The role of zooplankton in cyanobacteria bloom development in Australian reservoirs

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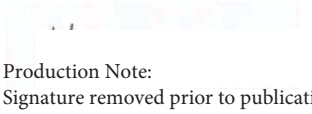
CERTIFICATE

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.

Signed 

Ying Hong (Ph D Candidate)

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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.

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CHAPTER 1

Introduction and thesis outline

Reservoirs support Australia's primary industries, supply essential drinking water for human and animal consumption, and provide significant recreational amenity. Blue-green algae (referred to as cyanobacteria) bloom recurrently in these water systems and threaten the entire spectrum of users. In particular, toxic algae cause substantial risk to human and animal health. For water suppliers, algal blooms cause a significant increase in water treatment costs (Atech, 2000). Limnologists have therefore been preoccupied with the processes leading to cyanobacteria blooms.

Cyanobacteria blooms are regulated by both top-down and bottom-up processes (Figure 1). Bottom-up processes generally refer to physical and chemical environmental conditions including nutrient supply that influence algal growth. Alternatively, top-down processes include biotic interactions between cyanobacteria and higher trophic levels. Zooplankton can play a key role in cyanobacteria growth and bloom development through species-specific production (top-down) or by altering the temporal and the spatial supply of nutrients (bottom–up) (Sommer, 1988). These processes provide a useful framework for investigating the role of zooplankton in cyanobacteria bloom development in Australia and are discussed in detail below.

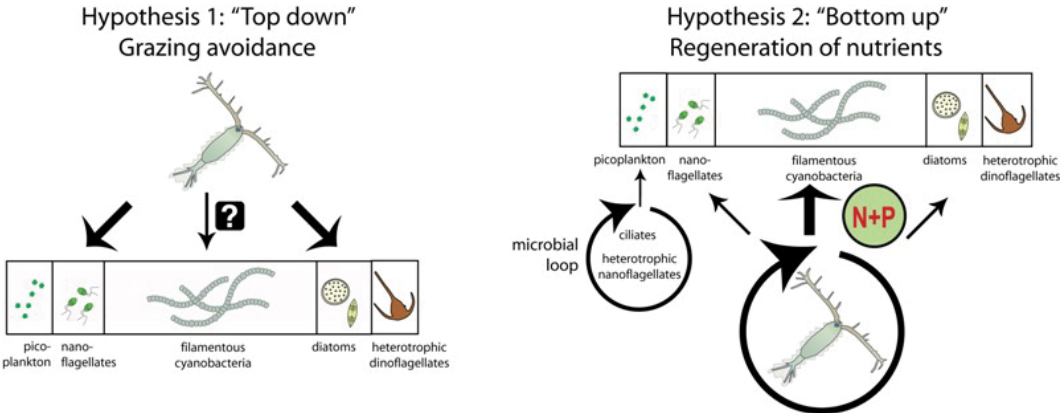

Figure 1 Schematic diagram of bottom-up or top-down processes that affect cyanobactierial growth.

1.1. Top-down control of cyanobacteria blooms

The concept of top–down control is based on the trophic cascade theory, where each trophic level is considered to be controlled by the next higher one and top predators determine the structure of the entire food web (Carpenter and Kitchell, 1996). Cyanobacteria blooms develop when biomass accumulation exceeds the rate of dispersal or consumption through physical (bottom-up) and biological (top-down) processes, respectively (Sommer, 1988). The common cyanobacteria bloom forming species have filamentous and colonial growth forms, as shown in Figure 2.

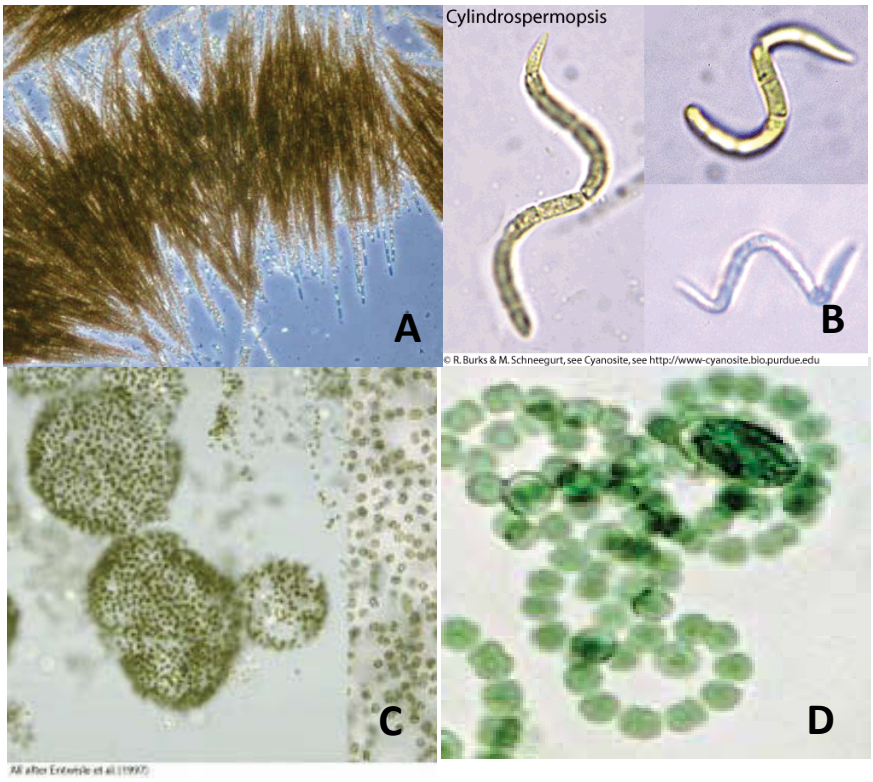

Figure 2 Examples of cyanobacteria genera that bloom in Australia freshwater reservoirs; A: *Aphanizomenon*; B: *Cylindrospermopsis;* C: *Microcystis*; D: *Anabaena*. Image sources: http:///www.cyanosite.bio.purdue.edu.

Zooplankton grazing is a loss process for cyanobacteria. Most research with respect to top-down control has focused on zooplankton size and feeding behaviour. Feeding behaviour is different between three major zooplankton groups, including rotifers and two subclasses of the Crustacea (Figure 3), the cladocera and copepoda. Rotifers feed largely by the moving action of coronal cilia that push particles into the mouth orifice. Cladocera, also known as cladocerans are small crustaceans commonly called water fleas. The most common genus *Daphnia* is filter feeding, usually not able to prevent unsuitable foods from entering the filtering chamber, while the genus *Bosmina* has two modes of feeding, combining passive filtering with active capture of particles (DeMott, 1982; Vanderploeg, 1990). Copepods are highly selective raptorial feeders, and can discriminate prey by size and taste (DeMott and Moxter, 1991;

Vanderploeg, 1994). Major groups include the *Calanoida*, *Cyclopoida* and *Harpacticoida.*

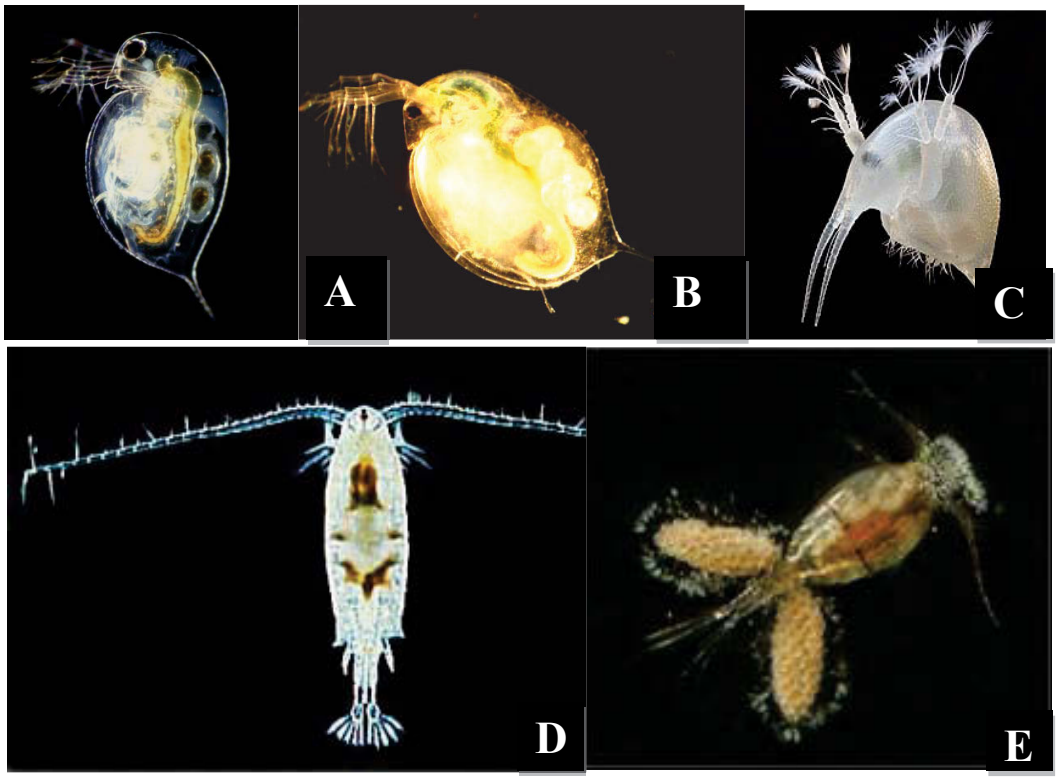

Figure 3 Examples of common genera of crustacean zooplankton*;* A: *Daphnia* (image source: http://upload.wikimedia.org), B: *Monina* (image source: http://upload.wikimedia.org), C: *Bosmina*, (www.notempire.com), D: *Diaptomus* (image source: http://www.bioresurs) E: a representative of *Cyclopoida* (image source: http:// lh4.ggpht.com).

Both predator (zooplankton) characteristics (e.g., size and feeding behaviour) and prey (cyanobacteria) quantity and quality (e.g., nutrient value, morphology and toxin) will affect the outcome of top down control of cyanobacteria populations. Below, currently known consequences of cyanobacteria-herbivore interactions based on one or more regulatory factors will be reviewed.

1.1.1. The impact of zooplankton size and feeding behaviour on top-down control

Large zooplankton (0.2-5mm) like *Daphnia* play a vital role in top-down control (Carpenter and Kitchell, 1996; Cottingham et al., 1997; Ives et al., 1999). Their high filtration rate and ability to ingest a broad size spectrum of food results in a greater capacity to consume algal biomass than small zooplankton (Maron and Crone, 2006). This is demonstrated by field observations of a shift from turbid to clear water when the small sized *D. cucullata* were replaced by the larger *D. galeata. and D. hyalina* (Scharf, 2008). Also, field mesocosm experiments in a eutrophic water body showed that zooplankton dominated by large-sized *D. longispina* increased grazing rate on *Anabaena flos-aquae* (0.5-1 mm) by a factor of 350 times compared to the control with fish (Pogozhev and Gerasimova, 2001).

However, zooplankton size is not always advantageous for effective top-down control. Large zooplankton with greater carapace openings may have greater sensitivity to algal toxins, due to a higher probability of exposure to toxic cells (Hawkins and Lampert, 1989; Attayde and Hansson, 1999). The small cladocerans *Moina* and *Ceriodaphnia* were more tolerant of toxic *Microcystis* than large *Daphnia* (Guo and Xie, 2006). In the field, the impact of cyanobacteria toxins on the zooplankton size spectrum has also been demonstrated. Microcystin concentration was negatively correlated with biomass of large *Daphnia* and calanoid copepods, whereas there was no correlation with biomass of small cladocerans and cyclopoid copepods (Ganf, 1983, Cottingham et al. 2004).

The feeding behaviour of different zooplankton is important in controlling nuisance blooms of cyanobacteria in freshwaters (Agrawal and Agrawal, 2011a). For example, *Daphnia pulicaria* filters the colonial toxic *Microcystis* at the same rate as suitable food *Scenedesmus* (Ghadouani et al., 2003) While the copepod *Diaptomus ashlandi* is highly selective, avoiding ingestion of toxic or poor-quality cyanobacteria in a mix of prey types (Carpenter et al., 1985). Therefore, both size and feeding behaviour lead to changes in zooplankton species composition and size frequency, and this affects top down control.

1.1.2. Food concentration limitation of top-down control

The strength of top-down control varies according to food concentration, which mainly determines zooplankton feeding and growth capacity (Attayde and Hansson, 1999, Sahuquillo et al., 2007). Zooplankton feeding can be expressed as ingestion rate, which is defined as the amount of food ingested per unit of time, and consumption or clearance rate is defined as the volume of water cleared of food by a consumer per unit time and per consumer mass (Båmstedt et al. 2000). The ingestion rate of some zooplankton on cyanobacteria is a function of cyanobacteria abundance, and clearance rate and cyanobacteria concentrations are positively correlated at low cyanobacteria concentration but become negatively correlated as cyanobacteria reach high biomass (Gliwicz and Lampert, 1990). Another study also showed that *Daphnia magna* was not able to grow when *Aphanizomenon flos-aquae* concentration exceeded a threshold concentration of 6.7×10^3 filaments ml^{-1} (Attayde and Hansson, 1999).

The concentration of cyanobacteria also can cause a shift in the zooplankton community structure and thus influence the nature of top-down control of phytoplankton. Large *Daphnia carinata* was replaced by small *Monina micrura*, *Daphnia brachyurum* and *Ceriodaphnia cornuta* when the algal composition was dominated (50:1) by colonial *Microcystis* sp with a small proportion of the chlorophyte *Scenedesmus obliquus*. In contrast, small cladocerans were replaced by large *Daphnia carinata* as the concentration of *Microcystis* sp. decreased to a fifth of its original biomass (Sereda and Hudson, 2011). Therefore, cyanobacteria concentration impacts the strength of top-down control by either inhibiting zooplankton growth or causing a shift from large zooplankton with relatively high grazing capacity to small zooplankton with less grazing capacity.

1.1.3. Food nutrient value and its effect on top-down control

Cyanobacteria are generally considered poor food for zooplankton due to their deficiency in essential fatty acids (DeMott et al., 2004; Martin-Creuzburg and Von Elert, 2009). The growth and reproduction as well as survivorship of cladocerans were inhibited if fed cyanobacteria only (Hochstädter, 2000; Touratier et al., 2001), but the

inhibition often disappeared in a mixture of cyanobacteria and green algae because green algae normally have high essential fatty acids (Hochstädter, 2000). Therefore, poor nutrient quality of cyanobacteria influences the strength of top-down control by limiting the growth of zooplankton.

Top-down control can also be influenced by food quality limitation (Agrawal and Agrawal, 2011). Most studies have focused on elemental and lipid deficiencies. Phosphorus (P) has been the centre of attention due to its limited availability in lakes, and its role in the development of cyanobacteria blooms (Kleebert and Kozerski, 1997). There is evidence from both lab and field data that zooplankton will be P limited with P-deficient food, leading to low growth, survivorship and decreased P-content of zooplankton (DeMott and Gulati, 1999). Experimental data demonstrates zooplankton fecundity steadily decreases with declining dietary P (Mauchline, 1998), resulting in less top-down pressure on phytoplankton. For example, *Daphnia magna* suffered a loss of P-content when feeding on an increasingly poor P diet, which eventually limited its growth (Elser and Urabe, 1999). Similarly, field data showed growth *of Daphnia cucullata* on a phosphorus deficient diet decreased egg production and biomass (DeMott and Gulati 1999; DeMott et al., 2001; DeMott, 2003), and the declines coincided with seasonal increases in the particulate C:P ratio (DeMott et al., 2001). The C: P ratio of the diet can be an indicator of P quality of food, with the threshold for *Daphnia* growth ranging from 200 to 300. Therefore, phytoplankton with low P-content can limit the growth of zooplankton and consequently affect the strength of top-down control.

1.1.4. Food size and shape influences top-down control

Top-down control by zooplankton can be affected by the size and shape of prey. Cyanobacteria morphology not only determines whether zooplankton can directly ingest cells, but also whether they disturb the ingestion of other algae. The grazing rate of zooplankton on cyanobacteria can be decreased because zooplankton have difficulty in handling and ingesting filaments and colonies of bloom-forming cyanobacteria such as *Microcystis* and *C. raciborskii* (Anderson, 1992; Hessen, 1992; DeMott and Gulati, 1999; Elser and Urabe, 1999). For example, the grazing rate of

Daphnia magna was significantly compromised when filamentous cyanobacteria were abundant (Degans and De Meester, 2002). It was suggested that 1.5 mm is the maximum size of *Aphanizomenon* colonies that *D. pulicaria* can handle (Matveev et al., 2000). The impact of prey size to their predator also depended on the size of zooplankton species. Large colonies (> 110um) of *Microcystis aeruginosa* did not depress the filtering rate of small *Ceriodaphnia reticulata* (Wen and Peters, 1994), but reduced the filtering rate of larger herbivores *D. hyalina* (Sereda and Hudson, 2011). This was because large cladocerans allowed more filamentous cyanobacteria to enter the filter chamber. Consequently, large filamentous or colonial cyanobacteria interfered with the filtering apparatus, and compromised the cladocerans filtration capacity (Attayde and Hansson, 1999; Hochstädter, 2000). The cyanobacteria filament interference was supported by comparing the growth and feeding rates of four *Daphnia* taxa that varied in body size. The results showed that the two largest species, *D. magna* and *D. galeata*, failed to grow in natural seston consisting of filamentous cyanobacteria but the smaller zooplankton grew well (DeMott et al., 2001).

Filaments and colonies of cyanobacteria however, may not always negatively affect zooplankton. They can serve as a food source for zooplankton (Gulati and DeMott, 1997), e.g. *Anabaena* can be consumed by tropical cyclopoid copepods (Hall 2009; Kâ et al., 2012) and single filaments and small colonies of *Aphanizomenon* can be consumed by *Daphnia pulex* (Touratier et al., 2001). Hence, the size and morphology of cyanobacteria affect the zooplankton grazing rate, but there are some exceptions. One species of cyanobacteria may be consumed by zooplankton when filaments are short or colonies are small, but might inhibit zooplankton feeding when filaments are long and colonies are large (Elser and Urabe, 1999). Therefore, food quality, size and abundance together shape the magnitude of potential zooplankton grazing control of cyanobacteria.

1.1.5. Food toxin inhibition of top down control

Cyanobacteria toxins also affect top-down control of phytoplankton. Several cyanobacteria have been reported to be toxic (Paerl and Fulton, 2006), and inhibit zooplankton ingestion rate or decrease their survivorship (DeMott et al., 1991). Most

data showing zooplankton toxicity is related to the cyanobacteria genus *Microcystis*. It was reported that *Daphnia* mortality was greater in the presence of toxic *Microcystis* compared to controls with no food (Lampert, 1987), but different strains of *Microcystis* vary in their impact on zooplankton (Nizan, 1986, Hanazato, 1984). Similarly, zooplankton may have different sensitivity to the same strain of *Microcystis*. For example, *Microcystis* reduced the clearance rate for a range of zooplankton, but the effectiveness of the toxin was less pronounced in *Ceriodaphnia* and *Bosmina* compared to *Daphnia* (Lampert, 1987).

The second important toxic cyanobacteria genus in freshwater is *Anabaena*. The survival of *Daphnia magna* in the presence of a toxic strain of *Anabaena* was shorter than the control without food (Porter, 1980). Also, the filtrate of a toxic *Anabaena* culture inhibited *Daphnia* clearance rate, survivorship and reproduction (Starkweather, 1983). Another genus, *Aphanizomenon*, seems rarely to be toxic to zooplankton, but it was found that an extract of *Aphanizomenon flos-aquae* was toxic to *Bosmina longirostris* (Holm, 1984).

Within the past decade, toxic *C. raciborskii* has attracted a lot of attention (Britton, 2009), as this species can produce a suite of highly potent neurotoxic and hepatotoxic alkaloids (Orr et al., 2010) which threatens the health of humans, animals, and aquatic ecosystems. Among these toxins, cylindrospermopsin (CYN) is rapidly being recognised as one of the most globally important of the freshwater algal toxins (Kinnear , 2010). Cylindrospermopsin producing blooms of *C. raciborskii* have been shown to pose negative effects on *D. magna* (Nogueira et al., 2006).

The response of zooplankton to toxic cyanobacteria in some cases seems to be an inherent character. For example, comparison of purified toxins from *Microcystis* on the survival and feeding of a copepod and three species of *Daphnia* showed that the copepod *Diaptomus birgie* was most sensitive, *D. pulicaria* was least sensitive, and *D. hyalina* and *D. pulex* were intermediate in their sensitivity (DeMott et al., 1991).

1.1.6. The impact of zooplankton and phytoplankton composition on top-down control

The strength of top-down control can also be affected by both zooplankton and cyanobacteria species composition. Firstly, zooplankton species in the same genus may have different grazing capacity on cyanobacteria. For example, *Bosmina meridionalis* scarcely ingested cyanobacteria, yet *B. longirostris* readily ingested both cyanobacteria species *Anabaena* spp. and *Microcystis aeruginosa*. *Bosmina coregoni* ingested *M. aeruginosa* at a high rate, while this species was strongly avoided by *Daphnia magna* (Langeland and Reinertsen, 1982; Reinertsen et al., 1986; Havens et al., 1996; Hillebrand et al., 2008). Secondly, the same cyanobacteria species may be assimilated differently by different zooplankton species. With respect to *Microcystis*, assimilation efficiency (defined as the proportion of ingested nutritive substance in food that is absorbed by an animal during passage through the digestive system) is only 20% for *D. longispina* and *D. magna*, but 50% for brackish water copepods (Zhang et al., 2007). Additionally, *Moina macropoda*, *D. obtusa* and *Daphnia hyaline* (Gulati and DeMott, 1997) assimilated *Microcystis* as efficiently as single celled *Chlorella*. So the strength of top-down control on *Microcystis* is different depending on the dominant zooplankton species.

1.1.7. Top-down control of cyanobacteria blooms in the Southern Hemisphere

The efficiency of top-down control has primarily been studied in the temperate lakes of North America and Europe. Using top-down control as a management tool to mitigate algal blooms in aquatic systems has been termed biomanipulation (Shapiro, 1975). However, the application of top–down control in the management of cyanobacteria blooms in Australia freshwater systems has been intensively debated (Boon et al., 1994). The key arguments against using biomanipulation in Australia include the different zooplankton species composition compared to the Northern Hemisphere and their reduced capacity to control cyanobacteria (Arnér et al., 1998).

The most common zooplankton in Australia inland waters are calanoid copepods and rotifers (Boon et al., 1994), while large cladocerans dominate Northern Hemisphere freshwater systems (Arnér et al., 1998). Grazing of native zooplankton is not strong enough to consume toxic cyanobacteria at a rate required for controlling cyanobacteria blooms (Shiel, 1990, Vanderploeg, 1990). Although some studies have shown zooplankton grazing on cyanobacteria in Australian freshwaters (Sinistro et al., 2007), others have shown little impact of zooplankton grazing on cyanobacteria due to interference by large cyanobacteria colonies and filaments (Kobayashi, 1992). Utilization inefficiency of zooplankton on cyanobacteria was supported by gut content analysis of zooplankton during an intense bloom of *Anabaena circinalis* and *A. spiroides* in the Darling River (Boon et al., 1994). Although *Daphnia* had ingested cyanobacteria when cyanobacteria filaments were abundant, fewer zooplankton ingested cyanobacteria at extremely high concentrations because of their clogged feeding apparatus.

In contrast, some scientists support biomanipulation for managing cyanobacteria blooms in Australia freshwaters (Matveev, 1998; Matveev, 2003). They argue that large *Daphnia* can be dominant in some Australian systems. An analysis of zooplankton in eleven reservoirs along a trophic and latitudinal gradient in Queensland showed that Daphnia *carinata* with high grazing rate dominated in cold, less productive lakes while calanoid copepods of the genus *Boeckella* with low grazing rate dominated in warm, more productive reservoirs (Matveev, 2003). Additional data show zooplankton can graze on cyanobacteria with nutritional benefits (Hessen et al., 1992; Sinistro et al., 2007; Knoll et al., 2009). Therefore, it is still unclear whether cyanobacteria are readily consumed food for zooplankton in Australian subtropical freshwater systems.

1.2. Bottom-up control of cyanobacteria blooms

The bottom-up approach that restricts the supply of essential nutrients for growth of phytoplankton is a favoured management tool to decrease the frequency and intensity of cyanobacteria blooms (Boon et al., 1994). The emphasis on controlling cyanobacteria blooms in freshwater lakes has been to decrease the input of

phosphorus (Paerl and Fulton 2006). However, algal blooms are not always successfully controlled by this management strategy (Sager and Richman, 1991; Bolch and Blackburn, 1996). The nutrient recycling by fish, zooplankton and invertebrates may change the nutrient regime such that phosphorus is still available and leads to algal growth. With their dual role as nutrient recyclers as well as consumers, zooplankton can therefore be a nutrient source or sink.

While much attention has focused on grazing affects, the role of zooplankton in nutrient regeneration has received far less attention. Below, nutrient cycling by zooplankton will be reviewed, emphasizing macronutrients such as phosphorus, as well as the factors which regulate the zooplankton contribution to total nutrient availability and the role of zooplankton- derived nutrients in cyanobacteria blooms.

1.2.1. Nutrient regeneration by zooplankton

Zooplankton can contribute to phytoplankton biomass and productivity by excretion of inorganic and organic nutrient (McCauley and Briand, 1979; Roth and Horne, 1981; Lehman and Sandgren, 1985; De Bernardi and Giussani, 1990; Panosso et al., 2003; Sommer and Sommer, 2006). While zooplankton assimilate some proportion of their diet, nutrients are released partly as metabolic by–products through excretion (into the particulate pool) and well as via “sloppy feeding” (into the dissolved pool). The dissolved pool includes nitrogen and phosphorus and can be directly used by phytoplankton as essential nutrients to support growth (Rocha and Duncan, 1985; Sterner, 1990). Zooplankton-derived nutrients can potentially provide up to 100% of the daily nutrient requirement of phytoplankton (Mills, 1986; Persson et al., 1988; Elser et al., 1990; Bormans et al., 2004), but this is variable between lakes (Table 1). Generally, more nitrogen is released than phosphorus in freshwater systems

Table 1. Contribution of zooplankton-derived nutrients to phytoplankton daily nutrient requirements in different lakes worldwide. Values are average percentages; values in brackets represent the range.

Lake	Contribution (%)		Nutrient	References
	N	P		
Tahoe, USA	0.5		Oligotrophic	Carney & Elser 1990
Titicaca, Chile	11.5		Oligotrophic	Carney & Elser 1990
Michigan, USA	58		Mesotrophic	Carney & Elser 1990
Washington,	24	33	Mesotrophic	Lehman 1980
Biwa, Japan	3 (3-104)	15 (1-36)	Mesotrophic	Urabe 1995
Latvian,		16 (2-34)	Eutrophic	Gutelmakher 1995
Loosdrecht,		30	Eutrophic	Den Oude & Gulati 1988
Kinneret, Israel	26 (3-46)	43 (6-94)	Eutrophic	Bruce et al. 2006

Table 2: Nitrogen and phosphorus release rates by different zooplankton species. Units are µg of N or P per dry mass of zooplankton per hour.

Zooplankton	N (µg N	P (µg P	Source	References
Daphnia magna	0.27-1.05	0.04-0.30	Funada-ike Pond,	Urabe, 1993
Daphnia longispina		0.14-1.06	Lake Mekkojarvi, USA	James and Salonen,1991
Daphnia pulicaria	2.80-1.10		Cornell Experimental pond, USA	Hambright, 2007
Skistodiaptomus pallidus	3.40-4.60		Cornell Experimental pond, USA	Hambright, 2007

1.2.2. Nutrient regeneration by zooplankton compared to total nutrient loading

In nutrient–poor lakes, zooplankton nutrient regeneration can be an important source of nutrients supporting algal growth, but in nutrient-rich lakes, zooplankton derived nutrients are typically a small fraction of the total nutrient loading (James, 1991, Table 3). The quantity of nutrient recycling also seems to be different for major zooplankton groups. For example, in a *Daphnia*–dominated lake, phosphorus excretion was approximately 23% of the total nutrient loading, while in a copepod dominated lake it was only approximately 8% (Hessen and Lyche, 1991; Rothhaupt, 1995).

However, zooplankton driven nutrient supply not only depends on the lake's nutrient status, but also the hydrodynamic processes that govern it. Bruce et al. (2006) showed that the annual hydrodynamic cycle of stratification and mixing had a profound influence on the relative contribution by zooplankton to phosphorus fluxes and inorganic nutrients in the surface layer. During the mixing period in winter, the phosphorus flux from zooplankton was low while phosphorus fluxes from sediments were high. During periods of stratification however, nutrient inflows were low and the proportion of zooplankton excretion compared to total nutrient inputs was the highest (62%). These diminished to 2% during the breakdown of stratification. Therefore, the hydrodynamics of lakes is an important factor determining the magnitude of zooplankton nutrient regeneration and its resultant impact on phytoplankton communities.

N: P ratio of zooplankton-regenerated nutrients. Zooplankton can influence algal competition and hence cyanobacteria bloom development through the ratios of nutrients they excrete (DeMott, 1989; Jeppesen et al., 2005). Different elements are recycled by zooplankton with different efficiency, resulting in different nitrogen and phosphorus ratios. Cyanobacteria can fix nitrogen and grow well when the dissolved N:P ratio is lower than 16 (Olsen et al., 1986). Once N_2 fixers are established, they may coexist with non-N_2 fixers (Paerl, 2006). Accordingly, excessive P favours the development of the N_2-fixing cyanobacteria blooms by decreasing the total and

dissolved N:P ratio (Nogueira et al., 2004). For example, an investigation of dissolved nitrogen and phosphorus stoichiometry in a eutrophic lake demonstrated that the N:P ratio increased when *Daphnia* abundance peaked, leading to a decline of N-fixing cyanobacteria (Mitra et al., 2007).

So far, only a few studies have attempted to measure the N:P of zooplankton derived nutrients in freshwater systems (Mills, 1986; Panosso et al. 2003; Hansson et al., 2007) even though the balance between N and P released by crustacean zooplankton has been long studied in marine systems (Friedman and Strickler, 1975, Sterner 1990; Carpenter et al., 1992; Burford et al., 2007). Interestingly, the skewing of the N:P ratio is 4-6 times greater in freshwater than in seawater (Table 3). Therefore, phytoplankton in freshwater is more phosphorus limited and cyanobacteria blooms may develop when the quantity of phosphorus recycling changes (DeMott, 1990).

Table 3: The stoichiometry of phytoplankton and zooplankton in marine and freshwater ecosystems. Data derived from Elser (1995).

	Marine	Freshwater
Phytoplankton N:P	lower	higher
Zooplankton N:P	higher	lower
N:P released by zooplankton	lower	higher
Dominant zooplankton group	copepod	cladoceran

1.2.3. The controlling factors for elemental ratios released from zooplankton

Some researchers have examined endogenous and exogenous factors affecting zooplankton nutrient regeneration, including zooplankton species and food quality (Friedman and Strickler, 1975; Rocha and Duncan, 1985; Bayly, 1992; Mauchline, 1998; Smayda, 2008). It is widely accepted that the recycling rate of an element in a pelagic system depends on the physiological properties of individual zooplankton

species (Sterner, 1990). Studies have demonstrated that the N:P ratio is constant within individual zooplankton species, and zooplankton maintain their elemental composition by excreting nutrients at a lower N:P if their body issue has relatively high N:P ratio and vice versa (Sterner, 1990; Urabe, 1993; Smayda, 2008). A comparison of the nutrient regeneration rate by daphnid cladocerans and diaptomid copepods indicated that cladocerans exhibit high N:P regeneration, while copepods exhibit low N:P regeneration (Hessen and Lyche, 1991). Consequently, copepod–dominated systems are expected to be more vulnerable to blooms of N-fixing cyanobacteria because of N rather than P limitation for phytoplankton growth (Hessen and Lyche, 1991; Rothhaupt, 1995).

Recent studies have examined the effect of the prey elemental ratio (N:P) on the elemental ratios released by zooplankton (Urabe, 1993; Mauchline, 1998; Cembella, 2003; Mitra and Flynn, 2006). N:P released by zooplankton was correlated with the N:P ratio in the food. The grazer recycled whichever element appeared in the food in relatively low proportions with relatively low efficiency (Sterner, 1990), and the N:P release rate was linked to maintaining the elemental composition of body tissues (Smayda, 2008). A linear relationship between both the N:P and C:P ratio of the food and the N:P and C:P ratios released by *Daphnia* was demonstrated (Irigoien et al., 2005). More recently, a more complex curvilinear relationship was found between phosphorus release and P-deficient diets (Mauchline, 1998).

In summary, zooplankton can alter the N:P ratio of available dissolved nutrients within pelagic ecosystems through excretion, and may therefore play a key role in cyanobacteria dominance and bloom formation. The importance of zooplankton mediated nutrient regeneration depends on zooplankton species, food quality, and hydrodynamic conditions in a given aquatic system.

1.2.4. Nutrient regime and *Cylindrospermopsis raciborskii* blooms in the Southern Hemisphere

The promoting factors of cyanobacteria blooms may be expected to be different in the Southern Hemisphere due to different dominant cyanobacteria species and nutrient regulation of blooms. Cyanobacteria often dominate the summer phytoplankton in

freshwater systems and represent an important part of phytoplankton in sub-tropical and tropical environments due to the relatively constant annual solar input and air temperature which favour cyanobacteria dominance (Bouvy et al., 2000). In addition, cyanobacteria blooms are usually promoted by high phosphorus loading, but most of the reservoirs experiencing *C. raciborskii* blooms are strongly phosphorus deficient (Bouvy et al., 2000). Therefore, *C. raciborskii* blooms may be influenced by nutrients differently compared to other cyanobacteria in the Northern Hemisphere (e.g. *Microcystis*).

1.3. Synergism of top-down and bottom-up processes on cyanobacteria bloom

Although researchers now recognize the importance and interaction of both top-down grazing mechanisms and bottom-up nutrient loading on cyanobacteria bloom formation, studies which investigate both mechanisms have been rare (Sommer; 1988; Rothhaupt, 1995). A prominent example of both selective grazing and grazer-derived nutrient regeneration on cyanobacteria bloom is the recurrence of *Microcystis* blooms in the Great Lakes after phosphate was reduced for decades. Since the 1960s, scientists have recognized that cyanobacteria blooms in Lake Erie were due to eutrophication, and have reduced the P loading in Lake Erie by approximately 60% (Vanderploeg, et.al., 2001). This led to a reduction in cyanobacteria from 1970 to 1985 (Makarawicz and Bertram, 1991). However, *Microcystis* blooms reappeared in Lake Erie in the 1990s, which was proposed to involve both grazing mortality and grazer-mediated nutrient recycling (Sager and Richman, 1991). The blooms were promoted and maintained by zebra mussels selectively filtering and rejecting the *Microcystis* (Vanderploeg et al., 2001), while grazer-mediated nutrient recycling provided a supply of available N and P to *Microcystis*. The soluble and reactive phosphorus (SRP) concentration was higher than NH_4 in lakes with zebra mussels, which favoured phytoplankton species that are better competitors for N like *Microcystis*. Therefore, selective grazing and low N:P ratio together may promote cyanobacteria blooms in freshwater lakes (Sager and Richman, 1991).

1.4. Research directions in cyanobacteria blooms in Australian reservoirs

The knowledge gaps with respect to managing cyanobacteria blooms in Australian freshwater systems are summarized here. First, there is a lack of empirical grazing data of zooplankton on the cyanobacterium *C. raciborskii*. Toxic *C. raciborskii* is a dominant cyanobacterium in Australian subtropical reservoirs but there have been no studies on the importance of copepods in regulating its abundance. Secondly, there are no empirical data of phosphorus recycling by zooplankton in Australian reservoirs, and lack of knowledge to evaluate the contribution of zooplankton derived nutrients to cyanobacteria bloom development. Finally, there is a lack of studies which assess the relative importance of top-down and bottom-up processes on cyanobacteria blooms. Planktonic systems are regulated by both top-down and bottom-up controls and the two controls are in a dynamic balance (Gilbert, 1998). However, these processes may become decoupled such that algae being consumed are not the same algae that benefit from zooplankton-recycled nutrients (a food web subsidy). The estimation of how, and to what degree, the bottom up and top down factors are coupled, is essential to determining the fate of nutrients in reservoirs.

1.5. Objectives and thesis outline

The goal of this study was to test a conceptual model that predicts *C. raciborskii* abundance depends on the dominance of copepods to cladocerans in the zooplankton. *C. raciborskii* concentration decreases when cladoceran abundance increases because these zooplankton consume algae unselectively and they have relatively high P body content, thus release less P into the environment to support algal growth. In contrast, *C. raciborskii* abundance increases when copepods dominate because copepods are selective consumers, but also have relatively low P body content, thus release more P to support algal growth.

Specifically, the aims of this thesis were to:

1. Examine zooplankton grazing on *C. raciborskii* and assess under which conditions grazing losses would be at a maximum;
2. Assess the indirect effects of zooplankton nutrient regeneration versus the direct effect of zooplankton grazing on *C. raciborksii* growth;
3. Scale-up the laboratory experiments and examine the net outcome of zooplankton-phytoplankton interactions within *in-situ* mesocosms; and
4. Assess the generality of zooplankton-*C. raciborskii* interactions by examining the zooplankton composition, size structure and biomass in reservoirs experiencing *C. raciborksii* blooms and those which do not.

The associated scientific hypotheses are:

Hypothesis I: Meso-zooplankton copepods such as *Boeckella in* particular, will not consume *C. raciborskii* in complex natural mixtures when alternative algae are abundant or when the nutritional status of other prey types is higher than *C. raciborskii*.

Hypothesis II: The importance of recycled phosphorus (P) in sustaining *C. raciborskii* growth depends on food quality P (quota) and total P availability, and *C. raciborskii* has competitive advantage to use recycled P compared to other algae.

Hypothesis III: Selective zooplankton grazing and nutrient regeneration act synergistically to facilitate the accumulation of *C. raciborskii in situ.*

Hypothesis IV: Zooplankton composition, biomass and size structure will be different in reservoirs with *C. raciborskii*. *C. raciborksii* will be positively correlated with copepods because these zooplankton are better able to consume cyanobacteria and tolerate their toxins.

CHAPTER 2

Zooplankton facilitate toxic cyanobacterium *Cylindrospermopsis raciborskii* persistence in aquatic habitats

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ABSTRACT

Blooms of the toxin-producing cyanobacterium *Cylindrospermopsis raciborskii* occur in tropical and subtropical lakes during spring-summer, but the mechanisms behind bloom formation are unclear. This study tests the hypothesis that development of *C. raciborskii* blooms is facilitated by selective zooplankton grazing. Consumption by zooplankton was examined in a series of experiments designed to evaluate the strength of feeding discrimination between *C. raciborskii* and the green alga, *Chlamydomonas reinhardtii,* as well as within natural phytoplankton assemblages. Clearance rates of the copepod *Boeckella* sp. on a *C. raciborskii* diet were 2-4 times lower than that of a common cladoceran *Ceriodaphnia* sp. when both grazers had prey choice. More *C. raciborskii* was consumed by *Boeckella* sp. when in mixed natural phytoplankton assemblages, but the clearance rate declined when nutrient replete *C. reinhardtii* was added, demonstrating that when alternate "high quality" algae were present, so did *C. raciborskii* consumption. The clearance rates of *Boeckella* sp. on two toxic *C. raciborskii* strains were significantly lower than on a non-toxic strain, and on *C. raciborskii* with low cellular P content. When we tested the grazing preference of a mixed zooplankton community on *C. raciborskii* during the early bloom period, clearance rates were relatively low (0.05-0.20 ml individual^{-1} h^{-1}), and decreased significantly as the proportion of *C. raciborskii* increased above 5%. These results suggest that *C. raciborskii* persistence could be promoted by copepods preferentially grazing on other algae, with significant loss of top-down control as *C. raciborskii* abundance increases.

1. INTRODUCTION

Cylindrospermopsis raciborskii is a toxic cyanobacterium that produces neurotoxic and hepatoxic alkaloids, including cylindrospermopsins (Ohtani et al., 1992; Lagos et al., 1999; Molica et al., 2002; Wood and Stirling, 2003; Molica et al., 2005). It blooms (i.e., abundance exceeds 1 x 10^5 cells ml^{-1}; WHO 2000) under a variety of environmental conditions (Padisák, 1997), making it difficult to predict its proliferation. Several attributes have been suggested to contribute to this cyanobacterium's ecological success: (Sarma et al., 2005) its competitive nutrient acquisition and storage mechanisms, including its N_2-fixing ability, high affinity for phosphorus (P) and ammonium, high P-storage capacity (Padisák, 1997, Istvánovics et al., 2000); (2) wide thermal tolerance (Briand et al., 2004; O'Neil et al., 2011; Sinha et al., 2011), superior shade tolerance and buoyancy (Briand et al., 2002; O'Brien et al., 2009); and (3) resistance to grazing (Padisák, 1997). 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 influencing blooms has been little investigated (Padisák, 1997; Figueredo et al ., 2007).

In the laboratory, both straight and spiral filaments of *C. raciborskii* are readily consumed by meso-zooplankton such as cladocerans (Hawkins and Lampert, 1989; Soares et al., 2009; Panosso and Lurling, 2010; Kâ et al., 2012) and rotifers (Soares et al., 2010; Kâ et al., 2012). However, it is unclear if copepods, particularly calanoid copepods, which are the dominant meso-zooplankton in Australian and South American inland waters (Boon et al., 1994), can consume *C. raciborskii* and thereby influence bloom formation. While copepods can shorten filaments of *C. raciborskii* and then ingest them (Bouvy et al., 2001; Kâ et al., 2012), there is limited evidence of *C. raciborskii* consumption within complex algal assemblages (Lehman, 1980).

Copepods are well adapted to respond to variability within their environment (Kleppel, 1993), shifting their feeding behaviour depending on prey availability and (DeMott and Watson, 1991;). When food is abundant, calanoid copepods are selective consumers, but when food is more limited, they are less discriminate (DeMott, 1989). In

addition, copepods make prey choices based on taste or perceived nutritional value, as opposed to cladocerans which tend to choose prey based on size (DeMott ,1993).

In subtropical Australian reservoirs, there is a strong seasonality in food quantity and quality for zooplankton. Algal abundance is relatively low during the winter dry season (May to October) and phytoplankton growth is limited by temperature (Muhid, 2010). However, from October to May (spring-summer), seasonal warming results in a 2-3 fold increase in algal abundance (Muhid, 2010). These changes in algal prey quantity (and potentially quality) are coincident with increasing abundance of *C. raciborskii* (Burford and O'Donohue, 2006; Burford et al., 2007), and suggest that selective consumption by zooplankton could facilitate *C. raciborskii* bloom formation. To test this hypothesis, we set up a series of experiments to examine copepod grazing preferences under different environmental conditions. In feeding trials with cultures and mixed natural algal assemblages, we measured zooplankton consumption of *C. raciborskii* at different relative abundances of *C. raciborskii* and under different concentrations of total phytoplankton biomass (mg carbon (C) L^{-1}). We also examined whether grazing preferences were related to cellular P-content or toxicity of *C. raciborskii* strains. We predicted that meso-zooplankton, and *Boeckella* sp. in particular (compared to a subtropical cladoceran, *Ceriodaphnia* sp.), would not consume *C. raciborskii* in complex natural mixtures when *C. raciborskii* was in low relative abundance, or when the nutritional status of other prey types were higher than *C. raciborskii*. Furthermore, we predicted that clearance rates on P-deplete or toxic *C. raciborskii* strains would be lower than the P-replete or non-toxic strains, and that *C. raciborskii* consumption would increase if copepods were pre-exposed to limiting food concentrations.

2. MATERIALS AND METHODS

2.1. Experimental organisms

Feeding experiments were conducted using zooplankton collected from Manly Dam (34° 46'3" S, 151º 14'52" E) and Wivenhoe Dam (27° 30' S and 152° 45' E), situated in

New South Wales and southeast Queensland, Australia, respectively. Animals were sampled by vertical net hauls (diameter 0.5 m; mesh size 165 μm), with the copepod *Boeckella sp.* being the dominant taxon, typical of many Australian lakes (Bayly, 1992; Boon *et al.,* 1994). Natural phytoplankton assemblages were collected from the surface using a clean bucket. On return to the laboratory, zooplankton were maintained in 10-20 L containers of lake water at ambient temperature (21°C) under 10 μmol photons m^{-2} s^{-1} with a 12:12 h light-dark cycle. Phytoplankton were maintained at 21°C under 40 μmol photons m^{-2} s^{-1}. Zooplankton were isolated individually using wide-bore plastic pipettes under a dissecting microscope, with similar sized *Boeckella* sp. individuals (0.83 ± 0.06 mm) selected for experiments.

