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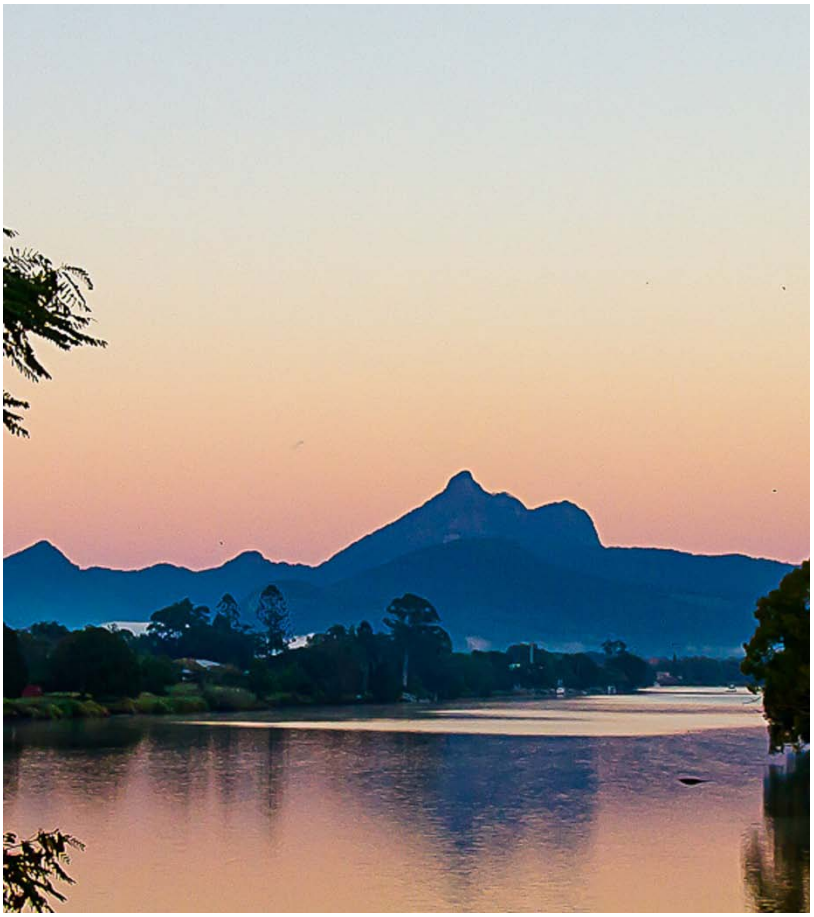

The Tweed River and Mt Warning situated in the Tweed Shire, located on the north eastern corner of New South Wales on Australia's eastern coastline (photo by Sally Everson 2011).

Dedication

I dedicate this thesis to the memory of Genifer and Allan Hinton, both passed away during the period of my research, sadly neither is here in person to see the end. Mum and Dad's undaunted belief in my ability and perseverance; their guidance, encouragement and support throughout my formative years and beyond made me the person I am today and in turn made this thesis possible. I miss you both so very much - this is for you.

Phytoplankton ecology of the Tweed River catchment with special reference to toxin-producing cyanoprokaryotes

Sally Ruth Everson

Thesis submitted in accordance with the requirements of Central Queensland University for the degree of Doctor of Philosophy

Centre for Environmental Management

Faculty of Sciences, Engineering and Health

Central Queensland University

Australia

July, 2013

Statement of Originality

I declare that the research and discussion presented in this thesis are my own original work and have not been submitted in any form to any university or tertiary institution for any other award. Contributions by others are acknowledged, where appropriate, in the relevant chapter and are separately detailed in the 'Statement of Contributions by Others'.

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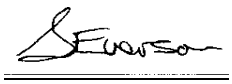

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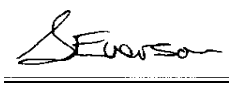


22/11/2013

Sally Ruth Everson

Statement of Contributions by Others

Others who contributed to this thesis by providing specialist support services are listed hereunder, together with notes on the level of their contributions.

