularly in relation to nutrient loads, should take this into account, as efforts to reduce P loads may not lead to a decrease in *C. raciborskii* cell number or phytoplankton dominance.
Acknowledgements

It has been a long and bumpy ride and the completion of this thesis would not have been possible without the help and support from a number of people. Firstly to my supervisors, Dr. Michele Burford and A/Prof. Glen Shaw, we’ve had our moments, well you patiently stood by while I had mine but we got there in the end. I will be forever grateful for your patience and guidance over the past 5 years.

I would like to acknowledge my industry partner SEQ Water. Particularly the following people for their help with data acquisition, field sampling or interpretation of results; Suzi Johnson, Phill Orr, James Udy, Ben, Simon, Andrew, Lewis and Jason.

There are many colleagues from the Australian Rivers Institute who have joined me on this journey and I would like to thank them all for sharing the beers, the tears and the cheers along the way. Particularly I would like to thank Susie Green, Andrew Cook, Priya Muhid, James Fawcett, Pat Laceby, Deslie Smith, Lacey Shaw, Cath Leigh, Jason Kerr, Dave Roberts, Emily Saeck, Jeff Argo

I would like to thank the following organisations for their financial assistance to attend conferences: Australian Society for Limonlogy (ASL Annual Conference, Albury 2006); the Australian Water Association (Enviro, Melbourne 2006) and; the Australian Rivers Institute (ICTC, Brazil 2007).

I would like to acknowledge the comments and recommendations from two anonymous reviewers.

I would also like to acknowledge the following people for their technical assistance;

Rene Diocares (Australian Rivers Institute) for help with analysing cellular carbon and phosphorus.

The late Dan Wruck (Queensland Health) for nutrient analysis.

Geoff Eaglesham (Queensland Health) for toxin analysis.

Dr. Glen McCgreggor (Department of Natural Resources and Water) for suppling the NPD Strain of C. raciborskii.

Dave Reubart (EnTox) for suppling the AWT/205 Strain of C. raciborskii (originally isolated by Peter Hawkins).

And lastly I would like to thank my family, who have stood by me and supported me until the end, always believing in me even if I couldn’t do it myself. In particular my Mum, Carol and my Husband Matt. Mum you’re my rock and my role model, I couldn’t have done it without you. Matt, you bore the brunt of the Amanda storm for the entirety of this thesis, and you’re still around. I love you.
Publications arising from this thesis


**Oral presentations**

Australian Water Association Young Water Professionals National Conference (February 2008) Understanding the relationship between nutrients and toxic cyanobacterial blooms in SEQ. Brisbane, Queensland

Australian Society for Limnology National Conference (September 2006) *Cylindrospermopsis raciborskii* and low phosphate combine for reservoir domination! Albury, N.S.W

**Poster presentations**

International Conference on Toxic Cyanobacteria (August 2007) Phosphorus addition affects *C. raciborskii* dominance within the phytoplankton community of a subtropical reservoir. Rio de Janeiro, Brazil
Contents

STATEMENT OF ORIGINALITY ....................................................... I

LIST OF TABLES ........................................................................... XVI

LIST OF ABBREVIATIONS .............................................................. XVIII

CHAPTER 1. INTRODUCTION .......................................................... 1
  1.1 Evolution of the Research Question ......................................... 1
  1.2 Toxic Cyanobacteria ................................................................ 1
    1.2.1 Cylindrospermopsis raciborskii ....................................... 3
  1.3 Factors promoting growth of Cylindrospermopsis raciborskii ........ 6
    1.3.1 Temperature .................................................................. 6
    1.3.2 Light ............................................................................ 7
    1.3.3 Carbon ......................................................................... 9
    1.3.4 Nitrogen ...................................................................... 9
    1.3.5 Phosphorus .................................................................. 11
    1.3.5.1 Current knowledge of the relationship between phosphorus and Cylindrospermopsis raciborskii .................................................. 14
  1.3.6 Other physico-chemical factors ......................................... 15
  1.4 Aims and Objectives ............................................................. 18