Monocultures of the green alga *Chlamydomonas reinhardtii* (diameter 5.4 ± 1.2 μm) and three strains of *Cylindrospermopsis raciborskii* (diameter 2.9 ± 0.5 μm; filament length 115 ± 89 μm) were maintained in MLA medium at 25 °C under 40 μmol photons m^{-2} s^{-1} (see Table 1 for strain information). *C. reinhardtii* was used primarily because it has been used in previous studies (DeMott and Moxter, 1991; Burns and Hegarty, 1994). Pilot studies revealed *C. reinhardtii* is readily consumed by *Boeckella sp.*, with clearance rates being saturated at ~1.0 mg C L^{-1} (equivalent to 7.4 x 10^4 cells ml^{-1}). The *C. raciborskii* strains were chosen because they are morphologically similar (all have straight filaments), with one strain being non-toxic (CS-508) and two strains, CS-505 and NPD, producing toxin (Saker *et al.,* 2004; Davis, unpublished data). Strain NPD was originally isolated from Lake Samsonvale (Queensland, Australia) where *C. raciborskii* occurs regularly in high abundance during the austral summer (Burford and O'Donohue, 2006).

In experiments testing the effect of P-content on copepod prey selection, *C. raciborskii* (NPD strain) was cultured with different concentrations of inorganic phosphate. P-sufficient algae were maintained in MLA medium at 25°C, 40 μmol photons m^{-2} s^{-1} with a 12:12 h light-dark cycle, and were transferred into fresh medium every 5 d. P-deficient *C. raciborskii* was prepared with a step-wise series of transfers into P-deplete MLA medium; i.e. exponentially growing cells were transferred from 100% P into 10% P medium, and after 5 days, they were transferred into 0% P medium. P-sufficient and P-deficient cultures were both centrifuged (3500 rpm for 10 min) and the pellets were suspended in fresh medium on each day for 5 d prior to experimentation.

To test whether the nutrient status caused a change in morphology or whether selectivity was affected by filament length (see Panosso and Lurling, 2010), filaments of *C. raciborskii* (n = 100) were measured using a compound microscope (Olympus BX50, Hamburg, Germany) before experiments.

The elemental (C, N, P) content of natural phytoplankton communities and cultures of *C. raciborskii* and *C. reinhardtii* used in the experiments was estimated by filtering known volumes onto pre-combusted glass fibre filters which were stored frozen at -20 °C until analysis. Samples for total phosphorus (TP) were digested using a persulfate digestion procedure. After digestion, TP was analyzed based on the ascorbic acid reduction of phosphomolybdate (Cottingham, 1997). For total carbon and nitrogen analyses, the filters were dried at 50 °C overnight, packaged into tin cups and analysed using an Elemental Analyser and 20-20 IRMS (Europa Scientific). The cell abundance of each strain was estimated using a Sedgwick Rafter cell at 400 x magnification under a compound microscope. Cell quotas of C, N and P were then calculated (Table 2).

2.2. Experimental design

2.2.1. Strength of selective grazing

Experiments testing for selective grazing were performed in two ways: by offering zooplankton two prey types with consumption estimated using a multiple excitation wavelength fluorometer at the end of 2-3 h feeding trials. Alternatively, one prey type (or the entire algal assemblage) was labelled with the radiotracer ^{33}P (1.85 - 3.70 MBq L^{-1}; PerkinElmer, MA, USA), with consumption estimated by tracking radioactivity into zooplankton after 30 min or 3 h, similar to Fulton and Pearl (1988) (Fig. 1).

In the first experiment, treatments had the same amount of food (1.0 mg C L^{-1}), but the proportion of *C. raciborskii* compared to *C. reinhardtii* was 0, 20, 40, 60, or 100 %. In the second experiment, treatments had the same amount of *C. raciborskii* (0.5 mg C L^{-1}), but the total food concentration was increased by adding *C. reinhardtii* (to achieve a total food concentration of 1.5, 2.0, or 2.5 mg C L^{-1}). One adult copepod was placed in a 10 ml test tube, following the method of Panosso and Lürling (2010), and each treatment had four replicates. There were also three controls with no animals for

each treatment. Cyanobacterial and chlorophyte abundance in each tube were monitored simultaneously using a PhytoPAM (Walz GmBH, Effeltrich, Germany). Three ml subsamples were removed at the start and end of the experiment (after 2.5 h), and dark adapted for 10 min before measuring their Chl-*a* fluorescence. Algal signatures were set up in the blue and green channels using unialgal cultures of *C. raciborskii* and *C. reinhardtii*, respectively, grown under experimental conditions, and fluorescence was validated with cell counts. Clearance rates (CR) were estimated from the difference in Chl-a fluorescence between the start and end of the experiment and comparison with no zooplankton controls according to the following equation:

$$CR = Ln (F_c - F_t) * V/T$$

where F_t is the final Chl-a fluorescence in the zooplankton treatment and F_c is the Chl-a fluorescence in the control after the feeding period T; V is the volume of the incubation (10 ml).

The selection coefficient (α) was calculated according to Burns and Hegarty (1994) as the ratio of clearance rates:

$$\alpha = CR_{cyn}/(CR_{cyn} + CR_{green})$$

where CR_{cyn} and CR_{green} are the clearance rates on *C. raciborskii* and *C. reinhardtii*, respectively.

2.2.2. Mechanisms of selective grazing

After observing selection coefficients < 0.5 in the above studies (indicating feeding preference against *C. raciborskii*), further experiments were conducted to understand the mechanisms driving prey selection under different conditions.

The first experiment measured clearance rates of meso-zooplankton on a natural phytoplankton assemblage containing *C. raciborskii*, and was designed to simulate pre-bloom conditions. Zooplankton and surface phytoplankton were collected on 7 September 2010 from Lake Wivenhoe and brought back to the laboratory where they were maintained under ambient conditions. A compound microscope (Olympus BX50,

Hamburg, Germany) was used to estimate total algal abundance, and feeding treatments (triplicates) were then set up by replacing a proportion of cells in the natural phytoplankton community with cultured P-deficient *C. raciborskii*. Final treatments therefore had 0, 5, 15 or 100% *C. raciborskii* with relatively constant total food concentration. The entire algal assemblage was labeled with ^{33}P for 1 h, then provided to zooplankton (approximately 15 animals in 50 ml). Zooplankton consumption was monitored by collecting animals after 30 min and measuring their radioactivity using a liquid scintillation counter (Perkin Elmer, Massachusetts, USA).

To examine whether similar sized adult copepods (length: 83 ± 6 mm) and cladocerans (length: 77 ± 12 mm), rather than a mixed meso-zooplankton community consumed *C. raciborskii* under non-saturating and saturating phytoplankton biomass, they were offered ^{33}P-labelled *C. racibor*skii (0.5 mg C L^{-1}) in 0.7 µm GF/F filtered (Whatman, New Jersey, USA) Manly Dam water, (2) a natural phytoplankton community (Manly Dam water containing 0.7 mg C L^{-1}), and (3) a natural phytoplankton community (Manly Dam water) enriched with 0.5 mg C L^{-1} *C. reinhardtii* (total 1.2 mg C L^{-1}). *C. raciborskii* comprised 100, 42 and 29% of the carbon biomass in the treatments, respectively. In this and all subsequent experiments, six adult zooplankton were added into 100 ml plastic jars with 50 ml algal food and acclimated for at least 1 h before each experiment was initiated.

Further experiments tested whether copepod clearance rates were different on *C. raciborskii* strains of different toxin and nutritional status. Copepods were provided one of three ^{33}P-labelled *C. raciborskii* strains (two toxic and one non-toxic, each 0.5 mg C L^{-1}), in the presence of unlabelled *C. reinhardtii* (1.0 mg C L^{-1}) and clearance rate was estimated by tracking ^{33}P in copepod consumers. In another experiment, copepods were provided a mixture of unlabelled *C. reinhardtii* (1.0 C mg L^{-1}) and labelled *C. raciborskii* (0.5 C mg L^{-1}) which was either P-sufficient or P-deficient. Because nutrient status of *C. raciborskii* filaments have been shown to affect filament length (Panosso and Lürling, 2010), we measured filament length before experiments to confirm that toxicity and P status did not affect *C. raciborskii* morphology and confound our results.

To examine whether selection against *C. raciborskii* changed depending on copepod gut fullness, copepods were held for approximately 6 h in 0.7 µm GF/F filtered Manly Dam water, unfiltered Manly Dam water (~0.7 mg C L^{-1}), or a solution of *C.*

raciborskii (0.5 mg C L^{-1}), or *C. reinhardtii* (0.5 mg C L^{-1}). After the pre-conditioning period, animals were offered ^{33}P labelled *C. raciborskii* (0.5 mg C L^{-1}) mixed with unlabelled *C. reinhardtii* (0.5 mg C L^{-1}), and clearance rates on *C. raciborskii* were quantified after 30 min. It was expected that copepods preconditioned on filtered lake water and unialgal *C. raciborskii* would have lower gut content than those feeding on unfiltered lake water and *C. reinhardtii*, and that they would consequently feed less selectively and have elevated *C. raciborskii* clearance rates compared to other treatments.

2.3. Experimental procedure with ^{33}P labeled prey

Cultured algae were sampled in exponential phase the day before each experiment and centrifuged at 3500 rpm for 10 min. The pellets were resuspended into P-free MLA medium, and then spiked with carrier-free ^{33}P orthophosphate (1.85 - 3.70 MBq L^{-1}; PerkinElmer, MA, USA) with the initial activity ranging from 50 to 100 µCi L^{-1}. After 24 to 48 h, when cells were considered uniformly labelled, labelled algae were centrifuged and rinsed with P-free MLA medium to remove any unincorporated ^{33}P, and were then resuspended into 10 ml P-free MLA medium. Initial radioactivity (> 10,000 DPM) was checked by adding 0.5 ml of labelled algae into 4.5 ml scintillation cocktail (Ready Safe™, Beckman Coulter, CA, USA) and ^{33}P activity (dpm) was measured using a liquid scintillation counter (Packard Tri-Carb 2100TR, CT, USA). Unlabelled algae were processed the same way (minus the labeling).

Feeding experiments were initiated by adding 50 ml of ^{33}P labelled algae food into the container. After 10 min, the animals were collected onto 100 µm cell strainers (BD Falcon™ New Jersey, USA), rinsed several times with MilliQ water and were pipetted into 6 ml vials (Pico Prias PerkinElmer, MA, USA). Five ml aliquots of food were filtered through 25 mm diameter, 0.6 µm pore size polycarbonate membranes (Steritech, WA, USA). Both animals and algae were digested with 0.5 ml of 0.5 M NaOH, and then 4.5 ml scintillation cocktail was added. The ^{33}P activity of the samples was measured using a liquid scintillation counter.

Zooplankton clearance rates on ^{33}P labelled diets were calculated using the following equation (Bamstedt et al., 2000):

$$F = (dpm_{animal} \times V) / (dpm_{algae} \times T)$$

Where dpm_{animal} is the radioactivity associated with each animal, dpm_{algae} is the radioactivity of V ml of algal food, and T is the incubation time in h.

2.4. Statistical analyses

Clearance rates were compared between treatments using one-way analysis of variance followed by a Tukeys post-hoc test. To meet the assumption of normality for an ANOVA, the data were either square root or natural log transformed and Levene's test was used to check for homogeneity of variances. The level of significance for all tests was 0.05.

3. RESULTS

Copepod clearance rates on *C. raciborskii* were consistently low irrespective of *C. raciborskii* relative abundance (0 - 0.3ml ind^{-1} h^{-1}, $F = 1.68$, $p = 0.20$). They were also relatively low with fixed *C. raciborskii* abundance under increasing total food concentration (0.0 ml ind^{-1} h^{-1}, $F = 1.82$, $p = 0.19$), and showed a clear selection preference for *C. reinhardtii* in all treatments (Table 3).

Similarly, zooplankton community clearance rates declined with an increasing proportion of *C. raciborskii* in a mixed natural algal assemblage (Fig. 1), ranging from 0.13 ± 0.16 ml ind^{-1} h^{-1} when *C. raciborskii* comprised 5 or 15% of the total food biomass, to 0.06 ± 0.02 ml ind^{-1} h^{-1} when *C. raciborskii* was the only available prey ($F = 19.84$, $p = 0.038$).

The cladoceran *Ceriodaphnia sp.* cleared *C. raciborskii* more rapidly (3.14 – 6.87 ml ind^{-1} h^{-1}) than the copepod *Boeckella sp.* (1.16 – 1.97 ml ind^{-1} h^{-1}) under both limiting (filtered and unfiltered lake water) and non-limiting food conditions (unfiltered lake water supplemented with *C. reinhardtii*; Fig. 2). When total food abundance increased, cladoceran clearance rates on *C. raciborskii* increased, but decreased for the copepod ($F = 31.60$, $p = 0.001$).

Experiments designed to test whether copepod feeding preference was different amongst *C. raciborskii* strains of different toxic status or P content showed variable clearance rates. While filament length was slightly shorter in strain NPD (mean ± standard error: 90 ± 13 µm) compared to strain CS-508 (111 ± 10 µm) and CS-505 (133 ± 15 µm), the filament lengths of toxic strain CS-505 and non-toxic train CS-508 were not significantly different ($p = 0.837$) (Fig. 3A). *Boeckella sp.* cleared the non-toxic strain CS-508 (0.85 ± 0.06 ml ind^{-1} h^{-1}) approximately 20% faster than the toxic strain CS-505 (0.61 ± 0.06 ml ind^{-1} h^{-1}) and more than 100% faster than toxic NPD (0.25 ± 0.09 ml ind^{-1} h^{-1}) (Fig. 3B). Furthermore, the clearance rate of *Boeckella sp.* on *C. raciborskii* (toxic NPD strain) was lower (0.35 ± 0.02 ml ind^{-1} h^{-1}) when the cells had very low P content (0.02 pg $cell^{-1}$), but there was no consistent correlation of clearance rate with a gradient in *C. raciborskii* C:P ratio (Fig. 4B). Furthermore, there was no difference in filament length amongst the cultures with different P status ($F = 13.702$; $P = 0.839$), averaging 90 ± 13 and 104 ± 10 µm for P-replete and P-deplete cultures, respectively (Fig. 4A), indicating the results were not confounded by filament length.

Copepods held in filtered lake water for 6 h resulted in animals consuming more *C. raciborskii* (NPD) compared to when they were held in unfiltered lake water or lake water with *C. reinhardtii* ($F = 40.68$, $p < 0.001$, Fig. 5). Similarly, *Boeckella sp.* pre-conditioned with *C. raciborskii* had similar clearance rates to copepods that hadn't eaten for ~6 h.

4. DISCUSSION

Copepods from subtropical and tropical regions are expected to consume cyanobacteria because cyanobacteria comprise the major food source throughout the year (Haney, 1987; Boon *et al.,* 1994). However, this study revealed generally low consumption of *C. raciborskii* by the copepod *Boeckella sp.*, the dominant (59-67% of total zooplankton biomass) meso-zooplankter in freshwater reservoirs of subtropical Queensland (Matveev, 2003). This suggests that copepod consumers could facilitate *C. raciborskii* accumulation by their preference for grazing other algae.

Our *a priori* expectation was that zooplankton would consume *C. raciborskii*,

partly based on the relatively high (> 60%) proportion of this species in Australian subtropical and tropical freshwaters (McGregor and Fabbro, 2000, Burford and O'Donohue, 2006; Burford et al., 2007) but also because both copepod and cladoceran functional groups are capable of handling large prey, including filamentous cyanobacteria (Fulton, 1988; Sommer, 2003). Copepods in particular prefer large particles when prey quality is similar (Boon *et al.,* 1994; Price and Paffenhofer, 1985; Vanderploeg et al., 1988) with the optimum prey size ranging from 10 to 100 µm (Bruce, 2006). *C. raciborskii* (length: 40 - 150 µm) is within this preferred size range, but copepods showed a clear preference for consuming other algae. *C. raciborskii* composed of straight and coiled forms in samples collected from Wivenhoe Dam. The coiled trichome could present some defense against grazer as it occupies two dimensions, and make it more difficult to handle (Vanderploeg et al., 1988). However, cultured *C. raciborskii* became straight trichome in this study, and unlikely defend against grazer through two dimension morphology. The greatest consumption of *C. raciborskii* by copepods occurred when food was limiting—clearance rates of *C. raciborskii* were at a maximum when prey biomass was low or when animals had empty guts. However, under non-limiting conditions, *Boeckella sp.* consumed the relatively small, spherical cells of *C. reinhardtii* (diameter 5 - 10 µm*)* almost 10-fold faster than the large filaments of *C. raciborskii*. Furthermore, consumption of *C. raciborskii* by a mixed zooplankton assemblage also diminished with an increasing proportion of *C. raciborskii*. This result suggests that food quality instead of prey size was a more important influence on prey selection (Fulton, 1988). Generally, cyanobacteria, such as *C. raciborskii*, lack important fatty acids and may produce toxins (Reynolds, 1984; Reynolds, 1987; Nogueira et al., 2004). Increasing the quantity and quality of food by supplementing a natural phytoplankton community with *C. reinhardtii* resulted in a decline of *C. raciborskii* consumption by copepods, consistent with the optimal diet model that predicts zooplankton discriminate most strongly against low-quality food when high-quality food is abundant (Burns and Hegarty, 1994; DeMott and Moxter, 1991). The selection against *C. raciborskii* by copepods was further verified by comparing the feeding behavior of the cladoceran *Ceriodaphnia sp.* on *C. raciborskii* under increasing food biomass. In contrast to *Boeckella sp*., *Ceriodaphnia sp.* increased consumption of *C. raciborskii* when total prey abundance increased, demonstrating less selection against *C. raciborskii.*

Our study also demonstrated that copepods distinguished between morphologically similar *C. raciborskii* strains with different toxic status. Clearance rates on two toxic strains were significantly lower than the non-toxic strain, and support the notion that copepods perceive strain-specific signals related to toxicity to avoid ingestion of harmful food. While it is well established that toxins affect the consumption of cyanobacteria by zooplankton (Burns and Hegarty, 1994; DeMott, 1993; Vanderploeg, 1990), cyanobacterial toxins are less problematic for calanoid copepods (Burns and Xu 1990) or certain rotifers (Fulton and Paerl, 1987; Gilbert and Durand ,1990; Gilbert 1998) compared to cladocerans. Indeed, there is increasing evidence that copepods tolerate exposure to cyanobacterial toxins, showing peaks in abundance in the presence of toxic cells *in situ* (Bouvy et al., 1999). In this study, pre-exposure to *C. raciborskii* resulted in similar rates of *C. raciborskii* consumption compared to animals kept for the same length of time with no food, suggesting that copepods may not be affected by ~6 h exposure to toxins.

Although the cellular P content of *C. raciborskii* was expected to be important in regulating the prey preference of the copepod *Boeckella sp.,* our experiments showed variable results. Copepod clearance rates on *C. raciborskii* were 2-4 times lower when *C. raciborskii* was strongly P depleted compared to cells with greater P content, but it was not consistent with the C:P ratio. Copepods consumed *C. raciborskii* with the highest C:P ratio at a relatively low rate, suggesting not only P content, but other factors (such as toxin concentration) were influencing prey choice by *Boeckella sp*. We focussed on phosphorus in our experiments because previous studies have found algal growth in many Australian subtropical freshwaters to be P-limited and that *C. raciborskii* dominates under periodic low-level dissolved inorganic P enrichment (Posselt et al., 2009). Phosphorus is an important element in food quality for consumers because it is used to construct new biomass (e.g., phospholipid membranes), is involved in cellular energy processes, and therefore affects zooplankton growth and reproduction (Elser *et al.,* 2001; van Donk *et al.,* 2008). In addition, P-starved algae can thicken their cell walls, making them less digestible to zooplankton (Lürling and van Donk, 1997). While we saw no obvious change in *C. raciborskii* morphology under P-deplete conditions, we did note P-starved cells were a different colour, suggesting low P had an effect on photosynthetic pigment production, and could therefore have influenced prey perception and palatability by copepods. Dickmann et al. (2008) showed that the trophic

transfer efficiency between phytoplankton and zooplankton was lower when algal C:P was high. Furthermore, we observed no mortality during our short-term feeding experiments, but subsequent observations have shown *Boeckella sp.* are susceptible to mortality in the presence of P-deficient *C. raciborskii* (Y. Hong, unpublished data).

Clearance rates of *Boeckella sp.* on *C. raciborskii* ranged from 0.2 to 2 ml ind^{-1} h^{-1}, at the same order of magnitude as rates of phytoplankton consumption by other freshwater copepod taxa (DeMott and Moxter, 1991). However, under most circumstances, the clearance rates of *Boeckella sp.* on *C. raciborskii* observed in this study were less than 0.6 ml $individual^{-1}$ h^{-1}, significantly lower than clearance rates of *Boeckella sp.* on *Microcystis sp*. (>1 ml ind^{-1} h^{-1} ; Matveev and Matveeva, 1997). Based on our results, we would therefore predict low consumption of *C. raciborskii* when *Boeckella sp.* dominates the zooplankton community, and preferential consumption of other algae by copepods when *C. raciborskii* comprises a small to moderate proportion (15%) of the phytoplankton community. Together, this suggests that subtropical copepods facilitate *C. raciborskii* accumulation in aquatic habitats, with selective grazing increasing the risk of bloom occurrence.

Conclusion

Our study found that:

- The copepod *Boeckella sp.* showed strong preference against *C. raciborskii* consumption under most conditions tested;
- The cladoceran *Ceriodaphnia sp*. was a more effective consumer of *C. raciborskii*, indicating the potential for zooplankton community composition to affect the strength of top-down processes regulating this toxic cyanobacterium;
- *Boeckella sp.* had greater clearance rates on non-toxic and P-replete *C. raciborskii* compared to toxic and P-deplete cells and showed maximum clearance rate of *C. raciborskii* under limiting food conditions;
- Collectively, these results indicate that subtropical systems are at risk of *C. raciborskii* blooms when copepods are the dominant meso-zooplankton, and when algal biomass is non-limiting (> 1.0 mg C L^{-1}).

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REFERENCES

Båmstedt, U., Gifford D., Atkinson, A., Irigoien, X., Roman, M., 2000. Feeding. In: *ICES Zooplankton Methodology Manual* (Ed. Harris R.). Academic Press, San Diego, pp. 297-399.

Bayly, Y. I., 1992. Fusion of the genera Boeckella and Pseudoboeckella. *Revista Chilena de Historia Natural*, 65:17-63.

Boon, P. I., Stuart, E. B., Green, J. D., Shiel, R. J., 1994. Consumption of Cyanobacteria by Freshwater Zooplankton: Implications for the Success of 'Top-down' Control of Cyanobacterial Blooms in Australia. *Aust. J. Mar. Freshwater Res.,* 45:875-887.

Bouvy, M., Molica, R., De Oliveira, S., Marinho M., Beker, B., 1999. Dynamics of a toxic cyanobacterial bloom (*Cylindrospermopsis raciborskii*) in a shallow reservoir in the semi-arid region of northeast Brazil. *Aquatic Microbial Ecology,* 20: 285-297.

Bouvy, M., Pagano, M., Troussellier, M., 2001. Effects of a cyanobacterial bloom (*Cylindrospermopsis raciborskii*) on bacteria and zooplankton communities in Ingazeira reservoir (northeast Brazil). *Aquatic Microbial Ecology*, 25:215-227.

Briand, J. F., Leboulanger, C., Humbert, J. F., Bernard, C., Dufour, P., 2004. *Cylindrospermopsis raciborskii* (cyanobacteria) invasion at mid latitudes: selection, wide physiological tolerance, or global warming? *Journal of Phycology*, 40:231-238.

Briand, J. F., Robillot, C., Quiblier-Lloberas, C., Humbert, J. F., Coute, A., Bernard, C., 2002. Environmental context of *Cylindrospermopsis raciborskii* (Cyanobacteria) blooms in a shallow pond in France. *Water Research*, 36:3183–3192.

Bruce, L. C., Hamilton, D., Imberger, J., Gal, G., Gophen, M., Zohary, T., Hambright, K.D., 2006. A numerical simulation of the role of zooplankton in C, N and P cycling in Lake Kinneret, Israel. *Ecological Modelling,* 193:412-436.

Burford, M. A., Davis T. W., 2011. Physical and chemical processes promoting dominance of the toxic cyanobacterium *Cylindrospermopsis raciborskii*. *Chinese Journal of Oceanology and Limnology,* 29: 883-891.

Burford, M. A., O'Donohue, M. J., 2006. A comparison of phytoplankton community

assemblages in artificially and naturally mixed subtropical water reservoirs. *Freshwater Biology,* 51: 973-982.

Burford, M. A., Johnson, S. A., Cook, A. J ., Packer, T. V., Taylor, B. M., Townsley, E. R., 2007. Correlations between watershed and reservoir characteristics, and algal blooms in subtropical reservoirs. *Water research,* 41:4105-4114.

Burns, C. W., Hegarty, B., 1994. Diet selection by copepods in the presence of cyanobacteria. *Journal of Plankton Research,* 16: 1671-1690.

Burns, C. W., Xu, Z., 1990. Calanoid copepods feeding on algae and filamentous cyanobacteria: rates of ingestion, defaecation and effects on trichome length. *Journal of Plankton Research,* 12:201-213.

Cottingham, K. L., Knight, S.E., Carpenter, S.R., Cole, J.J., Pace, M.L., Wagner, A.E., 1997. Response of phytoplankton and bacteria to nutrients and zooplankton: a mesocosm experiment. *J. Plankton Res.,* 19:995-1010.

Cowles, T., 1979. The feeding response of copepods from the Peru upwelling system: food size selection. *J. Mar. Res.,* 37:601-622.

DeMott, W. R., 1982. Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina*. *Limnology and Oceanography*, 27: 518-527.

DeMott, W. R., 1988. Discrimination between algae and artificial particles by freshwater and marine copepods. *Limnology and Oceanography,* 33: 397-408.

DeMott, W. R., 1993. Hunger dependent diet selection in suspension-feeding zooplankton. In: *Diet Selection: An Interdisciplinary Approach to Foraging Behavior* (Ed R.N. Hughes). Wiley-Blackwell, London, pp. 102-122.

DeMott, W. R., Kerfoot, W. C., 1982. Competition among cladocerans: nature of the interaction between *Bosmina* and *Daphnia*. *Ecology*, 63: 1949-1966.

DeMott, W. R., Watson, M. D., 1991. Remote detection of algae by copepods: responses to algal size, odors and motility. *Journal of Plankton Research,* 13: 1203-1222.

DeMott, W. R., 1995. Optimal foraging by a suspension-feeding copepod responses to short-termed seasonal-variation in food resources. *Oecologia*, 103:230-240

DeMott, W. R., Moxter, F., 1991. Foraging cyanobacteria by copepods: responses to chemical defense and resource abundance. *Ecology*, 782:1820-1834.

Dickman, E. M., Newell J. M., González, M. J., Vanni, M. J., 2008. Light, nutrients, and food-chain length constrain planktonic energy transfer efficiency across multiple trophic levels. *Proceedings of the National Academy of Sciences,* 105: 18408-

18412.

Dufour, P., Sarazin, G., Quiblier, C., Sané, S., Leboulanger, C., 2006. Cascading nutrient limitation of the cyanobacterium *Cylindrospermopsis raciborskii* in a Sahelian lake (North Senegal). *Aquatic Microbial Ecology*, 44:219-230.

Elert, E., Martin-Creuzburg, D., Le Coz, J. R., 2003. Absence of sterols constrains carbon transfer between cyanobacteria and a freshwater herbivore (*Daphnia galeata*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270:1209-1214.

Elser, J. J., Hayakawa, K., Urabe, J., 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. *Ecology*, 82: 898-903.

Engström, J., Koski, M., Viitasalo, M., Reinikainen, M., Repka, S., Sivonen, K., 2000. Feeding interactions of the copepods *Eurytemora affinis* and *Acartia bifilosa* with the cyanobacteria *Nodularia sp*. *Journal of Plankton Research,* 22: 1403-1409.

Ferrão Filho, A. S., da Costa, S. M., Ribeiro, M. G. L., Azevedo, S., 2008. Effects of a saxitoxin-producer strain of *Cylindrospermopsis raciborskii* (cyanobacteria) on the swimming movements of cladocerans. *Environmental Toxicology,* 23: 161-168.

Ferrão-Filho, A. D. S., Soares, M. C. S., de Freitas Magalhães, V. , Azevedo, S. M. F. O., 2009. Biomonitoring of cyanotoxins in two tropical reservoirs by cladoceran toxicity bioassays. *Ecotoxicology and Environmental Safety*, 72: 479-489.

Figueredo, C. C., Giani, A., Bird, D. F., 2007. Dose alleopathy contribute to *Cylindrospermopsis raciborskii* (cyanobacteria) bloom occurrence and geographic expansion? *Journal of Phycology,* 43: 256-265.

Fulton, R. S., Paerl, H. W., 1987. Toxic and inhibitory effects of the blue-green alga *Microcystis aeruginosa* on herbivorous zooplankton. *Journal of Plankton Research,* 9: 837-855.

Fulton, R. S., 1988. Grazing on filamentous algae by herbivorous zooplankton. *Freshwater Biology*, 20:263-271.

Ger, K. A., Panosso, R., LÃrling, M., 2011. Consequences of acclimation to *Microcystis* on the selective feeding behavior of the calanoid copepod *Eudiaptomus gracilis*. *Limnology and Oceanography*, 56: 2103-2114.

Gilbert, J. J., Durand, M. W., 1990. Effect of *Anabaena flos aquae* on the abilities of *Daphnia* and *Keratella* to feed and reproduce on unicellular algae. *Freshwater Biology,* 24: 577-596.

Gliwicz, Z. M., Lampert ,W., 1990. Food thresholds in *Daphnia* species in the absence and presence of blue-green filaments. *Ecology*, 71: 691-702.

Graham, J. L., Loftin, K. A., Kamman, N., 2009. Monitoring recreational freshwaters. *Lakeline*, 29:16-22.

Gulati, R. D., Van Donk, E., 2002. Lakes in the Netherlands, their origin, eutrophication and restoration: state-of-the-art review. *Hydrobiologia,* 478: 73-106.

Hambright, K. D., Hairston, N. G. J., Schaffner, W. R. , Howarth, R. W., 2007. Grazer control of nitrogen fixation: phytoplankton taxonomic composition and ecosystem functioning. *Fundamental and Applied Limnology Archiv fuer Hydrobiologie,* 170: 103-124

Hambright, K. D., Zohary, T., Easton, J., Azoulay, B. , Fishbein, T., 2001. Effects of zooplankton grazing and nutrients on the bloom-forming, N2-fixing cyanobacterium *Aphanizomenon* in Lake Kinneret. *Journal of Plankton Research,* 23: 165-174.

Haney, J. F., 1987. Field studies on zooplankton-cyanobacteria interactions. *New Zealand Journal of Marine and Freshwater Research*, 2: 467-475.

Hawkins, P., Lampert, W., 1989. The effect of *Daphnia* body size on filtering rate inhibition in the presence of a filamentous cyanobacterium. *Limnology and Oceanography,* 34: 1084-1089.

Hawkins, P. R., 1988. The zooplankton of a small tropical reservoir (Solomon Dam, North Queensland). *Hydrobiologia*, 157:105-118.

He, X., Wang, W. X., 2007. Kinetics of phosphorus in *Daphnia* at different food concentrations and carbon: phosphorus ratios. *Limnology and Oceanography,* 52: 395-406.

Isvánovics, V., Shafik, H. M., Présing, M., Juhos, S., 2000. Growth and phosphate uptake kinetics of the cyanobacterium, *Cylindrospermopsis raciborskii* (Cyanophyceae) in throughflow cultures. *Freshwater Biology*, 43:257-275.

James, M. R., Salonen, K., 1991. Zooplankton-phytoplankton interactions and their importance in the phosphorus cycle of a polyhumic lake. *Archiv fuer Hydrobiologie,* 123: 37-51.

Kâ, S., Mendoza-Vera, J. M., Bouvy, M., Champalbert, G., N'Gom-Kâ, R., Pagano, M., 2012. Can tropical freshwater zooplankton graze efficiently on cyanobacteria? *Hydrobiologia*, 679: 119-138

Kleppel, G., 1993. On the diets of calanoid copepods. *Marine Ecology-progress Series,* 99:183-183.

Kobayashi, T., Church, A., Hardiman, S., Gallagher, L., 1998. Grazing by a resident macrozooplankton community and non-resident *Daphnia carinata* King: A preliminary in situ incubation study. *Lakes & Reservoirs: Research and Management,* 3:193-203.

Lagos, N., Onodera, H., Zagatto, P. A., Andrinolo, D., Azevedo, S. M. F. Q., Oshima, Y., 1999. The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii,* isolated from Brazil. *Toxicon,* 37:1359-1373.

Lehmam, J. T., 1980. Release and cycling of nutrients between planktonic algae and herbivores. *Limno. Oceanogr.* 25:620-632.

Leonard, J. A., Paerl, H. W., 2005. Zooplankton community structure, micro-zooplankton grazing impact, and seston energy content in the St. Johns river system, Florida as influenced by the toxic cyanobacterium *Cylindrospermopsis raciborskii*. *Hydrobiologia*, 537:89-97.

Lürling, M., van Donk, E., 1997. Morphological changes in *Scenedesmus* induced by infochemicals released in situ from zooplankton grazers. *Limnology and Oceanography,* 42: 783-788.

Matveev, V., Matveeva, L., 1997. Grazer control and nutrient limitation of phytoplankton biomass in two Australian reservoirs. *Freshwater Biology*, 38: 49-65.

McGregor, G. B., Fabbro, L. D., 2000. Dominance of *Cylindrospermopsis raciborskii* (Nostocales, Cyanoprokaryota) in Queensland tropical and subtropical reservoirs: Implications for monitoring and management. *Lakes & Reservoirs: Research & Management*, 5:195-205.

Priyanesh, M., 2010. The role of nutrients with respect to promoting phytoplankton growth and species composition in subtropical reservoir. Diss thesis. Griffith University. Griffith, Print.

Nogueira, I. C. G., Lobo-da-Cunha, A., Vasconcelos, V. M., 2006. Effects of

Cylindrospermopsis raciborskii and *Aphanizomenon ovalisporum* (cyanobacteria) ingestion on *Daphnia magna* midgut and associated diverticula epithelium. *Aquatic Toxicology,* 80: 194-203.

Nogueira, I. C. G., Saker M. L., Pflugmacher, S., Wiegand, C., Vasconcelos, V. M., 2004. Toxicity of the cyanobacterium *Cylindrospermopsis raciborskii* to *Daphnia magna*. *Environmental Toxicology*, 19: 453-459.

Nogueira, I. C. G., Saker, M. L., Pflugmacher, S., Wiegand, C., Vasconcelos, V. M., 2004. Toxicity of the cyanobacterium *Cylindrospermopsis raciborskii* to *Daphnia magna*. *Environmental toxicology*, 19:453-459.

O'Brien, K. R., Burford, M. A., Brookes, J. D., 2009. Effects of light history on primary productivity in a phytoplankton community dominated by the toxic cyanobacterium *Cylindrospermopsis raciborskii*. *Freshwater Biology* 54:272-282.

Ohtani, I., Moore, R. E., Runnegar, M.T. C., 1992. Cylindrospermopsin: a potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*. *Journal of the American Chemical Society,* 114:7941-7942.

O'Neil, J., Davis, T., Burford, M., Gobler, C., 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae,* 14:313-334.

Padisák, J., 1997. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and a review of its ecology. *Arch. Hydrobiol. Suppl*. 107, *Monogr. Stud*.: 563–593.

Paerl, H. W., Fulton III, R. S., Moisander P. H., Dyble J., 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World Journal,* 1: 76-113.

Panosso, R., Lurling, M., 2010. *Daphnia magna* feeding on *Cylindrospermopsis raciborskii*: the role of food composition, filament length and body size. *Journal of Plankton Research*, 32:1393-1404.

Panosso, R., Carlsson, P., Kozlowsky-Suzuki, B., Azevedo, S. M. F. O., Granéli, E., 2003. Effect of grazing by a neotropical copepod, *Notodiaptomus*, on a natural cyanobacterial assemblage and on toxic and non-toxic cyanobacterial strains. *Journal of Plankton Research*, 25:1169-1175.

Price, H. J., Paffenhofer, G. A., 1985. Perception of food availability by calanoid

copepods *Arch.Hydrobiol. Beih. Ergeb. Limnol.,* 21: 115-124.

Reynolds, C. S., 1984. Loss Processes. In: *The Ecology of Freshwater Phytoplankton* (Eds E. Beck; H. J. b. Birks, E. F. Connor). Cambridge University Press, New York, pp. 225-274.

Saker, M. L., Nogueira, I. C. G., Vasconcelos, V. M., 2004. Distribution and toxicity of *Cylindrospermopsis raciborskii* (Cyanobacteria) in Portuguese freshwaters. *Limnetica,* 23: 145-152.

Sarma1, S., Nandini, S., Gulati, R., 2005. Life history strategies of cladocerans: comparisons of tropical and temperate taxa. *Aquatic Biodiversity II: Developmetns in hydrobiology*, 180: 315-333.

Scavia, D., Gardner, W.S., 1982. Kinetics of nitrogen and phosphorus release in varying food supplies by *Daphnia magna*. *Hydrobiologia,* 96:105-111.

Shiel, R.J., 1990. Zooplankton. In: *The Murray* (Eds N. Mackay , D. Eastburn), Murray-Darling Basin Commission. Canberra, pp. 275-286.

Sinha, R., Pearson, L. A., Davis, T. W., Burford, M. A., Orr, P. T., Neilan, B. A., 2012. Increased incidence of *C. raciborskii* in temperate zones-is climate change responsible? *Water research,* 46:1408-1419.

Soares, M. C. S., Lürling, M., Huszar, V. L. M., 2010. Responses of the rotifer Brachionus calyciflorus to two tropical toxic cyanobacteria (*Cylindrospermopsis raciborskii* and *Microcystis aeruginosa*) in pure and mixed diets with green algae. *Journal of Plankton Research,* 32:999-1008.

Soares, M. C. S., Lürling, M., Panosso, R., Huszar, V., 2009. Effects of the cyanobacterium *Cylindrospermopsis raciborskii* on feeding and life-history characteristics of the grazer *Daphnia magna*. *Ecotoxicology and Environmental Safety*, 72:1183-1189.

Sommer, U., 1989. The role of competition for limiting nutrients in phytoplankton species replacements.In: *Plankton Ecology; Succession in Plankton Communities* (Ed U. Sommer). Springer, Berlin, pp. 57-106.

Sommer, U., Sommer, F., Santer, B., Zollner, E., Jurgens, K., Jamieson, C., Boersma, M., Gocke, K., 2003. *Daphnia* versus copepod impact on summer phytoplankton: functional compensation at both trophic levels. *Oecologia,* 135: 639-647.

Towns, T. G., 1986. Determination of aqueous phosphate by ascorbic acid reduction of phosphomolybdic acid. *Analytical Chemistry,* 58:223-229.

Van Donk, E., Hessen, D. O., Verschoor, A. M., Gulati, R. D., 2008. Re-oligotrophication by phosphorus reduction and effects on seston quality in lakes. *Limnologica-Ecology and Management of Inland Waters*, 38: 189-202.

Vanderploeg, H. A., 1990. Feeding mechanisms and their relation to particle selection and feeding in suspension-feeding zooplankton In: *The Biology of Particles in Aquatic Systems*, R.S. Wotton (ed.).CRC Press, Boca Raton, FL, pp. 183-212.

Vanderploeg, Henry A.; Paffenhöfer, Gustav-Adolf; Liebig, James R., 1988. *Diaptomus* vs. Net Phytoplankton: Effects of Algal Size and Morphology on Selectivity of a Behaviorally Flexible, Omnivorous Copepod. *Bulletin of Marine Science*, 43: 377-397.

Wang, X., Qin, B., Gao, G. , Paerl, H. W., 2010. Nutrient enrichment and selective predation by zooplankton promote *Microcystis* (Cyanobacteria) bloom formation. *Journal of Plankton Research*, 32: 457-470.

Table 1. Origin of algal strains used in this study

Strain ID	Species	Toxicity	Source	Toxin authority
CS-51	*C. reinhardtii*	none	ANACC[1]	
CS-508	*C. raciborskii*	none	ANACC	
CS-505	*C. raciborskii*	toxic[2]	ANACC	Saker and Griffiths 2000
NPD	*C. raciborskii*	toxic	ARI/GU[3]	Davis (unpublished data)

1. Australian National Algae Culture Collection
2. cylindrospermopsin
3. Australian Rivers Institute, Griffith University

Table 2. Cellular carbon, nitrogen and phosphorus content (pg cell^{-1}) of *C. raciborskii* (strain NPD) used in experiments. Note that *C. raciborskii* results are cited per cell but that each filament (the morphological form used in experiments) comprises an average of 15 cells per filament.

Strain	culture medium	C content (pg cell^{-1})	N content (pg cell^{-1})	P content (pg cell^{-1})
C. raciborskii (NPD)	0% P MLA	3.62 ± 0.13	0.70 ± 0.00	0.02 ± 0.00
C. raciborskii (NPD)	10% P MLA	5.05 ± 0.22	0.96 ± 0.02	0.07 ± 0.00
C. raciborskii (NPD)	100% P MLA	3.19 ± 0.14	0.70 ± 0.01	0.06 ± 0.00
C. reinhardtii	100% MLA	15.70 ± 1.03	2.21 ± 0.02[1]	NM[2]
C. raciborskii (NPD)	Filtered LWW[3]	5.14 ± 0.04	0.91 ± 0.06	0.14 ± 0.00
Lake Wivenhoe water		3.09 ± 0.02[3]	0.39 ± 0.03	0.13 ± 0.01

1. N content at limit of detection
2. not measured
3. Cell quota estimated by microscopic enumeration of total phytoplankton cell abundance

Table 3. The selective coefficient (α) of copepod *Boeckella sp.* on the green alga *Chlamydomonas reinhardtii* and the cyanobacterium *Cylindrospermopsis raciborskii* when *C.* raciborskii was mixed with *C. reinhardtii* in various proportions, keeping total phytoplankton biomass constant at 1.0 mg C L^{-1}, or alternatively, when *C. raciborskii* (0.5 mg C L^{-1}) was mixed with different amounts of *C. reinhardtii* with increasing total phytoplankton biomass. Data are mean of three replicates ± standard deviation.

Biomass (mg C L^{-1})			Clearance rate (ml ind^{-1} h^{-1})		Selection Coefficient (α)	
Total	*C. raciborskii*	*C. reinhardtii*	*C. raciborskii*	*C. reinhardtii*	*C. raciborskii*	*C. reinhardtii*
1.0	0.0	1.0	n/a	1.1 0.22		
1.0	0.2	0.8	0.0	1.4 ± 0.24	0.0	1.0
1.0	0.4	0.6	0.0	0.9 ± 0.43	0.0	1.0
1.0	0.6	0.4	0.2 ± 0.03	1.5 ± 0.06	0.1	0.9
1.0	1.0	0.0	0.3 ± 0.08	n/a		
1.5	0.5	1.0	0.0	1.5 ± 0.08	0.0	1.0
2.0	0.5	1.5	0.0	1.3 ± 0.02	0.0	1.0
2.5	0.5	2.0	0.0	0.8 ± 0.07	0.0	1.0

Figure legends

Figure 1. Experiment design

Figure 2. Clearance rates of a mixed zooplankton community on a natural Lake Wivenhoe phytoplankton community with different proportions of *C. raciborskii*. Data are mean ± SD for three replicates. Different letters above the columns indicate significant differences between treatments.

Figure 3. Clearance rates of copepod *Boeckella sp.* and cladoceran *Ceriodaphnia sp*. on *C. raciborskii* when they were fed with filtered lake water , unfiltered lake water with a natural phytoplankton community (0.7 mg C L^{-1}) and lake water enriched with *C. reinhardtii* (1.2 mg C L^{-1}) respectively. Data are mean ± SD for three replicates. Different letters above the columns indicate significant differences between treatments.