Chapter 3 - Section 3.4

Flow Injection Analyses (FIA) of samples for nutrient concentrations and inductively coupled plasma-optical emission spectroscopy (ICP-OES) metal analyses were performed by experienced technicians with relative NATA signatory status at the Tweed Laboratory Centre. This facility has NATA accreditation according to ISO/IEC 17025 with Chemical NATA accreditation No 12754 and Biological NATA accreditation No 13538.

Chapter 3 - Section 3.8

Cyanotoxin analyses of water samples were carried out by Queensland Health Forensic and Scientific Services, Brisbane, Australia on a paid consultancy basis.

Chapter 3 - Section 3.10

The Polymerase Chain Reaction (PCR) analyses on depth profile samples from Cobaki Lake were carried out by the Co-Operative Research Centre for Water Quality and Treatment, Australian Water Quality Centre in Adelaide.

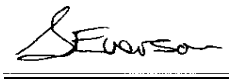
The Polymerase Chain Reaction (PCR) *pks*-F2 and *pks*-R1forward and reverse primers and the *pks* probe used during this study were modifications by Barbara Sendall from Queensland Health Forensic and Scientific Services.

Melt Curve analysis was conducted at Tweed Laboratory Centre to analyse the laboratory cultured strains of *Aphanizomenon ovalisporum* (Strains A and B) from Cobaki Lake. These strains were isolated by Lindsay Hunt at Queensland Health Forensic and Scientific Services, Brisbane, Australia.

Chapters 1 to 10 inclusive

My supervisors, Associate Professor Larelle D. Fabbro, Dr Susan Kinnear and Dr Paul Wright, supervised my work to ensure scientific rigour in design and performance in accordance with the Code of Conduct for Research at Central Queensland University. They provided critical appraisal of manuscripts to ensure correctness for submission to scientific journals. The contributions of these persons are recognised through co-authorship of the papers arising from this thesis.

Excepting the above, all work was performed, and all the manuscripts and chapters were written, by the undersigned candidate.



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Sally Ruth Everson

Abstract

The management of human health risks in drinking water supplies is a global challenge, particularly in the context of urban population growth, increased water demands and climate change. Cyanoprokaryotes and their toxins may present a serious risk to the quality of drinking water supplies. Understanding the ecology of algal assemblages and their accompanying risks is therefore critically important for effective management of those water bodies that experience problematic cyanoprokaryote blooms.

This project was conducted within the Tweed River catchment, a coastal subtropical area under pressure from population growth, ongoing development and climate change. The three study sites chosen in the catchment were: (1) a small artificial lake with a maximum depth of 19 m and covering an area of 2.5 hectares (Cobaki Lake); (2) a water storage facility with a maximum depth of 20 m, a destratification unit installed, and an area of 340 hectares (Clarrie Hall Dam); and (3) a flowing river with a maximum depth of 4 m (Tweed River at Bray Park). Water column dynamics, water chemistry and algal assemblages were investigated. Vertical depth profile data were logged and samples collected at one metre intervals each month over a twenty month period (January 2007 to August 2008). Detailed multivariate statistical analyses of both environmental and biological data were carried out using PRIMER V6 statistical software, and the data was then discussed in relation to the relevant Australian Drinking Water and Recreational Water Guidelines (National Health and Medical Research Council - NHMRC). Physical and chemical data recorded during this study were also discussed in relation to the relevant Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC).

During the monitoring of phytoplankton populations and the corresponding physical and chemical conditions over twenty months (January 2007 to August 2008) the occurrence of the known toxin-producing cyanoprokaryote species *Aphanizomenon ovalisporum* and

Cylindrospermopsis raciborskii was detected, and this prompted a closer examination of the associated cylindrospermopsin (CYN) and deoxy-cylindrospermopsin (deoxy-CYN) toxin concentration profiles.