CHAPTER 2. EXPERIMENTAL DESIGN – THE THREE LEVELLED APPROACH .............................................. 19
  2.1 Whole System (Lake) ............................................................ 20
    2.1.1 Lake Samsonvale ......................................................... 21
  2.2 Enclosure (in situ bioassays) .................................................. 23
    2.2.1 Assessment of dialysis tube bioassays as a method for determining nutrient pulse effects within the phytoplankton community ......... 24
  2.3 Organism (cell culture) .......................................................... 26
  2.4 The advantage of a multi-levelled approach .............................. 28

CHAPTER 3. PROFILING CYLINDROSPERMOPSIS RACIBORSKII IN LAKE SAMSONVALE .................................................... 29
  3.1 Introduction ........................................................................ 29
  3.2 Methods ............................................................................ 29
    3.2.1 Profiling Cylindrospermopsis raciborskii and nutrients before and during artificial destratification in Lake Samsonvale ......... 29
    3.2.1.1 Analysis of total and dissolved nutrients ...................... 30
    3.2.1.2 Counting phytoplankton cell numbers using microscopy .... 31
  3.2.2 Profiling a Cylindrospermopsis raciborskii summer bloom in Lake Samsonvale ................................................................. 32
  3.2.3 Phytoplankton phosphate uptake and regeneration in Lake Samsonvale ... 33
  3.2.4 Analysis of general physico-chemical water quality parameters .... 38
  3.2.5 Statistics ....................................................................... 39
  3.3 Results ............................................................................... 39
    3.3.1 Profiling Cylindrospermopsis raciborskii and phosphorus in relation to artificial destratification ......................................... 39
CHAPTER 4. THE ROLE OF PHOSPHATE IN PROMOTING
DOMINANCE OF CYLINDROSPERMOPSIS RACIBORSKII
WITHIN THE PHYTOPLANKTON COMMUNITY....................... 66
4.1 Introduction........................................................................ 66
4.2 Methods............................................................................. 67
4.3 Results.............................................................................. 70
4.4 Discussion......................................................................... 79
4.5 Conclusions..................................................................... 83

CHAPTER 5. UNDERSTANDING THE RELATIONSHIP
BETWEEN CYLINDROSPERMOPSIS RACIBORSKII AND
PHOSPHORUS IN CULTURE..................................................... 84
5.1 Introduction....................................................................... 84
5.2 Methods........................................................................... 87
  5.2.1 Batch Culture Experiments ........................................ 87
     5.2.1.1 Calculating phosphate uptake .............................. 90
  5.2.2 Through-flow Culture Experiments ......................... 91
     5.2.2.1 Analysis of toxin .................................................. 93
     5.2.2.2 Analysis of alkaline phosphatase activity .......... 94
5.3 Results............................................................................. 96
  5.3.1 Batch Culture Experiments ....................................... 96
     5.3.1.1 Phosphorus uptake rates .................................. 99
  5.3.2 Through-flow Culture Experiments ....................... 101
     5.3.2.1 Phosphorus/Phosphate starvation ..................... 101
     5.3.2.2 Growth rate with increasing concentrations of phosphorus.... 102
     5.3.2.3 Toxin production ............................................ 105
5.4 Discussion...................................................................... 110
  5.4.1 Phosphorus/Phosphate dependant growth rate ........... 110
  5.4.2 Phosphate uptake ....................................................... 112
  5.4.3 Phosphorus and toxin ............................................... 113
  5.4.4. Dissolved Organic Phosphorus and Alkaline Phosphatase...... 115
CHAPTER 6 CONCLUSIONS – IS PHOSPHORUS A KEY DRIVER IN PROMOTING BLOOMS OF CYLINDROSPERMOPSIS RACIBORSKII IN LAKE SAMSONVALE? .................................................................................................................. 120

6.1 The relationship between Cylindrospermopsis raciborskii and phosphorus – summary of the main findings .................................................................................................................. 120

6.1.1 The relationship between Cylindrospermopsis raciborskii and phosphorus .................................................................................................................. 121

6.2 Potential management implications ........................................................................................................ 123

6.3 Relevance for local and international reservoirs .................................................................................. 124

6.4 Suggested further work ......................................................................................................................... 125

REFERENCES ....................................................................................................................................... 128
List of Figures

Figure 1.1 *Cylindrospermopsis raciborskii* filament showing terminal heterocyst............ 3

Figure 1.2 Factors that can potentially affect *C. raciborskii* dominance in L. Samsonvale. .......................................................... 6