Figure 4. Filament length (A) and copepod *Boeckella sp.* clearance rates (B) on different *C. raciborskii* strains of different toxic status. Data are mean ± SD for three replicates. Different letters above the columns indicate significant differences between treatments.

Figure 5. Filament length (A) and copepod *Boeckella sp.* clearance rates (B) on different *C. raciborskii* strains of different P content and C:P status. Data are mean ± SD for three replicates. Different letters above the columns indicate significant differences between treatments.

Figure 6. Clearance rates of *Boeckella sp.* on *C. raciborskii* after animals were preconditioned for ~6 h in filtered Manly Dam lake water, lake water containing a natural phytoplankton community (0.7 mg C L^{-1}), filtered lake water with *C. reinhardtii* (0.5 mg C L^{-1}) or *C. raciborskii* (0.5 mg C L^{-1}). Data are mean ± SD for three replicates. Different letters above the columns indicate significant differences between treatments.

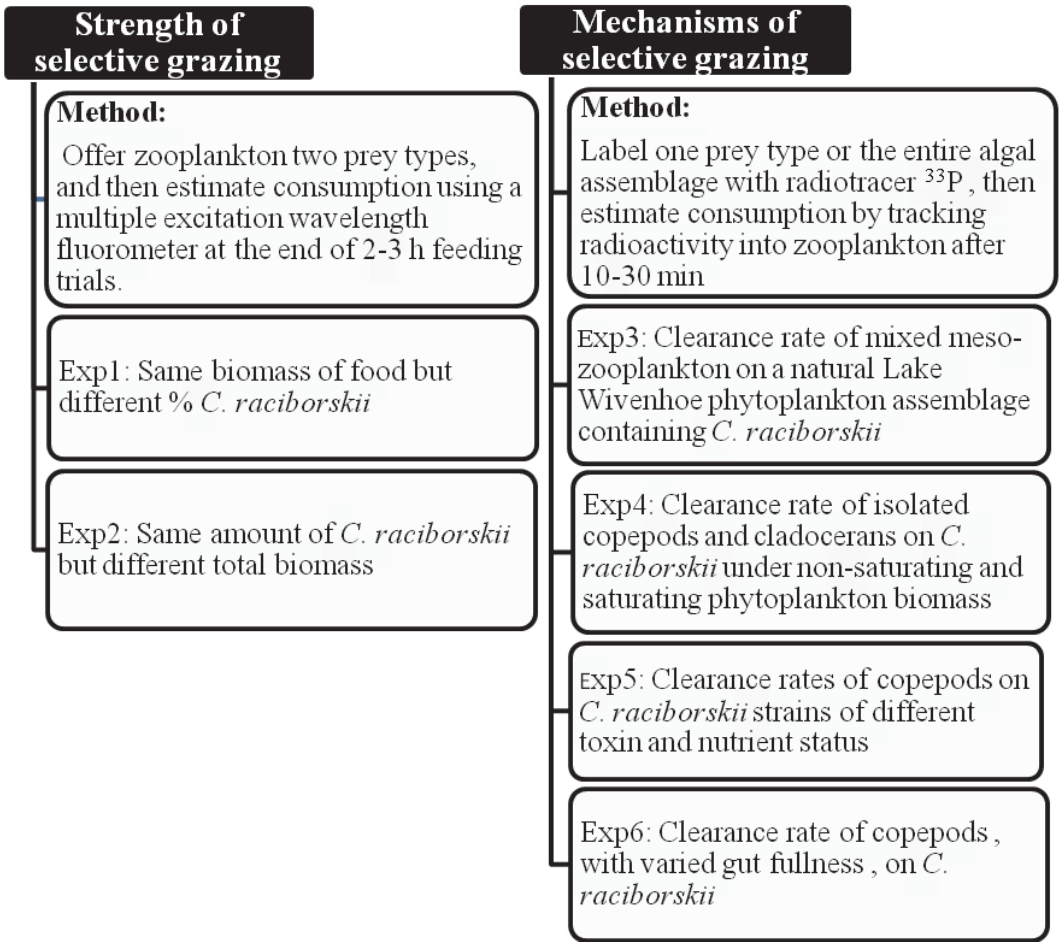
Strength of selective grazing
Method:
Offer zooplankton two prey types, and then estimate consumption using a multiple excitation wavelength fluorometer at the end of 2-3 h feeding trials.
Exp1: Same biomass of food but different % C. raciborskii
Exp2: Same amount of C. raciborskii but different total biomass
Mechanisms of selective grazing
Method:
Label one prey type or the entire algal assemblage with radiotracer 33P , then estimate consumption by tracking radioactivity into zooplankton after 10-30 min
Exp3: Clearance rate of mixed meso-zooplankton on a natural Lake Wivenhoe phytoplankton assemblage containing C. raciborskii
Exp4: Clearance rate of isolated copepods and cladocerans on C. raciborskii under non-saturating and saturating phytoplankton biomass
Exp5: Clearance rates of copepods on C. raciborskii strains of different toxin and nutrient status
Exp6: Clearance rate of copepods , with varied gut fullness , on C. raciborskii

Figure 1

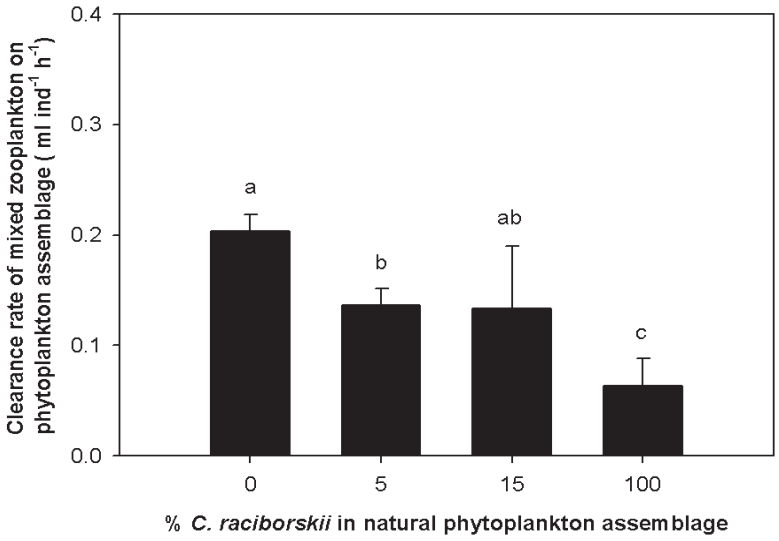
Clearance rate of mixed zooplankton on phytoplankton assemblage (ml ind-1 h-1)
0.4
0.3
0.2
0.1
0.0
a
b
ab
c
0
5
15
100
% C. raciborskii in natural phytoplankton assemblage

Figure 2

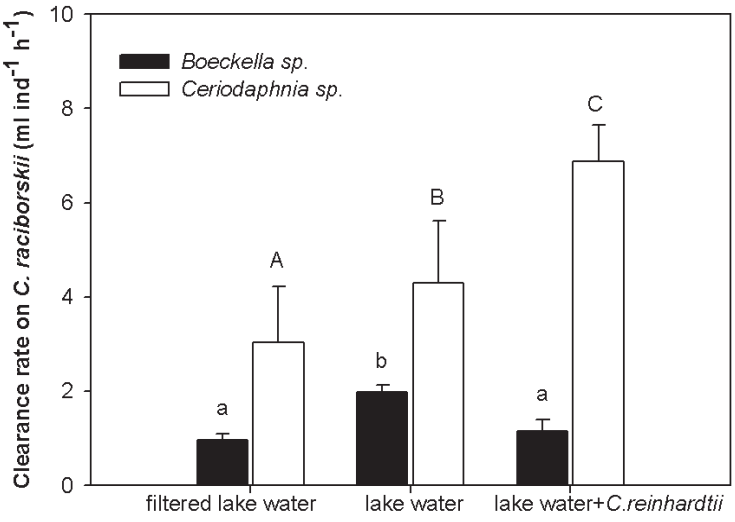
Clearance rate on C. raciborskii (ml ind-1 h-1)
10
8
6
4
2
0
Boeckella sp.
Ceriodaphnia sp.
a
A
b
B
a
C
filtered lake water
lake water
lake water+C.reinhardtii

Figure 3

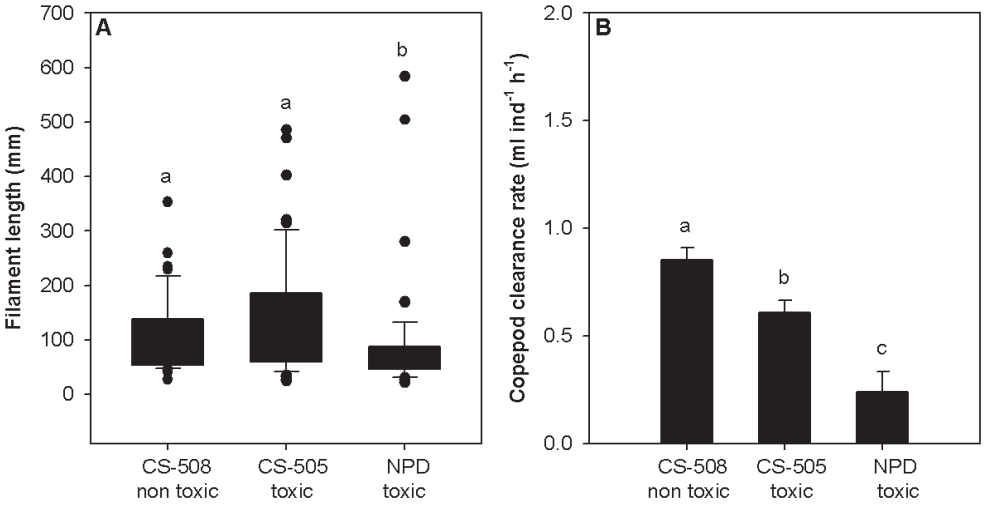
A
Filament length (mm)
700
600
500
400
300
200
100
0
a
a
b
CS-508
non toxic
CS-505
toxic
NPD
toxic
B
Copepod clearance rate (ml ind-1 h-1)
2.0
1.5
1.0
0.5
0.0
a
b
c
CS-508
non toxic
CS-505
toxic
NPD
toxic

Figure 4

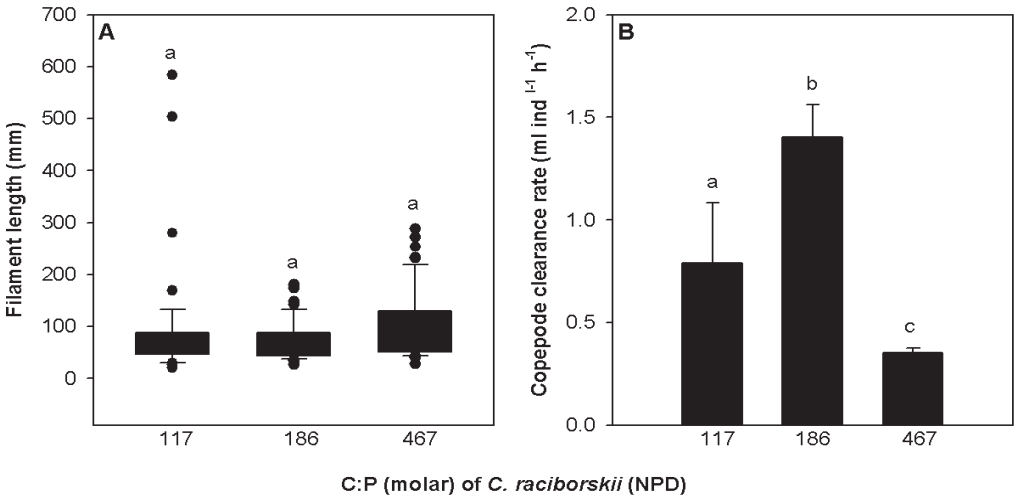
A
B
Filament length (mm)
Copepode clearance rate (ml ind $^{l-1}$ h^{-1})
700
600
500
400
300
200
100
0
2.0
1.5
1.0
0.5
0.0
a
a
a
b
c
117
186
467
117
186
467
C:P (molar) of C. raciborskii (NPD)

Figure 5

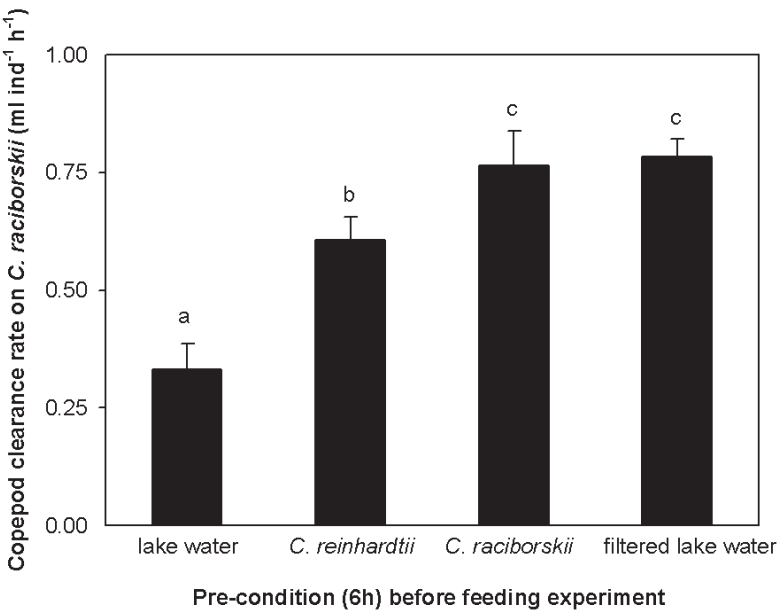
Copepod clearance rate on C. raciborskii (ml ind-1 h-1)
1.00
0.75
0.50
0.25
0.00
a
b
c
c
lake water
C. reinhardtii
C. raciborskii
filtered lake water
Pre-condition (6h) before feeding experiment

Figure 6

CHAPTER 3

Nutrient excretion by herbivorous zooplankton promotes the growth of the cyanobacterium *Cylindrospermopsis raciborskii*

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ABSTRACT

Cylindrospermopsis raciborskii is a major summer bloom-forming cyanobacterium in the water supplies of subtropical Australia and other regions. This cyanobacterium can be highly toxic and form blooms even when the concentration of phosphorus (P) in ambient waters is below the detection limit. To explain the bloom formation of *C. raciborskii* in low nutrient concentrations, we tested the hypothesis that zooplankton nutrient recycling facilitates *C. raciborskii* growth. *C. raciborskii* can fix nitrogen, so we focused on P as the growth-limiting nutrient. Experiments with dialysis tubing 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 40% greater in the presence of a green algal competitor when both algae were in dialysis tubes, indicating that *C. raciborskii* may also access nutrients released by co-existing algae. 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 promoted both directly and indirectly by zooplankton, suggesting that foodweb interactions can facilitate blooms of this toxic cyanobacterium.

1. INTRODUCTION

Herbivores affect the growth, reproduction, and survival of primary producers via multiple pathways (Danel, 2006; Maron and Crone, 2006). Grazing suppresses primary producers, while nutrient recycling by herbivores can increase dissolved nutrient pools and enhance the growth of primary producers (Hillebrand et al., 2008). For example, catfish presence in a riverine ecosystem increased dissolved N concentration and recycling rates, leading to higher periphyton N:P, and increased periphyton growth by 1- to 2-fold (Knoll et al., 2009).

Consumer-mediated nutrient cycling has been demonstrated as an important driver of primary production in the oligotrophic ocean (Banse, 1995). There is, however, more uncertainty about the impact of this process in freshwater systems. Despite much speculation, there is little experimental evidence for indirect facilitation of algal growth by zooplankton consumers. Hunt and Matveev (2005) showed strong direct impacts of grazing that were partially offset by nutrient regeneration, but few studies have tested the strength or relative magnitude of nutrient recycling by zooplankton in aquatic systems (Sterner, 1986; Vanni and Layne, 1997; Vanni, 2002). This is partly due to the difficulty in separating these effects experimentally.

Ecological stoichiometry theory (Sterner and Elser, 2002) provides a useful framework for understanding herbivore-plant interactions by consumer-driven nutrient recycling (CNR) (Sterner, 1990; Hillebrand et al., 2008). The theory predicts that herbivores of contrasting body stoichiometry will remineralise nutrients differently to maintain their elemental ratios. Copepods with relatively high N:P in their body tissue tend to release nutrients with low N:P compared to cladocerans with relative low body N:P (Rothhaupt, 1995; Elser and Urabe, 1999). The theory also predicts that elemental assimilation by grazers is driven by the elemental ratio of prey. He and Wang (2007) demonstrated this in a laboratory study using the cladoceran *Daphnia magna* and the green alga, *Chlamydomonas reinhardtii*, showing that P released from the herbivores decreased when dietary C:P declined.

Consumer-driven nutrient recycling has the potential to favour certain phytoplankton groups due to algal cell preference for organic versus inorganic nutrients

(Glibert and Burkholder, 2006) or contrasting nutrient uptake efficiencies (Persson et al., 1988). For example, the harmful algal bloom species *Aureococcus anophagefferens* (Pelagophyceae), a eukaryotic picoplankton that blooms in estuaries and coastal bays (Berg et al., 2003), can outcompete coexisting algae because of its ability to use dissolved organic nitrogen. In freshwaters, the toxic cyanobacterium *Cylindrospermopsis raciborskii* is characterized by a very high affinity for phosphorus (Isvánovics et al., 2000; Burford and O'Donohue, 2006), showing higher phosphate uptake rate and higher P storage capacity than other algae, including other cyanobacteria (i.e., *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*) (Wu et al., 2009).

C. raciborskii is a major summer bloom-forming cyanobacterium in the water supplies of subtropical Australia, Brazil and other temperate locations worldwide including Greece, Germany, Hungary, Slovakia, Spain, and North America (Sinha et al., 2011), when phosphate concentrations are below detection limit (McGregor and Fabbro, 2000) and where zooplankton are typically dominated by copepods and rotifers (Bayly, 1992; Jeppesen et al., 2005). Given the competitiveness of *C. raciborskii* for P uptake and the potential for proportionally higher P release by herbivores with high N:P body content (such as copepods), we predicted that the copepod-dominated zooplankton community would facilitate *C. raciborskii* dominance through both direct and indirect effects.

In this study we aimed to: 1) quantify the growth response of *C. raciborskii* to zooplankton-derived nutrients; 2) measure the competitive advantage of *C. raciborskii* in using recycled P compared to other algae; and 3) evaluate to what degree food quality (P quota) affects the importance of recycled P in sustaining *C. raciborskii*, and 4) quantify the selective feeding of copepods on *C. raciborskii* and a green alga, *Chlamydomonas reinhardtii*.

2. MATERIALS AND METHODS

2.1. Experimental design

Experiments were designed to separate the direct (grazing) and indirect (nutrient regeneration) effects of zooplankton-algal interactions, with dialysis tubing nested

inside a jar enabling zooplankton to access food outside the dialysis tubing, while algae inside the dialysis tubing had access to zooplankton-derived nutrients for growth. A basic experimental unit comprised a 60 mm diameter jar (200 ml volume) in which dialysis tubing (25 mm diameter; 50 ml volume) was suspended (Fig. 1). The dialysis tubing had a flat width of 50 - 54 mm and molecular weight cutoff (MWCO) of 12,000 - 14,000 Daltons (Visking, Medical International, London, UK). The tubing was soaked in distilled water for 2 hours prior to the experiments before the ends were folded and clamped with plastic clips (Visking, Medical International, London, UK). Each dialysis tube was filled with 50 ml cultured algae (Table 1) which was collected by centrifugation at 3500 r.p.m. for 10 min and then resuspended into 0% P MLA medium or as otherwise noted. Dialysis tubes were then suspended in jars containing filtered lake water, with each jar containing 10 adult copepods (*Boeckella fluvialis*) and prey algae equivalent to 2.0 mg C L^{-1}. A second series of identical jars with no copepods formed the controls.

Experiment 1: Quantifying the growth response of *C. raciborskii* to zooplankton-derived nutrients

The first experiment assessed whether zooplankton-derived nutrients could alleviate P-limitation of a non-toxic strain *C. raciborskii* and stimulate growth (Fig. 1). P-replete and P-deplete *C. raciborskii* were placed inside the dialysis bags and copepods outside the dialysis bags were provided with the P-replete green alga, *C. reinhardtii*, which are readily consumed by *Boeckella* sp. (Hong, unpublished data).

Experiment 2. Assessing the uptake of regenerated nutrients and inorganic P by *C. raciborskii* and other algae

A follow-up experiment compared growth of P-deplete *C. raciborskii* and the green alga, *C. reinhardtii*, when grown together with access to inorganic P versus zooplankton-derived nutrients, and was designed to evaluate the competitiveness of each alga to obtain either nutrient source (Fig. 2).

Experiments 3 and 4. Effect of food quality on zooplankton regeneration and its impact on growth of a non-toxic and toxic strain of *C. raciborskii*.

Experiment 3 tested the impact of algal prey stoichiometry (carbon: phosphorus, C:P) on the magnitude of zooplankton nutrient regeneration by feeding copepods with P-deplete or P-replete algae, and its subsequent impact on growth of P-deplete *C.*

raciborskii (non-toxic strain) and *C. reinhardtii* inside dialysis tubes (Fig. 3). In addition, the growth of each alga when present on its own in the dialysis bags or in a mixture was compared to assess the competitiveness of each alga to obtain zooplankton-derived nutrients.

A similar experiment (4) was performed with a toxic strain of *C. raciborskii* which was extremely P-deplete (reflecting its likely condition in natural oligotrophic habitats). Algal prey was either P-replete ("r") or P-deplete ("d") and was available to copepods in rr, rd, dr and dd combinations to allow additional assessment of selective grazing (Fig. 4). The rr treatment rr represented eutrophic conditions so that all algae were P-replete. The dd treatment represented extremely P limited conditions, while dr reflected conditions when there is a nutrient pulse in oligotrophic habitats such that *C. raciborskii* is richer in P than other algae due to its high affinity for P.

2.2. Experiment procedures

2.2.1. Collection of animals

Zooplankton were collected from Manly Dam (34° 46'3" S, 151º 14'52" E) situated in NSW, Australia. Animals were sampled by vertical net hauls (diameter 0.5 m; mesh 165 µm), with *Boeckella fluvialis* Henry (copepod) and *Ceriodaphnia* sp. (cladoceran) being the dominant meso-zooplankton taxa, typical of south-east Australian lakes and reservoirs (Kobayashi, 1993; Boon *et al.,* 1994). On return to the laboratory, zooplankton were maintained in 10-20 L containers of lake water at ambient temperature (21 °C) under 10 µmol photons m^{-2} s^{-1} with a 12:12 h light-dark cycle.

2.2.2. Algal strains

The green alga *Chlamydomonas reinhardtii* and a non-toxic strain of *C. raciborskii* (CS-508) were obtained from the Australian National Algae Culture Collection (ANACC), and maintained in MLA medium (Bolch and Blackburn, 1996) at 25 °C under 40 µmol photons m^{-2} s^{-1}. *C. reinhardtii* was used because previous studies indicated it is readily consumed by zooplankton (DeMott & Moxter, 1991; Burns & Hegarty, 1994; Hong et al., submitted). While *C. raciborskii* CS-508 was used for

Experiments 1-3, a toxic strain of *C. raciborskii* (NPD) was used in Experiment 4. The NPD-strain was obtained from Griffith University, Queensland, Australia and was originally isolated from Lake Samsonvale (QLD, Australia) where *C. raciborskii* occurs regularly in high abundance during the austral summer (Burford & O'Donohue, 2006).

2.2.3. Preparation of P-replete and P-deplete algae

P-replete *C. raciborskii* and *C. reinhardtii* were maintained by transferring cells into fresh nutrient-replete medium every 5 days. P-deplete cells were prepared by a step-wise series of transfers into phosphorus-depleted MLA medium; i.e., exponentially growing cells were transferred from 100% P into 10% P medium, and after 5 days, they were transferred into 1 % or 0% P medium. For Experiment 1, P-deplete cells were prepared by putting *C. raciborskii* in 0% medium for 1 week, but this was modified to 1% medium for 1 week in Experiment 2 and 3 because *C. reinhardtii* looked visibly P limited (cells were yellow rather than green). For Experiment 4, P-deplete cells were kept in 0% P medium for 30 days to ensure they had depleted their internal P stores.

2.2.4. Measurement of elemental content (C, N and P) of algae

The elemental (C, N, P) content of prey (*C. raciborskii* and *C. reinhardtii*) used in experiments was estimated by filtering known volumes of cultures onto pre-combusted 25 mm quartz filters (Whatman, UK) which were stored frozen at -20 °C until analysis. Samples for Total Phosphorus (TP) were digested using a persulfate digestion procedure. After digestion, TP was analysed based on the ascorbic acid reduction of phosphomolybdate (Towns, 1986). For C and N analyses, the filters were dried at 50 °C overnight, packaged into tin cups and analysed using an Elemental Analyser with 20-20 IRMS (Europa Scientific). The cell abundance of each algal species was estimated using a Sedgwick Rafter cell and compound microscope (Olympus BX50, Hamburg, Germany) at 400 x magnification. Cell quotas of C, N and P were then calculated.

2.2.5. Measurement of algal growth rates

All jars were kept at 21 °C under 40 µmol photons m^{-2} s^{-1} with a 12:12 h light-dark cycle and were gently shaken（~ 45 o.p.m.）on an orbital shaker (Paton Scientific, Victor Harbor, Australia) to ensure homogenous mixing and exchange of recycled nutrients across the dialysis membrane. Subsamples (5 ml) were removed from dialysis tubes every 24 h, and chlorophyll-a (Chl-a) fluorescence (F) and maximum quantum yield of PSII was measured after 30 min dark adaptation using a Phyto-PAM (Walz, Effeltrich, Germany) equipped with a "Phyto-ED" emitter-detector unit. To distinguish between the Chl-a fluorescence of the cyanobacterium *C. raciborskii* and the chlorophyte, *C. reinhardtii*, a reference spectrum was recorded for each alga in the blue and green channel, respectively. The time course of Chl-a fluorescence of both algae was estimated and growth rate (K, d^{-1}) calculated according to the following equation:

$$K = (\ln F_t - \ln F_0)/T$$

where F_t is the final Chl-a fluorescence and F_0 is the initial Chl-a fluorescence, and T the incubation period in days.

Cell counts of T_0 and T_f samples were also conducted to verify that changes in fluorescence reflected changes in cell abundance, not changes in algal cell physiology. Due to extreme nutrient limitation during experiment 4, cell count data instead of Chl-a fluorescence was used to estimate algal growth rates.

2.3. Statistical analyses

In each experiment, the indirect effect of zooplankton nutrient regeneration was quantified by comparing growth of the algae inside the dialysis tubes when copepods were present or absent from jars. This took into account the nutrients released from algal cells in the absence of herbivores. The direct effect of zooplankton grazing (grazing loss) was quantified by comparing growth of the algae outside the dialysis tubing in the presence and absence of copepods. When two prey types were present, one-way analysis of variance (ANOVA) tested whether the grazing losses of the two prey types was significantly different.

Growth rates of algae inside dialysis bags were compared between controls and relevant treatments using a paired t-test (T(df) = t-value, p = p-value). The data was not included if dead zooplankton were found in jars. Growth was compared between treatments using ANOVA (F = F-value, p = p-value) followed by a Tukeys post-hoc test. To meet the assumption of normality for an ANOVA, the data were either square root or natural log transformed and Levene's test was used to check for homogeneity of variances. The level of significance for all tests was 0.05.

3. RESULTS

Experiment 1

The growth rates assessed by Chl-a fluorescence were comparable to the ones estimated by cell counts for both P-deplete *C. raciborskii* ($R^2 = 0.83$, p = 0.002) and *C. reinhardtii* ($R^2 = 0.83$, p = 0.017). Thus, fluorescence based estimates of growth are presented for experiments 1 to 3.

A t-test confirmed that P-deplete *C. raciborskii* growth inside the dialysis tubing was higher in the presence of copepods compared to the no copepod control (T(4) = 4.309, p = 0.013; Fig. 5, with difference indicated by asterisk *), but there was no significant difference between the control and treatment for P-replete *C. raciborskii* (T(4) = 1.393, p = 0.236). ANOVA confirmed that the growth of P-deplete *C. raciborskii* exposed to zooplankton derived nutrients was significantly higher than P-replete cells inside the dialysis tubing ($F_{3,8} = 6.224$, p = 0.017; Fig. 5 with difference indicated by letters).

Experiment 2

There was a significant positive effect of inorganic phosphate enrichment and zooplankton-derived nutrients on the growth of P-deplete *C. raciborskii* (T(4) = -9.400, p = 0.005; T(4) = -15.886, p <0.001, respectively) relative to controls. However, *C. raciborskii* grew fastest (0.28 ± 0.06 d^{-1}) (mean ± SD) when cells had access to nutrients regenerated by zooplankton ($F_{1,4} = 23.038$, p = 0.017), followed by phosphate

enrichment (0.23 ± 0.02 d^{-1}), and was ~40% greater than each of the controls with no zooplankton or added phosphate (0.17 ± 0.08 d^{-1} and 0.14 ± 0.01 d^{-1}, respectively, Fig. 6A).

In comparison, growth of *C. reinhardtii* was greatly stimulated by zooplankton-derived nutrients (T(4) = -7.754, p = 0.001, Fig. 6B), but not by inorganic P addition (T(4) = 2.924, p = 0.01). Growth was greatest when cells had access to zooplankton-derived nutrients (0.16 ± 0.03 d^{-1}), with no growth in the paired control without zooplankton (-0.01 ± 0.03 d^{-1}), and ~80% slower growth in the P enriched and unamended control treatments (0.01 ± 0.04 d^{-1} and 0.03 ± 0.03 d^{-1}, respectively). Overall, *C. raciborskii* grew faster than *C. reinhardtii* ($F_{1,4}$ = 38.112, p = 0.003) when both species accessed regenerated nutrients.

Experiment 3

Indirect effect (algal growth inside dialysis tubing)

When copepods fed on P-replete algae, the growth of *C. raciborskii* inside the dialysis tubing was stimulated relative to the control without zooplankton (T(4) = -4.789, p = 0.009). However, when zooplankton consumed P-deplete prey, there was no stimulation of growth relative to the control (T(4) = 0.147, p = 0.890; Fig 7A). Furthermore, growth of *C. raciborskii* in the presence of *C. reinhardtii* was ~40% greater compared to *C. raciborskii* on its own (0.32 ± 0.07 d^{-1} versus 0.19 ± 0.02 d^{-1}, respectively, $F_{1,4}$ = 89.144, p = 0.001).

In contrast, growth of the chlorophyte *C. reinhardtii* was stimulated in the presence of zooplankton-derived nutrients when copepods accessed both P-replete (T(4) = -0.065, p = 0.04) or P-deplete prey (T(4) = -4.607, p = 0.010; Fig 7B). When zooplankton were provided with P-replete prey, growth of *C. reinhardtii* was 23% greater than the controls without zooplankton (0.34 ± 0.01 d^{-1} versus 0.26 ± 0.03 d^{-1}) and 32% greater than the controls when zooplankton had P-deplete prey (0.34 ± 0.03 versus 0.23 ± 0.02 d^{-1}). Unlike *C. raciborskii*, the growth of *C. reinhardtii* was ~30% greater when it was on its own in dialysis tubing compared to when it was grown with *C. raciborskii* (0.48 ± 0.02 d^{-1} versus 0.34 ± 0.01 d^{-1}, respectively; $F_{1,4}$ = 91.987, p = 0.001).

Direct effect (algal growth outside dialysis tubing)

Outside the dialysis tubes, P-replete and P-deplete *C. raciborskii* grew in the absence of zooplankton grazers (0.22 ± 0.05 d^{-1} and 0.25 ± 0.08 d^{-1}, respectively) but net growth was negative in the presence of zooplankton (-0.08 ± 0.16 d^{-1} and -0.24 ± 0.13 d^{-1} respectively). Therefore, there was a significant grazing effect in P-replete and P-deplete treatments (T(4) = 5.421, p =0.006 and T(4) = 3.504, p =0.038, respectively). Copepods cleared a similar amount of P-replete and P-deplete *C. raciborskii* ($F_{1,4}$ = 1.631, p = 0.271, Fig. 8A), indicating no selection preference.

Unlike *C. raciborskii*, *C. reinhardtii* showed negative growth even when copepods were absent, possibly due to the negative impact of coexisting *C. raciborskii* or physical disturbance through agitation (Fig. 8B). Overall, growth rates of *C. reinhardtii* were more negative when cells were P-replete compared to P-deplete ($F_{3,6}$ = 18.023, p = 0.004), indicating a selection preference by copepods for P-rich prey.

Comparing the grazing losses of the two algae, more *C. reinhardtii* was cleared (-0.4 ± 0.17 d^{-1}) compared to *C. raciborskii* (-0.05 ± 0.17 d^{-1}) when both prey were P-replete (F1,4 = 12.648, p = 0.038). When algae were P-deplete however, the grazing losses of *C. reinhardtii* (-0.23 ± 0.13 d^{-1}) and *C. raciborskii* (-0.30 ± 0.10 d^{-1}) were similar ($F_{1,4}$ = 2.329, p = 0.224).

Experiment 4

Indirect effect (algal growth in dialysis tubing)

Extremely P-deplete toxic *C. raciborskii* inside dialysis tubing showed positive growth in all treatments, but growth was only stimulated in the presence of zooplankton when copepods fed on prey that were P-replete (rr treatment; (T(4) = -3.654, p = 0.022). Growth of *C. raciborskii* was not stimulated by zooplankton-regenerated nutrients when copepods had access to mixtures of P-replete and P-deplete or only P-deplete cells (dr, rd, dd; Fig. 9A). In contrast, *C. reinhardtii* showed negative growth inside dialysis tubing in all treatments (Fig. 9B). Similar to *C. raciborskii,* the growth of *C. reinhardtii* in dialysis tubing increased relative to the no copepod control (but was still negative) when cells accessed nutrients regenerated from P-replete prey (T(4) = -3.994, p = 0.016).

Direct effect (algal growth outside dialysis tubing)

The growth of toxic *C. raciborskii* outside the dialysis tubing was positive in all cases, and showed no difference in the presence and absence of zooplankton except for when both prey types were P-deplete (dd). *C. raciborskii* was consumed by zooplankton only in the dd treatment ($T(4) = -2.536$, $p = 0.064$, Fig. 10A) where *C. raciborskii* growth declined from 0.161 ± 0.053 d^{-1} in the no zooplankton control to -0.049 ± 0.133 d^{-1} in the presence of copepods.

In contrast, *C. reinhardtii* showed positive growth only in the absence of zooplankton, when prey cells were both P-replete, or when *C. raciborskii* was P-deplete (rr and rd treatments; Fig. 10B). There was significant zooplankton clearance of *C. reinhardtii* in both the rd and dr treatments, with growth declining by 200% and 80% relative to controls ($T(4) = -14.721$, $p = 0.002$ and $T(4) = -6.232$, $p = 0.003$, respectively). When both prey types were P-deplete, growth of *C. reinhardtii* was negative and not affected by zooplankton (-0.33 ± 0.07 d^{-1} compared to -0.32 ± 0.06 d^{-1} in the control; $T(4) = -0.176$, $p = 0.869$).

Comparing the grazing losses of the two algal prey types, the loss of C. *reinhardtii* cells was significantly greater than toxic *C. raciborskii* in treatments rr ($F_{1,4} = 13.208$, $p = 0.022$), rd ($F_{1,4} = 103.667$, $p = 0.001$) and dr ($F_{1,4} = 21.959$, $p = 0.001$). In treatments rr, rd and dr, the grazing losses of *C. reinhardtii* were -0.22 ± 0.05 d^{-1}, -0.57 ± 0.06 d^{-1} and -0.42 ± 0.11 d^{-1}, while the grazing losses of toxic *C. raciborskii* were -0.05 ± 0.06 d^{-1}, -0.00 ± 0.07 d^{-1} and -0.05 ± 0.09 d^{-1}, respectively. In contrast, in treatment dd there was no significant difference ($F_{1,4} = 5.437$, $p = 0.080$) between the grazing loss of *C. reinhardtii* (-0.01 ± 0.07 d-1) and toxic *C. raciborskii* (-0.21 ± 0.13 d-1).

4. DISCUSSION

This study found that copepods promoted growth of *C. raciborskii* through both nutrient regeneration and grazing avoidance. *C. raciborskii* was therefore affected both indirectly and directly by zooplankton, and had greater net growth than its green algal competitor under P-replete conditions, suggesting that foodweb interactions can facilitate blooms of this toxic cyanobacterium.

The importance of regenerated nutrients on algal growth

To the best of our knowledge, this study is the first to evaluate the importance of recycled nutrients on *C. raciborskii* growth by separating nutrient regeneration and grazing processes using dialysis tubing. A similar experimental design was used by Sterner (1990), who provided evidence that phytoplankton growth was higher in the presence of *Daphnia pulex* grazers than in their absence.

This study indicated that the importance of consumer-driven nutrient recycling (CNR) to growth of *C. raciborskii* and *C. reinhardtii* depended on the nutrient status of cells taking up recycled nutrients and the nutrient status of prey cells. *C. reinhardtii* had the most benefit of CNR when it was P-deplete both inside and outside the dialysis bags. In contrast, CNR was most important to both non-toxic and toxic *C. raciborskii* when cells inside the dialysis tubing were P-deplete, but when cells outside the dialysis tubing were P-replete. Furthermore, the growth enhancement by CNR from zooplankton depended on the degree of P deficiency. *C. raciborskii* growth was significantly enhanced when cells were moderately P depleted (i.e. maintained in 0% P medium for 7 days as in Experiment 2). However, when the P content of *C. raciborksii* was only slightly reduced (i.e., in 1% P medium for 7 days as in Experiment 3) or very strongly reduced (i.e., in 0% P medium for 30 days as in Experiment 4), the growth rates were not significantly different between the treatments with CNR and the controls without CNR. In the former situation, *C. raciborskii* might not have been P limited to respond to the small amount of P released by zooplankton, while in the latter situation, zooplankton

may have released very little P into the environment when feeding on strongly P-deplete food.

The competitiveness of *C. raciborskii* to access zooplankton derived nutrients compared to coexisting species depended on the different degree of P limitation of both algae. When *C. raciborskii* and *C. reinhardtii* were cultured in the same low P condition, *C. reinhardtii* become more P-depleted than *C. raciborskii*, evident by its yellow rather than green appearance, and explained by the high capacity for P retention in *C. raciborskii* (Isvánovics et al., 2000). Thus CNR promoted the growth of *C. reinhardtii* but was less important to overall growth of *C. raciborskii*. However, when both algae were cultured under extremely P limited conditions, *C. raciborskii* as well as *C. reinhardtii* growth was enhanced by CNR. These observations are consistent with previous studies which showed that the magnitude of nutrient uptake depends on the nutritional status of the algae, with nutrient-deficient algae displaying faster rates of uptake than nutrient-sufficient algae (Lehman, 1980; Rocha and Duncan, 1985; Vanni, 2002).

The importance of zooplankton direct (grazing) and indirect (nutrient regeneration) effects on *C raciborskii* dominance

It was expected that the grazing rates of copepods on *C. raciborskii* would be low, due to filament feeding interference and the potential negative impact of toxins (Leonard, and. Paerl, 2005). When algae were P-replete, *Boeckella* consumed more *C. reinhardtii* than *C. raciborskii*. However, when prey was increasingly P-limited, *Boeckella* switched its preference from *C. reinhardtii* to *C. raciborskii*. This is likely to have been due to *C. raciborskii*'s higher nutritional quality for zooplankton consumers (due to high P retention; (Isvánovics et al., 2000). Toxic *C. raciborskii* cells were almost completely avoided by *Boeckella* except when other available prey were of very low quality (i.e., in the dd treatment in Experiment 4, when *C. reinhardtii* was in poor condition due to strong P-limitation).

Overall, our experiments demonstrate that *C. raciborskii* growth is influenced more by direct grazing processes than indirect nutrient recycling. In most situations tested, the benefits to *C. raciborskii* growth from nutrient regeneration were low, but the

gains due to low grazing losses were significant, particularly for toxic (as opposed to non-toxic) *C. raciborskii*. Furthermore, our experiments also show that *C. raciborskii* is more competitive than *C. reinhardtii* in a P-limited environment. Firstly, *C. raciborskii* showed less signs of P-limitation when grown in the absence of P (cells did not turn yellow and growth rate was not reduced to almost zero), an observation consistent with its high affinity for phosphorus and high-phosphorus storage capacity (Isvánovics et al., 2000; Burford and O'Donohue, 2006). This means that under moderate P-limitation, *C. raciborskii* cells retained more P and had higher net growth rates in the absence of zooplankton compared to *C. reinhardtii*. In addition, *C. raciborskii* might negatively affect the growth of its competitors – growth of *C. reinhardtii* was 30% slower when it was in dialysis bags with *C. raciborskii*, and growth of *C. raciborskii* was ~40% higher when grown together with *C. reinhardtii*, presumably due to nutrient contributions from its competitor or allelopathy. Allelopathic interactions play an important role in phytoplankton ecology, and many cyanobacteria genera have been shown to produce compounds with allelopathic activity. The exudate from *C. raciborskii* showed a strong inhibitory effect on co-existing algae from Lagos Santa, and allelopathy was suggested as one reason for the relatively long-lived bloom of *C. raciborskii* in the lake (Figueredo et al., 2007).

Conclusion

This study was designed to test the relative strength of direct and indirect effects on *C. raciborksii* growth, and clearly showed that under P-replete conditions this cyanobacterium benefits most from low grazing losses rather than gains in growth due to rapid uptake of regenerated nutrients. As P limitation strengthens however, *C. raciborksii* is consumed more readily than co-existing cells, and is at increased risk of consumption which is not offset by increased growth via uptake of regenerated nutrients. These results therefore indicate that *C. raciborksii* net growth is possible when P supply rates are such that co-existent phytoplankton are the preferred food for copepod consumers.

REFERENCES

Banse, K.,1995. Zooplankton: Pivotal role in the control of ocean production I. Biomass and production. *ICES Journal of Marine Science*, 52:265-277.

Bayly, I. A. E., 1992. *The non-marine Centropagidae (Copepoda: Calanoida) of the world*. The Hague, Netherlands. SPB Academic, pp. 1-30.

Berg, G. M., Repeta, D. J., LaRoche, J., 2003. The role of the picoeukaryote *Aureococcus anophagefferens* in cycling of marine high-molecular weight dissolved organic nitrogen. *Limnology and Oceanography,* 48:1825-1830.

Bolch, C. J. S., I. Blackburn, S., 1996. Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kütz. *Journal of Applied Phycology,* 8:5-13.

Boon, P. I., Stuart, E. B., Green, J. D., Shiel, R. J., 1994. Consumption of Cyanobacteria by Freshwater Zooplankton: Implications for the Success of 'Top-down' Control of Cyanobacterial Blooms in Australia. *Aust. J. Mar. Freshwater Res*., 45:875-887.

Burford, M. A., O'Donohue, M. J., 2006. A comparison of phytoplankton community assemblages in artificially and naturally mixed subtropical water reservoirs. *Freshwater Biology*, 51:973-982.

Burns, C. W., Hegarty, B., 1994. Diet selection by copepods in the presence of cyanobacteria. *Journal of Plankton Research*, 16: 1671-1690.

DeMott, W. R., Moxter, F., 1991. Foraging cyanobacteria by copepods: responses to chemical defense and resource abundance. *Ecology*, 72:1820-1834.

Elser, J. J., Urabe, J., 1999. The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. *Ecology*, 80:735-751.

Figueredo, C. C., Giani, A., Bird , D. F., 2007. Does alleopathy contribute to *Cylindrospermopsis raciborskii* (cyanobacteria) bloom occurrence and geographic expansion? *Journal of Phycology*, 43:256-265.

He, X., Wang, W. X., 2007. Kinetics of Phosphorus in *Daphnia* at Different Food Concentrations and Carbon: Phosphorus Ratios. *Limnology and oceanography*, 52:395-406.

Hillebrand, H., Frost, P., Liess, A., 2008. Ecological stoichiometry of indirect grazer effects on periphyton nutrient content. *Oecologia*, 155:619-630.

Hunt, R. J., Matveev, V., 2005. The effects of nutrients and zooplankton community structure on phytoplankton growth in a subtropical Australian reservoir: An enclosure study. *Limnologica-Ecology and Management of Inland waters*, 35:90-101.