Quite different phytoplankton assemblages were present at each study site. Samples from the Tweed River at Bray Park contained reduced populations, with species of *Oscillatoriales* and *Cryptomonas* present during periods of muddy, high flow and *Anabaena* (now *Dolicospermum* (Wacklin *et al.* 2009)) species present during periods of low flow and warm temperatures. Samples from Clarrie Hall Dam contained a larger range of phytoplankton species, with chlorophytes dominating. Low concentrations of the cyanoprokaryotes *Anabaena circinalis* (now *Dolicospermum circinale* (Wacklin *et al.* 2009)), *Microcystis aeruginosa, Aphanizomenon ovalisporum* (now *Chrysosporum ovalisporum* (Zapomělová *et al.* 2012)) and *Geitlerinema amphibium* were detected. The data collected in this study have shown that the presence of different artificially-created habitats is also important in the development of different phytoplankton assemblages. This suggests that ongoing management of water bodies containing potentially toxic phytoplankton should be informed not only by the climatic and geographic positioning of sites but also by the presence and nature of any introduced (man-made) structures.

Aphanizomenon ovalisporum and *C. raciborskii* were the dominant toxigenic cyanoprokaryotes present in Cobaki Lake. Both species produced specialised cells during the bloom. As the water chemistry changed, *A. ovalisporum* produced akinetes before experiencing a rapid decline in cell numbers. In contrast, *C. raciborskii* continued to bloom without producing detectable akinetes although heterocytes were produced. Peak *C. raciborskii* cell concentrations (83,160 cells mL^{-1}) occurred in the late autumn, when surface water temperatures were 19.1ºC, and were accompanied by concentrations of total cylindrospermopsin in the hypolimnion exceeding 100 µg L^{-1}. Water quality analyses indicated that stratification and oxygenation of the water column were of considerable

influence in both the distribution of the cyanoprokaryote populations and their associated toxin concentrations. The relative distribution of CYN and deoxy-CYN paralleled the distribution of ammonium nitrogen (NH_4N) and oxides of nitrogen (NO_x) within the water column, with oxygenated chemical species dominating above 15 m and de-oxygenated species dominating below 15 m. Cyanoprokaryote cell concentrations were highest in the oxic, warm and low conductivity waters of the epilimnion, and cyanoprokaryote species succession was associated with nutrient and trace-metal depletion in the surface layer. These findings are directly relevant to the management of water supplies affected by toxin producing blooms, particularly with respect to the considered placement of off-take devices to avoid layers of cyanoprokaryote cell and toxin concentrations. This is the first field study to provide evidence that *C. raciborskii*, despite being traditionally considered a tropical species, can be highly toxic in cooler waters when accompanied by strong stratification involving an anoxic semi-saline hypolimnion. This has serious implications for both water quality management and human health risks in those subtropical and temperate climates where *C. raciborskii* is present.

Real-time PCR and cultured isolates were used to determine that both *A. ovalisporum* and *C. raciborskii* sampled from Cobaki Lake between January and June 2007 were genetically capable of producing CYN. Environmental depth profile analyses from Cobaki Lake revealed a lack of correlation between molecular results and traditional toxin concentrations at depth, suggesting that PCR assays should not be used as a proxy to measure extracellular toxin in water. For example, the highest concentration of CYN was in the hypolimnion (for example, 64.4 µg mL^{-1} at 16 m and 101.4 µg mL^{-1} at 17 m) whereas the PCR *pks* assay did not detect any CYN genes below 10 m.

The findings reported from Cobaki Lake were significant in both their international as well as local (Tweed Shire) application. This small, strongly stratified lake presented a unique set of physical and chemical parameters which were conducive to the growth of toxin-producing

cyanoprokaryotes. The comparison between Cobaki Lake and the other two sites studied in this project (Clarrie Hall Dam and the Tweed River at Bray Park) has given insight and direction into the future management of the raw water supply of the Tweed Shire catchment. However, Cobaki Lake is also a good example of some of the issues that could potentially compromise health with coastal development that involves artificial and decorative lakes.