Figure 2.1 Location of L. Samsonvale within Australia and south east Queensland indicating two sampling and experimental sites A and B as well as the destratification unit. ........................................................................... 22

Figure 2.2 Diagram of dissolved material flux in the closed bottle and dialysis tube bags........................................................................... 24

Figure 2.3 The photosynthetic activity of the phytoplankton community in L. Samsonvale under different conditions: within closed bottles (bottle), within dialysis tubing (tube) and in ambient conditions (ambient)........................... 26

Figure 3.1 Map showing location of L. Borumba in relation to L. Samsonvale and Brisbane ........................................................................... 34

Figure 3.2 Mean (±SD) concentrations of a) *C. raciborskii* (cells mL⁻¹), b) *M. aeruginosa* (cells mL⁻¹), c) NO₃ (µM) and d) NH₄ (µM) at site A before (5 October) and after (17 October) the destratification system was turned on in L. Samsonvale........ 40

Figure 3.3 Phytoplankton composition (a) cell abundances (cells mL⁻¹) and (b) biovolume (%) of major phytoplankton genera during the summer 2007/08 in L. Samsonvale. ................................................................................................. 42

Figure 3.4 Mean (±SD) P fractions (µM) in L. Samsonvale over the summer 2007/08 PO₄: phosphate DOP: Dissolved Organic phosphorus, PP: particulate phosphorus (Total P minus DOP and PO₄). Method detection limit 0.064 µM....................................................... 43

Figure 3.5 Composition of the N fraction (µM) in L. Samsonvale over the summer 2007/08. DIN: Dissolved Inorganic Nitrogen, DON: Dissolved Organic Nitrogen, PN: particulate nitrogen (Total N minus DON and DIN). Method detection limit 0.064 µM........................................................................................................... 44

Figure 3.6 Molar N:P ratio for surface (0-3 m) and bottom waters in L. Samsonvale over the summer 2007/08. N:P ratio calculated from total N and P concentrations........ 44

Figure 3.7 Mean daily (24 h from midnight to midnight) temperatures (°C) at L. Samsonvale during the summer 2007/08. ........................................................................................................... 46

Figure 3.8 Daily rainfall (mm) and lake level (% full capacity) of L. Samsonvale during the summer 2007/08. ........................................................................................................... 46

Figure 3.9 Concentrations of silicate, manganese and iron in L. Samsonvale during the summer 2007/08. Samples taken from site A (Fig 2.2). ........................................................................................................... 47
Figure 3.10 Mean daily solar radiation at L. Samsonvale during the summer 2007/08. Mean taken from sunrise to sunset. The dot (9 January 2008) indicates when C. raciborskii cell abundances were highest.............................................................. 47

Figure 3.11 Mean (± SD) dissolved oxygen concentrations (% saturation and mg L⁻¹) at approximately 10am in L. Samsonvale during the summer 2007/08. Samples taken from site A (Fig 2.2). ................................................................. 48

Figure 3.12 Phosphate uptake rates of L. Samsonvale phytoplankton community calculated from 19 February and 5 March 2008 data .............................................................. 51

Figure 3.13 Mean concentrations (±SD) of ³²P within the cells of L. Samsonvale lake water after a spike of ³³P (1.3 pM). a) Sample collected on 19 February 2008, b) Sample collected on 5 March 2008. Linear curves fitted to the initial increase in phosphate concentration ................................................................. 52

Figure 3.14 Mean concentrations (±SD) of ³³P within the cells of L. Borumba water after a spike of ³³P (1.3 pM). a) Sample collected on 9 March 2008, b) Sample collected on 19 March 2008 Linear curves fitted to the initial increase in phosphate concentration ................................................................. 52

Figure 3.15 Concentrations of ³³P within the cells of L. Samsonvale lake water after a spike of ³¹P (3.2 µM) as indicated by the arrow. a) Sample collected on 19 February 2008, b) Sample collected on 5 March 2008 .............................................................. 53

Figure 3.16 Concentrations of ³³P within the cells of L. Borumba water after a spike of ³¹P (3.2 µM) as indicated by the arrow. a) Sample collected on 9 March 2008, b) Sample collected on 19 March 2008 .............................................................. 54