Isvánovics, V., Shafik, H. M., Présing, M., Juhos, S., 2000. Growth and phosphate uptake kinetics of the cyanobacterium, *Cylindrospermopsis raciborskii* (Cyanophyceae) in throughflow cultures. *Freshwater Biology,* 43:257-275.

Jeppesen, E., Soendergaard, M., Jensen, J. P., Havens, K. E., Anneville, O. , Carvalho, L., Coveney, M. F., Deneke, R., Dokulil, M. T., Foy. B., 2005. Lake responses to reduced nutrient loading–an analysis of contemporary long-term data from 35 case studies. *Freshwater Biology*, 50:1747-1771.

Knoll, L. B., McIntyre, P. B., Vanni, M. J., Flecker, A. S., 2009. Feedbacks of consumer nutrient recycling on producer biomass and stoichiometry: separating direct and indirect effects. *Oikos*, 118:1732-1742.

Kobayashi, T., 1993. Filtering rates of *Daphnia carinata* King (Crustacea: Cladocera) on the blue-green algae *Microcystis aeruginosa* Kütz. and *Anabaena cylindrica* (Lemm.). *Australian Journal of Ecology*, 18: 231-234.

Lehman, J. T., 1980. Release and cycling of nutrients between planktonic algae and herbivores Limno. *Oceanogr.*, 25:620-632.

Leonard, J. A., Paerl, H. W., 2005. Zooplankton community structure, micro-zooplankton grazing impact, and seston energy content in the St. Johns river system, Florida as influenced by the toxic cyanobacterium *Cylindrospermopsis raciborskii*. *Hydrobiologia*, 537:89-97.

Maron, J. L., Crone, E., 2006. Herbivory: effects on plant abundance, distribution and population growth. *Proceedings of the Royal Society B: Biological Sciences*, 273:2575-2584.

Persson, L., Andersson, G., Hamrin, S. F., Johansson, L., 1988. Predator regulation and primary production along the productivity gradient of temperate lake ecosystems. In. Stephen R. Carpenter (Ed.) *Complex interactions in lake communities*. Springer New York, pp. 45-65.

Leonard, J. A., Paerl, H. W., 2005. Zooplankton community structure, micro-zooplankton grazing impact, and seston energy content in the St. Johns river

system, Florida as influenced by the toxic cyanobacterium *Cylindrospermopsis raciborskii*. *Hydrobiologia*, 537:89-97.

Rocha, O., Duncan, A., 1985. The relationship between cell carbon and cell volume in freshwater algal species used in zooplanktonic studies. *Journal of Plankton Research*, 7:279-294.

Rothhaupt, K. O., 1995. Algal nutrient limitation affects rotifer growth rate but not ingestion rate. *Limnology and Oceanography*, 40:1201-1208.

Sinha, R., Pearson, L. A., Davis, T. W., Burford, M. A., Orr, P. T., Neilan, B. A., 2011. Increased incidence of *C. raciborskii* in temperate zones-is climate change responsible? *Water Res*, 46: 1408-1419

Sterner, R. W., 1986. Herbivores' direct and indirect effects on algal populations. *Science*, 231:605-607.

Sterner, R. W., 1990. The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algae comkpetitive arena. *The American Naturalist,* 136:209-229.

Sterner, R. W., Elser, J. J., (eds) 2002. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton Univ Press.

Towns, T. G., 1986. Determination of aqueous phosphate by ascorbic acid reduction of phosphomolybdic acid. *Analytical Chemistry*, 58:223-229.

Vanni, M. J., 2002. Nutrient cycling by animals in freshwater ecosystems. *Annu. Rev. Ecol. Syst*., 33: 341-370.

Vanni, M. J., Layne, C. D., 1997. Nutrient recycling and herbivory as mechanisms in the “top-down” effect of fish on algae in lakes. *Ecology*, 78:21-40.

Wu, Z., Shi, J., Li R., 2009. Comparative studies on photosynthesis and phosphate metabolism of *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*. *Harmful Algae*, 8:910-915.

Figure legends

Figure 1. Setup of experiment 1, which compared the impact of zooplankton-derived nutrients on growth of P-deplete and P-replete *C. raciborskii* cells inside the dialysis tubing. Symbols are courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

Figure 2. Setup of experiment 2 which was designed to estimate the growth response of P-deplete *C. raciborskii* and *C. reinhardtii* to addition of external inorganic P and zooplankton-derived nutrients. Symbols are courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

Figure 3. Setup of experiment 3 which was designed to test the impact of zooplankton nutrient regeneration on the growth of P-deplete *C. raciborskii* and *C. reinhardtii* depending on P content of algal prey, as well as the growth of each alga when present on its own in the dialysis bags versus in mixed culture. Symbols are courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

Figure 4. Setup of experiment 4 which was designed to test the impact of regenerated nutrients on the growth of extremely P-deplete toxic *C. raciborskii* and *C. reinhardtii* inside the dialysis tubes when copepods outside the dialysis tubes were fed with mixed prey of different P content. The setup also tested copepod food preference outside the dialysis bags. Symbols are courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

Figure 5. Growth rates of P-replete and P-deplete *C. raciborskii* inside dialysis tubes. Columns are paired to demonstrate the influence of nutrients released from algal prey in the absence of zooplankton (Control) compared to nutrients released from algal prey and zooplankton grazing activities (+Zoo). Data are mean ± SE (n = 3). Asterisk * indicates significant difference in *C. raciborskii* growth between control (white bar) and paired zooplankton (black bar) treatments. Letters indicate comparison of algal growth in all treatments by ANOVA; different letters above the columns indicate significant differences between treatments.

Figure 6. The growth rates of P-deplete *C. raciborskii* (A) and *C. reinhardtii* (B) inside dialysis tubes with different nutrient conditions outside the dialysis tubes: nutrients released from algal prey (Control) or nutrients released from algal prey and zooplankton grazing activities (+Zoo), as well as in the absence and presence of inorganic phosphate (Control without algae and DIP, respectively). Data are mean ± SE (n = 3). Asterisk * indicates significant difference in algal growth rate in the control (white bar) and paired zooplankton or P treatment (black bar). Letters indicate comparison of algal growth between treatments by ANOVA; different letters above the columns indicate significant differences between treatments.

Figure 7. The growth rates of P-deplete *C. raciborskii* (A) and *C. reinhardtii* (B) inside dialysis tubes with different nutrient conditions outside the dialysis tubes: nutrients released from algal prey (Control) or nutrients released from algal prey and zooplankton grazing activities (+Zoo). Outside the dialysis tubes either P-replete or P-deplete prey were provided. For the P-replete treatment, growth is also shown for cells inside dialysis tubes in the absence of their competitor (+Zoo (mono)). Data are mean ± SE (n = 3). Asterisk * indicates significant difference in growth between paired control and zooplankton treatment. Letters indicate comparison of algal growth in all treatments by ANOVA; different letters above the columns indicate significant differences between treatments.

Figure 8. Growth rates of *C. raciborskii* (A) and *C. reinhardtii* (B) outside dialysis tubes in controls without zooplankton (white bars) and with zooplankton (black bars). Data are mean ± SE (n = 3). Asterisk * indicates a significant difference in growth between paired control and zooplankton treatment. Letters indicate comparison of algal growth in all treatments by ANOVA; different letters above the columns indicate significant differences between treatments.

Figure 9. Growth rates of extremely P-depleted toxic *C. raciborskii* (A) and *C. reinhardtii* (B) inside dialysis tubes with nutrients released from algal prey (Control) or nutrients released from algal prey and zooplankton grazing activities (+Zoo). Algal prey (*C. reinhardtii* and *C. raciborskii*) were provided in the following combinations: rr: both algae P-replete; rd: P-replete *C. reinhardtii* and P-deplete *C. raciborskii*; dr: P-deplete *C. reinhardtii* and P replete *C. raciborskii*, and dd: both algae P deplete. Data are mean ± SE (n = 3). Asterisk * indicates a significant difference in growth between

paired control and zooplankton treatment. Letters indicate comparison of algal growth in all treatments by ANOVA; different letters above the columns indicate significant differences between treatments.

Figure 10. Growth rates of toxic *C. raciborskii* (A) and *C. reinhardtii* (B) outside dialysis tubes in controls without zooplankton (white bars) and with zooplankton (black bars). Algal prey (*C. reinhardtii* and *C. raciborskii*) were provided in the following combinations: rr: both algae P-replete; rd: P-replete *C. reinhardtii* and P-deplete *C. raciborskii*; dr: P deplete *C. reinhardtii* and P replete *C. raciborskii*, and dd: both algae P-deplete. Data are mean ± SE (n = 3). Asterisk * indicates a significant difference in growth between paired control and zooplankton treatment. Letters indicate comparison of algal growth in all treatments by ANOVA; different letters above the columns indicate significant differences between treatments.

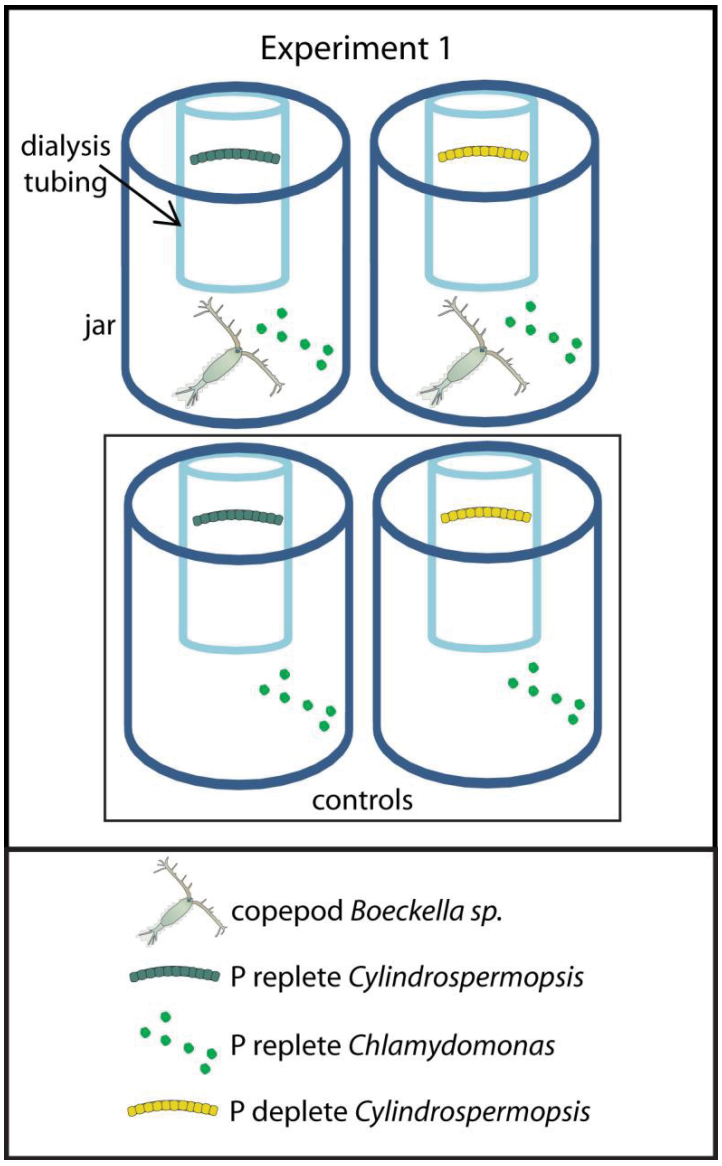
Experiment 1
dialysis tubing
jar
controls
copepod Boeckella sp.
P replete Cylindrospermopsis
P replete Chlamydomonas
P deplete Cylindrospermopsis

Figure 1

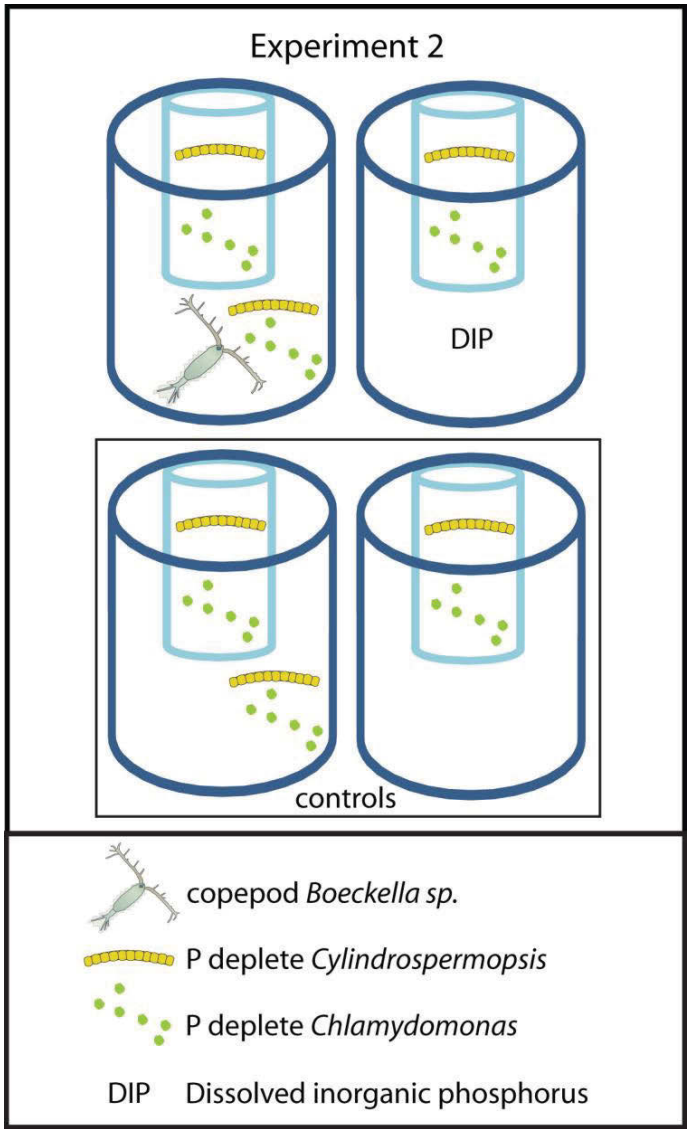
Experiment 2
DIP
controls
copepod *Boeckella sp.*
P deplete *Cylindrospermopsis*
P deplete *Chlamydomonas*
DIP Dissolved inorganic phosphorus

Fig. 2

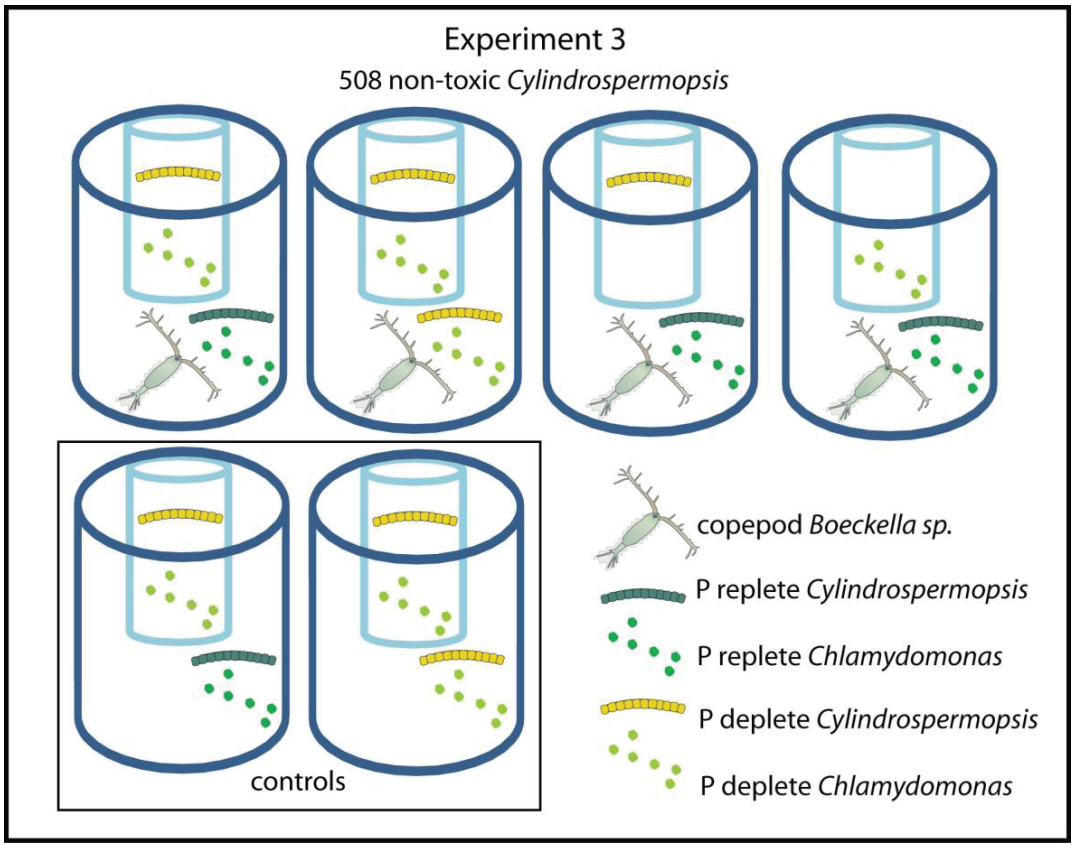
Experiment 3
508 non-toxic Cylindrospermopsis
controls
copepod Boeckella sp.
P replete Cylindrospermopsis
P replete Chlamydomonas
P deplete Cylindrospermopsis
P deplete Chlamydomonas

Figure 3

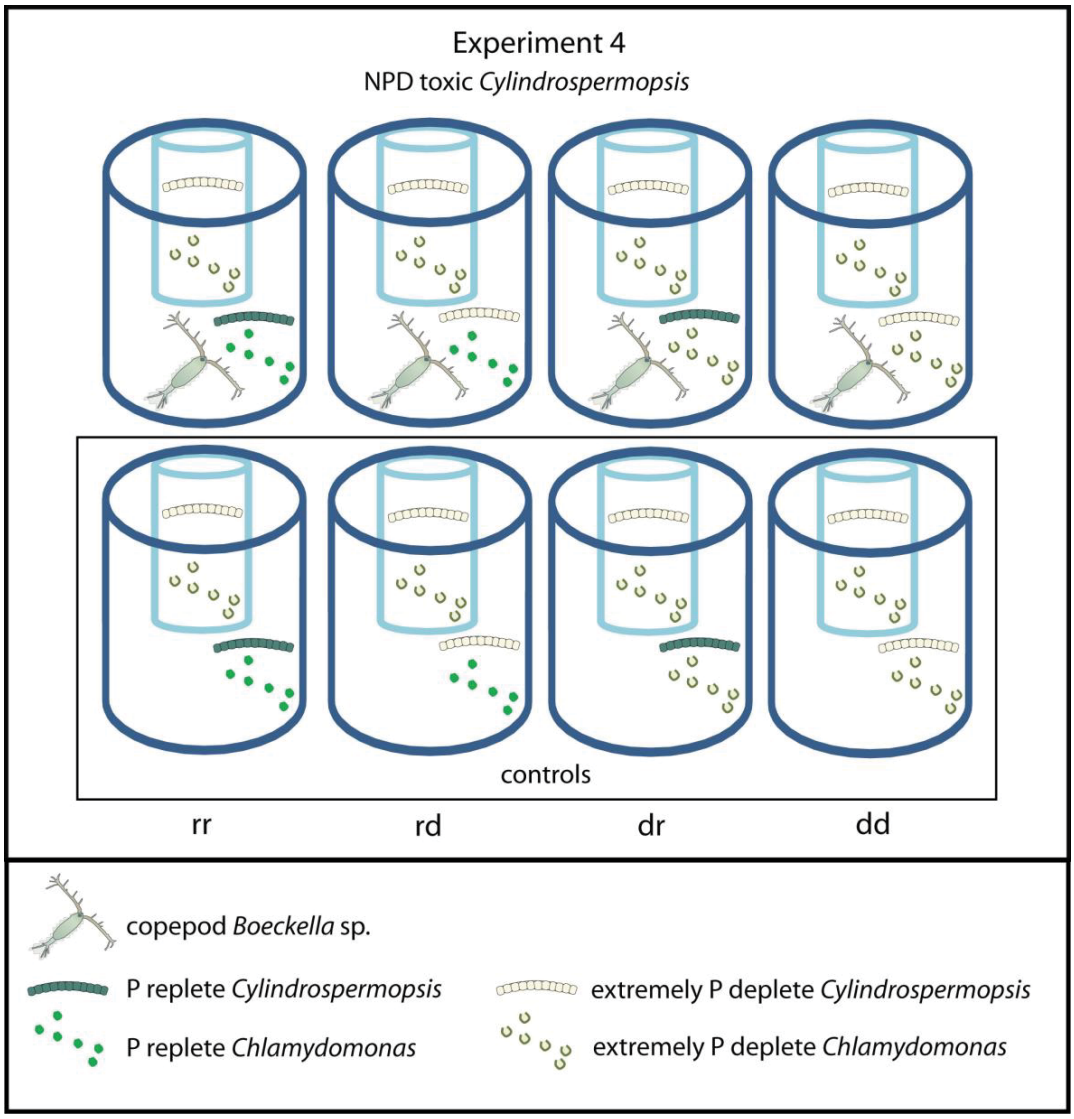
Experiment 4
NPD toxic Cylindrospermopsis
controls
rr
rd
dr
dd
copepod Boeckella sp.
P replete Cylindrospermopsis
P replete Chlamydomonas
extremely P deplete Cylindrospermopsis
extremely P deplete Chlamydomonas

Figure 4

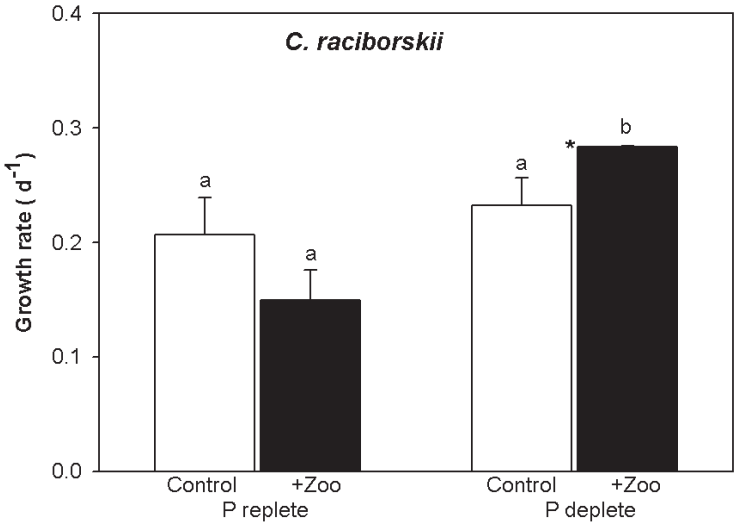
C. raciborskii
Growth rate (d-1)
0.4
0.3
0.2
0.1
0.0
a
a
a
*
b
Control
+Zoo
Control
+Zoo
P replete
P deplete

Figure 5

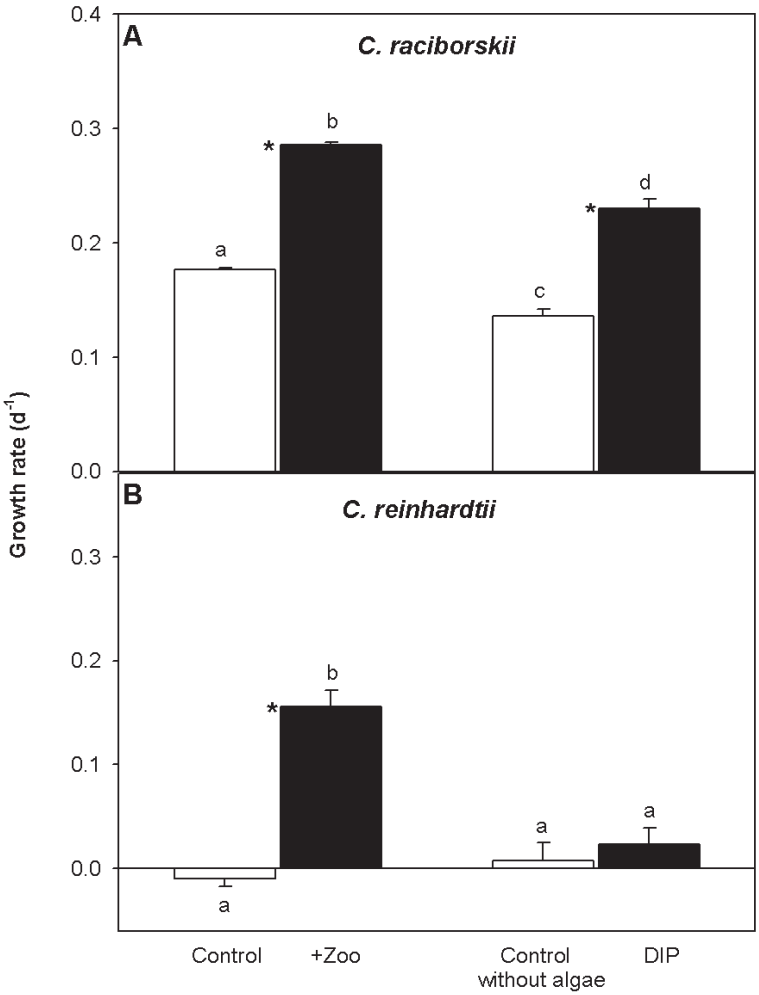
A
C. raciborskii
B
C. reinhardtii
Growth rate (d-1)
0.4
0.3
0.2
0.1
0.0
a
b
c
d
*
Control
+Zoo
Control
without algae
DIP

Figure 6

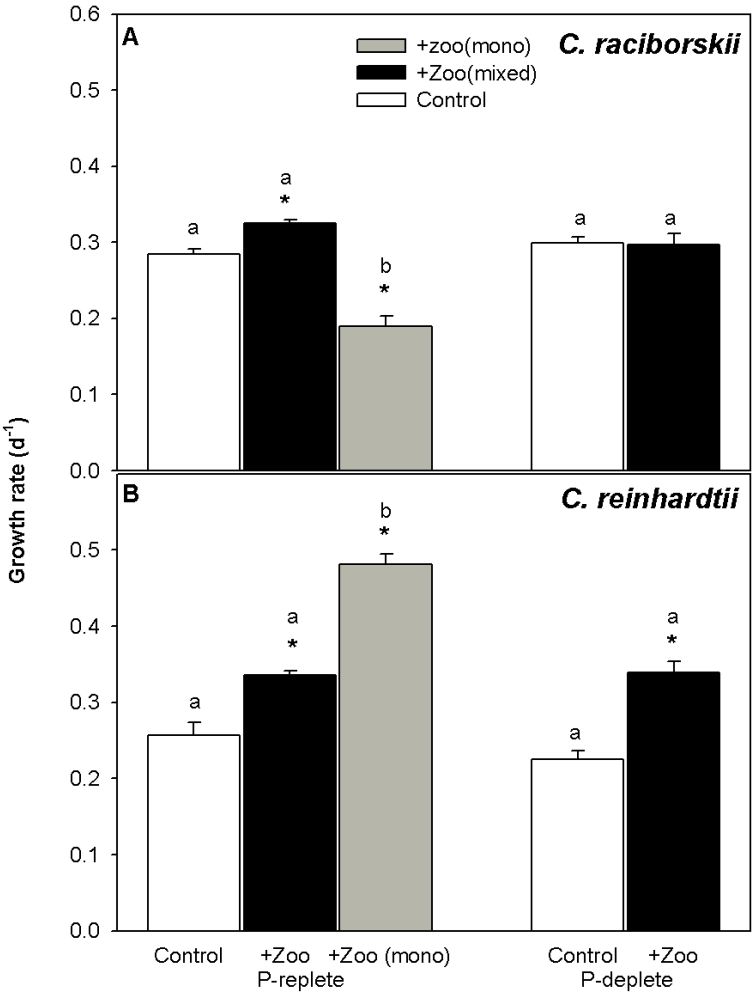
A
C. raciborskii
+zoo(mono)
+Zoo(mixed)
Control
B
C. reinhardtii
Growth rate (d-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
a
a
*
b
*
a
a
b
*
a
*
a
a
*
Control
+Zoo
+Zoo (mono)
P-replete
Control
+Zoo
P-deplete

Figure 7

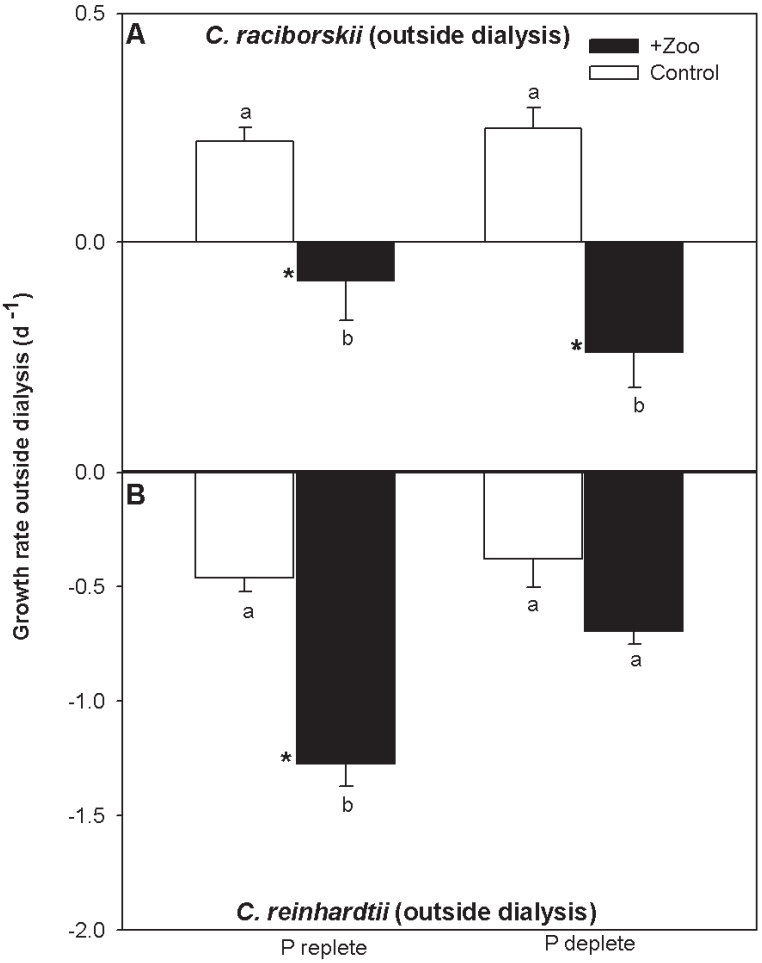
A
C. raciborskii (outside dialysis)
+Zoo
Control
B
C. reinhardtii (outside dialysis)
Growth rate outside dialysis (d -1)
0.5
0.0
0.0
-0.5
-1.0
-1.5
-2.0
a
b
a
b
a
b
a
a
*
*
*
P replete
P deplete

Figure 8

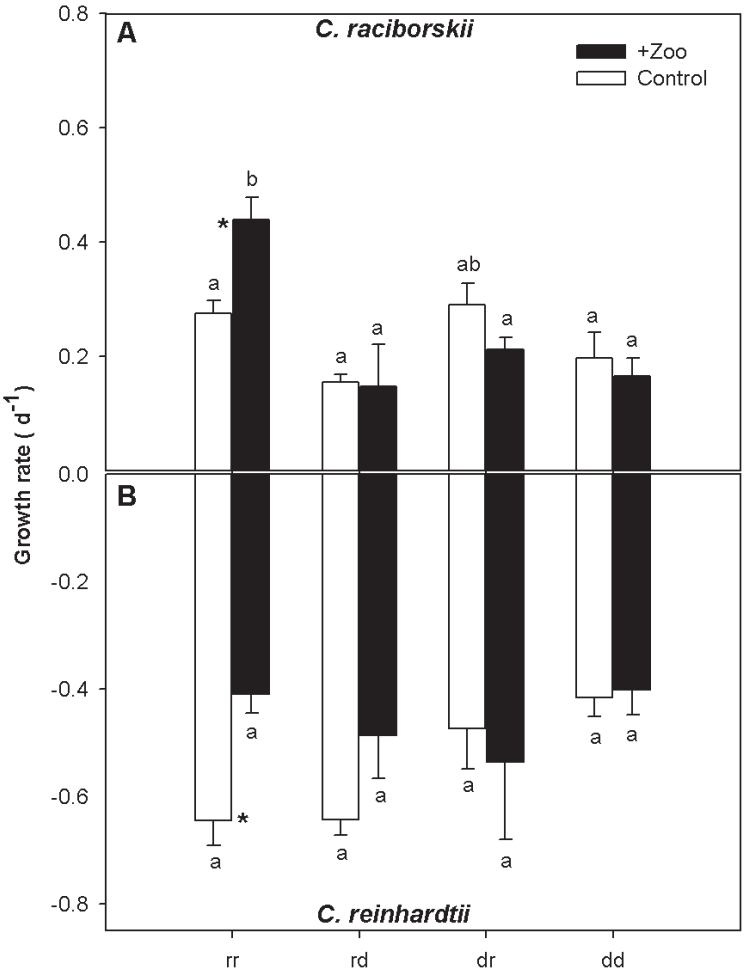
A
C. raciborskii
+Zoo
Control
B
C. reinhardtii
Growth rate (d-1)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
rr
rd
dr
dd
a
b
*
ab

Figure 9

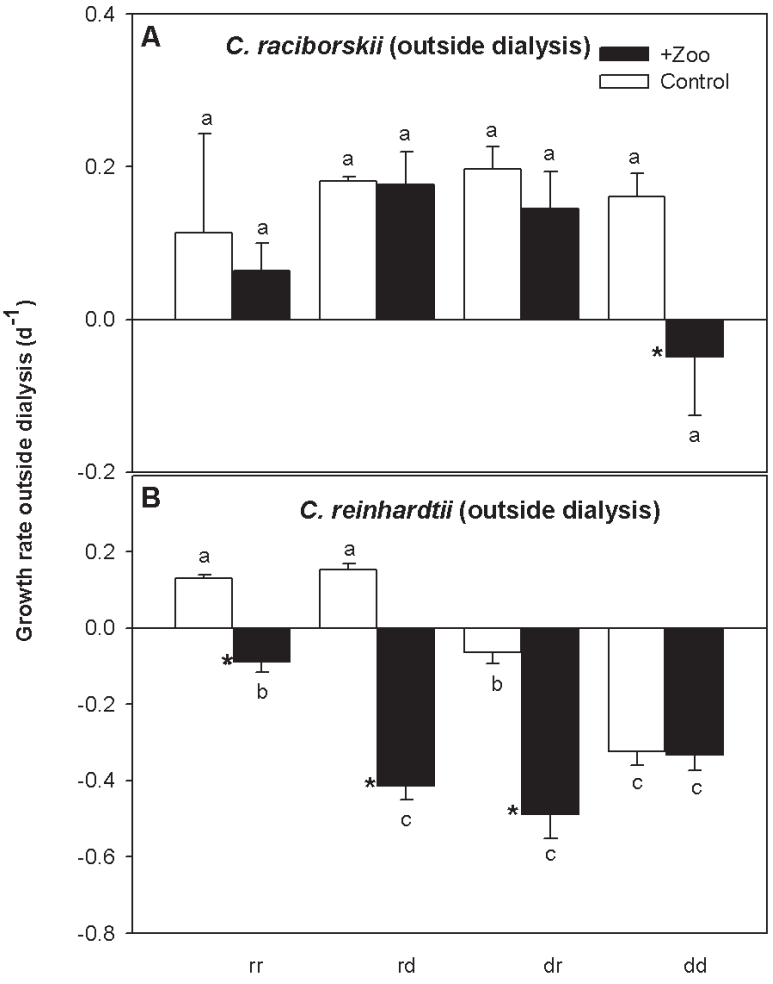
A
C. raciborskii (outside dialysis)
+Zoo
Control
B
C. reinhardtii (outside dialysis)
Growth rate outside dialysis (d-1)
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
rr
rd
dr
dd

Figure 10

CHAPTER 4

Subtropical zooplankton community promotes *Cylindrospermopsis raciborskii* in a mesocosm experiment

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ABSTRACT

Harmful algal blooms (HAB) with public health impacts threaten freshwater ecosystems, including drinking water reservoirs, globally. These systems are often dominated by filamentous and colonial cyanobacteria, and are therefore assumed to support zooplankton growth. While laboratory studies have shown meso-zooplankton can consume the filamentous cyanobacterium *C. raciborskii*, there have been no larger scale studies to test the outcome of such biotic interactions *in situ*. Using ~800 L bags suspended in the upper 2 m of the water column, we examined changes in abundance of *C. raciborskii* amidst a natural phytoplankton community in subtropical Australia. We had four treatment conditions: 1x ambient zooplankton abundance, 5x zooplankton, 5x zooplankton plus inorganic P addition and a no amendment control (1Z, 5Z, 5ZP, control, respectively). We predicted that meso-zooplankton addition would potentially have a stimulatory effect on *C. raciborskii*, and that P enrichment would elevate P content of the phytoplankton, increase their growth and hence alter the outcome of plankton interactions. After 4 days, it was evident that *C. raciborskii* relative abundance doubled in the 5Z and 5ZP treatments compared to the control and 1Z treatments, and P addition had caused an order of magnitude increase in N-fixing phytoplankton. Elemental analyses showed a decrease in the particulate C:P ratio with zooplankton addition, suggesting that meso-zooplankton facilitated P transfer to algae. Consistent zooplankton C:P ratios amongst treatments indicated that zooplankton regulated the elemental ratios in their tissues despite differences in the P content of their food, and hence altered the amount of P they regenerated. In summary zooplankton had an overall neutral impact on the total abundance of phytoplankton, but increased P availability to phytoplankton and promoted *C. raciborskii* abundance.

1. INTRODUCTION

Cyanobacteria are the predominant HABs in inland waters, with harmful effects occurring through toxin production, oxygen depletion, shading or smothering of habitats, and disruption of energy flow through aquatic food webs (Havens, 2008; Paerl and Huisman, 2008). Apart from being a global threat to potable water supply, cyanobacteria remain an important research priority due to rising concern over their increased competitiveness under climate change (O'Neil et al., 2011).

Meso-zooplankton play an important role in aquatic food webs, and influence the phytoplankton community directly through grazing pressure, and indirectly through nutrient regeneration (Sterner, 1990). While laboratory experiments (volume ≤ 1 litre) have been valuable in assessing the outcomes of herbivore-prey interactions and the potential for top-down control of cyanobacteria (Panosso et al., 2003), there have been relatively few *in situ* experiments that scale up small volume investigations. By increasing the experimental volume by 2-3 orders of magnitude, mesocosms can capture natural variability in aquatic habitats and a wider spectrum of plankton behaviour (e.g., vertical migration), and can therefore provide different insights into foodweb interactions. Most mesocosm studies to understand regulation of cyanobacteria by zooplankton have been carried out in temperate lakes (Sommer and Sommer, 2006). However, patterns of zooplankton-phytoplankton interactions in tropical and subtropical lakes may deviate from those in temperate lakes due to differences in physical, chemical, and biological characteristics (Havens et al., 1996; Low and Ng, 2010). In terms of the biological characteristics, temperate lakes generally have higher abundances of relatively large crustacean zooplankton such as *Daphnia sp*., but most tropical and subtropical lakes are dominated by relatively small copepods and rotifers (Bayly, 1992; Jeppesen et al., 2005; Sommer and Sommer, 2006). With respect to phytoplankton, cyanobacteria often dominate freshwater ecosystems, but bloom taxa are different between tropical and temperate systems: species of *Cylindrospermopsis*, *Limnothrix* and *Planktolyngbya* bloom in the tropics, whereas *Anabaena* and *Microcystis* are the most common bloom forming temperate species (Bormans et al., 2004). Not all of these cyanobacteria bloom under high nutrient conditions, some (e.g. *Anabaena* and *Cylindrospermopsis*) fix nitrogen and are present in high abundance when ambient

nutrient concentrations are relatively low (Briand et al., 2002). Under these conditions, zooplankton interactions with cyanobacteria may have different outcomes, such that the indirect benefits of nutrient regeneration are greater than the direct negative consequences of consumption (Persson et al., 1988; Elser et al., 1990). Zooplankton may also transfer nutrients to toxic cyanobacteria at the expense of competing algal species (Mitra and Flynn, 2006). Furthermore, under nutrient enrichment, grazing losses may be offset by allowing previously limited phytoplankton to grow faster (Hunt and Matveev, 2005).

C*ylindrospermopsis raciborskii* is a filamentous cyanobacterium which blooms in subtropical and tropical lakes and reservoirs in the Southern and Northern Hemisphere (Sinha et al., 2011), including northern Australia (Harris and Baxter, 1996; Saker et al., 1999; McGregor and Fabbro, 2000), South America (Bouvy et al., 2001; Figueredo and Giani, 2009), North America (Chapman and Schelske, 2008), and Thailand (Li et al., 2001). While biological control of this species by zooplankton does not look favourable, based on relatively low clearance rates observed in small scale laboratory experiments (Panosso et al., 2003; Ka et al. 2012; Hong et al., in preparation), copepods have been shown to cut *C. raciborskii* filaments, effectively shortening them to an edible size for other zooplankton (Bouvy et al. 2001). This type of facilitation has never been tested at larger scales, and together with *C. raciborskii*‘s ability to efficiently take up and store dissolved inorganic phosphorus (DIP) (Padisák, 1997; Shafik, 2003), suggests that this cyanobacterium may be more susceptible to grazing than previously thought, yet attract a greater share of zooplankton-derived nutrients than co-existing algae (Hong et al., in preparation). In this study, we tested whether *C. raciborskii* would increase in abundance under zooplankton or P enriched conditions, or both. Our hypothesis was that selective zooplankton grazing and nutrient regeneration would act synergistically to facilitate the accumulation of *C. raciborskii*, and that enrichment with inorganic phosphate would stimulate growth of other algae and thereby reduce *C. raciborskii*’s competitiveness.

2. MATERIALS AND METHODS

2.1 Study site and experimental design

The mesocosm experiment was carried out at Lake Wivenhoe (Figure 1) in southeast Queensland, a subtropical, oligotrophic reservoir with 0.50 ± 0.08 mg L^{-1} total nitrogen and 0.01 ± 0.00 mg L^{-1} total phosphorus across surface and bottom waters (Burford et al., 2007*)*. The abundance of the toxic cyanobacterium *C. raciborskii* typically increases in austral spring and peaks in summer (Posselt et al., 2009), so the experiment was conducted from 19 – 25 January, 2010 when *C. raciborskii* abundance was ~2.0 x 10^4 cells ml^{-1} (pre-bloom). The mesocosms consisted of clear 150 µm thick bags (Redblade Pty Ltd, Albion, Australia) made of 1 x 1 x 3 m polyethylene sheeting with ~800 L capacity. Each was sealed with a heat sealer at the lower end and had its top end sewed onto a square frame. The final configuration involved fitting four mesocosm bags onto a floating PVC framework for support. On deployment, bags sat in the upper 2 m of the water and bird netting was put on top of the frames to prevent birds from disturbing the experiment. The experiment had four treatments including an unamended control (surface water with ambient zooplankton), a 1Z zooplankton treatment (addition of ~ 60 individuals L^{-1}), a 5Z zooplankton treatment (addition of ~ 280 individual L^{-1}). The fourth treatment (5ZP) had 5Z zooplankton with phosphorus (P) addition, spiked daily in the form of inorganic KH_2PO_4 (18 µg L^{-1}) to maintain dissolved N:P concentrations close to the Redfield (1958) ratio (16:1). Each treatment had triplicate bags. The mesocosms were filled with surface reservoir water using bilge pumps. Zooplankton were collected from the mesocosm site with vertical net tows (to 12m) using a 75 µm net (20 cm diameter, 0.5 m length). Collected zooplankton were pooled into a 20 L container, gently mixed and were then added into each treatment bag accordingly. The zooplankton biomass added to the 1Z treatment was 1.42 ± 0.20 mg carbon L^{-1} and 7.09 ± 1.01 mg carbon L^{-1} for both 5Z and 5ZP treatments.

2.2. Sampling and analysis

Daily measurements of temperature, dissolved oxygen concentration (DO), pH, conductivity and turbidity were made in mesocosm bags and adjacent reservoir water at the surface and at 1 m with a multiparameter instrument and automated logger

(handheld YSI 6600 Sonde and YSI 650; Yellow Springs, Ohio, USA). Additionally, daily irradiance profiles were measured through the water column from 0 to 2 m using a 4-pi PAR sensor (LiCor, NB, USA) and the Secchi depth was also recorded.