This research has important implications for water quality management, and human and ecological health risks in subtropical climates where *Cylindrospermopsis raciborskii* is present. For example, this includes implications for the placement of off-take devices and routine sampling regimes.

This study has also revealed that a gap in knowledge exists relative to the reason why cylindrospermopsins accumulate at depth away from the cyanoprokaryote cells which produce them. If these dynamics can be understood, then methods to manage and reduce these risks could be devised. Further consideration of the potential impact that groundwater intrusions may have on stratification of coastal freshwater lakes (and the subsequent concentrations of cylindrospermopsins) is also needed. These issues may have adverse health effects and consequently impact on sustainable development in coastal cities.

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Acknowledgements

I wish to thank all those people who have advised and encouraged me during the period of this study.

In particular, I wish to indicate my appreciation to my principal supervisor, Associate Professor Larelle D. Fabbro, and to my co-supervisors Dr Susan Kinnear and Dr Paul Wright, for their interest, advice, guidance, encouragement, and endless patience.

This research was conducted under a Commonwealth Research Training Scheme place. I am grateful for the additional financial and in-kind support provided to the project by the Central Queensland University, Centre for Environmental Management, the Tweed Shire Council and the Tweed Laboratory Centre, as well as the assistance from staff past and present of the Tweed Shire Council Water Unit, the Tweed Laboratory Centre, the Bray Park Water Treatment Plant, and the management and residents of the Cobaki Village Park. My project would not have been possible without the support of these organisations and personnel.

I sincerely thank Dr Noel Sammon for proof reading and reviewing my thesis and for his valued advice and continued encouragement. I would also like to thank Dr Leonie Barnett for proof reading and reviewing my thesis.

I am grateful to Dr Geoff Eaglesham, Dr Barbara Sendall and Lindsay Hunt from QFHSS for providing assistance with toxin analyses, culture isolates and PCR primer and probe sequences. I appreciate their valuable contribution.

My thanks are extended to Dr Paul Monis and his team at South Australia Water for assistance in real-time PCR analyses and ongoing advice and recommendations.

Finally I wish to acknowledge my deep appreciation for the emotional support and encouragement given by my family and friends throughout the course of my project.

Publications arising

Published papers

Everson SR, Fabbro LD, Kinnear S, Eaglesham G, Wright P (2009) Distribution of the cyanobacterial toxins cylindrospermopsin and deoxycylindrospermopsin in a stratified lake in north-eastern New South Wales, Australia, *Marine and Freshwater Research* **60**, 25-33. (This manuscript comprises Chapter 7 of this thesis).

Everson SR, Fabbro LD, Kinnear S, Wright P (2011) Extreme differences in akinete, heterocyte and cylindrospermopsin concentrations with depth in a successive bloom involving *Aphanizomenon ovalisporum* (Forti) and *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju, *Harmful Algae* **10**, 265-276. (This manuscript comprises Chapter 8 of this thesis).

Manuscripts in preparation

Everson SR, Wright P, Fabbro LD, Kinnear S, Monis P, Sendall B, Hunt L (2013) Practical application of real-time PCR in a field based CYN producing mixed population of *Aphanizomenon ovalisporum* (Forti) and *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju. (This manuscript relates to Chapter 9).

Zohary T, Wood S, Crossetti L, Fabbro L, Everson SR, Paul W, Hamilton D (2013) Investigation into heterocyte formation in Nostocalean species from Lake Linneret, Israel; Karori Reservoir, New Zealand; Garcas Lake, Brazil; Fitzroy River, Australia and Cobaki Lake, Australia. (This manuscript relates to Chapter 8).

Conference presentations

Everson SR, International oral presentation - "Akinete development in a toxin producing bloom of *Aphanizomenon ovalisporum* and *Cylindrospermopsis raciborskii* (Nostocales/Cyanobacteria) in a small monomictic lake in northern NSW, Australia." Presented at the Vth Asian Pacific Phycological Forum (convened by ASPAB - Australasian Society for Phycology and Aquatic Botany), Wellington, New Zealand, November 10-14, 2008, highlighting "Algae in a Changing World".