Figure 3.17 Modified from Burford et al. (2006). N and P pools (T) and phytoplankton demand (T year⁻¹) in the water column in L. Samsonvale, based on measurements at the reservoir wall site. Pink numbers represent calculations from current study, black numbers represent calculations from Burford et al. 2006. NH₄, ammonium; NO₃, nitrate; PN, particulate nitrogen; DON, dissolved organic nitrogen; TN, total nitrogen; TP, total phosphorus; PO₄, phosphate .............................................................. 61

Figure 3.18 Sources of P in L. Samsonvale showing measured phosphate uptake and regeneration rates and water column P concentrations with potential concentrations from catchment inflow (Harris and Baxter 1996) and sediment (Longmore 2001) DOP: Dissolved Organic Phosphorus, PO₄: Phosphate, PP: Particulate Phosphorus...... 62

Figure 4.1 Method for securing the dialysis tube bioassays 2.5 m below the surface in L. Samsonvale .............................................................. 68

Figure 4.2 Sampling the dialysis tube bags .............................................................. 69

Figure 4.3. Constant release phosphate concentrations in both laboratory and field experiments. Laboratory = release rate from the constant release pellet (concentration within the dialysis tube) in DI water under laboratory conditions. Bioassay = release rate from the constant release pellet (concentration within the dialysis tube) in lake
water under field conditions. Bioassay line shows diffusion out of dialysis tubes under field conditions...

Figure 4.4. Concentration of phosphate taken up within the dialysis tube bags. Black line indicates mean and grey shading indicates 1 SD from the mean.

Figure 4.5. Concentration of the daily nutrient pulse (μM) in situ in dialysis tube bioassays over time showing relative uptake and regeneration of N and P. The 0.32 μM phosphate concentration was below detection limit after 4 min.

Figure 4.6. Composition of the a. cell abundances (cells mL⁻¹) and b. biovolume (mm³ L⁻¹) of the phytoplankton community in L. Samsonvale at time zero of each experiment month.

Figure 4.7. Percent change in *C. raciborskii* dominance (±SD) (percentage of the total phytoplankton biovolume) relative to time zero (T0). Results show change after 4 d in *C. raciborskii* dominance within nutrient bioassays in L. Samsonvale. C: control, N+P: daily pulse of ammoniacal N (7.1 μM N) and phosphate (0.3 μM P), DS LP: daily pulse of low phosphate (0.3μM P), DS HP: daily pulse of high phosphate (16 μM P), CRP: constant release phosphate (> 6.4 μM) ns: not significant, *p<0.05, **p<0.01, ***p<0.005. Statistics represent change in *C. raciborskii* dominance after 96 h from time 0.

Figure 4.8. Change in *C. raciborskii* and other phytoplankton biovolume (mm³ L⁻¹ ±SD) in in-situ bioassays with added nutrients at time zero (T0) and after 96 h. C: control, N+P: daily pulse of 7.1 μM ammoniacal N and 0.3 μM P, DS LP: daily pulse of low phosphorus (0.3 μM P), DS HP: daily pulse of high phosphorus (16 μM P), CRP: constant release phosphate (> 6.4 μM) ns: not significant, *:p<0.05, **:p<0.01, ***:p<0.005. Statistics represent change in biovolume after 96 h from time 0.

Figure 4.9. Cell abundance (Log cells mL⁻¹ ±SD) for *C. raciborskii* in L. Samsonvale at the Dam wall site (near the bioassays) over the 2006/07 Summer. Shaded bars represent time and duration of bioassay.

Figure 5.1. Standard curve for AWT/205 used to calculate cell abundances from optical density.

Figure 5.2. Standard curve for NPD used to calculate cell abundances from optical density.

Figure 5.3 Schematic diagram of through-flow culture set up.

Figure 5.4 Schematic diagram of the 4-methylumbelliferone method for alkaline phosphatase quantification.