To assess phytoplankton biomass and species composition as well as nutrient chemistry, water samples were collected from each mesocosm bag and the adjacent reservoir water using a 3 m long depth-integrated sampler (volume = 0.5 L). Samples were collected at daily intervals from days 1 to 5 and at the end of the experiment on day 7. Dissolved inorganic nitrogen and phosphorus (DIN and DIP) were measured in 0.45 µm filtered water (Millipore, Cork, Ireland) filtered on site using a hand pump. Unfiltered water was used for total nitrogen and total phosphorus (TN and TP, respectively) analyses. Total particulate carbon and nitrogen samples (TPC and TPN respectively) were also prepared on site by filtering water samples onto pre-combusted GF/F filters (Whatman, Piscataway, USA). All chemical samples were immediately stored on ice and on return to the laboratory were frozen at –20°C until analysis. Samples for TPP were digested using a persulfate digestion procedure. After digestion, TPP was analysed based on the ascorbic acid reduction of phosphomolybdate (Towns 1986). For TPC and TPN analyses, the GF/F filters with suspended particulate matter (SPM), and zooplankton were dried at 50 °C overnight, and analysed using an Elemental Analyser with 20-20 IRMS (Europa Scientific). Unfortunately, our particulate samples were lost during sample processing, so the carbon content of phytoplankton was estimated using conversion factors described in Hendrickson (2011).

Phytoplankton samples were fixed with Lugols solution (1%) solution on site, and samples from day 1, 2, 4 and 7 were enumerated. Phytoplankton were identified and counted using a Lund cell under 400x magnification on a compound microscope (Olympus BX50, Hamburg, Germany). One short traverse, with more than 400 units (single cells or filaments) was counted for each sample. For colonial and filamentous cyanobacteria, cell numbers in each filament or colony were estimated by counting cells in an average of at least 30 units. The number of *C. raciborskii* cells per filament was determined by multiplying the number of filaments by 14. This value was previously determined by counting cells in an average of 400 filaments from Lake Samsonvale (Glenn McGregor, personal communication). Size classes were defined as nanoalgae (2 – 20 µm) and microalgae (20 - 200 µm) (Sommer and Sommer, 2006). Two further

functional groups were identified, with species having heterocysts grouped as N_2-fixers (*Anabaena* and *Aphanizomenon*) and potentially toxic genus (*Anabaena, Aphanizomenon Cylindrospermopsis* and *Geitlerinema)*. Phytoplankton biovolume was determined on an individual cell basis, where length, width and depth were used to estimate the basic shape and calculate the biovolume according to Hillebrand et al. (1999). Phytoplankton pigment concentrations were estimated using High Performance Liquid Chromatography (HPLC). Fifty ml of mesocosm water was filtered under low vacuum (e.g. ≤ 100 mm Hg) onto 25 mm GF/F filters in low light (< 10 µmol photons · m^{-2} · s^{-1}). Filters were folded in half, blotted dry on absorbent paper, placed into screw-capped cryovials and stored frozen at minus 80 °C until HPLC pigment analysis. In the laboratory, pigments were extracted at 4 °C in the dark over 15–18 h in 3 mL acetone (100%, diluted to 90% for analysis, Mallinkrodt, HPLC grade) then sonicated on ice for 15 minutes. Samples were recovered using filtration (0.45 µm, Whatmann) and centrifugation (2500 rpm, 5 min at 4°C). The samples were analysed by HPLC (Waters – Alliance comprising a 2695XE separations module with column heater and refrigerated autosampler) using a C_8 column (Zorbax Eclipse XDB-C8, Agilent Technologies) and binary gradient system with an elevated column temperature (55° C) following a modified version of the Van Heukelem and Thomas (2001) method. Pigments were identified by their retention time and absorption spectrum from a photo-diode array detector (Waters- Alliance 2996 PDA). Concentrations of pigments were determined from commercial and international standards (Sigma; DHI, Denmark). The HPLC system was also calibrated using phytoplankton reference cultures (Australian National Algae Culture Collection) whose pigment composition has been documented in the literature (Mantoura and Llewellyn, 1983; Barlow et al., 1993; Jeffrey et al., 1997). Chlorophyll-a cell quotas were calculated by dividing the total number of phytoplankton cells (> 10 µm) by the Chl-a concentration estimated using HPLC.

Zooplankton were collected from each mesocosm at the end of the experiment (day 7) by repeated vertical tows using a 75 µm, 20 cm diameter (1 m long) net. Zooplankton samples were divided into three equal parts for identification (preserved in ethanol, 70% final concentration), biomass determination (preserved in formaldehyde, 4% final concentration) and elemental analyses (stored frozen at -20 °C). Zooplankton collected from adjacent water at the beginning and end of the experiment were processed using the same method. Zooplankton were enumerated into functional groups

(cladocerans, copepods, rotifers and juveniles which included immature forms of cladocerans and copepods) using a compound microscope (Olympus BX50, Hamburg, Germany). Zooplankton biovolume was determined using an Optical Plankton Counter (Focal Technologies, Inc., Dartmouth, Canada) configured as described by Moore and Suthers (2006). The concentration of particles (zooplankton) is expressed as number per litre. The biomass (mm^3 L^{-1}) was calculated as the sum of the products of volume (mm^3 $ind.^{-1}$) and concentration (ind. L^{-1}) of particles over all equivalent spherical diameter (ESD) values between 240 and 3000 µm ESD. The volume of water sampled for zooplankton was calculated using the following equation: Volume = pi * (diameter of net/2)^2 * depth of tow (12 m in ambient water and 3 m in mesocosm).

2.3. Data Analysis

Repeated measures analysis of variance was used to compare differences in total and functional group (nanoalgae, microalgae, toxic algae, N_2-fixer) phytoplankton abundance between treatments over time. If there was a significant treatment X time interaction, phytoplankton abundance among the treatments was compared on the relevant day using one-way analysis of variance. One-way ANOVA was also performed to test the difference abundance of individual phytoplankton species among four treatments (Control, 1Z, 5Z and 5ZP) on day 4. Tukey's multiple comparison tests were used when significant differences were found between treatments. Data were examined for normality and were ln (x + 1) transformed when normalization was required. When data did not meet the assumptions of normality even after transformation, they were analysed using a non-parametric Kruskal-Wallis one-way analysis of variance by ranks to test for the difference between population medians. Results are expressed as X^2 (df, n = number of replicates) (Green and Salkind, 2008). ANOVAs were performed in SPSS version 8.0 ® (1997 SPSS Inc.) and the significance level for all tests was p = 0.05.

Multivariate analyses of phytoplankton composition data were undertaken with PRIMER 5.2.9 software ® (2002 PRIMER-E Ltd., Plymouth, UK). Transformed abundance data ($\log_{10}$ + 1) were used to generate a Bray-Curtis similarity matrix. The Bray-Curtis similarity matrix was then used to ordinate samples (visualised using an nMDS plot) and undertake analysis of similarity (ANOSIM). The ANOSIM test statistic, R, is based on the ratio of the between treatment to within-treatment similarity ranking

and ranges from 0 to 1, with the value indicating the degree of dissimilarity (1 = completely dissimilar; 0 = completely similar).

3. RESULTS

3.1. Physical and chemical data

The physical and chemical factors including light, temperature, conductivity, turbidity and pH were similar among treatments and relatively constant with time (Table 1). Despite inorganic P addition to the 5ZP treatment, dissolved inorganic phosphorus and nitrogen (DIP and DIN, respectively) concentrations at day 7 were similar across treatments ($F_{3,8} = 3.176$, $p = 0.085$, and $F_{3,8} = 0.790$, $p = 0.533$, respectively), averaging 0.01 ± 0.00 mg L^{-1} and 0.41 ± 0.01 mg L^{-1}, respectively. The molar DIN:DIP ratios averaged 60 ± 5 (SE) and were similar in all treatments on day 7 ($F_{3,8} = 0.685$, $p = 0.601$, Fig. 2A), and substantially higher than the Redfield ratio (16). In contrast, the particulate C:P ratio differed among the treatments ($F_{3,6} = 0.202$, $p = 0.012$, Fig. 2B), and was lower in 5Z and 5ZP treatments (128 ± 16 and 95 ± 16, respectively) compared to the control (212 ± 7) and 1Z (197 ± 48) treatments. Zooplankton phosphorus content per individual was similar between treatments by non-parametric Kruskal-Wallis analysis due to data not meeting the assumptions of normality (X^2 (df = 3, n = 12) = 7.650, p =0.055 (Fig. 3A), as was the zooplankton C:P ratio (X^2 (df = 3, n = 12) = 6.231, p =0.101 (Fig. 3B).

3.2. Phytoplankton

On day 7, chlorophyll-a (Chl-a) concentration in the 5ZP treatment (8.4 ± 1.0 µg L^{-1}) was twice as high as all the other treatments ($F_{4,10} = 5.674$, $p = 0.012$; Fig. 4A), which were not significantly different from T_0 (4.2 ± 0.4 µg L^{-1}). Total phytoplankton abundance differed between treatments on day 4 ($F_{3,12} = 7.41$, $p = 0.011$), being lowest in the 1Z and highest in the 5ZP treatment (6.02 ± 0.25 versus $8.88 \pm 0.65 \times 10^4$ cells ml^{-1}, respectively). However, at the end of the experiment (day 7), total phytoplankton

abundance was the same amongst treatments ($F_{3,7}$ = 1.780, p = 0.238). Chl-a cell quotas were the same among the treatments ($F_{4,9}$ = 0.604, p = 0.669; Fig. 4B). However, Chl-a concentration per biovolume of phytoplankton was significantly higher in the 5ZP treatment (0.12 ± 0.04 µg Chl-a μm^{-3}) than in the control (0.05 ± 0.01 µg Chl-a μm^{-3}, p=0.047), and 1Z treatment (0.04 ± 0.06 µg Chl-a μm^{-3}, p=0.029). However, Chl-a was only marginally higher than that in 5Z treatment (0.05 ± 0.01 µg Chl-a μm^{-3}, p=0.072), and was not different from T_0 (Fig. 4C).

Cyanobacteria comprised most of the phytoplankton community biovolume in the mesocosms at T_0 (84 ± 5%), with *Planktolyngbya, Cylindrospermopsis* and *Limnothrix* being the dominant taxa (Table 2). Cyanobacteria biovolume and abundance was stable during the experiment ($F_{3.7}$ = 1.775, p = 0.239), however treatments had a significant effect on the abundance of phytoplankton functional groups. Nano-algae decreased over time in all treatments (Fig. 5; $F_{3.7}$ = 6.732, p = 0.017) but were least abundant in the 5Z treatment on day 7 (4.20 ± 0.63 x10^2 cells ml^{-1}; $F_{3,5}$ = 11.364, p = 0.011). Microalgae abundance (including all filamentous and colonial cyanobacteria) was significantly higher in the 5ZP treatment (8.77 ± 0.64 x10^4 cells ml^{-1}) compared to the 1Z and control (5.84 ± 0.24 and 6.61 ± 0.33 x10^4 cells ml^{-1}, respectively) on day 4 ($F_{3.7}$ = 7.262, p = 0.011), but microalgae abundance was not significantly different between 5Z and 5ZP treatments. The abundance was the same among all treatments on day 7 ($F_{3.7}$ = 1.667, p = 0.260). The abundance of potentially toxic algae was higher in the 5ZP and 5Z treatments (2.73 ± 0.79 and 2.30 ± 0.79 x10^2 cells ml^{-1}, respectively) than in the 1Z and the control (1.85 ± 0.12 and 1.82 ± 0.12 x 10^2 cells ml^{-1}, respectively) on day 4 ($F_{3.7}$ = 12.608, p = 0.002). By the end of the experiment, the 5ZP treatment had the greatest abundance of potentially toxic algae (9.42 ± 1.00 x10^4 cells ml^{-1}) and the control treatment the least (3.51 ± 0.06 x10^4 cells ml^{-1}) ($F_{3.7}$ = 4.80, p = 0.049). N_2-fixers increased steadily in the 5ZP treatment and had the greatest abundance amongst all other treatments on day 7 (2.04 ± 0.32 x10^4 cells ml^{-1}; $F_{3,6}$ = 18.736, p = 0.008).

The abundance of *C. raciborskii* was similar in all bags at the start of the experiment, ranging from 1.82 to 2.44 x 10^4 cells ml^{-1} (approximately 22% of the total biovolume). However, on day 4, *C. raciborskii* abundance had doubled in the 5Z and 5ZP treatments ($F_{3,8}$ = 14.763, p = 0.001, Fig. 6A) and increased its relative abundance from 15% to 37% ($F_{3,8}$ = 8.235, p =0.008). On day 7, *C. raciborskii* relative abundance

was still greater in the 5ZP compared to other treatments (marginally significant, $F_{3,7} = 4.054$, $p = 0.058$, Fig. 6B), but its absolute abundance was the same amongst treatments ($F_{3,7} = 1.523$, $p = 0.291$).

Considering the total phytoplankton species composition on day 4, the ANOSIM showed strong significance (Global R: 0.429, $p = 0.019$). SIMPER analysis revealed four species including *Cylindrospermopsis*, *Planktolygbya*, *Limnothrix* and *Geitlerinema* were the major contributors to the dissimilarity amongst treatments. Potentially toxic *Geitlerinema* cell abundance in 5Z and 5ZP *(*1.40 ± 0.54 and 2.31 ± 0.27 x 10^4 cells ml^{-1}) was greater than in the control and 1Z (0.45 ± 0.45 and 0.48 ± 0.40 x 10^4 cells ml^{-1}) ($F_{3,8} = 0.056$, $p = 0.019$), while *Anabaena* cell abundance in 5ZP (5.38 ± 0.54 x 10^3 cells ml^{-1}) was significantly higher than in the 5Z treatment (0.99 ± 0.65 x 10^3 cells ml^{-1}) ($F_{3,8} = 14.763$, $p = 0.001$). In contrast, *Limnothrix* cell abundance in the control (2.4 ± 0.5 x 10^4 cells ml^{-1}) was higher than that in the 5Z (0.7 ± 0.6 x 10^4 cells ml^{-1}) and 5ZP treatments (0.1 ± 0.1 x 10^4 cells ml^{-1}; $F_{3,8} = 14.763$, $p = 0.001$). The phytoplankton species composition showed no significant difference on day 7 (Global R: 0.027, $p = 0.40$).

3.3. Zooplankton

As expected, zooplankton biomass ($F_{3,7} = 9.394$, $p = 0.008$, Fig. 9A) and abundance ($F_{3,7} = 9.458$, $p = 0.007$, Fig. 9B) was higher in the 5Z and 5ZP treatments compared to the control and 1Z treatments at the end of the experiment. Microscope counts revealed that copepods (37 ± 3%) and rotifers (39 ± 4%) dominated the zooplankton community throughout the experiment (Fig. 10) and did not differ significantly between treatments. However, the proportion of juveniles decreased 20% from day 0 to 7 ($F_{3,7} = 26.197$, $p < 0.001$), and the predator midge larva *Chaoborus* sp. comprised 16 ± 3% of zooplankton abundance on day 7 when it was virtually absent on day 0 ($F_{3,7} = 7.819$, $p = 0.010$).

4. DISCUSSION

The results of our mesocosm experiment show that zooplankton can promote dominance of the cyanobacterium *C. raciborskii* amongst a subtropical phytoplankton community. We demonstrated the relative proportion of *C. raciborskii* to total phytoplankton abundance increased from 15% to 37% under elevated mesozooplankton concentrations, and doubled its abundance from 2.60 ± 0.96 to 7.44 ± 0.52 x10^4 cells ml^{-1}over 4 days, suggesting that zooplankton can increase the risk of *C. raciborskii* blooms.

Grazing selection by zooplankton

We expected the zooplankton community to avoid *C. raciborskii* consumption based on our small-scale laboratory grazing studies, which showed very low clearance rates (<0.3 ml ind $^{-1}$ h^{-1}) on *C. raciborskii* under most conditions, but particularly when total prey abundance exceeded 1.0 mg C L^{-1} (equivalent to 3.15 x 10^5 *C. raciborskii* cells ml^{-1}, non-limiting for copepods; Hong et al. in preparation). The mean total phytoplankton abundance was 4.93 x 10^5 cells ml^{-1} at the commencement of this mesocosm experiment, which was in the non-limiting prey concentration range. Decreased phytoplankton abundance in the 1Z treatment compared to the control on day 4 showed that meso-zooplankton grazing occurred. However, the doubling of *C. raciborskii* abundance in the 5Z and 5ZP treatments over the same period suggests that the copepod and rotifer dominated zooplankton assemblage avoided consumption of *C. raciborskii*, or that grazing was not enough to stop its accumulation amongst a mixed natural phytoplankton assemblage. Notably, *C. raciborskii* cells in the natural phytoplankton assemblage were spiral filaments which could have presented some defence against grazing.

While filamentous *C. raciborskii* increased in the elevated zooplankton treatments, the abundance of single cells and other microalgae > 20 µm (e.g. colonial forms) declined or stayed constant over the 7 day experiment. Amongst the four dominant cyanobacteria (*C. raciborskii*, *Limnothrix sp.*, *Planktolyngbya sp.* and *Geitlerinema sp.*), two are potentially toxic. Australian strains of *C. raciborskii* produce

cylindrospermopsin (Stüken and Jakobsen, 2010) and *Geitlerinema sp.* produces microcystin (Richardson et al. 2007), but no toxin has been detected in Australian *Limnothrix* or *Planktolyngbya* strains (Stüken and Jakobsen, 2010). We found these dominant algae responded differently to zooplankton and inorganic P enrichment. The potentially toxic *C. raciborskii* and *Geitlerinema sp.* were significantly more abundant in 5Z and 5ZP treatments, while *Limnothrix sp*. was considerably less abundant compared to the unamended control. *C. raciborskii* and *Limnothrix sp.* are morphologically similar with filament lengths ranging from 18 to 153 µm and 27 to 177 µm, respectively. This suggests that food selection by mesozooplankton was unlikely based on cell size, and may have been more related to toxicity.

Phosphorus enrichment (5ZP treatment) caused an order of magnitude increase in the concentration of N_2-fixing taxa such as *Anabaena* relative to the 5Z treatment, and no change to the abundance of other phytoplankton functional groups. Growth of N_2-fixing algae can be limited under low external P concentrations due to a decrease in intracellular pools of nucleotides and low nucleic acid content, especially RNA (Karl et al., 2002). While N-fixing cells could have been consumed by grazers during this experiment, it was evident that P enrichment stimulated growth of N_2 fixing cells. P enrichment also elevated Chl-a quotas (µg Chl-a μm^3), suggesting that it promoted growth of larger cells that may have escaped grazing.

Facilitation of *C. raciborskii* dominance by nutrient regeneration

Concurrent with the direct effects of grazing, zooplankton also have indirect effects on phytoplankton through nutrient regeneration and the ratio of elements they regenerate (Elser et al., 1988; Sterner, 1990). Thus, grazing impacts on phytoplankton could be offset by enhanced growth due to nutrient recycling (Hunt and Matveev, 2005). The present study was designed to compare the outcome of elevated zooplankton with and without P enrichment. We predicted that zooplankton addition would increase P regeneration and lead to greater P content in phytoplankton, and that additional dissolved P enrichment would potentially disadvantage *C. raciborskii*, a species known for its rapid P uptake (Padisák, 1997; Shafik et al., 2001). Our study showed that zooplankton enrichment had no effect on the dissolved inorganic nutrient (DIN and DIP) pools or elemental ratios among the treatments, suggesting that algae in this P limited

environment rapidly took up available nutrients. As anticipated, there was a decrease in the particulate C:P ratio in the 5Z compared to 1Z and control treatments, suggesting that meso-zooplankton facilitated P transfer to algae and elevated their P content. Furthermore, consistent zooplankton C:P ratios amongst treatments indicated that zooplankton regulated the elemental ratios in their tissues despite differences in the P content of their food, just as the ecological stoichiometry theory would have predicted (Sterner, 1990). Thus, zooplankton in the 5Z and 5ZP treatments must have increased the amount of P they released, with potential flow on benefits to phytoplankton.

It is unclear whether *C. raciborskii* acquired a greater share of these regenerated nutrients compared to other cell types, but its abundance in the 5ZP treatment was the same as the 5Z treatment on day 4 and day 7, indicating little net benefit of external P on growth. However, the relative abundance of *C. raciborskii* was greatest in the 5ZP treatment at the end of the experiment, which suggests that the increase in P may have elevated the P content of other algae which were then more readily consumed by grazers. It seems that P addition had an indirect benefit for *C. raciborskii*, at the expense of coexistent algae. This would best be confirmed by including a P-only treatment, but inclusion of this additional treatment was outside the scope of this study for logistic reasons.

Net effects on *C. raciborskii* growth

Our large-scale mesocosm experiment demonstrated that a copepod and rotifer dominated mesozooplankton assemblage had a net positive impact on *C. raciborskii* dominance *in situ*. Our earlier laboratory studies found that toxic *C. raciborskii* was hardly consumed by the copepod *Boeckella* sp. and appeared to suppress the utilization of regenerated nutrients by coexistent algae (Hong et al., submitted). Promotion of *C. raciborskii* growth in the presence of zooplankton at the meso-scale observed in this study is therefore consistent with smaller scale studies and supports the hypothesis that *C. raciborskii* abundance is facilitated both directly and indirectly by zooplankton. Similar observations have been made for *Microcystis*. In a subtropical lake in China, *Microcystis aeruginosa* dominated the phytoplankton community when the water initially contained zooplankton, nitrogen and phosphorus, but no bloom formed after the

zooplankton were removed from the water, regardless of external nutrient additions (Wang et al., 2010).

This study also demonstrated an overall neutral impact of the zooplankton community on the *total* abundance of phytoplankton in an oligotrophic subtropical lake, consistent with the results from other studies in tropical and subtropical systems (Havens et al., 1996; Spencer and Ellis, 1998; Hunt and Matveev 2005; Von Rückert and Giani, 2008). The taxon-specific effects observed in this study show that zooplankton influence phytoplankton in opposite ways through grazing and nutrient excretion, and different phytoplankton taxa respond differently to these processes. Monitoring zooplankton impacts at the phytoplankton community level (e.g., by measuring Chl-a concentration), is therefore not sufficient to determine their role in the development of algal blooms.

Conclusion

Few mesocosm experiments have examined zooplankton-phytoplankton interactions in subtropical freshwater systems where the phytoplankton are dominated by filamentous cyanobacteria and the zooplankton community is dominated by copepods and rotifers rather than cladocerans. This study indicates that meso-zooplankton promote the accumulation of *C. raciborskii* directly through low grazing pressure. They also benefit *C. raciborskii* indirectly through regeneration of nutrients that increase the nutritional quality of the entire phytoplankton community, making coexistent algae more attractive for zooplankton consumption. Both processes lead to an increase in the relative abundance of *C. raciborskii* and effectively facilitate bloom formation.

REFERENCES

Ali Ger, K., Panosso, R., Lürling, M., 2011. Consequences of acclimation to *Microcystis* on the selective feeding behavior of the calanoid copepod *Eudiaptomus gracilis*. *Limnology and Oceanography*, 56:2103-2114.

Barlow, R.G., Mantoura, R.F.C., Gough, M.A., Fileman, T.W., 1993. Pigment signatures of the phytoplankton composition in the northeastern Atlantic during the 1990 spring bloom. *Deep Sea Research Part II: Topical Studies in Oceanography,* 40:459–477.

Bayly, I. A. E., 1992. T*he non-marine Centropagidae (Copepoda: Calanoida) of the world* by I.A.E. Bayly. The Hague, Netherlands, SPB Academic, pp. 1-30.

Bergquist, A. M., Carpenter, S., 1986. Limnetic herbivory: effects on phytoplankton populations and primary production. *Ecology,* 57:1351-1360.

Bormans, M., Ford, P.W., Fabbro, L., Hancock, G., 2004. Onset and persistence of cyanobacterial blooms in a large impounded tropical river, Australia. *Marine and Freshwater Research,* 55:1-15.

Bouvy, M., Pagano, M., Troussellier, M., 2001. Effects of a cyanobacterial bloom (*Cylindrospermopsis raciborskii*) on bacteria and zooplankton communities in Ingazeira reservoir (northeast Brazil). *Aquatic Microbial Ecology*, 25:215-227.

Briand, J. F., Robillot, C., Quiblier-Lloberas, C., Humbert, J. F., Coute, A., Bernard, C., 2002. Environmental context of *Cylindrospermopsis raciborskii* (Cyanobacteria) blooms in a shallow pond in France. *Water Research*, 36:3183–3192.

Burford, M. A., Johnson, S. A., Cook, A. J., Packer, T. V., Taylor, B. M., Townsley, E. R., 2007. Correlations between watershed and reservoir characteristics, and algal blooms in subtropical reservoirs. *Water research*, 41:4105-4114.

Burford, M. A., O'Donohue, M. J., 2006. A comparison of phytoplankton community assemblages in artificially and naturally mixed subtropical water reservoirs. *Freshwater Biology,* 51:973-982.

Chapman, A. D., Schelske, C. L., 2008. Recent appearance of *Cylindrospermopsis* (cyanobacterium) in five hypereutrophic Florida lakes. *Journal of Phycology*, 33:191-195.

DeMott, W. R., 1988. Discrimination between algae and detritus by freshwater and marine zooplankton. *Bulletin of Marine Science*, 43:486-499.

DeMott, W. R. *1990*. Retention efficiency perceptual bias, and active choice as mechanisms of food selection by suspension-feeding zooplankton. In Hughes, R. N. (ed.), *Behavorial Mechanisms of Food Selection*. NATO ASI Series, Series G: Ecological Series. Springer, New York, pp. 569–594.

Elser, J. J., Elser, M. M., MacKay, N. A., Carpenter. S. R., 1988. Zooplankton-mediated transitions between N-and P-limited algal growth. *Limnology and Oceanography*, 33:1-14.

Elser, J. J., H. J., Carney, H. J., Goldman, C. R., 1990. The zooplankton-phytoplankton interface in lakes of contrasting trophic status: an experimental comparison. *Hydrobiologia*, 200:69-82.

Ganf, G., 1983. An ecological relationship between *Aphanizomenon* and *Daphnia pulex*. *Marine and Freshwater Research*, 34:755-773.

Green, S. B., Salkind, N. J., 2008. Using SPSS for Window and Macintosh: Analyzing and understanding data (5th ed.). *Upper Saddle River*, NJ: Pearson Prentice Hall.

Hambright, K. D., Zohary, T., Easton, J., Azoulay, B., Fishbein, T., 2001. Effects of zooplankton grazing and nutrients on the bloom-forming, N2-fixing cyanobacterium *Aphanizomenon* in Lake Kinneret. *Journal of plankton research*, 23:165-174

Harris, G.P., Baxter, G., 1996. Interannual variability in phytoplankton biomass and species composition in a subtropical reservoir. *Freshwater biology*, 35:545-560.

Havens, K. E., 2008. Cyanobacteria blooms: effects on aquatic ecosystems. Cyanobacterial *Harmful Algal Blooms*: *State of the Science and Research Needs*, 619:733-747.

Havens, K., East, T., B. J. 1996. Experimental studies of zooplankton–phytoplankton–nutrient interactions in a large subtropical lake (Lake Okeechobee, Florida, USA). *Freshwater Biology*, 36:579-597.

Hendrickson, J. C., 2011. Appendix 8.D. CE-QUAL-ICM Setup, Calibration and Withdrawal Scenario Results. http://www.sjrwmd.com/technicalreports.

Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollingher, U., Zohary.T., 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology* ,35:403-424.

Hunt, R. J., Matveev V. F., 2005. The effects of nutrients and zooplankton community structure on phytoplankton growth in a subtropical Australian reservoir: an enclosure study. *Limnologica-Ecology and Management of Inland Waters*, 35:90-101.

Jeppesen, E., Søndergaard, M., Mazzeo, N., Meerhoff, M., Branco, C., Huszar, V., Scasso, F., 2005. Lake restoration and biomanipulation in temperate lakes:relevance for subtropical and tropical lakes. Chapter 11. In Reddy, M. V. (ed.), *Tropical Eutrophic Lakes:Their Restoration and Management*. Science Publishers, Enfield, pp. 331–359.

Kâ, S., Mendoza-Vera, J. M., Bouvy, M., Champalbert, G., N'Gom-Kâ, R., Pagano, M., 2012. Can tropical freshwater zooplankton graze efficiently on cyanobacteria? *Hydrobiologia,* 979: 119-138.

Karl, D., Michaels, A., Bergman, B., Capone, D., Carpenter, E., Letelier, R., Lipschultz, F., Paerl, H., Sigman, D., Stal, L., 2002. Dinitrogen fixation in the world's oceans. *Biogeochemistry*, 57:47-98.

Lehman, J. T., Sandgren C. D., 1985. Species-specific rates of growth and grazing loss among freshwater algae. *Limnol. Oceanogr.,* 30: 34-46.

Li, R., Carmichael, W., Brittain, S., Eaglesham, G., Shaw, G., Mahakhant, A., Noparatnaraporn, N., Yongmanitchai, W., Kaya, K., and Watanabe. M.,2001. Isolation and identification of the cyanotoxin cylindrospermopsin and deoxy-cylindrospermopsin from a Thailand strain of *Cylindrospermopsis raciborskii* (Cyanobacteria). *Toxicon*, 39:973-980.

Low, E., Ng, P. K. L., 2010. Top-down control of phytoplankton by zooplankton in tropical reservoirs in Singapore? *The Raffles Bull of Zoology,* 58:311-322.

Mantoura, R. F. C., Llewellyn, C.A., 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Analytica Chimica Acta,* 151:297-314.

McGregor, G. B., Fabbro, L. D., 2000. Dominance of *C. raciborskii raciborskii*(Nostocales, Cyanoprokaryota) in Queensland tropical and

subtropical reservoirs: Implications for monitoring and management *Lake Reserv. Manage.,* 5: 195-205.

Main, T., Dobberfuhl, D., Elser, J., (1997) N: P stoichiometry and ontogeny of crustacean zooplankton: a test of the growth rate hypothesis. *Limnol. Oceanogr.,* 42: 1474-1478.

Mitra, A., Flynn, K. J., 2006. Promotion of harmful algal blooms by zooplankton predatory activity. *Biology letters,* 2:194-197.

Moore, S. K., Suthers, I. M., 2006. Evaluation and correction of subresolved particles by the optical plankton counter in three Australian estuaries with pristine to highly modified catchments. *J. Geophys. Res.*, 111: 1-14.

O'Neil, J., Davis, T., Burford, M., Gobler, C., 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae*, 14: 313-334.

Olsen, Y., Jensen, A., Reinertsen, H., Borsheim, K. Y., Heldal, M., Langeland, A., 1986. Dependence of the rate of release of phosphorus by zooplankton on the P: C ratio in the food supply, as calculated by a recycling model. *Limnol. Oceanogr.*, 31: 34-44.

Padisák, J., 1997. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding highly adaptive cyanobacterium: worldwide distribution and review of its ecology. *Arch. Hydrobiol., Suppl*. 107: 563-593.

Paerl, H. W., and Huisman, J., 2008. Blooms like it hot. *Science,* 320: 57-58.

Panosso, R., Carlsson, P., Kozlowsky-Suzuki, B., Azevedo, S. M. F. O., Granéli, E., 2003. Effect of grazing by a neotropical copepod, *Notodiaptomus*, on a natural cyanobacterial assemblage and on toxic and non-toxic cyanobacterial strains. *Journal of Plankton Research*, 25:1169-1175.

Pastorok, R. A., 1980. The effects of predator hunger and food abundance on prey selection by *Chaoborus* larvae. *Limnol. Oceanogr.*, 25: 910-921.

Persson, L., Andersson, G., Hamrin, S.F., Johansson, L., 1988. Predator regulation and primary production along the productivity gradient of temperate lake ecosystems. In: Carpenter, SR (ed.), *Complex interactions in lake communities.* Springer-Verlag, New York, pp. 45–65.

Posselt, A. J., Burford, M. A., Shaw, G., 2009. Pulses of phosphate promote dominance of the toxic *Cylindrospermopsis raciborskii* in a subtropical water reservoir *Journal of Phycology,* 45:540-546.

Redfield, A. C., 1958. The biological control of chemical factors in the environment. *American Scientist*, 46:205-221.

Richardson, L. L., Sekar, R., Myers, J. L., Gantar, M., Voss, J. D., Kaczmarsky, L., Remily, E. R., Boyer, G. L., Zimba, P. V., 2007. The presence of the cyanobacterial toxin microcystin in black band disease of corals. *FEMS microbiology letters,* 272:182-187.

Riebesell, U,, Lee, K., Nejstgaard, J. C., 2010. Pelagic mesocosms. In: Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.P (eds) ,*Guide to best practices for ocean acidification research and data reporting* . Publications Office of the European Union, Luxembourg, pp. 95-122

Saker, M.L., Thomas, A.D., Norton, J.H., 1999. Cattle mortality attributed to the toxic cyanobacterium *Cylindrospermopsis raciborskii* in an outback region of North Queensland. *Environmental Toxicology,* 14:179-182.

Shafik, H.M., Herodek, S., Presing, M., Vörös, L., 2001. Factors effecting growth and cell composition of cyanoprokaryote *Cylindrospermopsis raciborskii* (Wołoszyńska) Seenayya et Subba Raju. *Archiv für Hydrobiologie*, 140:75-93.

Shafik, H. M., 2003. Morphological characteristics of *Cylindrospermopsis raciborski*i (Woloszynska) Seenayya et Subba Raju in laboratory cultures. A*cta Biologica Hungarica*, 54:121-136.

Sinha, R., Pearson, L. A., Davis, T. W., Burford, M. A., Orr, P. T., Neilan, B. A., 2011. Increased incidence of *C. raciborskii* in temperate zones-is climate change responsible? *Water Res*, 46: 1408-1419

Sommer, U., Sommer, F., 2006. Cladocerans versus copepods: the cause of contrasting top–down controls on freshwater and marine phytoplankton. *Oecologia*, 147:183-194.

Spencer, C. N., Ellis, B. K., 1998. Role of nutrients and zooplankton in regulation of phytoplankton in Flathead lake (Montana, U.S.A.), a large oligotrophic lake. *Freshwater Biology*, 39:755-763.

Sterner, R. W., 1990. The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algae competitive arena. *The American Naturalist*, 136:209-229.

Stüken, A., Jakobsen, K. S., 2010. The cylindrospermopsin gene cluster of *Aphanizomenon* sp. strain 10E6: organization and recombination. *Microbiology*, 156:2438-2451.

Towns, T. G., 1986. Determination of aqueous phosphate by ascorbic acid reduction of phosphomolybdic acid. *Analytical Chemistry*, 58:223-229.

Trommer, G., Pondaven, P., Siccha, M, Stibor, H., 2012. Zooplankton-mediated nutrient limitation patterns in marine phytoplankton: an experimental approach with natural communities. *Marine Ecology Progress Series*, 449:83-94.

Van Heukelem, L., Thomas, C.S., 2001. Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. *Journal of Chromatography, A* 910:31-49.

Vanni, M. J., 2002. Nutrient cycling by animals in freshwater ecosystems. *Annu. Rev. Ecol. Syst*., 33: 341-370.

Von Rückert, G., Giani A., 2008. Biological interactions in the plankton community of a tropical eutrophic reservoir: is the phytoplankton controlled by zooplankton? *Journal of Plankton Research*, 30:1157-1168.

Wang, X., Qin, B., Gao, G., Paerl, H. W., 2010. Nutrient enrichment and selective predation by zooplankton promote *Microcystis* (Cyanobacteria) bloom formation. *J. Plankton Res.*, 32: 457-470.

Table 1: Water quality parameters mean (± SE) in mesocosms and adjacent ambient water at the beginning and end of the mesocosm experiment.

	Ambient day0 (n=3)	Ambient day7 (n=1)	Mesocosm day0 (n=12)	Mesocosm day7 (n=12)
Temperature surface (ºC)	29.6 ± 0.4	32.1	28.5 ± 0.2	29.8 ± 0.4
Temperature 1m (°C)	29.4 ± 0.4	29.6	28.4 ± 0.2	29.3 ± 0.2
Conductivity surface (uS/cm)	0.3 ± 0.0	0.3	0.3 ± 0.0	0.3 ± 0.0
Conductivity 1m (uS/cm)	0.3 ± 0.0	0.3	0.3 ± 0.2	0.3 ± 0.0
Turbidity surface (NTU)	2.3 ± 0.3	3.0	2.0 ± 0.1	2.9 ± 0.1
Turbidity 1m (NTU)	2.2 ± 0.0	3.2	1.8 ± 0.0	2.7 ± 0.1
pH surface	8.6 ± 0.1	8.7	8.5 ± 0.1	8.6 ± 0.1
pH 1m	8.7 ± 0.0	8.7	8.3 ± 0.2	8.3 ± 0.0
DO% surface	102.9 ± 1.8	101.9	94.6 ± 1.2	96.6 ± 1.4
DO% 1m	102.1 ± 2.3	100.5	91.5 ± 1.9	95.8 ± 1.7
Secchi depth (m)	1.8 ± 0.1	1.8	1.7 ± 0.0	1.7 ± 0.0

Table 2: Phytoplankton species composition at the beginning of the mesocosm experiment; N = non-N_2-fixing algae, F = N_2-fixing algae, T = potentially toxic algae, Microalgae 20-200 µm, Nanoalgae 2-20 µm.

Species name	Functional group	Size	Class	Species abundance (cells ml^{-1})	% Biovolume
Anabaena sp.	F, T	Micro	Cyanophyta	1491	0.9
Aphanocapsa sp.	N	Micro	Cyanophyta	4830	0.0
Aphanizomenon sp,	F, T	Micro	Cyanophyta	0	0.0
Cyanodictyon imperfectum	N	Micro	Cyanophyta	1344	0.0
Cylindrospermopsis raciborskii	F, T	Micro	Cyanophyta	25200	22.6
Geitlerinema sp.	T	Micro	Cyanophyta	378	0.1
Limnothrix sp.	N	Micro	Cyanophyta	27468	18.1
Merismopedia sp.	N	Micro	Cyanophyta	588	0.0
Planktolyngbya limnetica	N	Micro	Cyanophyta	12852	12.5
Planktolyngbya microspira	N	Micro	Cyanophyta	31983	23.2
Pseudanabaena limnetica	N	Micro	Cyanophyta	126	0.2
Merismopedia punctata	N	Micro	Cyanophyta	252	0.1
Cryptomonas sp.	N	Nano	Cryptophyta	42	1.2
Cyclotella sp.	N	Nano	Bacillariophyta	252	2.6
Synedra sp.	N	Micro	Bacillariophyta	84	0.0
Cosmarium sp.	N	Nano	Chlorophyta	42	0.2
Oocystis sp.	N	Micro	Chlorophyta	42	0.2
Scenedesmus sp.	N	Micro	Chlorophyta	42	0.0
Peridinium sp.	N	Nano	Dinophyta	105	7.5
Other					10

Figure legends

Figure 1. Lake Wivenhoe in Southeast Queensland, Australia, showing the location of the mesocosm experiment.

Figure 2. The dissolved (A) and particulate (B) molar elemental ratios in treatments on day 7. Treatments include: 1x ambient zooplankton abundance (1Z), 5x zooplankton abundance (5Z), 5x zooplankton with inorganic P addition (5ZP) and a no amendment control. Data are means ± SE (n = 3). Statistical comparisons indicated by letters above columns: a is different from b, ab is not different from both a and b.

Figure 3. Phosphorus content per individual (A) and molar elemental ratios of zooplankton: N:P (B) and C:P (C) sampled on day 7. Treatments include: 1x ambient zooplankton abundance (1Z), 5x zooplankton abundance (5Z), 5x zooplankton with inorganic P addition (5ZP) and a no amendment control. Data are means ± SE (n = 3). Statistical comparisons indicated by letters above columns.

Figure 4. (A) Chl-a concentration (µg L^{-1}) at the end of experiment; (B) Chl-a content per cell (µg Chl-a $cell^{-1}$) and (C) Chl-a per biovolume of phytoplankton (µg Chl-a $µm^3$). Treatments include: 1x ambient zooplankton abundance (1Z), 5x zooplankton abundance (5Z), 5x zooplankton with inorganic P addition (5ZP) and a no amendment control. T0 are values from the beginning of the experiment. Data are means ± SE (n = 3). Statistical comparisons indicated by letters above columns.

Figure 5. Phytoplankton functional group abundance in different mesocosm treatments during the mesocosm experiment. Treatments include: A: no amendment control; B: 1x ambient zooplankton abundance (1Z), C: 5x zooplankton abundance (5Z), and D: 5x zooplankton with inorganic P addition (5ZP). Data are means ± SE (n = 3).

Figure 6. Abundance of *C. raciborskii* (A) and the % *C. raciborskii* of total phytoplankton biovolume (v/v) (B) in different treatments during the mesocosm

experiment. Treatments include: 1x ambient zooplankton abundance (1Z), 5x zooplankton abundance (5Z), 5x zooplankton with inorganic P addition (5ZP) and a no amendment control. Data are means ± SE (n = 3). Asterisk * above the data point indicates significant difference between treatment and control at that time point. "*" means marginally significant (p =0.058).

Figure 7. MDS plot of algal species composition in the mesocosm treatments on day 4/7. Treatments include: 1x ambient zooplankton abundance (1Z), 5x zooplankton abundance (5Z), 5x zooplankton with inorganic P addition (5ZP) and a no amendment control.

Figure 8. Change in abundance of two major phytoplankton species in the mesocosm treatments between day 0 and 4. Treatments include: 1x ambient zooplankton abundance (1Z), 5x zooplankton abundance (5Z), 5x zooplankton with inorganic P addition (5ZP) and a no amendment control. Data are means ± SE (n = 3). Statistical comparisons indicated by letters above columns.

Figure 9. Zooplankton biomass (mm^3 L^{-1}) (A) and abundance (ind. L^{-1}) (B) in each treatment at the end of experiment. Treatments include: 1x ambient zooplankton abundance (1Z), 5x zooplankton abundance (5Z), 5x zooplankton with inorganic P addition (5ZP) and a no amendment control. Data are means ± SE (n = 3). Statistical comparisons indicated by letters above columns.

Figure 10. The proportion of zooplankton functional groups in mesocosm treatments at the beginning (T0) and end of the experiment (day 7). Treatments include: 1x ambient zooplankton abundance (1Z), 5x zooplankton abundance (5Z), 5x zooplankton with inorganic P addition (5ZP) and a no amendment control. Data are means (n = 3).

