The role of zooplankton in cyanobacteria bloom development in Australian reservoirs

Ying Hong

B.SC & M.SC (Ecology)

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, Plant Functional Biology and Climate Change Cluster,

School of Environment

University of Technology, Sydney

June 2013

CERTIFICATE

I certify that the work presented in this thesis and the research to which it pertains, are the product of my own work and to the best of my knowledge, original. Any quotations ideas or work conducted by others published or otherwise, are fully acknowledged in accordance with the standard referencing practices of the discipline. Co-authors of published, submitted papers or articles in preparation have been acknowledged for their contributions and for each publication herein, my personal contribution and role clearly described. Furthermore, I certify that this has not previously been submitted, in completely or in part, for a degree at this or any other university.

Production Note:
Signature removed prior to publication.

Signed ___________________________

Ying Hong (Ph D Candidate)

ACKNOWLEDGEMENTS

I would first like to acknowledge and immensely thank UTS International Research Scholarship, seqwater and the Plant Functional Biology and Climate Change Cluster at UTS for supporting my studies with both finances and materials during the past three and half years.

I want to express my gratitude to my principle supervisor, Dr. Martina Doblin, for giving me the opportunity to work with her and for her unreserved and committed guidance. Because of her dedicated assistance, I was able to come this far. My sincere thanks to Dr. Peter Ralph, my co-supervisor, who directed my research projects. Additionally, much appreciation is due to my co-supervisor, Dr. Michele Burford, for always being available to answer my experimental and field study questions.

I am also very grateful to Dr. Tsuyoshi Kobayashi at NSW Office of Environment and Heritage for his assistance with zooplankton taxonomy and experimental design. Thank you to Dr. Jessica Hill at UTS and Timothy Davis at Griffith University for their guidance in molecular techniques and to Ann Chuang at seqwater for introducing me to the world of zooplankton. Further, Prof. Iain Suthers and Dr. Jason Everett provided me with support in zooplankton analysis with the OPC at UNSW, and Dr. Ellen Van Donk and Dr. Steven Declerck shared with me their time, inspiring discussions and their experiments when I visited Netherlands Institute of Ecology; thank you all.

This research would not have been possible without the outstanding help I received in data and fieldwork. Thanks to B. Reynolds and D. Gale at seqwater for retrieving historical data to aid my understanding of research questions. I owe a very special thanks to I. Taylor, J. Singleton, S. Slamet and C. Buckley for always driving the boat and helping me with the Manly Dam collections for 2 consecutive years. Additionally, many thanks go to Stephen Faggotter, Matthew Whittle, and Matthew Prentice at Griffith University for their assistance with the Wivenhoe Dam field collections.

I am indebted to my family and friends, especially my husband and son, for standing by me and supporting me during these four years. Also, to all the brothers and sisters of Living Water Chinese Congregational Church: thank you for your consistent prayers and words of encouragement, especially thanks to Jason Zhang for his art work for conceptual models in Chapter 6.

Lastly but above all I would like to thank my God. Had it been without His help and His will I could have done nothing.

SUMMARY

Cyanobacteria occupy diverse aquatic habitats and their ecological success is expected to increase under predicted future climate scenarios. Managing cyanobacteria abundance in freshwaters is therefore critical for reducing risks to human and animal health. One species that is currently undergoing range expansion from subtropical to temperate habitats is *Cylindrospermopsis raciborskii*. *C. raciborskii* is ecologically successful because of its (1) competitive nutrient acquisition and storage mechanisms (e.g. high affinity for phosphorus (P) and ammonium, high P-storage capacity); (2) wide thermal tolerance, superior shade tolerance and buoyancy regulation; (Briand et al. 2002) and (3) resistance to grazing. To date, research to understand the formation of *C. raciborskii* blooms and toxicity have mostly focused on environmental factors, but the importance of food web interactions in regulating blooms has been little investigated. In particular, there is a need to examine these foodweb interactions in subtropical systems in the Southern Hemisphere because much of the current understanding about zooplankton-cyanobacteria interactions comes from temperate systems dominated by large-bodied cladocerans. Given that warmer subtropical systems are dominated by copepods and smaller-bodied individuals, it is likely that interactions between zooplankton and phytoplankton have different outcomes for cyanobacterial bloom formation.

To understand the mechanisms of toxic cyanobacterium *C. raciborskii* bloom formation in subtropical oligotrophic Australian lakes, a series of investigations were undertaken across multiple spatial and temporal scales to test the hypothesis that *C. raciborskii* growth is facilitated by meso-zooplankton. Specifically, small-scale laboratory experiments (~100 ml) examined zooplankton grazing and tested whether copepods avoid consumption of *C. raciborskii* under food saturating conditions (Chapter 2). Both the direct (grazing) and indirect (nutrient regeneration) effects of zooplankton on *C. raciborskii* were further examined in laboratory experiments (Chapter 3). These laboratory experiments were then scaled up to mesocosms (~500 litres), where *in situ C. raciborskii* growth was examined under different treatments (control, 1x and 5x ambient zooplankton abundance, 5x ambient zooplankton

abundance + inorganic P) (Chapter 4). Comparisons between zooplankton populations were also made at the reservoir scale, testing to see whether lakes experiencing *C. raciborskii* blooms had different zooplankton biomass, size structure and functional group composition compared to lakes that do not experience blooms (Chapter 5).