Figure 5.5 Standard curve of measured fluorescence of Shrimp Alkaline Phosphatase (SAP) units in 4-MUP solution after 6 h incubations at 25°C.
Figure 5.6. AWT/205 batch culture growth experiments showing the effect of P source (G-6-P and DIP), P concentration (0, 0.032, 0.16, 0.64 and 16 µM) and pre-condition (P-starved and non-starved) on mean (± SD) cell concentrations over time. ... 97

Figure 5.7. NPD batch culture growth experiments testing P source (G-6-P and DIP), P concentration (0, 0.032, 0.16, 0.64 and 16 µM) and pre-condition (P-starved and non-starved) on mean (± SD) cell concentrations over time. .............................................. 98

Figure 5.8. Growth rates of AWT/205 and NPD strains with different P concentrations (0, 0.032, 0.16, 0.64 and 16 µM), sources (G-6-P and DIP) and pre-condition (P-starved and non P-starved). .......................................................... 99

Figure 5.9. Phosphate uptake rate (pmol P cell⁻¹ min⁻¹) using radioisotope ³³P, of C. raciborskii (AWT/205 Strain) .............................................................................................................. 100

Figure 5.10. Phosphate uptake rate (pmol P cell⁻¹ min⁻¹) using radioisotope ³³P, of C. raciborskii (NPD Strain) .................................................................................................................. 100

Figure 5.11 Change in (a) cell abundances of C. raciborskii and (b) Photosynthetic yield during P starvation. On day 0 the P concentration entering the through-flow culture went from ca. 48 to 0 µM. .......................................................... 102

Figure 5.12. The C. raciborskii (strain NPD) cell abundance with changing concentrations of a) G-6-P and b) DIP in the inflow of a through flow culture. Shaded bars represent concentration of P (as DIP or G-6-P) in the inflow of the through-flow culture. .......................................................... 103

Figure 5.13. The C. raciborskii (strain AWT/205) cell abundance with changing concentrations of a) G-6-P and b) DIP in the inflow of a through flow culture. Shaded bars represent concentration of P (as DIP or G-6-P) in the inflow of the through-flow culture. .......................................................... 103

Figure 5.14 The concentration of particulate P with changing concentrations of inflow P within a through-flow culture.5.3.2.3 Alkaline phosphatase activity ...................................... 104

Figure 5.15 Specific alkaline phosphatase activity (fM phosphate cell⁻¹ h⁻¹) with increasing concentrations of Glucose-6-Phosphate for two strains for C. raciborskii (NPD and AWT/205). .................................................................................. 105

Figure 5.16. Total toxin (CYN and deoxy-CYN) concentrations (mg CYN mg C⁻¹) with increasing P concentrations (phosphate µM) in two strains of C. raciborskii (NPD and AWT/205) .................................................................................. 106

Figure 5.17. Intra-cellular and extra-cellular CYN concentrations (µg CYN mg C⁻¹) in two strains of C. raciborskii at increasing concentrations of P (phosphate µM) ............. 106

Figure 5.18. Total toxin (CYN and deoxy-CYN) concentrations (mg CYN mg C⁻¹) with increasing P concentrations (G-6-P µM) in two strains of C. raciborskii (NPD and AWT/205) ........................................................................... 107
Figure 5.19. Intra-cellular and extra-cellular CYN concentrations (µg CYN mg C\(^{-1}\)) in two strains of *C. raciborskii* at increasing concentrations of phosphorus (G-6-P µM). 108

Figure 5.20. Total toxin (CYN + deoxy-CYN) concentrations (±SD) produced by *C. raciborskii* with increasing concentrations of phosphate and Glucose-6-Phosphate. 109

Figure 5.21. Percentage of CYN (±SD) within a *C. raciborskii* cell compared to extra-cellular concentrations at increasing phosphate and Glucose-6-Phosphate. 109
# List of Tables

Table 2.1. Hierarchy of scale for phytoplankton studies. Adapted from Garnett (2005) originally from Hecky et al. (1988) ................................................................. 20

Table 3.1 Amount of $^{32}$P and $^{31}$P and percentage of $^{33}$P in phosphate uptake ............... 35

Table 3.2. Experimental design of regeneration rate experiments using phytoplankton populations from Lakes Borumba and Samsonvale. Y = sample taken. Time 0 = just before ‘cold’ $^{31}$P was added ......................................................................................... 37

Table 3.3 Water quality parameters integrated from surface to 8 m in L. Samsonvale during the summer 2007/08 .............................................................................. 48

Table 3.4 Correlation coefficients for parameters potentially affecting C. raciborskii biovolume dominance in Lake Samsonvale .................................................. 49