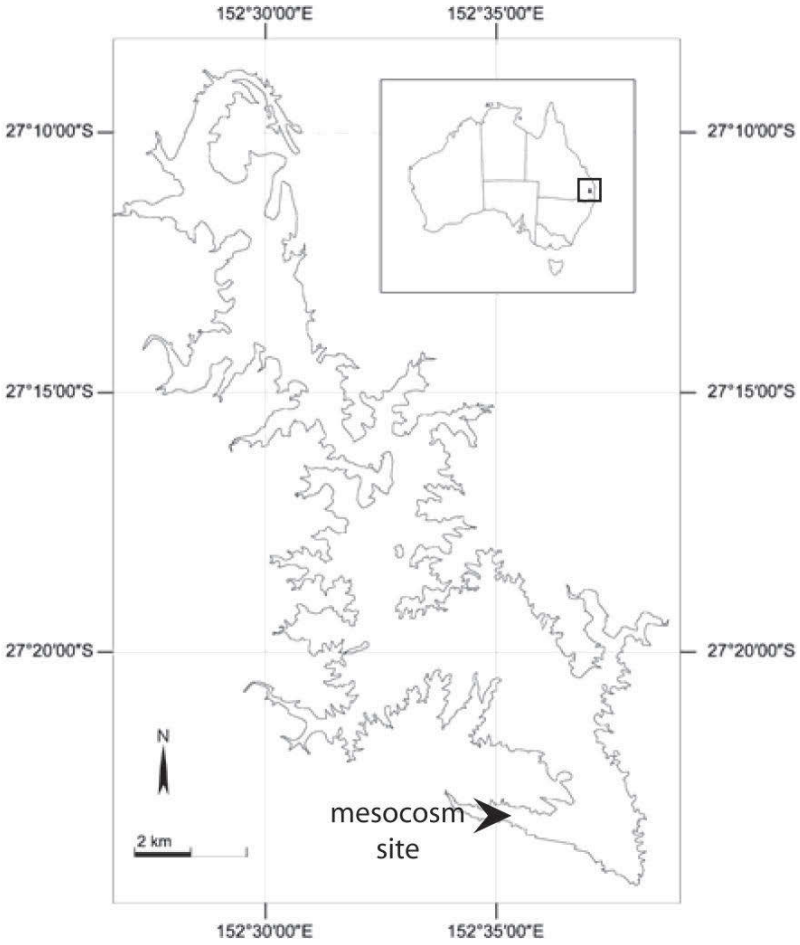
152°30'00"E
152°35'00"E
27°10'00"S
27°15'00"S
27°20'00"S
N
2 km
mesocosm
site

Figure 1

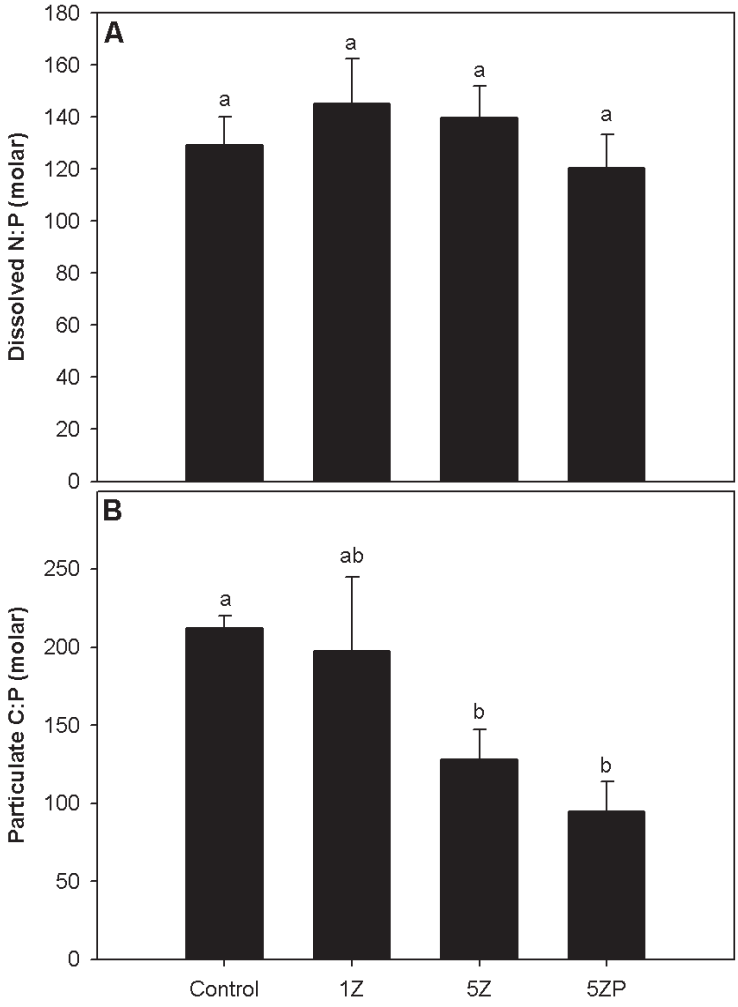
A
B
Dissolved N:P (molar)
Particulate C:P (molar)
180
160
140
120
100
80
60
40
20
0
250
200
150
100
50
0
a
a
a
a
a
ab
b
b
Control
1Z
5Z
5ZP

Figure 2

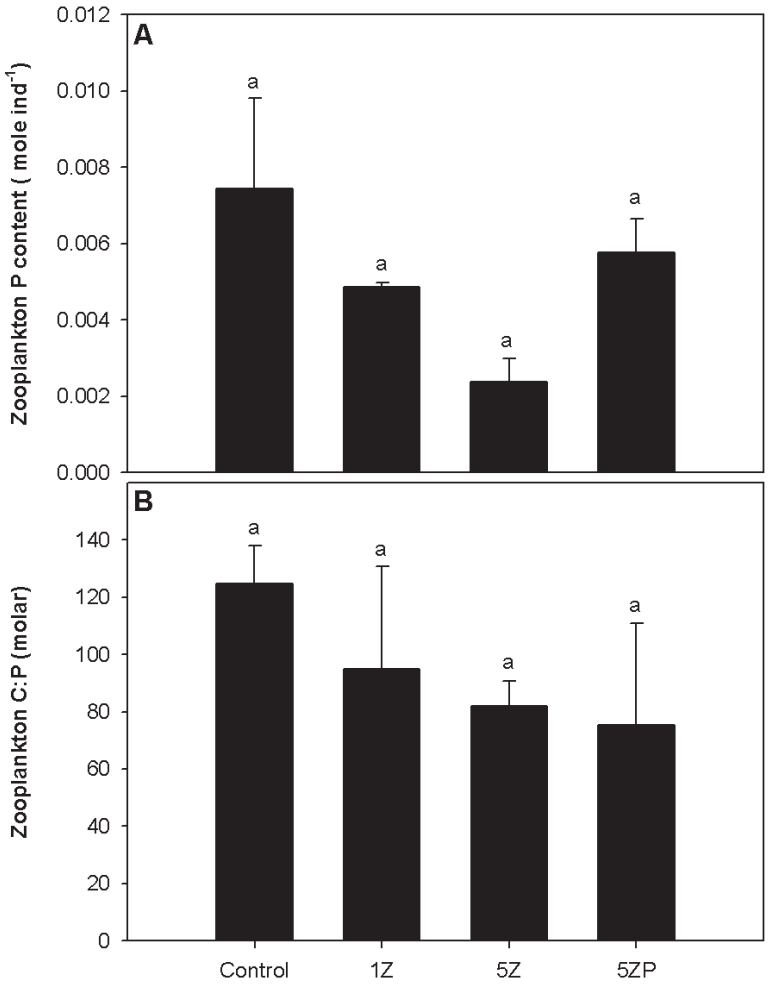
A
B
Zooplankton P content (mole ind-1)
Zooplankton C:P (molar)
0.012
0.010
0.008
0.006
0.004
0.002
0.000
140
120
100
80
60
40
20
0
a
Control
1Z
5Z
5ZP

Figure 3

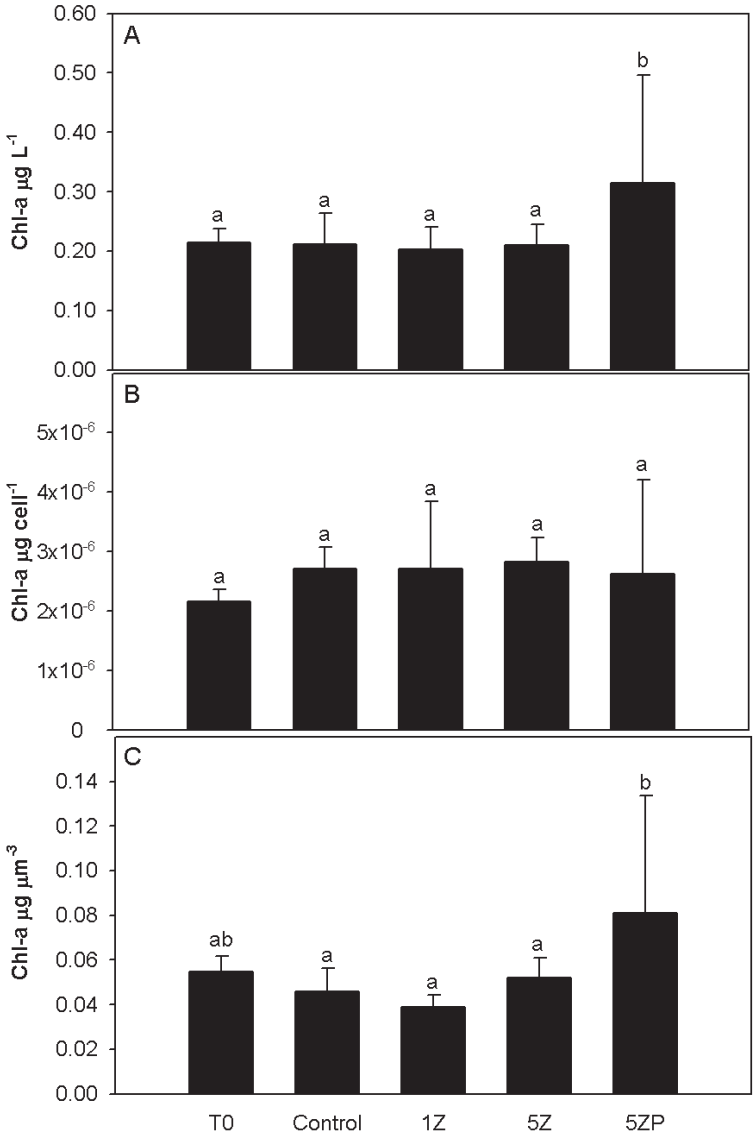
A
B
C
Chl-a µg L-1
Chl-a µg cell-1
Chl-a µg µm-3
0.60
0.50
0.40
0.30
0.20
0.10
0.00
5x10-6
4x10-6
3x10-6
2x10-6
1x10-6
0
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
a
a
a
a
b
a
a
a
a
a
ab
a
a
a
b
T0
Control
1Z
5Z
5ZP

Figure 4

.

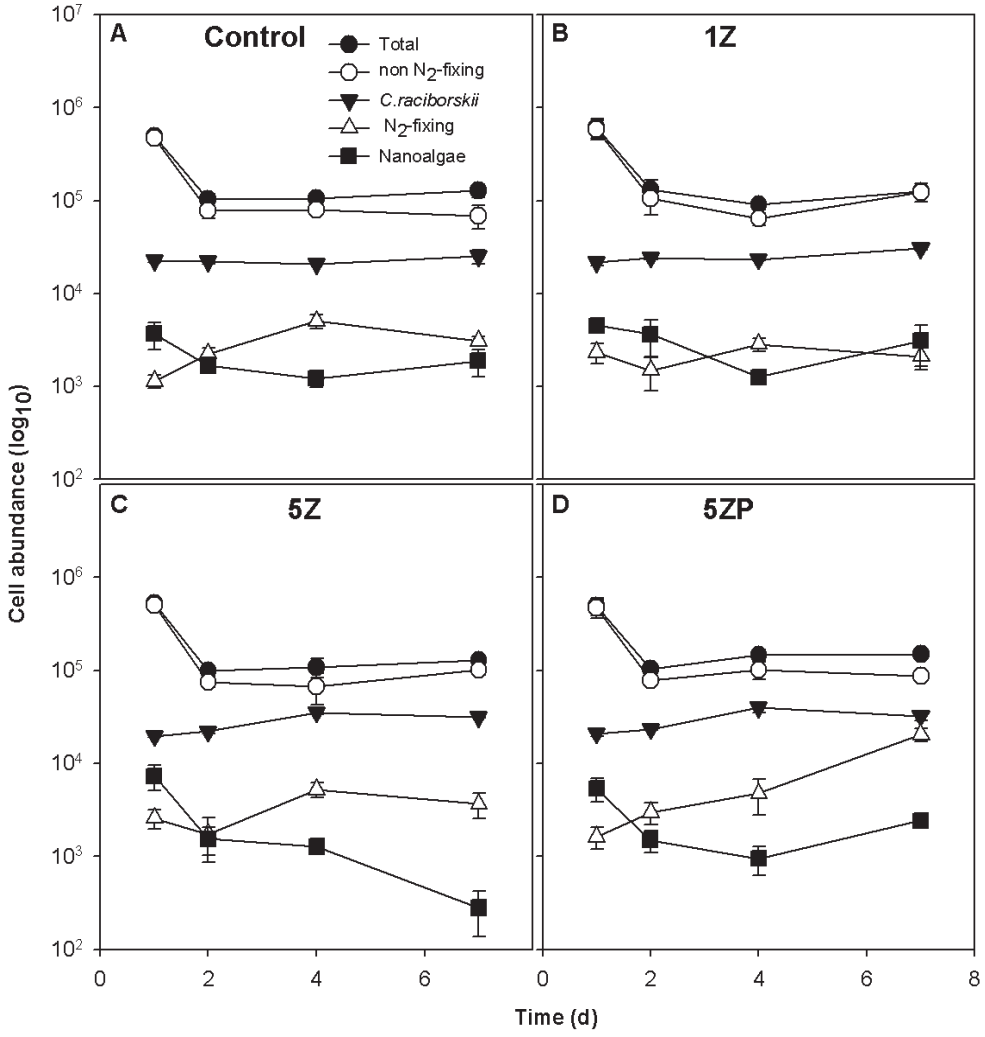
A
Control
Total
non N2-fixing
C.raciborskii
N2-fixing
Nanoalgae
B
1Z
C
5Z
D
5ZP
Cell abundance (log10)
Time (d)

Figure 5

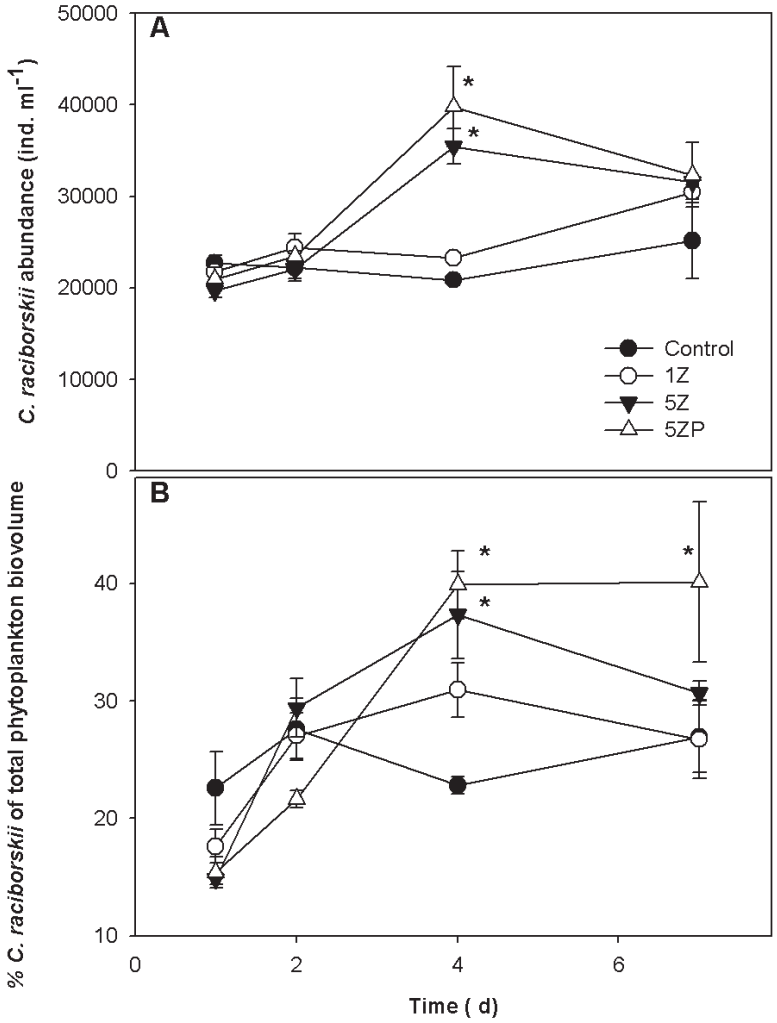
A
B
C. raciborskii abundance (ind. ml-1)
% C. raciborskii of total phytoplankton biovolume
Time (d)
Control
1Z
5Z
5ZP
50000
40000
30000
20000
10000
0
40
30
20
10
0
2
4
6
*

Figure 6

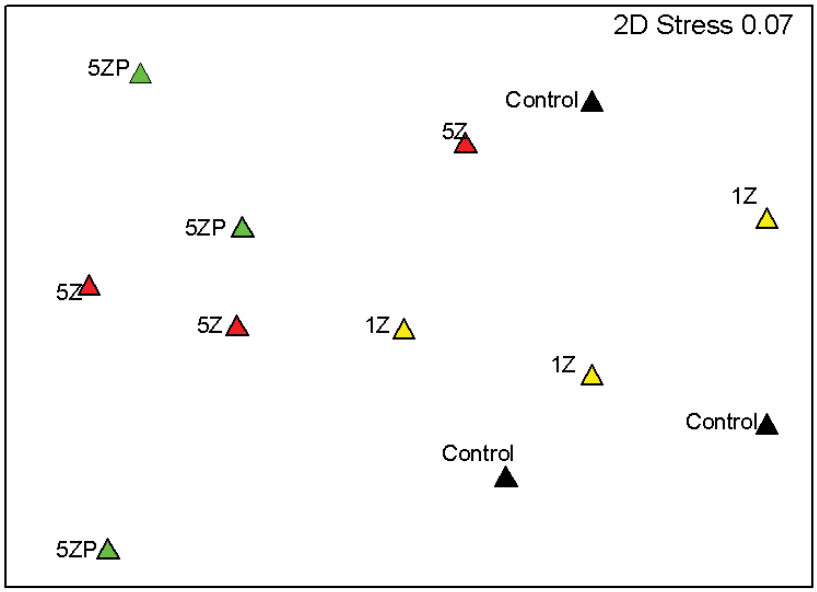
2D Stress 0.07
5ZP
Control
5Z
1Z
5ZP
5Z
5Z
1Z
1Z
Control
Control
5ZP

Figure 7

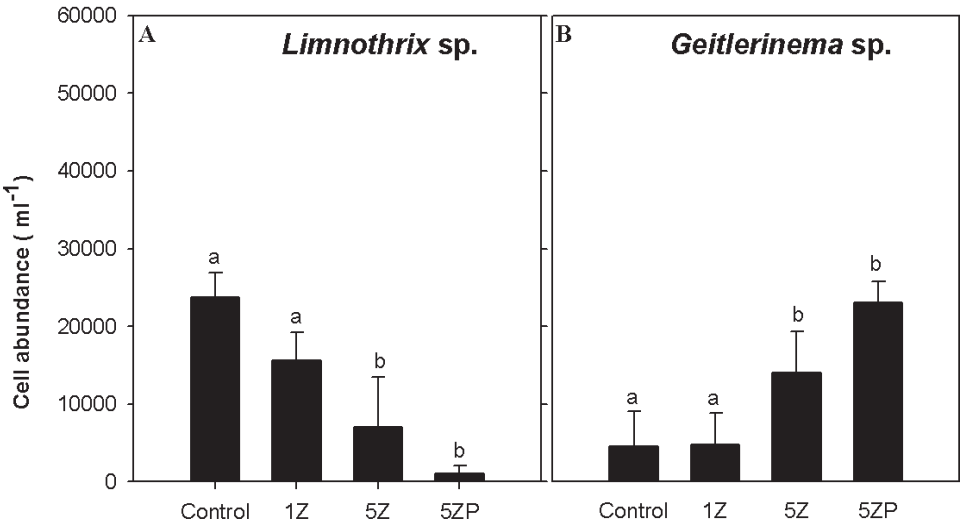
A
Limnothrix sp.
B
Geitlerinema sp.
Cell abundance (ml-1)
60000
50000
40000
30000
20000
10000
0
a
a
b
b
a
a
b
b
Control
1Z
5Z
5ZP
Control
1Z
5Z
5ZP

Figure 8

.

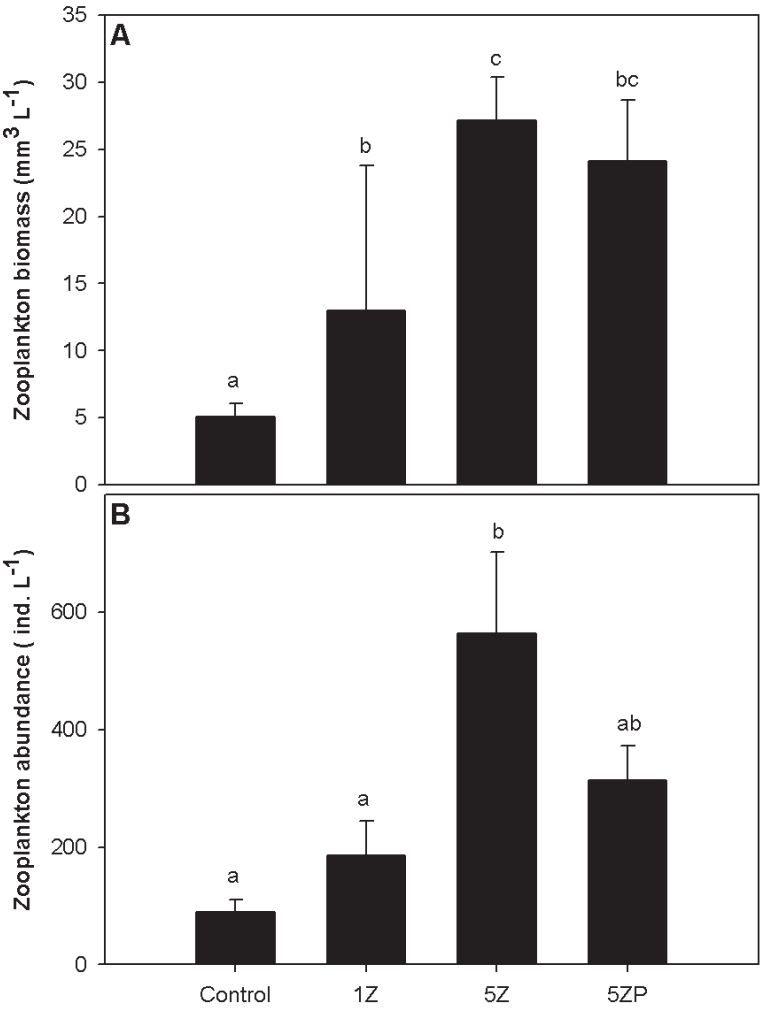
A
B
Zooplankton biomass (mm3 L-1)
Zooplankton abundance (ind. L-1)
35
30
25
20
15
10
5
0
600
400
200
0
a
b
c
bc
a
a
b
ab
Control
1Z
5Z
5ZP

Figure 9

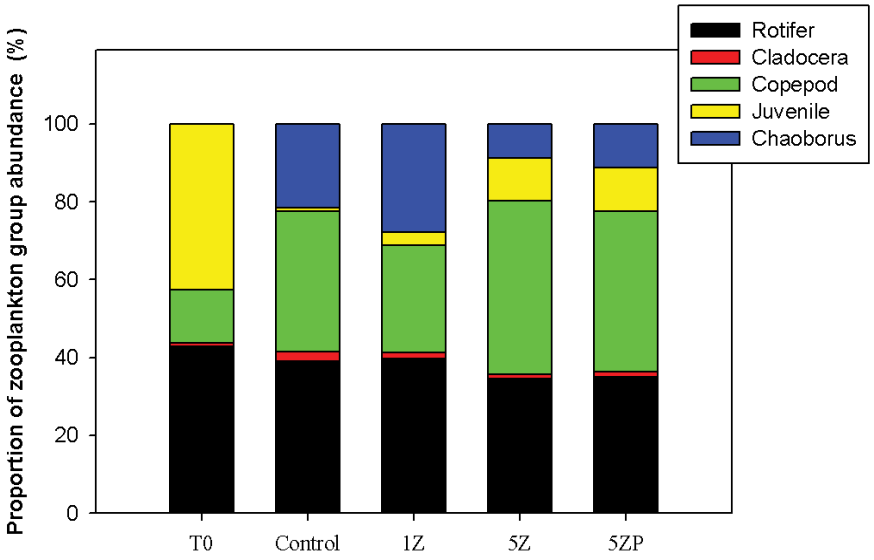
Proportion of zooplankton group abundance (%)
0
20
40
60
80
100
T0
Control
1Z
5Z
5ZP
Rotifer
Cladocera
Copepod
Juvenile
Chaoborus

Figure 10

CHAPTER 5

A comparison of zooplankton community structure in subtropical Australian reservoirs with and without blooms of *Cylindrospermopsis raciborskii*

ABSTRACT

This study examined meso-zooplankton biomass, size structure and functional group composition across 15 freshwater reservoirs in subtropical north-eastern Australia during an austral spring and summer. Reservoirs were split into two groups, those that experience *Cylindrospermopsis raciborskii* blooms and those that do not. Zooplankton communities were examined in both the dry (November 2009) and the wet (February 2010) season to capture seasonal variations of phytoplankton and zooplankton communities and associated environmental variables. The survey demonstrated that *C. raciborskii* cells present in these subtropical reservoirs at the time of sampling coincided with a shift in the functional group and community size structure of zooplankton. Reservoirs with *C. raciborskii* were positively correlated with a high proportion of copepods and rotifers, and a low proportion of cladocerans to total zooplankton abundance. Juvenile zooplankton abundance was negatively correlated with *C. raciborskii*, which suggests that *C. raciborskii* may reduce the fecundity of zooplankton.

1. INTRODUCTION

The interactions between zooplankton and phytoplankton play an important role in lake ecosystems, transferring energy through pelagic food webs and regulating the community structure (Elser et al. 1988). In some circumstances, zooplankton can substantially reduce total phytoplankton biomass (Lampert 1986, Sommer 2003) and regulate phytoplankton species composition (Elser et al. 1988, Sinistro et al. 2007). On the other hand, phytoplankton composition is able to influence the zooplankton community structure and the efficiency of top-down control by zooplankton (Ghadouani et al. 2003, Leonard and Paerl 2005, Hansson et al. 2007).

Cyanobacteria dominated phytoplankton communities appear to reduce the growth and reproduction of zooplankton due to the energy costs of low digestible and nutritionally deficient food (Brett and Muller-Navarra 1997). In addition, toxic cyanobacteria may cause a major disruption of energy flow and trophic structure in aquatic ecosystems by decreasing the diversity of the zooplankton community, often at the expense of large cladocerans (Attayde and Hansson 1999). Indeed, the adverse effect of cyanobacteria on zooplankton appears to be size dependent. For example, filamentous and colonial cyanobacteria negatively affect larger zooplankton by mechanical interference with food gathering (Webster and Peters 1978, Hawkins and Lampert 1989, Attayde and Hansson 1999, DeMott et al. 2001), and small-sized cladocerans develop stronger tolerance of cyanobacteria toxins than larger cladocerans, when both groups are exposed to cyanobacteria (Reinertsen et al. 1986, Guo and Xie 2006). In addition, the negative effect of cyanobacteria on zooplankton varies among zooplankton taxa. Rotifers can ingest some toxic cyanobacteria in mixed diets, although pure diets of toxic cyanobacteria restrict growth or caused mortality (Soares et al., 2010), while copepods are able to avoid ingestion of toxic cyanobacteria (Burns and Hegarty, 1994; DeMott and Moxter, 1991).

Most research on zooplankton-phytoplankton interactions in aquatic habitats has been conducted in eutrophic and mesotrophic temperate lakes. Cladocerans are typically

dominant in eutrophic and mesotrophic lakes, but less so in oligotrophic lakes (Sommer and Sommer 2006). Very few studies have been undertaken in the tropics and subtropics (Havens et al. 1996, Low and Ng 2010). Cladoceran-based generalizations restrict our understanding of plankton relationships in tropical and subtropical lakes where cyanobacterial blooms are more persistent and copepods dominate the zooplankton year-round (Ali Ger et al. 2011).

The interactions of copepods and the toxic cyanobacterium *C. raciborskii* were previously investigated in small–scale laboratory studies (Hong, unpublished data; Chapter 2 and 3), as well as *in situ* mesocosms (Hong, unpublished data; Chapter 4). These studies concluded that zooplankton acted synergistically (through selective grazing and nutrient regeneration) to facilitate the growth of *C. raciborskii*. The present study investigated 15 reservoirs located in subtropical north-eastern Australia which provided an opportunity to study the potential interaction of zooplankton and *C. raciborskii* within the context of an entire ecosystem. Blooms of the toxic cyanobacterium *C. raciborskii* typically occur in 8 of these 15 reservoirs (Leigh et al. 2010) and the abundance of *C. raciborskii* is generally higher in the summer due to relatively warm, vertically stratified conditions (Burford et al. 2007). Thus, November 2009 and February 2010 were chosen to represent the pre-bloom and bloom seasons, respectively. The objective of this investigation was to compare the zooplankton community, including biomass, size structure and functional group abundance between (1) the two groups of reservoirs and (2) pre-bloom and bloom seasons to test the prediction that zooplankton composition, biomass and size structure changes with *C. raciborskii* abundance. The expectation was that *C. raciborskii* would be positively correlated with the abundance or proportion of copepods because they are generally more selective against cyanobacteria, and that the zooplankton community would have greater representation of the smaller size classes and potentially lower biomass due to the presence of filamentous cyanobacteria.

2. MATERIALS AND METHODS

The study sites were 15 reservoirs located in southeast Queensland, Australia, within the wider Brisbane area (Fig. 1). These reservoirs supply drinking water to the urban and

semi-rural populations in the region and have a range of watershed and reservoir characteristics (e.g., reservoir surface area, volume, and depth; Table 1). For further details about the reservoirs, see Leigh et al. (2010).

2.1. Sampling

Reservoirs (Table 1) were sampled quasi-synoptically (i.e., within a two week period) in the spring (9 - 30 November 2009) and summer (10 - 25 February 2010). Physical parameters were profiled, including temperature, conductivity, pH, turbidity, fluorescence and dissolved oxygen (DO) at 1m depth intervals through the water column using a multi-parameter instrument (YSI 6920 Sonde). The sampling process was conducted by a team of researchers at University of Technology, Sydney, Griffith University and the seqwater Corporation. Three sites were sampled in each reservoir, except Somerset, Borumba, Hinze, Leslie Harrison and North Pine, where four sites were sampled. Samples for water quality and phytoplankton were collected and measured at all these sites, but only zooplankton samples from the dam wall site of each reservoir were counted. The dam wall site was typically the deepest part of the reservoir, adjacent to the water outflow gates and with the most abundant zooplankton. At each site, a 3 m depth-integrated sample of water was collected from the surface with a modified PVC pipe. The water was then transferred to a clean bucket from which samples for water quality analyses were taken. Water quality analyses included total nitrogen and phosphorus (TN, TP), dissolved inorganic nitrogen and phosphorus (DIN and DIP), filterable reactive phosphorus (FRP), chlorophyll a (Chl-a) concentration, Total Suspended Solids (TSS), Total Organic Carbon (TOC), as well as phytoplankton cell counts. Taxa identified included *C. raciborskii* and other potentially toxic cyanobacterial species (*Anabaena circinalis*, *Anabaena bergii*, *Aphanizomenon ovalisporum,* and *Microcystis aeruginosa*), yielding *C. raciborskii* abundance, total potentially toxic algae abundance, total cyanobacteria abundance, and total phytoplankton abundance (Leigh et al., 2010). Additional phytoplankton related parameters were calculated, such as TPN:TPP (molar), the proportion of cyanobacterial cells compared to total cells, and the proportion of potentially toxic cells compared to total cells. The chemical and biological parameters were analyzed using standard

methods and sampling equipment and protocols were consistent throughout the study (Leigh et al. 2010).

At each reservoir, zooplankton were collected with a collared net (85 µm mesh 20 cm diameter) towed vertically from depth to the surface. To standardize sampling efforts across reservoirs, vertical tows were 12 m deep; however, in shallow reservoirs, they were to within 1 m of the bottom (ranging from 5 to 8 m deep). At each site, four replicate tows were made: two were pooled and preserved in 5% formalin (v/v final concentration) for biomass analysis; the other two were pooled and preserved in 75% ethanol for species identification. All samples were kept in the dark at 4˚C until analyzed.

2.2. Zooplankton identification and enumeration

Zooplankton identification was undertaken using a compound microscope (Olympus BX50, Hamburg, Germany). The samples were concentrated (approximately 4 times) by sedimentation, homogenized and sub-sampled with a wide-mouth pipette before enumeration with a gridded Sedgwick-Rafter cell (Hausser Scientific, Germany). Zooplankton (85 to 750 µm diameter) were counted using 100x magnification to a minimum of 400 cells, otherwise the entire chamber was counted. Zooplankton were classified into four functional groups: rotifera, cladocera, copepoda and juveniles (copepodites and nauplii). Midge larvae, *Chaoborus* sp., appeared in some samples and were also enumerated. Calculated parameters included the ratio of cladocerans to copepods, as well as the proportion of juvenile copepods to total zooplankton.

2.3. Zooplankton biomass and structure analysis

Zooplankton total biomass and size structure was estimated on formalin fixed samples using an Optical Plankton Counter (OPC), set up according to Moore and Suthers (2006). Samples were initially prepared by filtering zooplankton onto mesh (165 µm) and rinsing the preservative off under running tap water. Animals were then re-

suspended in tap water and poured into a header tank, which fed into the OPC. The OPC measures and transmits the cross-sectional area of each particle passing through its beam and records it electronically, with a minimum detection of particles approximately 300 µm in diameter. Zooplankton biovolume was estimated using an ellipse geometric model: Volume (V) = (3.14 / 6) (ESD) (ESD/F) ^{2}, where ESD is the equivalent spherical diameter and F is the ratio of the major axis (longest linear dimension) over the minor axis of an ellipse. Biovolume (mm^3) was calculated as the sum of the particles over all ESD values between 240 and 3000 µm (Moore and Suthers 2006, Patoine et al. 2006). Plotting log transformed equivalent spherical diameter (ESD) and plankton biomass values for each size category yielded a normalized biomass size spectrum (NBSS, x = ESD; y = biomass) to indicate the zooplankton community structure (Moore and Suthers 2006). Over-representation of smaller size classes yields NBSS slopes greater than 1.0 and typically occurs as a result of nutrient enrichment, and low overall biovolume from top-down grazing pressure results in low NBSS intercepts. We have limited information about fishes in these reservoirs but acknowledge that large zooplankton are likely to experience increased fish predation during summer as lake productivity increases (Jeppesen et al. 2004, Moore and Suthers, 2006).

2.4. Data analysis

Two-way Analysis of Variance was performed to determine the effect of season (fixed factor) and reservoir type with and without blooms of *C. raciborskii* cells (fixed factor) on the abundance of zooplankton functional groups, the proportion of juvenile and copepods relative to total zooplankton abundance, the biovolume (intercept of size normalized biomass spectrum estimated with the OPC), as well as zooplankton size structure (slope of NBSS estimated with the OPC). The analyses were performed with SPSS Statistics 17 (SPSS Inc, Chicago, USA). Levene's test was used to check for the homogeneity of variances and the data were either square root or natural log transformed if the Levene's test was significant. The level of significance for all tests was $p < 0.05$. Spearman's rank correlation (rs) was used to determine the correlation between the direct microscope counts of zooplankton abundance and OPC estimates, reported as rs (n: sample size) = correlation, p= significance level.

Water quality data (i.e., temperature, conductivity, pH, dissolved oxygen (DO), NH_4, nitrite and nitrate (NOx), filterable reactive phosphorus (FRP), TPN:TPP, total organic carbon (TOC), and total suspended solids (TSS)) were analysed using multivariate techniques in PRIMER 6.0 software ® (2002 PRIMER-E Ltd., Plymouth, UK). After transformation with $\log_{10}$ (x + 1) and normalisation, a Euclidean-distance resemblance matrix was calculated. The resemblance matrix was used to ordinate samples, visualize the results using multidimensional scaling (MDS), and undertake analysis of similarity (ANOSIM). The ANOSIM R, is based on the ratio of the between group to within-group similarity ranking and ranges from 0 to 1, with the value indicating the degree of dissimilarity (1 = completely dissimilar; 0 = completely similar). A test of significance was done for season and reservoir type. The same analysis was done for the zooplankton data (including total abundance, abundance of functional groups, proportion of cladoceran:copepod and OPC biomass) between season and reservoirs with or without *C. raciborskii* cells. OPC slope and intercept were not included in the analysis because data was missing from Borumba and Little Nerang in November 2009 due to insufficient data (low zooplankton abundance) to estimate the OPC slope. Data were square root transformed and a Bray-Curtis resemblance matrix was calculated before MDS and ANOSIM analyses.

To test how much of the variation in the zooplankton composition and size structure could be explained by different environmental variables, multivariate redundancy analysis was undertaken using XLSTAT. These variables included: (1) reservoir physical characteristics, such as surface area (SA), water storage capacity (Vol) and depth (Table 1); (2) water quality characteristics, such as temperature, conductivity, DO (Tables 2 and 3); and (3) phytoplankton characteristics, such as Chl-a, *C. raciborskii* abundance, total abundance of cyanobacteria, total potentially toxic algae abundance, percentage of *C. raciborskii* relative to total phytoplankton abundance, percentage of cyanobacteria relative to total phytoplankton abundance and total phytoplankton abundance (Tables 4 and 5). These variables were considered proxies of total habitat availability (1), habitat quality (2) and prey abundance and quality (3), respectively.

Multivariate redundancy analysis (RDA) tested whether the correlation between the variation in the response variables (zooplankton community) and explanatory

variables (physical, water quality, phytoplankton factor) was significant or not by a permutation test. The permutation test examined the null hypothesis that there was no relationship between the response (zooplankton functional group, Y) and explanatory variables (environment factors, X), and that the model was not a significant representation of the response data. If the permutation test showed a significant relationship between the response variables and explanatory variables, then an RDA plot was made of how the inertia (variance) was spread between the explanatory variables (RDA axes), where the inertia is the Pearson's mean-square contingency coefficient, and is used as a measure of total variance. The sum of the squared loadings of the variables on a factor is known as the eigenvalue of the factor. Dividing the eigenvalue by the number of variables gives the proportion of variance explained by the factor. The higher the eigenvalue, the higher the proportion of variance explained by the factor.

3. RESULTS

Water quality results for each reservoir are summarized in Table 2 (spring) and Table 3 (summer). These data have been published in Leigh et al. (2010), but are provided here to show the significant effect of season (Global R = 0.087, p = 0.020) and reservoir type (Global R = 0.20, p = 0.001) on water quality.

3.1. Zooplankton functional group abundance among seasons and reservoir types

Across all reservoirs, zooplankton abundance was 26 ± 15 individuals L^{-1} (average ± SE ind. L^{-1}) in spring (November 2009) and 38 ± 30 ind. L^{-1} in summer (February 2010). The reservoir with the lowest zooplankton abundance was Little Nerang and that with the highest was Somerset (Fig. 2). Reservoirs with *C. raciborskii* blooms generally had zooplankton abundance similar to that of reservoirs without *C. raciborksii*. In general, rotifers contributed 30 - 40% of the total zooplankton abundance across reservoirs, averaging 33 ± 33% and 43 ± 28% in the spring and summer, respectively. They stood out as being the dominant zooplankton group (> 90%) in Lake Borumba and Lake

Macdonald. However, rotifers made a much smaller contribution to total zooplankton biomass due to their relatively small body size. Copepods comprised the most abundant mesozooplankton group in 8 of 15 reservoirs (Fig. 2), averaging 12 ± 3% and 26 ± 4% of total zooplankton abundance in the spring and summer, respectively. The ratio of cladocerans to copepods ranged from 0 to 100, averaging 22 ± 13% across reservoirs, and was higher in spring compared to summer in those reservoirs experiencing *C. raciborskii* blooms (24 ± 1% versus 4 ± 1%). However, there was no significant difference in the ratio of cladocerans to copepods between spring and summer in reservoirs without *C. raciborskii* (Fig. 3). Five reservoirs (i.e., Baroon Pocket, Leslie-Harrison, Maroon, North Pine and Wivenhoe) had a relatively high proportion of cladocerans, ranging from 17 to 43%.

Zooplankton functional group abundance also differed between reservoir types. Rotifer abundance was higher in reservoirs with *C. raciborskii* than those without (13 ± 5 versus 4 ± 1 ind L^{-1}, Table 4), but the proportion of copepods was consistent in the two reservoir types (27 ± 3% versus 23 ± 3%, with and without *C. raciborskii*; $F_{1,26}$ = 4.513, p = 0.043). Reservoirs with *C. raciborskii* had a lower proportion of juveniles (52 ± 5% and 34 ± 4%, Table 6). When examining the zooplankton community composition as a whole, there was no seasonal difference (ANOSIM test: Global R = 0.065, p = 0.076, Fig. 4A); however, there was a difference between reservoir type (ANOSIM test: Global R = 0.109, p = 0.032, Fig. 4B).

3.2. Normalized biomass size spectrum (NBSS) intercept and slope among seasons and reservoir types

OPC estimates of total zooplankton biovolume (biomass) showed a positive correlation with microscope counts (r_s = 0.470, p = 0.01, n = 29), and were positively correlated with y-intercepts of normalised biomass size- spectra (NBSS) (r_s = 0.350, p = 0.001). Overall, the NBSS (Fig. 5) had higher y-intercepts (greater zooplankton biomass) in summer than in spring ($F_{1,26}$ = 19.168, p = 0.000, Fig. 6), except for Borumba and North Pine (Figs. 5A, 5E); however, reservoirs with and without *C. raciborskii* had a similar y-intercept (Fig. 6).

NBSS slopes across reservoirs did not change significantly during spring and summer ($F_{1,26}$ = 0.270, p = 0.678, Fig. 6C) indicating no seasonal shift in the zooplankton size spectrum, while reservoirs experiencing *C. raciborskii* blooms had slightly steeper NBSS slopes than those without *C. raciborskii* blooms (1.4 ± 0.1 and 1.3 ± 0.1 Fig. 6D). Although the differences are marginally significant by Two-way Analysis of Variance (Table 7), the correlation between reservoirs with a history of *C. raciborskii* blooms and NBSS slope was significant (p = 0.045).

3.3. Variation in zooplankton community composition and structure explained by physical, water quality and phytoplankton parameters

Among the three groups of environmental variables, zooplankton functional groups were only correlated with phytoplankton variables (permutation test: p = 0.003), but not with physical or water quality variables (p = 0.388 and p = 0.674, respectively). The RDA plot between zooplankton and phytoplankton variables shows that the first two Factors were found to be a good quality projection of the multi-dimensional data table due to cumulative eigenvalues of Factor 1 and 2 (F1 and F2, respectively) corresponding to 76 % of the variance (Fig. 7). Table 8 summarizes the link between the Factors and each individual variable a score. The greater the score, the greater the link with the corresponding axis.

Rotifer abundance and the proportion of rotifers and cladocerans within the zooplankton community was most strongly associated with Total Cyanobacteria (TCY) and phytoplankton biomass (Chla) along Factor 1, and copepod abundance and zooplankton biovolume were most strongly associated with total potentially toxic algae abundance (TTX) and cyanobacteria percentage of phytoplankton abundance (B %) along Factor 2 (Table 8).

4. DISCUSSION

This study was conducted to gain insight into differences of zooplankton functional group abundance, biomass and size structure between reservoirs with and without *C. raciborskii* blooms and between spring pre-bloom and summer bloom seasons. As

expected, reservoirs with *C. raciborskii* had relatively high rotifer abundance and a high proportion of copepods but a relatively low proportion of juvenile zooplankton. Furthermore, *C. raciborskii* reservoirs had higher (i.e. steeper) NBSS slopes, indicating a greater representation of smaller-size classes than larger-size classes. The major differences between seasons were lower zooplankton biovolume and NBSS intercepts (proxy for zooplankton biomass) in spring than in summer.

Correlation of *C. raciborskii* and zooplankton functional group abundance

Subtropical and tropical reservoirs tend to be dominated by copepods and rotifers (Jeppesen et al., 2002), but this large-scale survey revealed that these zooplankton functional groups were also positively associated with *C. raciborskii* abundance, while cladocerans and juveniles (copepodites and nauplii) were negatively associated with *C. raciborskii*. These results are consistent with previous field and laboratory studies showing adverse impacts on cladocerans in the presence of *C. raciborskii*, and increased copepod biomass with *C. raciborskii* abundance (Hawkins, 1988; Bouvy et al., 2001). It has also been demonstrated that *C. raciborskii* is an inadequate sole food source for cladocerans due to feeding inhibition (Hawkins and Lampert, 1989), and the presence of filamentous cyanobacteria reduces filtering rates of numerous *Daphnia* species (Gliwicz and Lampert, 1990; Leonard and Paerl, 2005). Other studies have shown that rotifers are less likely to be mechanically disturbed by cyanobacteria (Low and Ng, 2010), and some rotifers seem resistant to cyanobacterial toxins (Fulton III and Paerl, 1987). Furthermore, Bouvy et al. (2001) demonstrated that rotifer abundance increased with *C. raciborskii* during a bloom. In this study, cladocerans comprised 22% and 49%, respectively of the zooplankton abundance/biomass, in the two reservoirs where no *C. raciborskii* cells were recorded (i.e., Leslie Harrison, and Maroon), while the two reservoirs with greatest *C. raciborskii* proportion (Borumba and Manchester) had the highest proportion of copepods, 42% and 39% respectively.