In Chapter 2, the hypothesis that copepod consumers discriminate against *C. raciborskii* was tested. Experiments were designed based on observed seasonal variation in food quantity and quality for zooplankton in subtropical Australian lakes and reservoirs, and tested whether clearance rates were dependent on the P-content of prey, the proportion of *C. raciborskii* present and the previous feeding history of zooplankton. The results indicated that the clearance rates of copepods on *C. raciborskii* were 2-4 times lower than that of a cladoceran *Ceriodaphnia* sp. when both grazers had prey choice. The copepod *Boeckella* sp. was found to select against *C. raciborskii* when alternative food was abundant, but selectivity declined when animals had been kept in low food conditions for 2-12 hours before experimentation. The clearance rates of *Boeckella* sp. on two toxic *C. raciborskii* strains were significantly lower than on a non-toxic strain. Clearance rates were also significantly lower on *C. raciborskii* with low cellular P content and when present at >5% relative abundance amongst natural phytoplankton assemblages. Together these results suggest that copepods largely avoid consumption of *C. raciborskii.*

In Chapter 3, the impact of zooplankton nutrient regeneration on *C. raciborskii* growth was evaluated. Indirect effects of zooplankton interactions may be relatively important seasonally when dissolved nutrient concentrations are low. Dialysis experiments were designed to simultaneously test the direct (grazing) and indirect effects (nutrient regeneration) of zooplankton-algal interactions, enabling zooplankton to access food outside the dialysis tubing, and for zooplankton-derived nutrients to be accessible to algae inside the tubing. Controls with no zooplankton were also set up to account for nutrient contributions from algal prey. Zooplankton-derived nutrients alleviated P-limitation of *C. raciborskii* inside the dialysis tubes and stimulated growth. Furthermore, *C. raciborskii* growth was favoured above a green algal competitor when both algae were in dialysis tubes, indicating *C. raciborskii* is more efficient at taking up P recycled by zooplankton. Outside the dialysis bags, zooplankton grazed a green alga in preference to *C. raciborskii* and selectively consumed P-replete cells. *C. raciborskii* growth was therefore affected both directly and indirectly by zooplankton, suggesting that foodweb interactions can facilitate blooms of this cyanobacterium.

In Chapter 4, zooplankton regulation of *C. raciborskii* dominance in a natural phytoplankton community was tested at a larger scale using mesocosms deployed in a subtropical reservoir. Laboratory studies often cannot account for diversity of natural assemblages, so treatments were set up to examine *C. raciborskii* growth under different zooplankton densities and P loading. To the best of our knowledge, this is the first field experiment to promote *C. raciborskii* through zooplankton manipulation. Zooplankton enrichment resulted in an increase in *C. raciborskii* relative abundance from 15% to 37% after four days. Simultaneously, elevated zooplankton lowered the C:P ratio of phytoplankton, supporting the notion that copepods tend to alleviate P limitation in the environment.

The generality of zooplankton-cyanobacteria interactions were examined in Chapter 5, which describes a survey of 15 subtropical reservoirs. Reservoirs were split into two groups (those experiencing *C. raciborskii* blooms and those that don't), and their zooplankton biomass, size structure and functional group composition were examined. The survey was carried out in both the wet and dry season to capture seasonal variations of phytoplankton and zooplankton communities and associated environmental variables. It was expected that *C. raciborskii* presence would be positively correlated to copepod abundance and negatively correlated to particulate N:P ratios. Ecological stoichiometry predicts that zooplankton with different body N:P content will differ in their relative rate of recycling of N and P. Copepods have low P content thus recycle nutrients with low N:P ratio into the environment. The survey demonstrated that reservoirs experiencing *C. raciborskii* blooms had a greater abundance of copepods compared to cladocerans, and a smaller proportion of juveniles. The correlation between environmental factors and *C. raciborskii* presence/absence was not statistically significant, but copepod abundance was negatively correlated to particulate N:P.

Together, these results suggest that *C. raciborskii* is most likely facilitated through a planktonic foodweb subsidy in copepod-dominated subtropical oligotrophic lakes, whereby copepods consume other algae in preference to *C. raciborskii* when alternative algae are abundant, then regenerate nutrients that are then rapidly taken up by low P-adapted *C. raciborskii*. In terms of management implications, this thesis has demonstrated that biomanipulation by increasing zooplankton abundance in reservoirs of subtropical Queensland where calanoid copepods are dominant would not be very effective. Based on the data collected and the major findings, recommendations are made for sustainable management of Australian subtropical reservoirs.

Table of Contents