Everson SR, National poster presentation – "A field-based comparison of toxin producing *Aphanizomenon ovalisporum* and *Cylindrospermopsis raciborskii*s they approach overwintering". Presented at the 2nd National (Australian) Cyanobacterial Workshop, convened by WQRA and Victoria Department of Sustainability & Environment and Department of Health, Melbourne, Australia, 2-3 August, 2010.

Everson SR, International oral and poster presentation – "Functional Group classification of phytoplankton assemblages as a consequence of hydrological alterations in the Tweed Shire in eastern Australia". Presented at the 16th Workshop of the International Association of Phytoplankton Taxonomy and Ecology (IAP), San Michele all'Adige, Italy 21-28 August 2011.

1 INTRODUCTION and FRAMING

1.1 Introduction to the Tweed River Catchment

The Tweed Shire is located in Eastern Australia and covers 1,303 km². It adjoins the New South Wales - Queensland border to its north where it divides the twin towns of Tweed Heads and Coolangatta (Figure 1). The Tweed Shire has a subtropical climate with average annual rainfall of 1,682 mm, mostly falling in the warmer months between January and March. Consequently, the winter is dry with the lowest average monthly rainfall of 60 mm in the cooler months of August and September. Water management is critical Australia-wide, and widespread drought, water shortages and water quality are paramount concerns. The Tweed Shire is no exception; it is a rapidly growing area that is a popular retirement and holiday destination. Given the current expansion of the area, water resources for the shire are becoming a precious commodity. There is, therefore, a critical need to understand and manage these resources so that they can be used in an effective way and with a minimum of risk to environmental and human health.

Figure 1. The Tweed Shire catchment in Eastern Australia situated on the border between Queensland and New South Wales. The sampling sites are indicated by red dots. (Source: Tweed Shire Council).

There has been no detailed study of the phytoplankton populations in the Tweed River catchment, nor has the prevalence of toxic blooms in the area been examined. However, a number of potentially toxic cyanoprokaryote species including *A. circinalis*, *M. aeruginosa*, *A. ovalisporum*, *C. raciborskii* and *G. amphibium* have been recorded in the catchment during routine monitoring (unpublished data). Two of these, *A. ovalisporum* and *C. raciborskii*, are historically tropical species, and their appearance in the subtropical waters of the Tweed Shire indicates the need for a detailed investigation into both the prevalence and toxicity of these species in the catchment. The movement of these species into cooler temperature zones, such as the Tweed Shire, may be a result of global warming (O'Neil *et al.* 2012; Sinha *et al.* 2012; Wiedner *et al.* 2007; Briand *et al.* 2004). The chemical, physical and microbiological parameters that contribute to the development of phytoplankton populations, and that specifically lead to blooms of toxic cyanobacteria in the Tweed catchment are poorly understood. This knowledge would contribute to the effective management and understanding of the natural water resources in the Tweed Shire. Most importantly, a deeper understanding of the ecology and toxicity of *A. ovalisporum* and *C. raciborskii* would support the development of best-practice management models for these species worldwide.

1.2 Aims

The main aims of this study were to:

- Collect and interpret data on the ecology of phytoplankton including physical and chemical properties of the three water bodies.
- Study the toxin-producing cyanoprokaryotes collected and identified from the sampling sites.
- Analyse patterns of toxin production through depth profiling at 1.0 m intervals.

- Present information that may be incorporated into existing management strategies for best-practice monitoring and risk reduction.
- Disseminate the findings through peer-reviewed journals, papers and other scholarly channels.

Subsidiary aims:

- Study the morphology of *A. ovalisporum* and *C. raciborskii* with respect to spatial (depth profiles) and temporal variability.
- Isolate and culture *A. ovalisporum* and *C. raciborskii* in the laboratory.
- Use real-time PCR to identify which species are genetically capable of producing the cyanotoxin cylindrospermopsin.
- Use real-time PCR analyses to evaluate the results from both spatial and temporal environmental samples.