Reservoirs with *C. raciborskii* also had a lower proportion of juveniles to total zooplankton compared to those without *C. raciborskii*. This suggests an inhibition of zooplankton reproduction with exposure to *C. raciborskii*. It has been documented that

cyanobacteria lack esssential compounds (e.g., sterols) that could hamper zooplankton reproduction (Von Elert et al., 2003). For example *Daphnia* juveniles were reported to decline during *C. raciborskii* blooms and only a few juvenile daphnids were found during a *C. raciborskii* bloom in the Solomon Dam (Hawkins 1988). Other toxic cyanobacteria such as *Anabaena* can cause a decline in the production of eggs and nauplii (Hunt and Matveev, 2005). Thus, a negative effect on zooplankton reproduction may be expected in the presence of *C. raciborskii*.

Correlation of *C. raciborskii* with zooplankton community structure

Toxic cyanobacterial blooms have major consequences for freshwater food web structure (Paerl et al., 2011). Toxic cyanobacteria can cause shifts in the size structure of the zooplankton community, which considerably affects water clarity, rates of nutrient regeneration, and fish abundance (Moore and Folt, 1993). The optical plankton counter (OPC), a powerful tool allowing a description of zooplankton size distribution, enabled us to compare different samples by estimating the slope of the normalized biomass size spectrum (NBSS). Generally, steeper slopes indicate an increase of zooplankton abundance in small-size classes, or a decrease in large-size classes (Moore and Suthers, 2006). Steeper slopes could also be caused by increased nutrients (bottom-up), which boosts the productivity and abundance of zooplankton in smaller classes, while selective predation by zooplanktivorous fish (top-down) removes zooplankton of large-size classes.

The present study revealed that reservoirs experiencing *C. raciborskii* blooms had steeper slopes than those without *C. raciborskii*, suggesting that *C. raciborskii* might lead the zooplankton community to shift from large to small species or individuals. The adverse effect of *C. raciborskii* on mesozooplankton, especially *Daphnia*, is body-size-dependent and more significant in large *Daphnia* species (Hawkins and Lampert, 1989; Attayde and Hansson, 1999). When three differently sized cladoceran species were exposed to toxic *Microcystis* cells, small-sized cladoceran species developed a stronger tolerance to toxic *Microcystis* than the large-sized cladocerans (Guo and Xie, 2006). In this study, NBSS intercepts were also significantly greater in summer compared to spring in most reservoirs. This indicates that

zooplankton biomass is generally greater in the summer when *C. raciborskii* usually blooms. Only two reservoirs showed that the NBSS intercepts were lower in summer than in spring. One of these reservoirs (North Pine) usually has high *C. raciborskii* abundance during summer (Orr et al., 2010). Increased nutrients could also boost the productivity and abundance of zooplankton in smaller classes (Moore and Suthers 2006), as some reservoirs with *C. raciborskii* had the highest concentration of TP and DOP in the bottom water and higher mean concentration of TN compared to the rest of the reservoirs (Leigh et al. 2010). However, our nutrient data from two seasons did not provide enough evidence to evaluate the nutrient impact on zooplankton size structure in this study. It is also possible that selective predation by planktivorous fish removed zooplankton in large-size classes but we cannot quantify its importance in this study due to the lack of fish data.

Correlation of environmental variables and zooplankton community structure

Abiotic factors such as temperature can regulate the composition and biomass of zooplankton communities (Kobayashi et al., 1998). We also expected the zooplankton abundance and community structure would be correlated with physical features of each reservoir because larger lakes have more littoral diversity, and deeper lakes could provide dark refuge for zooplankton from visual fish predators (Dodson, 1992). However, we found no significant correlation between physical reservoir characteristics (including reservoir depth) and zooplankton functional group abundance.

Nutrients like phosphate and nitrate serve as an important factor in controlling production, growth and distribution of zooplankton in freshwater ecosystems (Kumar, 2012). However, the nutrient status of reservoirs (e.g., NOx and FRP) appeared to have relatively little impact on the zooplankton community in this survey. The study by Sellami et al. (2011) suggested that local environmental parameters, hydrologic connections and dispersal factors all had a significant effect on the community structure of zooplankton in Kasseb Reservoir. In our study, phytoplankton variables such as food abundance (e.g., phytoplankton, TOC) and food quality (e.g., proportion of cyanobacteria and potentially toxic algae, total abundance of *C. raciborskii*) were much

more successful at predicting zooplankton structure than water quality and lake physical features (Fig. 7).

Conclusion

In summary, this large-scale survey supported our hypothesis that the proportion of copepods in the zooplankton community and abundance of *C. raciborskii* were positively correlated. We found that *C. raciborskii* presence in these subtropical reservoirs appeared to affect the functional group abundance and community size structure of zooplankton. Furthermore, it was found that biotic factors have a more significant impact on zooplankton abundance and community structure than abiotic factors in these southeast Queensland reservoirs.

5. REFERENCES

Ali Ger, K., Panosso, R., Lürling, M., 2011. Consequences of acclimation to *Microcystis* on the selective feeding behavior of the calanoid copepod *Eudiaptomus gracilis*. *Limnology and Oceanography*, 56:2103-2114.

Attayde, J. L., Hansson, L. A., 1999. Effects of nutrient recycling by zooplankton and fish on phytoplankton communities. *Oecologia*, 121:47-54.

Bouvy, M., Pagano, M., Troussellier, M., 2001. Effects of a cyanobacterial bloom (*Cylindrospermopsis raciborskii*) on bacteria and zooplankton communities in Ingazeira reservoir (northeast Brazil). *Aquatic Microbial Ecology*, 25:215-227.

Brett, M. T., Muller-Navarra, D. C., 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology*, 38:483-499.

Burford, M. A., Johnson, S. A., Cook, A. J., Packer, T. V., Taylor, B. M., Townsley, E. R., 2007. Correlations between watershed and reservoir characteristics, and algal blooms in subtropical reservoirs. *Water Research*, 41:4105-4114.

Carmichael, W. W., 1994. The toxins of cyanobacteria. *Scientific American,* 270:64-70.

DeMott, W. R., Gulati, R. D.,Van Donk, E., 2001. *Daphnia* food limitation in three hypereutrophic Dutch lakes: Evidence for exclusion of large-bodied species by

interfering filaments of cyanobacteria. *Limnology and Oceanography*, 46:2054-2060.

Elser, J. J., Elser, M. M., MacKay, N. A., Carpenter,S. R., 1988. Zooplankton-mediated transitions between N-and P-limited algal growth. *Limnology and Oceanography*, 33:1-14.

Fulton III, R. S., Paerl, H. W., 1987. Toxic and inhibitory effects of the blue-green alga *Microcystis aeruginosa* on herbivorous zooplankton. *Journal of Plankton Research*, 9:837-855.

Ghadouani, A., Pinel-Alloul, B., Prepas, E. E., 2003. Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. *Freshwater Biology*, 48:363-381.

Gliwicz, Z. M., Lampert, W., 1990b. Food thresholds in *Daphnia* species in the absence and presence of blue-green filaments. *Ecology*, 71:691-702.

Guo, N., Xie, P., 2006. Development of tolerance against toxic *Microcystis aeruginosa* in three cladocerans and the ecological implications. *Environmental Pollution,* 143:513-518.

Gustafsson, S., Rengefors, K., Hansson, L. A., 2005. Increased consumer fitness following transfer of toxin tolerance to offspring via maternal effects. *Ecology*, 86: 2561–2567.

Hansson, L. A., Gustafsson, S., Rengefors, K., Bomark, L., 2007. Cyanobacterial chemical warfare affects zooplankton community composition. *Freshwater Biology*, 52:1290-1301.

Havens, K., East, T., Beaver, J., 1996. Experimental studies of zooplankton–phytoplankton–nutrient interactions in a large subtropical lake (Lake Okeechobee, Florida, USA). *Freshwater Biology*, 36:579-597.

Hawkins, P., Lampert, W., 1989. The effect of *Daphnia* body size on filtering rate inhibition in the presence of a filamentous cyanobacterium. *Limnology and Oceanography*, 34:1084-1089.

Hawkins, P. R., 1988. The zooplankton of a small tropical reservoir (Solomon Dam, North Queensland): seasonal changes and the influence of water quality management measures. *Hydrobiologia*, 157:105-118.

Herman, A. W., 1988. Simultaneous measurements of zooplankton and light attenuance with a new optical plankton counter. *Cont. Shelf Res*., 8:205– 221.

Herman, A. W., 1992. Design and calibration of a new optical plankton counter capable of sizing small zooplankton, *Deep Sea Res*., 39:395–415.

Hunt, R. J., Matveev, V. F., 2005. The effects of nutrients and zooplankton community structure on phytoplankton growth in a subtropical Australian reservoir: an enclosure study. *Limnologica-Ecology and Management of Inland Waters,* 35:90-101.

Jeppesen, E., Jensen, J. P., Søndergaard, M., Fenger-Grøn, M., Bramm, M., Sandby, K., 2004. Impact of fish predation on cladoceran body weight distribution and zooplankton grazing in lakes during winter. *Freshwater Biology*, 49: 432-447.

Jeppesen, E., Søndergaard, M., Mazzeo, N., Meerhoff, M., Branco, C., Huszar, V., Scasso, F., 2005. Lake restoration and biomanipulation in temperate lakes: relevance for subtropical and tropical lakes. Chapter 11. In Reddy, M. V. (ed.), *Tropical Eutrophic Lakes: Their Restoration and Management*. Science Publishers, Enfield, pp. 331–359.

Lampert, W., Fleckner, W., Rai, H., Taylor, B. E., 1986. Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. *Limno. Oceanogr*., 31:478-490.

Leigh, C., Burford, M. A., Roberts, D. T., Udy, J. W., 2010. Predicting the vulnerability of reservoirs to poor water quality and cyanobacterial blooms. *Water research,* 44:4487-4496.

Leonard, J. A., Paerl, H. W., 2005. Zooplankton community structure, micro-zooplankton grazing impact, and seston energy content in the St. Johns river system, Florida as influenced by the toxic cyanobacterium *Cylindrospermopsis raciborskii*. *Hydrobiologia*, 537:89-97.

Low, E., NgP. K. L., 2010. Top-down control of phytoplankton by zooplankton in tropical reservoirs in Singapore? *The Raffles Bull of Zoology,* 58:311-322.

Moore, M., Folt, C., 1993. Zooplankton body size and community structure: Effects of thermal and toxicant stress. *Trends in Ecology & Evolution*, 8:178-183.

Moore, S. K., Suthers, I. M., 2006. Evaluation and correction of subresolved particles by the optical plankton counter in three Australian estuaries with pristine to highly modified catchments. *J. Geophys. Res.*, 111: 1-14.

Orr, P. T., Rasmussen, J. P., Burford, M. A., Eaglesham, G. K., Lennox, S. M., 2010. Evaluation of quantitative real-time PCR to characterise spatial and temporal

variations in cyanobacteria, *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya et Subba Raju and cylindrospermopsin concentrations in three subtropical Australian reservoirs. *Harmful Algae*, 9:243-254.

Paerl, H. W., Hall, N. S., Calandrino, E. S., 2011. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Science of the Total Environment*, 409:1739-1745.

Patoine, A., Pinel-Alloul, B., Méthot, G., Leblanc, M. J., 2006. Correspondence among methods of zooplankton biomass measurement in lakes: effect of community composition on optical plankton counter and size-fractionated seston data. *Journal of Plankton Research,* 28:695-705.

Reinikainen, M., Hietala, J., Walls, M., 1999. Reproductive allocation in *Daphnia* exposed to toxic cyanobacteria. *Journal of Plankton Research*, 21:1553-1564.

Reinertsen, H., Jensen, A., Langeland, A., Olsen, Y., 1986. Algal competition for phosphorus: the influence of zooplankton and fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 43:1135-1141.

Sellami, I., Elloumi, J., Hamza, A., Mhamdi, M. A., Ayadi, H., 2011. Local and regional factors influencing zooplankton communities in the connected Kasseb Reservoir, Tunisia. *Water SA* (Online), 37:201-212.

Sinistro, R., Sánchez, M. L., Marinone, M. C., Izaguirre, I., 2007. Experimental study of the zooplankton impact on the trophic structure of phytoplankton and the microbial assemblages in a temperate wetland (Argentina). *Limnologica-Ecology and Management of Inland Waters,* 37:88-99.

Smith, V. H., 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science,* 221:669-671.

Sommer, U., Sommer, F., 2006. Cladocerans versus copepods: the cause of contrasting top–down controls on freshwater and marine phytoplankton. *Oecologia*, 147:183-194.

Sommer, U., Sommer, F., Santer, B., Zollner, E., Jurgens, K., Jamieson, C., Boersma, M., Gocke, K.,2003. *Daphnia* versus copepod impact on summer phytoplankton: functional compensation at both trophic levels. *Oecologia*, 135:639-647.

Webster, K. E., Peters, R. H., 1978. Some size-dependent inhibitions of larger cladoceran filterers in filamentous suspensions. *Limnology and Oceanography*, 23:1238-1245.

Zohary, T., Pais-Madeira, A. M., Robarts, R., Hambright, K. D., 1996. Interannual phytoplankton dynamics of a hypertrophic African lake. *Archiv fuer Hydrobiologie*, 136:105-126.

Table 1. Several physical characteristics of the 15 subtropical reservoirs sampled in this study. Surface area (SA) is the reservoir surface area at full supply level (FSL), volume (Vol) is the water storage capacity at FSL, depth is mean depth at FSL.

Reservoir	Abbreviation	SA (km^2)	Vol (ML)	Depth (m)
Baroon	Bar	3.9	61,000	15.7
Borumba	Bor	3.8	46,000	12.2
Cooloolabin	Cool	1.4	14,200	9.9
Ewen Maddock	Ewen	2.3	16,600	7.4
Hinze	Hin	9.3	163,000	17.5
Kurwongbah	Kur	3.4	14,400	4.2
Leslie Harrison	LHD	0.6	9,280	16.6
Little Nerang	LN	4.2	24,800	5.9
Manchester	Man	2.6	26,000	10
Maroon	Mar	3.3	44,300	13.3
Mcdonald	Mac	2.6	8,000	3.1
Moogera	Moog	7.7	83,800	10.9
NorthPine	NPD	21.2	215,000	10.1
Somerset	Som	39.7	369,000	9.3
Wivenhoe	Wiv	110	1,150,000	10.5

Table 2. Water quality parameters measured in spring (November 2009) for each of the reservoirs sampled during this study.

Reservoir	Temperature °C	Conductivity (uS/cm)	pH	DO % sat	DOC mg/L	FRP mg/L	NH4 mg/L	NOX mg/L	TN:TP molar	TOC mg/L	TSS mg/L
Baroon (Bar)	24.8	117	9.2	90.4	3.9	0.0035	0.0010	0.0010	36	4.6	2.5
Borumba (Bor)	24.0	280	7.5	87.7	8.5	0.0025	0.0020	0.0010	53	8.8	3.5
Cooloolabin Cool)	28.0	70	7.5	85.7	8.4	0.0010	0.0035	0.0020	95	9.6	35.0
EwenMaddock (Ewen)	27.0	81	7.0	57.0	7.0	0.0010	0.0020	0.0010	72	7.9	20.5
Hinze (Hin)	27.3	99	8.2	80.2	4.0	0.0010	0.0020	0.0025	58	4.4	10.0
Kurwongbah (Kur)	27.7	124	8.1	79.9	7.9	0.0010	0.0025	0.0020	40	8.9	37.0
Leslie Harrison (LHD)	24.5	181	7.7	75.6	11.6	0.0025	0.0200	0.0710	28	12.5	4.5
Little Nerang (LN)	27.6	78	9.3	108.8	3.1	0.0025	0.0020	0.0000	35	3.8	3.0
Manchester (Man)	28.5	155	8.0	92.6	9.0	0.0015	0.0025	0.0010	51	9.6	2.0
Maroon (Mar)	23.9	256	9.4	91.5	6.5	0.0040	0.0010	0.0010	54	6.7	3.5
McDonald (Mac)	27.7	113	7.3	80.7	5.5	0.0010	0.0010	0.0010	46	6.1	5.5
Moogera (Moog)	24.3	263	9.0	85.6	7.6	0.0010	0.0010	0.0010	44	8.3	4.5
North Pine (NPD)	25.2	198	8.4	73.1	6.7	0.0010	0.0050	0.0075	49	7.4	3.0
Somerset (Som)	28.7	199	9.7	99.2	6.1	0.0015	0.0010	0.0015	41	7.3	4.0
Wivenhoe (Wiv)	25.8	298	9.3	99.7	6.7	0.0015	0.0025	0.0015	40	8.0	5.5

Table 3. Water quality parameters measured in summer (February2010) for each of the reservoirs sampled during this study.

Reservoir	Temperature °C	Conductivity (uS/cm)	pH	DO % sat	DOC mg/L	FRP mg/L	NH4 mg/L	NOX mg/L	TN:TP molar	TOC mg/L	TSS mg/L
Baroon (Bar)	27.1	113	7.7	94.1	5.3	0.0025	0.0040	0.0000	49	6.2	2.0
Borumba (Bor)	27.1	263	8.7	90.8	10.0	0.0035	0.0025	0.0000	89	12.9	9.0
Cooloolabin Cool)	27.1	68	6.6	87.7	8.1	0.0000	0.0030	0.0000	143	8.9	1.5
EwenMaddock (Ewen)	27.0	94	6.6	65.3	6.7	0.0000	0.0040	0.0000	75	7.0	1.0
Hinze (Hin)	27.3	101	7.8	101.7	5.2	0.0000	0.0030	0.0030	37	6.0	3.0
Kurwongbah (Kur)	27.7	206	7.2	44.3	12.3	0.0000	0.0190	0.0030	74	12.5	3.5
Leslie Harrison (LHD)	28.2	247	7.1	85.7	11.4	0.0000	0.0050	0.0020	65	11.9	1.0
Little Nerang (LN)	22.9	80	7.9	106.3	5.2	0.0025	0.0030	0.0160	24	5.7	5.0
Manchester (Man)	27.5	196	7.3	82.8	8.1	0.0000	0.0035	0.0000	82	9.8	2.0
Maroon (Mar)	28.6	259	9.1	103.6	8.0	0.0020	0.0000	0.0000	63	9.6	3.5
McDonald (Mac)	27.6	103	6.5	62.1	6.5	0.0020	0.0120	0.0000	32	6.8	3.5
Moogera (Moog)	28.9	235	8.8	121.5	9.8	0.0030	0.0030	0.0000	41	10.8	5.5
North Pine (NPD)	26.6	279	7.9	75.2	8.1	0.0025	0.0030	0.0445	43	8.4	1.0
Somerset (Som)	26.8	227	7.5	59.0	8.3	0.0030	0.0000	0.0000	57	9.1	1.0
Wivenhoe (Wiv)	26.9	376	8.3	98.8	7.5	0.0000	0.0000	0.0000	69	8.5	2.5

Table 4. Phytoplankton parameters measured in summer (February 2010) for each of the reservoirs sampled during this study.

Reservoir	Chl-a (μgL^{-1})	*C. raciborksii* (cells ml^{-1})	Cyano (cells ml^{-1})	Toxic (cells ml^{-1})	*C. raciborskii* %	Toxic %	Total abundance (cells ml^{-1})
Baroon (Bar)	6.0	8,325	67,815	8,815	11	12	73,200
Borumba (Bor)	47.5	270,000	325,575	270,000	40	40	674,000
Cooloolabin Cool)	7.5	0	3,968	0	0	0	10,800
EwenMaddock (Ewen)	9.5	0	20,218	0	0	0	25,000
Hinze (Hin)	7.5	0	23,612	0	0	0	29,100
Kurwongbah (Kur)	12.5	4,188	41,193	4,188	8	8	49,900
Leslie Harrison (LHD)	6.0	0	84,525	0	0	0	88,800
Little Nerang (LN)	27.5	0	83,200	0	0	0	101,000
Manchester (Man)	17.5	13,250	45,425	13,250	15	15	87,300
Maroon (Mar)	6.0	0	171,467	50	0	0	178,000
McDonald (Mac)	24.0	0	32,400	0	0	0	45,900
Moogera (Moog)	17.5	1,315	301,570	1,315	0	0	314,000
North Pine (NPD)	20.5	3,910	88,557	3,910	4	4	98,200
Somerset (Som)	10.0	615	160,454	615	0	0	168,000
Wivenhoe (Wiv)	13.0	14,175	271,600	14,700	5	5	301,000

Table 5. Phytoplankton parameters measured in spring (November 2009) for each of the reservoirs sampled during this study.

Reservoir	Chl-a (μgL^{-1})	*C. raciborksii* (cells ml^{-1})	Cyano (cells ml^{-1})	Toxic (cells ml^{-1})	*C. raciborskii* %	Toxic %	Total abundance (cells ml^{-1})
Baroon (Bar)	7.5	0	93,471	7,175	0	7	109,272
Borumba (Bor)	4.0	652	105,601	1,173	0	1	151,066
Cooloolabin Cool)	10.0	0	14,327	0	0	0	32,913
EwenMaddock (Ewen)	9.5	0	122,541	0	0	0	134,752
Hinze (Hin)	4.0	0	18,456	0	0	0	23,522
Kurwongbah (Kur)	18.0	100	39,380	150	0	0	102,617
Leslie Harrison (LHD)	4.5	0	886	0	0	0	4,721
Little Nerang (LN)	2.0	0	62,014	0	0	0	93,556
Manchester (Man)	9.5	0	365,346	125	0	0	392,508
Maroon (Mar)	5.5	0	3,128	1,199	0	15	7,854
McDonald (Mac)	18.0	0	60,055	0	0	0	91,831
Moogera (Moog)	8.5	782	430,507	964	0	0	450,647
North Pine (NPD)	14.5	0	61,669	801	0	1	72,112
Somerset (Som)	8.5	8,450	644,049	11,875	1	1	847,991
Wivenhoe (Wiv)	7.0	2,755	255,098	5,760	1	2	272,986

Table 6. Two-way analysis of variance of the abundance of zooplankton functional groups, total zooplankton abundance and biomass, as well as zooplankton size structure (slope of size normalized biomass spectrum estimated with the Optical Plankton Counter) across season and reservoir type (i.e. those reservoirs which had *C. raciborskii* cells at the time of sampling and those that did not).

Rotifer abundance	df	Mean Square	F	Sig.
Season	1	0.186	1.092	0.306
reservoir type	1	2.826	16.616	**0.000***
Error	26			
Cladoceran abundance				
Season	1	0.087	0.562	0.406
reservoir type	1	0.150	0.965	0.335
Error	26			
Copepod abundance				
Season	1	0.182	1.312	0.263
reservoir type	1	0.471	3.404	0.076
Error	26			
Juvenile abundance				
Season	1	0.012	0.122	0.730
reservoir type	1	0.001	0.008	0.931
Error	26			
Total abundance				
Season	1	0.029	0.277	0.603
reservoir type	1	0.314	2.983	0.096
Error	26			
% Juveniles				
Season	1	0.079	1.210	0.282
reservoir type	1	0.279	4.275	**0.049***
Error	26			
Total biovolume				
Season	1	1.582	5.360	**0.029***
reservoir type	1	0.174	0.589	0.450
Error	26			
OPC intercept (estimate of total biomass)				
Season	1	0.439	19.168	**0.000***
reservoir type	1	0.036	1.566	0.222
Error	26			
OPC slope (size structure)				
Season	1	0.002	0.270	0.608
reservoir type	1	0.008	0.921	0.346
Error	26			

Table 7. Two-way analysis of variance of the abundance of proportion of copepod and slope of size normalized biomass spectrum estimated with the Optical Plankton Counter across season and reservoirs with *C. raciborskii* or without historical *C. raciborskii* blooms.

% Copepods	df	Mean Square	F	Sig.
Season	1	0.303	2.255	0.145
reservoir type	1	0.607	4.513	**0.043 ***
Error	26			
OPC slope				
Season	1	0.005	0.582	0.453
reservoir type	1	0.029	3.526	0.072
Error	26			

Table 8. The scores of response variables (zooplankton) and explanatory variables (phytoplankton) corresponding to RDA factors. Factors are the linear combination of explanatory variables optimized by permutation. The greater the score, the greater the link with the corresponding axis. Red font indicates the explanatory variables that mostly contributed to F1 and F2. Blue font indicates the response variables that were most related to the Factors. Abbreviations of each variable are: phyto = total phytoplankton cell abundance, TB = total cyanobacteria abundance, TTX = total potentially toxic algae abundance, TCY = *C. raciborskii* abundance, %B = % cyanobacteria relative to total phytoplankton abundance; % Cy = % of *C. raciborskii* relative to total phytoplankton abundance, and LWC = reservoirs with *C. raciborskii* cells at the time of sampling and LHC = *C. raciborskii* bloom reservoirs.

Scores (Response variables):

Zooplankton variables	F1	F2	F3	F4
Rotifer abund	**0.763**	0.353	-0.156	-0.013
Cladoceran abund	-0.434	0.275	-0.215	-0.080
Copepod abund	0.086	**0.481**	-0.139	0.062
Juvenile abund	-0.102	0.146	-0.164	0.392
Total abund	0.089	0.402	-0.215	0.156
Cladoceran/Copepod	-0.198	0.090	-0.066	-0.034
Rotifer %	**0.913**	0.192	0.057	-0.116
Cladoceran %	**-0.636**	0.136	-0.337	-0.231
Copepod %	-0.005	0.167	0.030	-0.077
Juvenile %	-0.191	-0.245	0.041	0.254
OPC Intercept	-0.072	0.176	0.139	-0.004
OPC Slope	-0.015	0.082	0.055	0.056
Biovolume (biomass)	-0.475	**0.662**	0.465	0.007

Scores (Explanatory variables):

Phytoplankton variables	F1	F2	F3	F4
TN:TP	-0.020	0.111	0.175	0.406
TOC	0.141	0.277	0.168	0.377
TSS	0.041	-0.355	-0.226	0.265
Phyto	0.237	0.250	-0.175	-0.161
Chla	0.374	0.097	0.235	0.143
TB	0.218	0.321	-0.202	-0.174
TTX	0.143	**0.455**	0.040	-0.246
TCY	**0.478**	0.334	0.105	-0.041
%B	0.065	**0.478**	-0.197	-0.161
%Cy	0.250	0.090	0.446	-0.091
LWC	**0.542**	0.361	-0.068	-0.039
LHC	0.351	0.360	-0.108	-0.288

Figure legends

Figure 1. The Brisbane region in southeast QLD, showing the location of the 15 reservoirs sampled during this study (map sourced from Leigh et al. 2010).

Figure 2. The average abundance of zooplankton functional groups across all reservoirs in spring (November 2009) and summer (February 2010). Data are means ± SE (n = 15). X-axis labels include the reservoir abbreviation followed by a number (2 or 11), indicating the season (February or November, respectively). Left hand group shows reservoirs without *C. raciborskii* and right hand group shows reservoirs with *C. raciborskii*, with *C. raciborskii* abundance increasing from left to right.

Figure 3. The proportion of cladocerans to copepods in spring and summer and between reservoirs experiencing *C. raciborskii* blooms and reservoirs without historical *C. raciborskii* blooms. Data are means ± SE. Asterisk * indicates statistically significant ($p < 0.05$).

Figure 4. Normalized biomass size spectra (NBSS) of zooplankton in each reservoir during spring (November 2009; solid circle) and summer (February 2010; open circle). Plots A to H show NBSS of reservoirs with *C. raciborskii* and plots I to O show NBSS of reservoirs without historical blooms of *C. raciborskii*.

Figure 5. Ordination plot of zooplankton functional group abundance cross reservoirs (A) between seasons (blue symbols indicate spring; green symbols indicate summer) and (B) between reservoir type (blue symbols indicate reservoirs with *C. raciborskii* blooms, and green symbols indicate reservoirs without *C. raciborskii* blooms).

Figure 6. The intercept and slope of the normalized biomass size spectrum of zooplankton between spring and summer seasons (A, C), and reservoir groups (B, D). Reservoirs with and without *C. raciborskii* are shown as +Cylindro and –Cylindro, respectively. Data are means ± SE. Asterisk * indicates statistically significant ($p < 0.05$) for intercept, and < 0.1) for slope.

Figure 7. Redundancy analysis (RDA) ordination plot for zooplankton functional types fitted to phytoplankton variables. The RDA plot shows a projection of the zooplankton variables (open squares) along two Factors, F1 and F2 which are linear combinations of phytoplankton variables (red lines). The variables of LWC and rotifer abundance are far from the center and close to each other, so are likely strongly positively linked. Similarly, the variables of copepod abundance and %B and TTX (% cyanobacteria relative to total phytoplankton abundance and total potentially toxic algae abundance) are also close to each other, and are therefore likely positively linked. The abbreviations of the remaining variables are listed in Table 8.

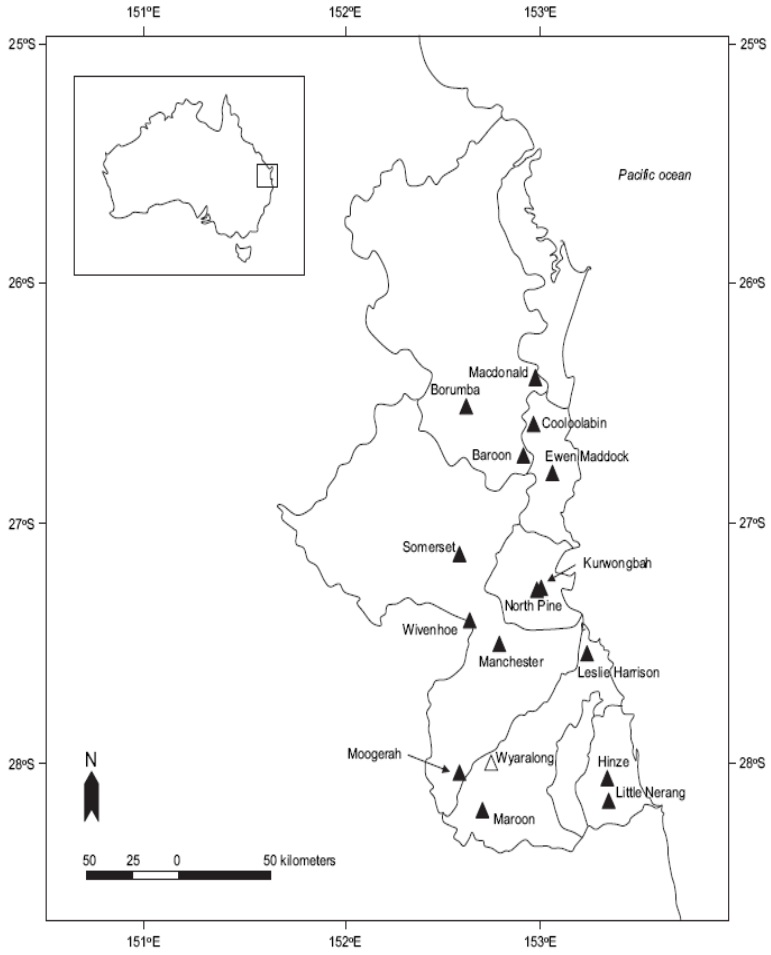
151ºE
152ºE
153ºE
25ºS
26ºS
27ºS
28ºS
Pacific ocean
Macdonald
Borumba
Cooloolabin
Baroon
Ewen Maddock
Somerset
Kurwongbah
North Pine
Wivenhoe
Manchester
Leslie Harrison
Moogerah
Wyaralong
Hinze
Little Nerang
Maroon
N
50 25 0 50 kilometers

Figure 1.

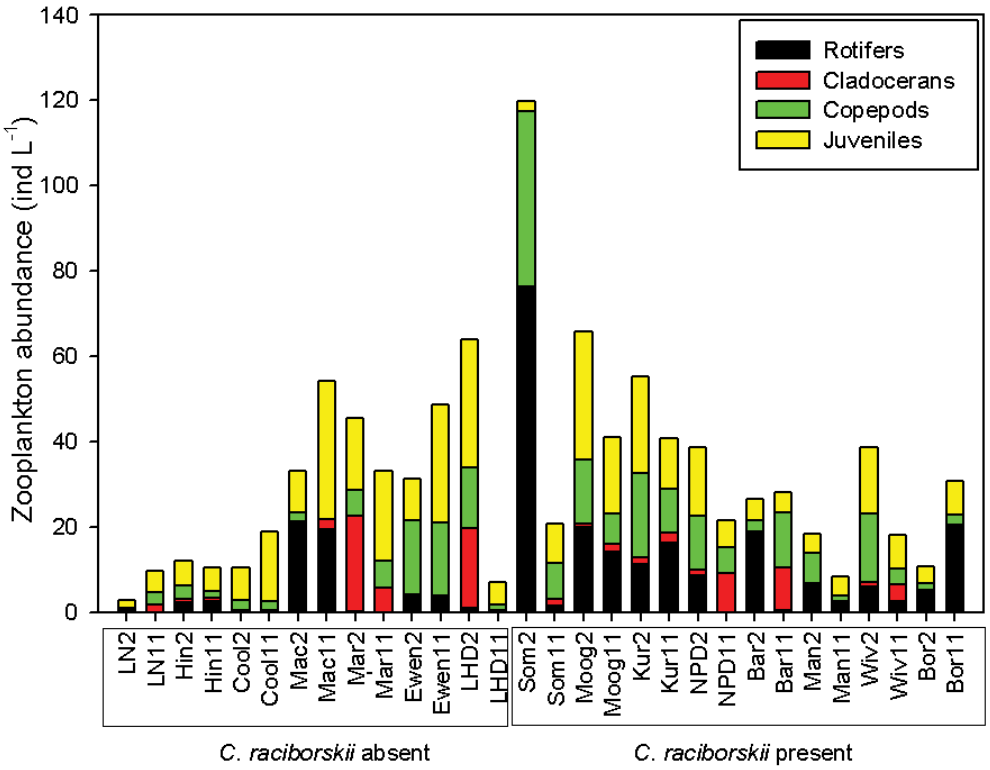
Zooplankton abundance (ind L-1)
140
120
100
80
60
40
20
0
Rotifers
Cladocerans
Copepods
Juveniles
LN2
LN11
Hin2
Hin11
Cool2
Cool11
Mac2
Mac11
Mar2
Mar11
Ewen2
Ewen11
LHD2
LHD11
Som2
Som11
Moog2
Moog11
Kur2
Kur11
NPD2
NPD11
Bar2
Bar11
Man2
Man11
Wiv2
Wiv11
Bor2
Bor11
C. raciborskii absent
C. raciborskii present

Figure 2.

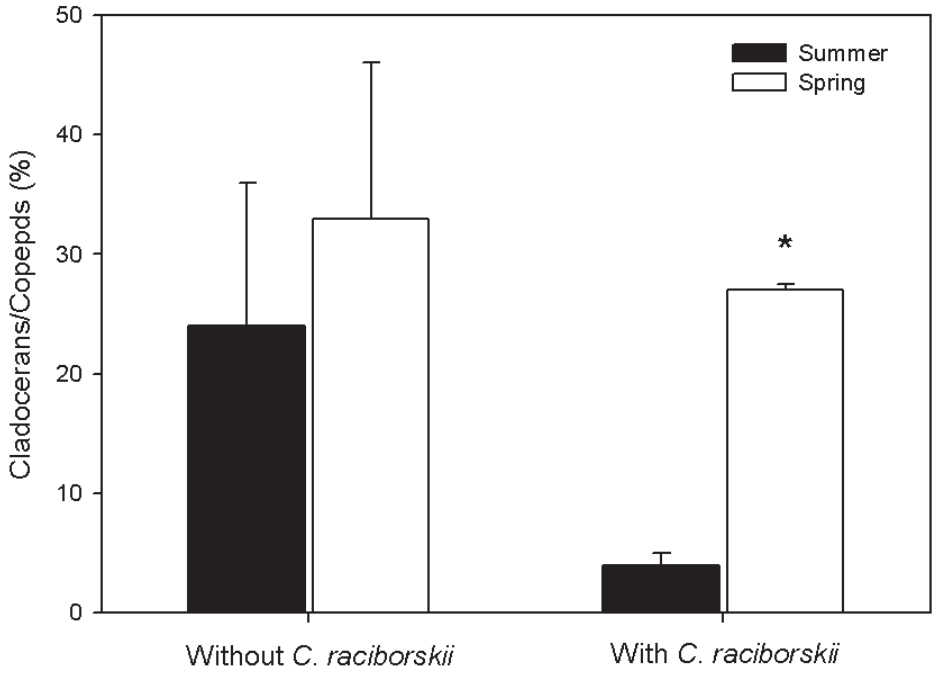
50
40
30
20
10
0
Cladocerans/Copepds (%)
Summer
Spring
*
Without C. raciborskii
With C. raciborskii

Figure 3.


A
2D Stress: 0.13
LN
Cool
Cool
LHD
LN
Mar
NPD
Som
Hin
Man
LHD
Ewen
Mar
Wiv
Bar
Wiv
Kur
NPD
Moog
Ewen
Moog
Mac
Kur
Man
Bor
Mac
Bar
Bor
Som
2D Stress: 0.13
LN
Cool
Cool
LHD
LN
Mar
NPD
Som
Hin
Man
LHD
Ewen
Mar
Wiv
Bar
Wiv
Kur
NPD
Moog
Ewen
Moog
Mac
Kur
Man
Bor
Mac
Bar
Bor
Som

Figure 4.

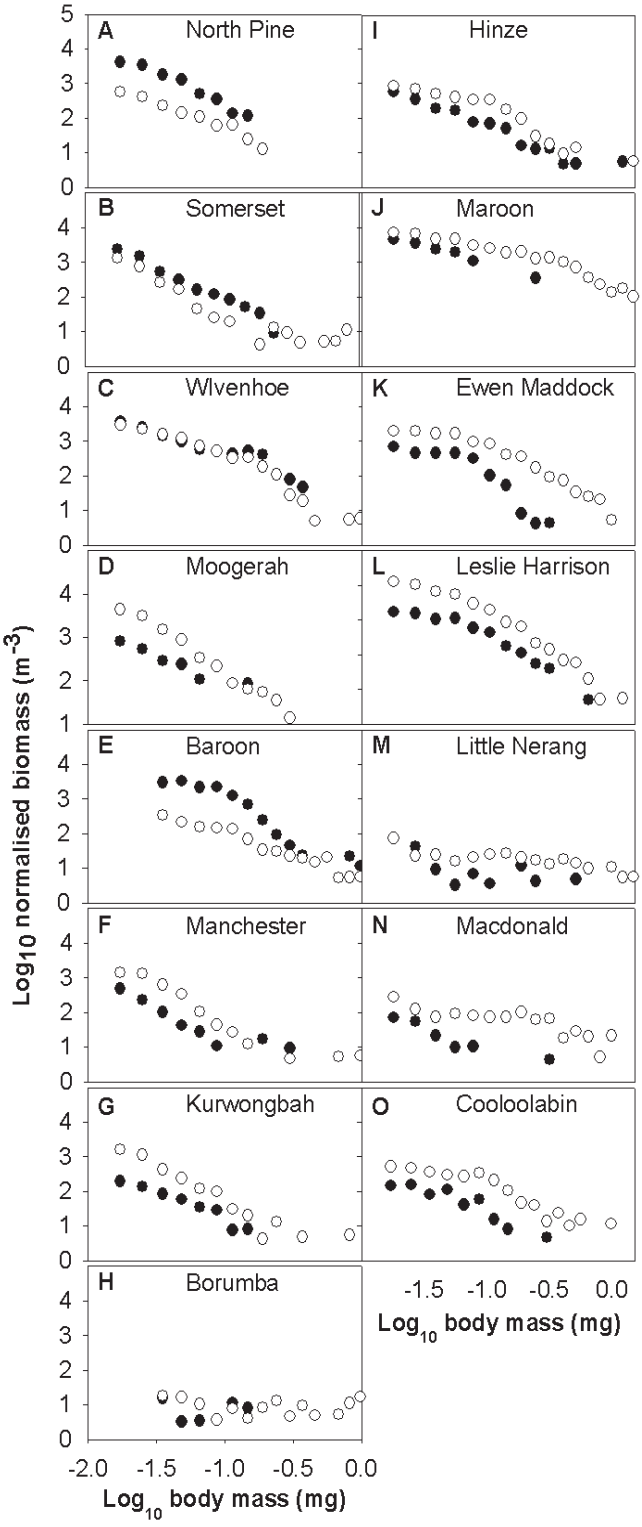
A North Pine
I Hinze
B Somerset
J Maroon
C Wivenhoe
K Ewen Maddock
D Moogerah
L Leslie Harrison
E Baroon
M Little Nerang
F Manchester
N Macdonald
G Kurwongbah
O Cooloolabin
H Borumba
Log10 normalised biomass (m-3)
Log10 body mass (mg)
-2.0 -1.5 -1.0 -0.5 0.0
-1.5 -1.0 -0.5 0.0

Figure 5

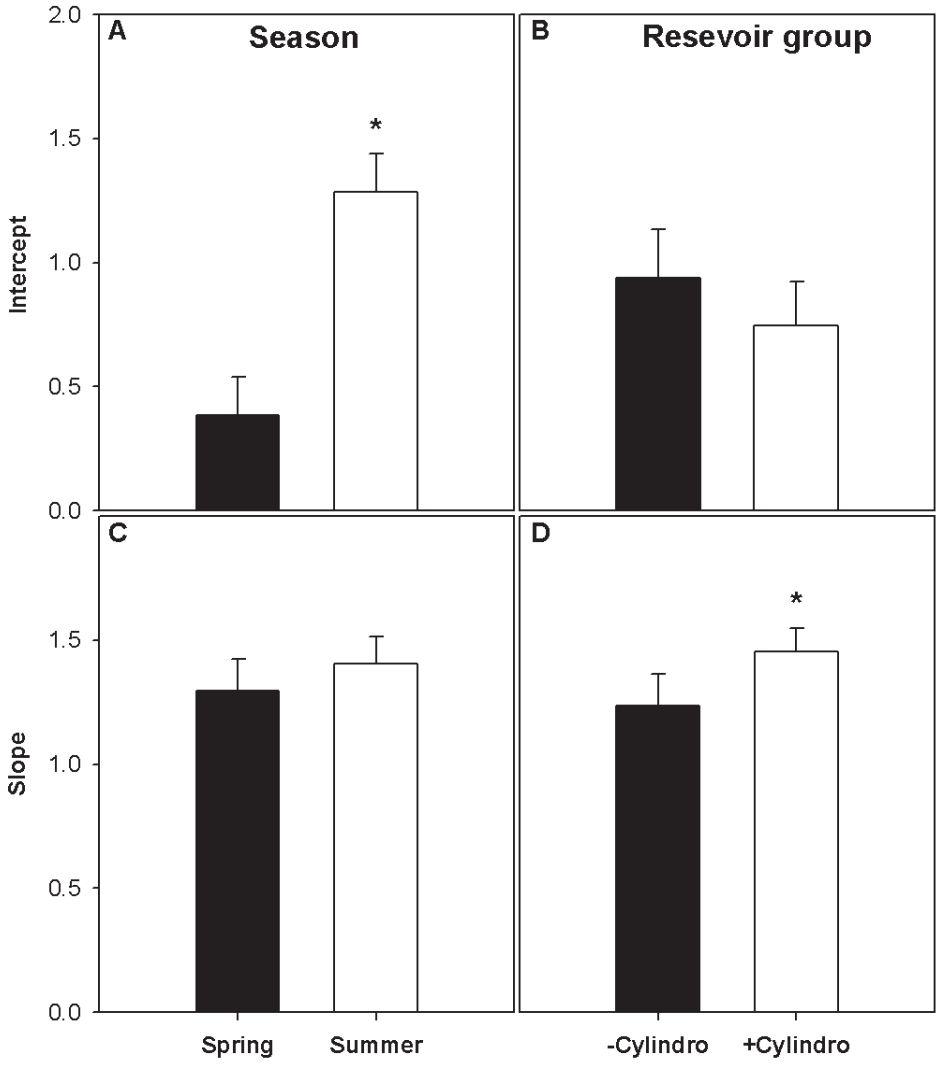
A
Season
B
Resevoir group
C
D
Intercept
Slope
2.0
1.5
1.0
0.5
0.0
*
*
Spring
Summer
-Cylindro
+Cylindro

Figure 6

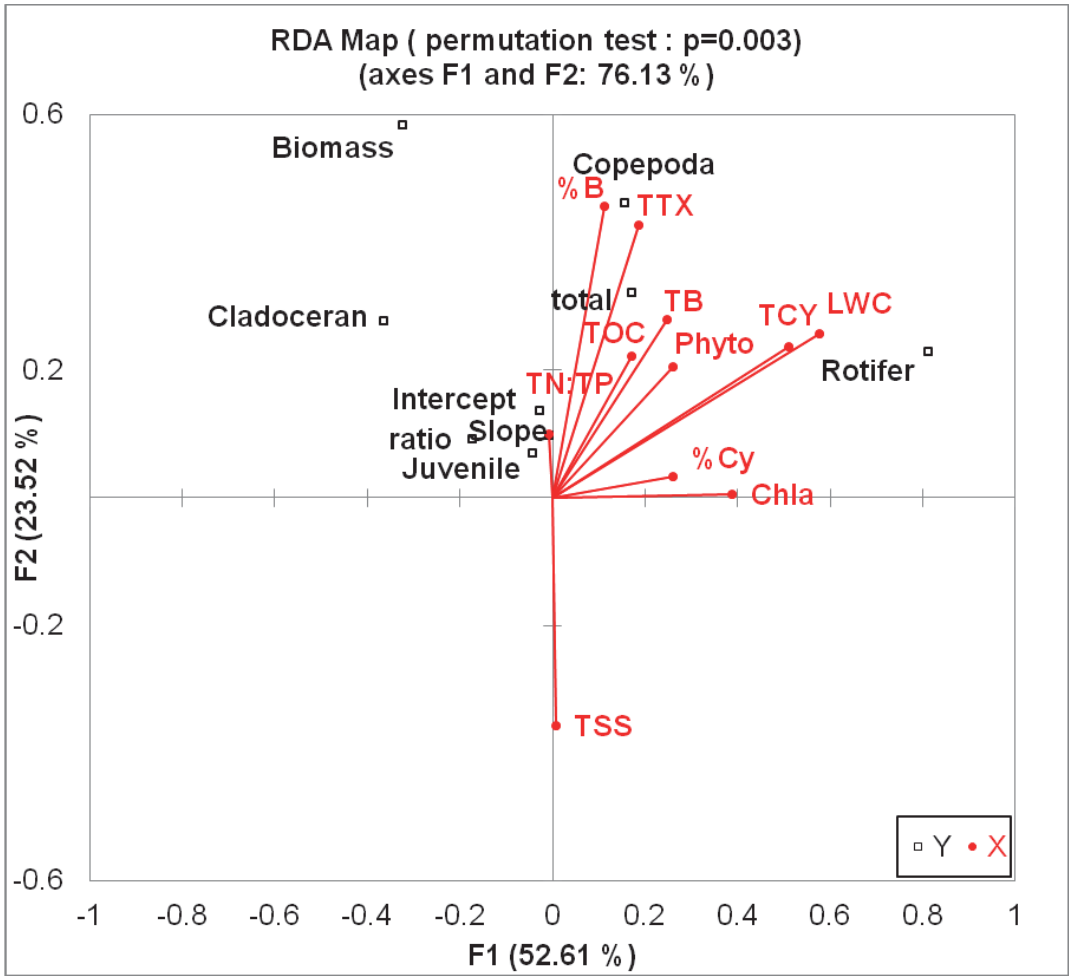
RDA Map (permutation test : p=0.003)
(axes F1 and F2: 76.13 %)
Biomass
Copepoda
%B
TTX
total
TB
TCY
LWC
Cladoceran
TOC
Phyto
Rotifer
TN:TP
Intercept
ratio
Slope
Juvenile
%Cy
Chla
TSS
Y
X
0.6
0.2
-0.2
-0.6
F2 (23.52 %)
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
F1 (52.61 %)

Figure 7

CHAPTER 6

General discussion

The primary focus of this thesis was to gain a better understanding of the zooplankton regulation of *C. raciborskii* blooms in subtropical reservoirs. Investigations were conducted at multiple scales to examine both the top-down (mortality by grazing) and bottom-up (growth enhanced by recycled nutrients) effects of zooplankton on *C. raciborskii* growth. The key findings are discussed below, followed by recommendations for management of Australian subtropical reservoirs with the aim of reducing the occurrence and toxicity of *C. raciborskii* blooms, as well as suggestions for future research.