Table 3.5. Comparison of mean water quality data and phytoplankton cell abundance from L. Samsonvale and L. Borumba ................................................................. 50

Table 3.6 Phosphate uptake rates of phytoplankton communities in Lake Samsonvale and Lake Borumba .............................................................................................. 52

Table 4.1 Nutrient enrichment in dialysis tube bioassays .............................................................. 69

Table 4.2. Ambient physico-chemical parameters and photosynthetic yield for the four experiment days during December 2006, January, February and March 2007 (mean ± SD). ................................................................................................................................. 71

Table 4.3. Dissolved inorganic nitrogen:phosphate (DIN:phosphate) molar ratios (in treatments) after pulses of nutrients were added to the bioassays (Mean ± SD, after 4 d). Values represent measured concentrations plus theoretical added pulse at time of addition. .............................................................................................................. 71

Table 5.1. Summary of the batch culture experiments .................................................................. 89

Table 5.2 Experimental design .................................................................................................. 91

Table 5.3 Sampling schedule in days from commencement and P concentration (μM) added to the four through-flow cultures (A, B, C, and D). Y = a sample was taken ........ 93

Table 5.4. Mean (±SD) Parameters of C. raciborskii during P starvation. 9 days after inflow was changed from excess phosphate concentration (48 µM) to 0. Dilution rate = approximately 0.2 d$^{-1}$ (n=6). Significance levels - *$P<0.05$ **$P>0.01$ ***$P>0.001$ . 102

Table 5.5 Maximum growth rates of cyanobacteria in culture in this study compared to other studies. $\mu$max d$^{-1}$:maximum growth rate per day. M/F: Marine or Freshwater Species ............................................. 111
Table 5.6 Phosphate uptake rates of phytoplankton. Adapted from Reynolds 2007 and Istávnovics et al. 2000. *assumes C content is 50% dry weight. ^ assumes 431 fg C cell$^{-1}$

Table 6.1 The effect of phosphorus source and concentration on C. raciborskii growth characteristics and toxin production

Table 6.2 Summary of the conclusions of the study
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIP</td>
<td>Dissolved Inorganic Phosphorus</td>
</tr>
<tr>
<td>G-6-P</td>
<td>Glucose-6-phosphate</td>
</tr>
<tr>
<td>DOP</td>
<td>Dissolved Organic Phosphorus</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>DS LP</td>
<td>Daily Spike Low Phosphate (10 µg L(^{-1}))</td>
</tr>
<tr>
<td>DS HP</td>
<td>Daily Spike High Phosphate (500 µg L(^{-1}))</td>
</tr>
<tr>
<td>CRP</td>
<td>Constant Release Phosphate</td>
</tr>
<tr>
<td>N+P</td>
<td>Nitrogen + Phosphorus</td>
</tr>
<tr>
<td>P(_{\text{cell}})(\mu=0)</td>
<td>Mass of P within the cell when growth rate = 0 (µg P mg C(^{-1}))</td>
</tr>
<tr>
<td>P(_{\text{cell}})(\mu=\text{max})</td>
<td>Mass of P within the cell when growth rate = maximum (µg P mg C(^{-1}))</td>
</tr>
<tr>
<td>R(_{\text{max}})</td>
<td>Maximum P uptake rate (µg P mg L(^{-1}) d(^{-1}))</td>
</tr>
<tr>
<td>R(_{\text{C,max}})</td>
<td>Maximum P uptake rate cell normalised (µg P mg C(^{-1}) d(^{-1}))</td>
</tr>
<tr>
<td>(\mu)</td>
<td>Growth rate (d(^{-1}))</td>
</tr>
<tr>
<td>K(_R)</td>
<td>Half saturation constant of P uptake (µM P)</td>
</tr>
<tr>
<td>P(_{\text{cell}})(\text{ex})</td>
<td>Mass of P within the cell at P saturation (16 µM P) (µg P mg C(^{-1}))</td>
</tr>
<tr>
<td>NH(_4)</td>
<td>Ammonia</td>
</tr>
<tr>
<td>NO(_X)</td>
<td>Nitrates and Nitrites</td>
</tr>
<tr>
<td>L</td>
<td>Lake</td>
</tr>
</tbody>
</table>