6.1 Understanding of the bio-regulation of *C. raciborskii*

A review by Elser (1999) suggested that cyanobacteria blooms are the end result of a series of key mechanisms involving nutrient loading, physical mixing conditions, and trophic interactions. Furthermore, each mechanism needs to be taken into account for successful management of lake and reservoir water quality. To date, research to understand *C. raciborskii* bloom formation has mostly focused on environmental factors (Padisák 1997, Figueredo et al. 2007). However, this study determined that trophic interactions facilitate growth of the cyanobacterium *C. raciborskii*, and that these could be important in bloom development.

The small-scale laboratory experiments showed that copepods did not consume toxic *C. raciborskii* in most experiments, while nutrients regenerated by copepods promoted P-deplete *C. raciborskii* growth. The large-scale mesocosm experiment with a natural phytoplankton community supported the findings of the laboratory studies, further corroborating the synergistic effects of selective grazing and nutrient regeneration by zooplankton to facilitate *C. raciborskii* growth. Finally, a field survey of zooplankton community structure in 15 reservoirs provided an opportunity to study

trophic interactions of zooplankton with *C. raciborskii* within the context of the whole ecosystem.

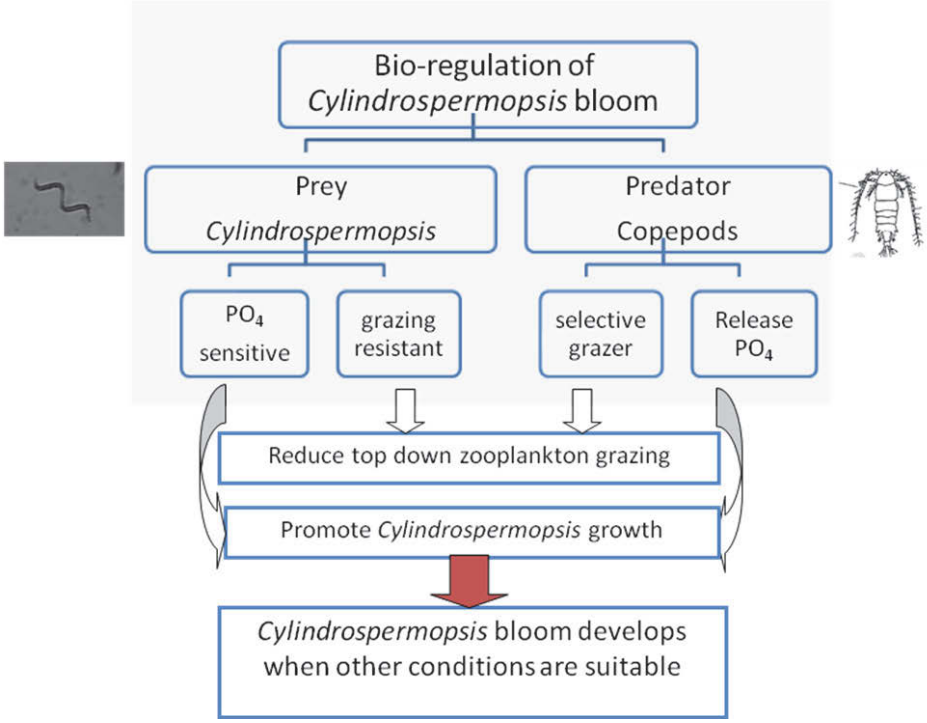

Fig.1. Hypothesis of the role of zooplankton in C. *raciborskii* bloom development in Australian reservoirs

6.2 Facilitation of *C. raciborskii* blooms by selective zooplankton grazing

This study supported Hypothesis I that the copepod *Boeckella sp.* demonstrates a strong preference against *C. raciborskii* consumption under most conditions (Fig. 1). Clearance rates of the copepod *Boeckella* sp. on a *C. raciborskii* diet were 2-4 times lower than that of a common cladoceran *Ceriodaphnia* sp. when both grazers (of similar length) had prey choice. However, copepods became less selective depending on their gut fullness and had greater clearance rates on non-toxic and P-replete *C. raciborskii* compared to toxic and P-deplete cells. Copepod consumption of *C. raciborskii* was maximized under food limited conditions.

The outcome of ^{33}P tracer experiments (Chapter 2) were confirmed by the dialysis experiments (Chapter 3) in which *Boeckella* sp. did not consume toxic *C. raciborskii* (NDP) when it was either P-replete or P-deplete, when *C. reinhardtii* was present. However, when copepods accessed non toxic *C. raciborskii*, food selection was based on the P-content of prey, showing a clear preference for P-replete compared to P-deplete cells. These detailed studies of copepod *Boeckella* sp. feeding behavior on *C. raciborskii* provide insight into how copepods regulate *C. raciborskii* growth in mixed natural communities. Collectively, these results indicate that Australian subtropical systems are at risk of toxic *C. raciborskii* blooms when copepods are the dominant meso-zooplankton, and when algal biomass is non-limiting (> 1.0 mg C L^{-1}). The flexibility in feeding behavior of copepods on *C. raciborskii*, depending on their food environment, may help to explain disparate field observations where copepods have been shown to be positively or negatively correlated with *C. raciborskii* abundance (Bouvy et al. 2001; Panosso et al. 2003; Leonard and Paerl 2005).

6.3 Facilitation of C. *raciborskii* growth by consumer-driven nutrient recycling (CNR)

The present study supported Hypothesis II, demonstrating that regenerated nutrients promoted growth of P-deplete *C. raciborskii* and that the importance of recycled phosphorus (P) in sustaining *C. raciborskii* growth depends on prey quality (P quota). The copepod *Boeckella* sp. feeding on P-replete food promoted the growth of P-deplete *C. raciborskii* in all experiments. This new information helps to identify the environmental conditions that most benefit *C. raciborskii* growth and suggests that, in an environment with very low P loading, the influence of nutrients regenerated by zooplankton to *C. raciborskii* is minor, and increased P loading will likely increase the impact of CNR on the growth of *C. raciborskii*.

This study provides laboratory evidence to support another field study in which daily spikes of DIP in a P-limited environment resulted in *C. raciborskii* dominance (Posselt et al. 2009). A monitoring study of Istvánovics (2008) also showed that *C. raciborskii* dominance depended on P loading, where the composition of the summer-

dominated phytoplankton switched from N_2-fixing cyanobacteria to *C. raciborskii* when P loading was reduced in eutrophic Lake Balaton. As P inputs were further decreased, *Ceratium hirundinella* replaced *C. raciborskii* to dominate the community (Istvánovics 2008).

This study also showed that *C. raciborskii* had a competitive advantage in the use of recycled P when it was in mixed culture with *C. reinhardtii*. The small green alga *C. reinhardtii* achieves higher rates of photosynthesis and has a higher specific growth rate compared to *C. raciborksii* when grown in unialgal culture (Hein et al. 1995). However, when both algae had access to nutrients recycled from *Boeckella sp*., *C. reinhardtii* showed 30% reduced growth in the presence of *C. raciborskii*, with the net result that both algae grew at the same rate. This finding suggests that *C. raciborskii* is more competitive among other algae in environments with low P and helps to understand why *C. raciborskii* blooms in P-limited systems.

6.4 Synergistic effects of top-down and bottom-up processes in promoting *C. raciborskii* dominance

The synergism of top-down and bottom-up processes to promote *C. raciborskii* (Hypothesis III) was tested by utilizing dialysis tubes. This enabled simultaneous examination of the direct (grazing) and indirect effects (nutrient regeneration) of copepods feeding on mixed *C. raciborskii* and *C. reinhardtii*. This study showed that, under P-replete conditions, *C. raciborskii* benefits most from lower grazing losses rather than gains in growth due to the rapid uptake of regenerated nutrients. In this situation, zooplankton could lead to the net growth of *C. raciborskii* when P supply rates are such that co-existent phytoplankton are the preferred food for copepod consumers.

The large-scale mesocosm study further confirmed the outcome of such biotic interactions *in situ* (Chapter 4). It was the first study to examine promotion of *C. raciborskii* dominance in a field experiment through the manipulation of zooplankton abundance. Zooplankton enrichment resulted in an increase of potentially toxic *C. raciborskii* abundance, but a decrease in non-toxic *Limnothrix*. Simultaneously, *C.*

raciborskii was negatively related to the C:P ratio of phytoplankton, indicating that elevated abundance of mesozooplankton (dominated by copepods) increased P recycling to benefit the growth of *C. raciborskii*. This study provided a good framework with which to test cyanobacteria bloom development based on phosphate-phytoplankton-zooplankton interactions.

The field survey (Chapter 5) of 15 subtropical reservoirs illustrated that reservoirs with *C. raciborskii* had a low proportion of cladocerans, but were positively correlated to abundances of rotifers and copepods. In the two reservoirs with the highest proportion of *C. raciborskii* (Borumba, 40% and Wivenhoe, 5%), as well as highest *C. raciborskii* abundance (Wivenhoe, 1.42 x 10^4 cells ml^{-1}), the zooplankton community was composed of 48% and 63% rotifers, respectively, but had no cladocerans. In contrast, in two reservoirs without *C. raciborskii* cells (Leslie Harrison and Maroon), cladocerans comprised 22% and 49% of the zooplankton community, respectively, while rotifers comprised only 1-2%. The snap shot view of zooplankton communities seen during the survey likely reflects a complex series of processes occurring at different temporal and spatial scales, but seems to have captured differences in phytoplankton and zooplankton community structure between the two reservoir types (i.e. those experiencing *C. raciborskii* blooms and those which don't) as well as the seasonal changes in physical-chemical factors.

6.5 Conceptual models of zooplankton regulation of *C. raciborskii* bloom development in Australian subtropical reservoirs

The new information generated in this thesis adds detail to the existing conceptual models on *C. raciborskii* bloom development (Fig. 2). In summer, increased temperature promotes algal growth and creates saturating food conditions where copepods will consume other algae in preference to *C. raciborskii*. The nutrients regenerated by zooplankton are potentially transferred from edible algae to promote toxic *C. raciborskii* growth. When reaching higher abundance, *C. raciborskii* might

adversely affect the zooplankton community leading to dominance by rotifers and small copepods, with a few juvenile zooplankton.

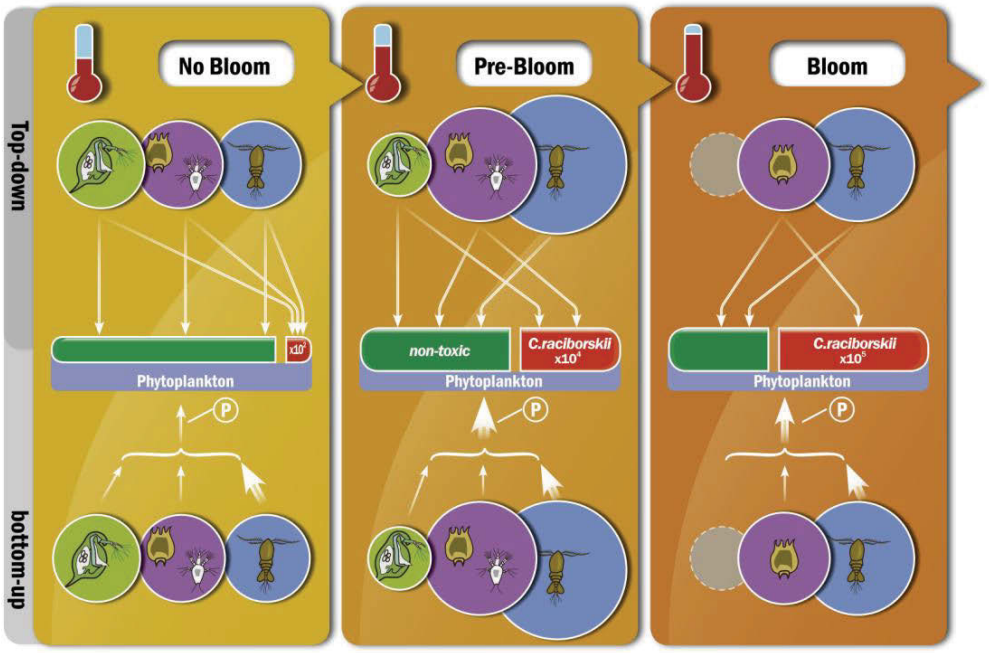

Figure 2. Conceptual model of zooplankton regulation of *C. raciborskii* abundance. High abundance of copepods facilitates *C. raciborskii* because of selective feeding and more P regeneration.

Concurrently, zooplankton also facilitate *C. raciborskii* through nutrient regeneration (Fig. 3). *C. raciborskii* has less chance of developing in an oligotrophic system (with very low P and N) due to unselective grazing of zooplankton and a low magnitude of zooplankton nutrient regeneration. When nutrient levels increase, *C. raciborskii* becomes more abundant because of selective grazing, and the magnitude of zooplankton nutrient regeneration increases. In eutrophic water, *C. raciborskii* growth is expected to be facilitated by zooplankton selective feeding with a minor impact of CNR.

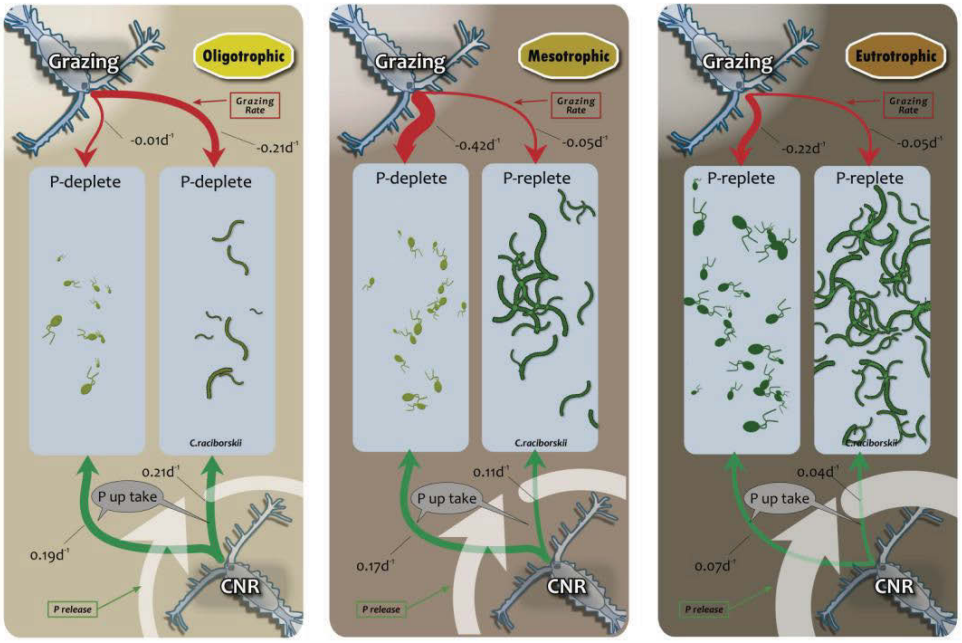

Figure.3. Conceptual model of zooplankton regulation of *C. raciborskii* abundance through synergistic top-down and bottom-up processes.

6.6 Significance and potential implications of this study

This study is significant because it has investigated previously untested hypotheses regarding the effects of zooplankton on growth and bloom development of the toxic cynobacterium *C. raciborskii*. It was the first to explore both bottom-up (nutrient regeneration) and top-down (grazing) of zooplankton in regulating *C. raciborskii* growth in Australian subtropical reservoirs. Importantly, this study has provided both laboratory and field study evidence to demonstrate that *C. raciborskii* dominance is facilitated through planktonic foodweb interactions that involve selective zooplankton grazing of co-existing algae and the nutritional subsidization of *C. raciborskii* growth through zooplankton nutrient recycling. The laboratory studies of grazing preference helped to understand that the mechanisms of feeding selection by copepods involve both prey P content and cell toxicity. This was further demonstrated in the mesocosm experiment where the zooplankton community selected against *C. raciborskii* and other

potentially toxin-producing cyanobacteria whilst consuming other algae whose size and morphology was similar to *C. raciborskii*.

These findings provide significant new knowledge in understanding the development of *C. raciborskii* blooms in subtropical freshwater ecosystems and provide insight for predicting cyanobacteria blooms in other P limited reservoirs and lakes.

Management implications

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, the following suggestions and recommendations for sustainable management of Australian subtropical reservoirs are made.

1. Controlling nutrient loads, such as reducing phosphate concentration, should be the management priority to diminish the risk of *C. raciborskii* (or other algal) blooms.
2. When reducing the external nutrient loads, managers should consider the balance of nutrients such as the N:P ratio. This is because *C. raciborskii* is able to efficiently use P released from zooplankton and fix N_2.
3. Biomanipulation might be considered if the intention is to increase zooplankton species diversity, because copepods might shorten filaments of *C. raciborskii* (Bouvy et al. 2001) which can then be efficiently consumed by cladocerans. This study found that the cladoceran/copepod ratio was higher in spring compared to summer; thus if biomanipulation were attempted, it should be at a time when cladocerans have a high relative abundance in the zooplankton community and *C. raciborskii* are at the first stage of bloom development.
4. Increasing temperatures could drive zooplankton size structure toward smaller species (Daufresne et al. 2009), and reduce the top-down grazing efficiency of zooplankton on phytoplankton. Building deeper reservoirs is another suggestion to provide more refuges for zooplankton, especially for larger species, which may be more effective consumers of *C. raciborskii*.

6.7 Perspectives on future research

This study distinguished the response of the small green alga *C. reinhardtii* and the filamentous cyanobacterium *C. raciborskii* to grazers and zooplankton-derived nutrients. However, filamentous cyanobacteria comprise most of the phytoplankton biomass in Australian subtropical reservoirs. To increase knowledge of *C. raciborskii* competitiveness, further research should focus on conducting experiments with natural phytoplankton assemblages, thus gaining a more comprehensive understanding of *C. raciborskii* dominance *in situ*. Furthermore, use of molecular tools to measure toxin production of phytoplankton would lead to a better understanding of selective grazing by zooplankton.

A novel part of this study involved use of dialysis tubing which enabled zooplankton to access food outside the dialysis tubing, and for zooplankton-derived nutrients to be accessible to algae inside the tubing. This experiment proved to be a good framework for testing simultaneously the direct (grazing) and indirect effects (nutrient regeneration) of zooplankton-algal interactions, with grazing effects being calculated through comparison with no zooplankton controls containing algal prey only. Further *in situ* nested design experimentation is needed to examine the phytoplankton community response to changes in zooplankton biomass and composition, to determine the contribution of CNR to *C. raciborskii* growth over other coexisting species in natural phytoplankton assemblages, as well as the food preference of zooplankton in the natural environment.

This study also revealed the grazing of zooplankton on different strains of *C. raciborskii*. The distribution of *C. raciborskii* is increasing due to its invasion of temperate lake systems (O'Neil, 2011). Further study is needed to determine whether zooplankton communities that have never been exposed to *C. raciborskii* are more susceptible to its negative impacts than zooplankton communities that have been exposed to *C. raciborskii* for some time. This data would provide valuable information to understand how *C. raciborskii* invades new environments successfully.

It is clear that the zooplankton have a strong effect on nutrient cycling and phytoplankton community structure in Australian subtropical reservoirs. Further studies

need to take a more holistic approach in terms of nutrient sources, stoichiometry of phytoplankton and zooplankton, and community-level consequences of animal-mediated nutrient cycling (including planktivorous fish), to have a better understanding of the importance of zooplankton in nutrient cycling in P limited subtropical reservoirs.

REFERENCES

Agrawal, M., Agrawal, M. K., 2011. Cyanobacteria-herbivore interaction in freshwater ecosystem. *Journal of Microbiology and Biotechnology Research,* 1:52-66.

Anderson, T. R., 1992. Modelling the influence of food C: N ratio, and respiration on growth and nitrogen excretion in marine zooplankton and bacteria. *Journal of Plankton Researc*h, 14:1645-1671.

Arnér, M., Koivisto, S., Norberg, J. , Kautsky, N., 1998. Trophic interactions in rockpool food webs: regulation of zooplankton and phytoplankton by *Notonecta* and *Daphnia*. *Freshwater Biology*, 39:79-90.

Atech , 2000. *Cost of algal blooms*. Report to land and water resources research and development corporation. ACT, Canberra. pp. 1-41.

Attayde, J. L., Hansson, L. A., 1999. Effects of nutrient recycling by zooplankton and fish on phytoplankton communities. *Oecologia*, 121:47-54.

Bayly, I. A. E., (ed) 1992. T*he non-marine Centropagidae (Copepoda: Calanoida) of the world.* The Hague, Netherlands, SPB Academic, pp. 1-10

Bolch, C. J. S., Blackburn, S. I., 1996. Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kütz. *Journal of Applied Phycology*, 8:5-13.

Boon, P. I., Stuart, E. B., Green, J. D., Shiel, R. J., 1994. Consumption of Cyanobacteria by Freshwater Zooplankton: Implications for the Success of 'Top-down' Control of Cyanobacterial Blooms in Australia. *Aust. J. Mar. Freshwater Res.,* 45:875-887.

Bormans, M., Ford, P. W., Fabbro, L., Hancock, G., 2004. Onset and persistence of cyanobacterial blooms in a large impounded tropical river, *Australia. Marine and Freshwater Research,* 55:1-15.

Bouvy, M., Falcao, D., Marinho, M., Pagano, M., Moura, A., 2000. Occurrence of *C. raciborskii* (Cyanobacteria) in 39 Brazilian tropical reservoirs during the 1998 drought. *Aquatic Microbial Ecology*, 23:13-27.

Bouvy, M., Pagano, M., Troussellier, M., 2001. Effects of a cyanobacterial bloom (*Cylindrospermopsis raciborskii*) on bacteria and zooplankton communities in Ingazeira reservoir (northeast Brazil). *Aquatic Microbial Ecology,* 25:215-227.

Burford, M. A., Davis, T. W., 2011. Physical and chemical processes promoting dominance of the toxic cyanobacterium *C. raciborskii*. *Chinese Journal of Oceanology and Limnology,* 29:883-891.

Burford, M. A., Johnson, S. A., Cook, A. J., Packer, T. V., Taylor, B. M., Townsley, E. R., 2007. Correlations between watershed and reservoir characteristics, and algal blooms in subtropical reservoirs. *Water research*, 41:4105-4114.

Carpenter, S. R., Kitchell, J. F., Hodgson, J. R., 1985. Cascading trophic interactions and lake productivity. *BioScience,* 35:634-639.

Carpenter, S. R., Kraft, C. E., Wright, R., He, X., Soranno, P. A., Hodgson, J. R., 1992. Resilience and resistance of a lake phosphorus cycle before and after food web manipulation. *American Naturalist*, 140:781-798.

Carpenter, S. R., Kitchell, J. F., (eds) 1996. *The trophic cascade in lakes*. Cambridge University Press, Cambridge, UK, pp. 1-385

Cembella, A. D., 2003. Chemical ecology of eukaryotic microalgae in marine ecosystems. *Phycologia,* 42:420-447.

Cottingham, K. L., Glaholt, S., Brown, A. C., 2004. Zooplankton community structure affects how phytoplankton respond to nutrient pulses. *Ecology*, 85:158-171.

Daufresne, M., Lengfellner, K., Sommer, U., 2009. Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences,* 106:12788-12793.

De Bernardi, R., Giussani, G., 1990. Are blue-green algae a suitable food for zooplankton? An overview. *Hydrobiologia*, 200:29-41.

Degans, H., De Meester, L., 2002. Top-down control of natural phyto- and bacterioplankton prey communities by *Daphnia magna* and by the natural zooplankton community of the hypertrophic Lake Blankaart. *Hydrobiologia,* 479:39-49.

DeMott, W. R., 1982. Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina*. *Limnol. Oceanogr* 27: 518-527.

DeMott, W. R., 1989. Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods. *Limnol. Oceanogr* 34:140-154.

DeMott, W. R., 1990. Retention efficiency perceptual bias, and active choice as mechanisms of food selection by suspension-feeding zooplankton. In Hughes, R. N. (ed.), *Behavorial Mechanisms of Food Selection*. NATO ASI Series, Series G: Ecological Series. Springer, New York, pp. 569–594.

DeMott, W. R., 2003. Implications of element deficits for zooplankton growth. *Hydrobiologia*, 491:177-184.

DeMott, W. R., Edington, J. R., Tessier, A. J., 2004. Testing zooplankton food limitation across gradients of depth and productivity in small stratified lakes. *Limnology and Oceanography,* 49:1408-1416.

DeMott, W. R., Gulati, R. D., 1999. Phosphorus limitation in *Daphnia*: Evidence from a long term study of three hypereutrophic Dutch lakes. *Limnology and Oceanography,* 44:1557-1564.

DeMott, W. R., Gulati, R. D., Van Donk, E., 2001. *Daphnia* food limitation in three hypereutrophic Dutch lakes: Evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. *Limnology and Oceanography*, 46:2054-2060.

DeMott, W. R., Zhang, Q. X., Carmichael, W. W., 1991. Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*. *Limnology and Oceanography*, 36:1346-1357.

Elser, J. J., 1999. The pathway to noxious cyanobacteria blooms in lakes: the food web as the final turn. *Freshwater Biology*, 42:537-543.

Elser, J. J., Carney, H. J., Goldman, C. R., 1990. The zooplankton-phytoplankton interface in lakes of contrasting trophic status: an experimental comparison. *Hydrobiologia*, 200:69-82.

Elser, J., Chrzanowski, T., Sterner, R., Schampel, J., Foster, D., 1995. Elemental ratios and the uptake and release of nutrients by phytoplankton and bacteria in three lakes of the Canadian Shield. *Microbial ecology,* 29:145-162.

Elser, J. J., Urabe, J., 1999. The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. *Ecology*, 80:735-751.

Figueredo, C. C., Giani, A., Bird, D. F., 2007. Dose alleopathy contribute to *Cylindrospermopsis raciborskii* (cyanobacteria) bloom occurrence and geographic expansion? *Journal of Phycology*, 43:256-265.

Friedman, M. M., Strickler, J. R., 1975. Chemoreceptors and feeding in calanoid copepods (Arthropoda: Crustacea). *Proceedings of the National Academy of Sciences*, 72:4185-4188.

Ganf, G., 1983. An ecological relationship between *Aphanizomenon* and *Daphnia pulex*. *Marine and Freshwater Research,* 34:755-773.

Ger, K. A., Panosso, R., Lürling, M., 2011. Consequences of acclimation to *Microcystis* on the selective feeding behavior of the calanoid copepod *Eudiaptomus gracilis*. *Limnology and oceanography,* 56: 2103-2114.

Ghadouani, A., Pinel-Alloul, B., Prepas, E. E., 2003. APPLIED ISSUES: Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. *Freshwater Biology,* 48:363-381.

Gilbert, P., 1998. Interactions of top-down and bottom-up control in planktonic nitrogen cycling. *Hydrobiologia*, 363:1-12.

Gliwicz, Z. M., Lampert, W., 1990. Food thresholds in Daphnia species in the absence and presence of blue-green filaments. *Ecology*, 71:691-702.

Gulati, R., DeMott, W. R., 1997. The role of food quality for zooplankton: remarks on the state of the art, perspectives and priorities. *Freshwater Biology,* 38:753-768.

Guo, N., Xie, P., 2006. Development of tolerance against toxic *Microcystis aeruginosa* in three cladocerans and the ecological implications. *Environmental Pollution,* 143:513-518.

Hall, S. R., 2009. Stoichiometrically explicit food webs: feedbacks between resource supply, elemental constraints, and species diversity. *Annual Review of Ecology, Evolution, and Systematics*, 40:503-528.

Hansson, L. A., Gustafsson, S. , Rengefors, K., Bomark, L., 2007. Cyanobacterial chemical warfare affects zooplankton community composition. *Freshwater Biology* , 52:1290-1301.

Havens, K. E., Elia, A. C., Taticchi, M. I., Fulton, R. S., 2009. Zooplankton–phytoplankton relationships in shallow subtropical versus temperate lakes Apopka (Florida, USA) and Trasimeno (Umbria, Italy). *Hydrobiologia,* 628:165-175.

Havens, K., East, T., Beaver, J., 1996. Experimental studies of zooplankton–phytoplankton–nutrient interactions in a large subtropical lake (Lake Okeechobee, Florida, USA). *Freshwater Biology*, 36:579-597.

Hawkins, P., Lampert, W., 1989. The effect of *Daphnia* body size on filtering rate inhibition in the presence of a filamentous cyanobacterium. *Limnology and Oceanography* , 34:1084-1089.

Hein, M., Pedersen, M. F., Sand-Jensen, K., 1995. Size-dependent nitrogen uptake in micro-and macroalgae. *Marine ecology progress series,* 118:247-253.

Hessen, D. O., 1992. Nutrient element limitation of zooplankton production. *American Naturalist,* 140:799-814.

Hessen, D., Andersen, T., Faafeng, B., 1992. Zooplankton contribution to particulate phosphorus and nitrogen in lakes. *Journal of Plankton Research*, 14:937-947.

Hessen, D., Lyche, A., 1991. Inter-and intra-specific variations in zooplankton element composition. *Archiv fur Hydrobiologie*, 121:343-353.

Hillebrand, H., Frost, P., Liess, A., 2008. Ecological stoichiometry of indirect grazer effects on periphyton nutrient content. *Oecologia*, 155:619-630.

Hochstädter, S., 2000. Seasonal changes of C: P ratios of seston, bacteria, phytoplankton and zooplankton in a deep, mesotrophic lake. *Freshwater Biology*, 44:453-463.

Irigoien, X., Flynn, K., Harris, R., 2005. Phytoplankton blooms: a 'loophole'in microzooplankton grazing impact? *Journal of Plankton Research*, 27:313-321.

Istvánovics, V., 2008. The role of biota in shaping the phosphorus cycle in lakes. *Freshwater Reviews*, 1:143-174.

Jeppesen, E., Søndergaard, M., Mazzeo, N., Meerhoff, M., Branco, C., Huszar, V., Scasso, F., 2005. Lake restoration and biomanipulation in temperate lakes: relevance for subtropical and tropical lakes. Chapter 11. In Reddy, M. V. (ed.), *Tropical Eutrophic Lakes: Their Restoration and Management*. Science Publishers, Enfield, pp. 331–359.

Kâ, S., Mendoza-Vera, J. M., Bouvy, M., Champalbert, G., N'Gom-Kâ, R., Pagano, M., 2012. Can tropical freshwater zooplankton graze efficiently on cyanobacteria? *Hydrobiologia,* 979: 119-138.

Kinnear, S., 2010. Cylindrospermopsin: a decade of progress on bioaccumulation research. *Marine Drugs,* 8:542-564.

Kleebert, A., Kozerski, H.P., 1997. Phosphorus release inlake Grosser Muggelsee and its implcations for lake resoration. *Hydrobiologia*, 342:9-26.

Knoll, L. B., McIntyre, P. B., Vanni, M. J., Flecker. A. S., 2009. Feedbacks of consumer nutrient recycling on producer biomass and stoichiometry: separating direct and indirect effects. *Oikos*, 118:1732-1742.

Kobayashi, T., 1992. Plankton of Lyell Reservoir, New South Wales. *Proceedings of the linnean Society of South Wales*, 113:245-261.

Langeland, A., Reinertsen, H., 1982. Interactions between phytoplankton and zooplankton in a fertilized lake. *Holarctic Ecology*, 5:253-272.

Lehman, J. T., Sandgren, C. D., 1985. Species-specific rates of growth and grazing loss among freshwater algae. *Limnology and Oceanography*, 30:34-46.

Leonard, J. A., Paerl, H. W., 2005. Zooplankton community structure, micro-zooplankton grazing impact, and seston energy content in the St. Johns River system, Florida as influenced by the toxic cyanobacterium *Cylindrospermopsis raciborskii*. *Hydrobiologia,* 537:89-97.

Makarawicz, J. C., Bertram, J. C., Bertram, P., 1991. Evidence for the restoration of the Lake Erie ecosystem. *Bioscience*, 41:216-223.

Maron, J. L., Crone, E., 2006. Herbivory: effects on plant abundance, distribution and population growth. *Proceedings of the Royal Society B: Biological Sciences,* 273:2575-2584.

Martin-Creuzburg, D., Von Elert, E., 2009. Good food versus bad food: the role of sterols and polyunsaturated fatty acids in determining growth and reproduction of *Daphnia magna*. *Aquatic Ecology,* 43:943-950.

Matveev, V., 1998. Evidence for biotic control of Australian reservoir phytoplankton communities and the potential for applied biomanipulation. *Lake and Reservoir Management*, 3:105-111.

Matveev, V., 2003. Testing predictions of the lake food web theory on pelagic communities of Australian reservoirs. *Oikos*, 100:149-161.

Matveev, V., Matveeva, L., Jones, G. J. , 2000. Relative impacts of *Daphnia* grazing and direct stimulation by fish on phytoplankton abundance in mesocosm communities. *Freshwater Biology,* 44:375-385.

McCauley, E., Briand, F., 1979. Zooplankton grazing and phytoplankton species richness: field tests of the predation hypothesis. *Limnology and Oceanography,* 24:243-252.

Mills, E. L., 1986. Trophic Relationships in Freshwater Pelagic Ecosystems. J. Fish. Aquat. Sci 43:1571-1581.

Mitra, A., Flynn, K. J. (2006) Promotion of harmful algal blooms by zooplankton predatory activity. *Biol. Letters,* 2:194-197.

Mitra, A., Flynn, K. J., 2006. Promotion of harmful algal blooms by zooplankton predatory activity. *Biology letters*, 2:194-197.

Mitra, A., Flynn, K. J., Fasham, M. J. R., 2007. Accounting for grazing dynamics in nitrogen-phytoplankton-zooplankton models. *Limnology and Oceanography,* 52:649-661.

Nogueira, I. C. G., Lobo-da-Cunha, A., Vasconcelos, V. M., 2006. Effects of *C. raciborskii* and *Aphanizomenon ovalisporum* (Cyanobacteria) ingestion on *Daphnia magna* midgut and associated diverticula epithelium. *Aquatic Toxicology,* 80:194-203.

Nogueira, I. C. G., Saker, M. L., Pflugmacher, S., Wiegand, C., Vasconcelos, V. M., 2004. Toxicity of the cyanobacterium *C. raciborskii* to *Daphnia magna*. *Environmental toxicology*, 19:453-459.

Olsen, Y., Jensen, A., Reinertsen, H., Borsheim, K. Y., Heldal, M., Langeland, A., 1986. Dependence of the rate of release of phosphorus by zooplankton on the P: C ratio in the food supply, as calculated by a recycling model. *Limnology and Oceanography,* 31:34-44.

Orr, P. T., Rasmussen, J. P., Burford, M. A., Eaglesham, G. K., Lennox, S. M., 2010. Evaluation of quantitative real-time PCR to characterise spatial and temporal variations in cyanobacteria, *C. raciborskii* (Woloszynska) Seenaya et Subba Raju and cylindrospermopsin concentrations in three subtropical Australian reservoirs. *Harmful Algae,* 9:243-254.

Padisák, J., 1997. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and a review of its ecology. A*rch. Hydrobiol. Suppl. Monogr. Stud.* 107: 563–593.

Paerl, H. W., Fulton, R. S., 2006. Ecology of harmful cyanobacteria. In. E.Granéli J.T. Turner (Eds.) E*cology of harmful algae*. Springer-Verlag Berlin Heidelberg, pp. 95-107.

Panosso, R., Carlsson, P., Kozlowsky-Suzuki, B., Azevedo, S. M. F. O., Granéli, E., 2003. Effect of grazing by a neotropical copepod, *Notodiaptomus*, on a natural cyanobacterial assemblage and on toxic and non-toxic cyanobacterial strains. *Journal of Plankton Research*, 25:1169-1175.

Persson, L., Andersson, G., Hamrin, S. F., Johansson, L., 1988. Predator regulation and primary production along the productivity gradient of temperate lake ecosystems. In. Stephen R. Carpenter (Ed.) *Complex interactions in lake communities*. Springer New York, pp. 45-65.

Pogozhev, P. I., Gerasimova, T. N., 2001. The Effect of Zooplankton on Microalgae Blooming and Water Eutrophication. *Water Resources/Vodnye Resursy*, 28:420-427.

Posselt, A. J., Burford, M. A., Shaw, G., 2009. Pulses of phosphate promote dominance of the toxic *Cylindrospermopsis raciborskii* in a subtropical water reservoir *Journal of Phycology*, 45:540-546.

Reinertsen, H., Jensen, A., Langeland, A., Olsen, Y., 1986. Algal competition for phosphorus: the influence of zooplankton and fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 43:1135-1141.

Rocha, O., Duncan, A., 1985. The relationship between cell carbon and cell volume in freshwater algal species used in zooplanktonic studies. *Journal of Plankton Research,* 7:279-294.

Roth, J., Horne, A., 1981. Algal nitrogen fixation and micro-crustacean abundance: An unregarded interrelationship between zooplankton and phytoplankton. *Verh. Internat. Verein. Limnol.*, 21:333-338.

Rothhaupt, K. O., 1995. Algal nutrient limitation affects rotifer growth rate but not ingestion rate. *Limnology and Oceanography*, 40:1201-1208.

Sager, P. E., Richman, S., 1991. Functional interaction of phytoplankton and zooplankton along the trophic gradient in Green Bay, Lake Michigan. *Canadian Journal of Fisheries and Aquatic Sciences,* 48:116-122.

Sahuquillo, M., Melao, M. G. G., Miracle, M. R., 2007. Low filtering rates of *Daphnia magna* in a hypertrophic lake: laboratory and in situ experiments using synthetic microspheres. *Hydrobiologia,* 594:141-152.

Scharf, W., 2008. The use of nutrient reduction and food-web management to improve water quality in the deep stratifying Wupper Reservoir, Germany. *Hydrobiologia,* 603:105-115.

Sereda, J. M., Hudson, J. J., 2011. Empirical models for predicting the excretion of nutrients (N and P) by aquatic metazoans: taxonomic differences in rates and element ratios. *Freshwater Biology,* 56:250-263.

Sinistro, R., Sánchez, M. L., Marinone, M. C., Izaguirre, I., 2007. Experimental study of the zooplankton impact on the trophic structure of phytoplankton and the microbial assemblages in a temperate wetland (Argentina). *Limnologica-Ecology and Management of Inland Waters*, 37:88-99.

Smayda, T. J., 2008. Complexity in the eutrophication-harmful algal bloom relationship, with comment on the importance of grazing. *Harmful Algae*, 8:140-151.

Sommer, U., 1988. Phytoplankton succession in microcosm experimemts under simultaneous grazing pressure and resource limitation. *Limnol. Oceanogr.,* 33:1037-1054.

Sommer, U., Sommer, F., 2006. Cladocerans versus copepods: the cause of contrasting top–down controls on freshwater and marine phytoplankton. *Oecologia*, 147:183-194.

Sterner, R. W., 1990. The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algae comkpetitive arena. *The American Naturalist*, 136:209-229.

Touratier, F., Field, J. G., Moloney, C. L., 2001. A stoichiometric model relating growth substrate quality (C: N: P ratios) to N: P ratios in the products of heterotrophic release and excretion. *Ecological Modelling,* 139:265-291.

Urabe, J., 1993. Seston stoichiometry and nutrient deficiency in a shallow eutrophic pond. *Archiv fuer Hydrobiologie,* 126:417-428.

Vanderploeg, H. A., G.-A. Paffenhofer, and J. R. Liebig. 1990. Concentration-variable interactions between calanoid copepods and particles of different food quality. In R. N. Hughes (ed.) *Behavioral mechanisms of food selection.* Springer Verlag, Heidelberg, New York, pp. 595-613

Vanderploeg, H. A., 1994. Feeding mechanisms and particle selection in suspension-feeding zooplankton. In R. S. Wotton (ed.) *The biology of particles in aquatic systems.* Lewis, Ann Arbor, Michigan, pp. 183-212.

Vanderploeg, H. A., Liebig, J. R., Carmichael, W. W., Agy, M. A., Johengen, T. H., Fahnenstiel, G. L., Nalepa, T. F., 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic Microcystis blooms in Saginaw Bay (Lake Huron) and Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences,* 58:1208-1221.

Wen, Y. H., Peters, R. H., 1994. Empirical models of phosphorus and nitrogen excretion rates by zooplankton. *Limnology and Oceanography*, 39:1669-1679.

Zhang, M., Kong, F., Xing, P., Tan, X., 2007. Effects of interspecific interactions between *Microcystis aeruginosa* and *Chlorella pyrenoidosa* on their growth and physiology. *International Review of Hydrobiology,* 92:281-290.