1.3 Thesis structure

This study comprised a series of fieldwork and laboratory based activities. Much of the data and analyses resulting from these were published during canditure in internationally available, peer-reviewed journals, with some manuscripts still awaiting publication. Consequently, the thesis is presented as a combination of the journal papers (as published), as well as other materials that provide additional detail, such as data and multi-variate analyses. Published papers are reproduced *verbatim* in Chapters 7 and 8 in the Journal's required style and format. Each paper contains its own list of references. The main bibliography presented as Chapter 11 lists all other references in the thesis. This approach is in accordance with Central Queensland University's Policy 'Publication of higher degree research work for inclusion in the thesis'.

Chapter 1 provides a general introduction and background to the thesis topic, sets out the aims of the study, and defines and explains the thesis structure. Chapter 2 is a review of the scholarly literature involving ecology, toxin dynamics and physico-chemical conditions in the main discipline areas of limnology and phycology. Chapter 3 details the materials and methods used to enumerate and characterise the phytoplankton assemblages and the corresponding physical-chemical data from the three sites in the Tweed River catchment. Chapter 4 examines in detail the physico-chemical data from the sampling sites, with the intent of comparing and contrasting the conditions recorded at each site. A comparison between the two sampling seasons (2007 and 2008) is also provided. Chapter 5 examines the phytoplankton assemblages found at the sites and also compares the data between sites and over the two seasons of the study. Chapter 6 presents a general overview of Cobaki Village Lake and provides supplementary information relative to the two published papers reproduced as Chapters 7 and 8. Chapter 7 is the published paper "Distribution of cyanobacterial toxins cylindrospermopsis and deoxycylindrospermopsin in a stratified lake in north-eastern New South Wales, Australia". Chapter 8 is the published paper "Extreme differences in akinete, heterocyte and cylindrospermopsin concentrations with depth in a successive bloom involving *Aphanizomenon ovalisporum* and *Cylindrospermopsis raciborskii*". Chapter 9 examines how real-time PCR can be a useful tool in predicting the genetic ability of cyanoprokaryote species to produce toxin. Chapter 10 provides a summary of findings and suggests areas for further research. A full list of references is provided in Chapter 11.

2 LITERATURE REVIEW

2.1 Phytoplankton

The term phytoplankton is defined as "the community of organisms adapted to suspension in the sea or in freshwaters and which are liable to passive movement by wind and current" (Reynolds 1993a p.2). Phytoplankton are autotrophic species, which produce complex organic compounds from simple substances using photosynthesis or inorganic reactions, thus eliminating the need for sources of organic carbon (Mauseth 2008). Freshwater phytoplankton includes several groups of algae, including the cyanoprokaryotes. The spatial and temporal distributions of phytoplankton are dependent on a multitude of factors, particularly the water chemistry and environmental parameters that are unique in each water body (Reynolds and Glaister 1993). Hence, the phytoplankton occurring in flowing rivers (lotic environments) is different to that occurring in stratified dams and lakes (lentic environments) (Reynolds *et al.* 1991). In rivers, phytoplankton generally have fast growth rates and the ability to survive in low light conditions (for example, turbid) and are able to use organic compounds to fix carbon rather than depending entirely on light (Reynolds *et al.* 1994). With increased turbidity, some genera are able to grow and thrive on the nutrients suspended in the water column (Reynolds *et al.* 1991). However, this assemblage can change as river conditions change and select or favour different species distributions (Reynolds 1993b). Phytoplankton have a very short life cycle and respond to nutrient and heavy metal concentrations in the water column and hence are a reliable and quick indicator of water quality (Hötzel and Croome 1999).

2.2 Phytoplankton, nutrients and soluble metals.

The availability of nutrients is a major limiting factor in the growth and development of phytoplankton in water bodies (Reynolds and Glaister 1993). The importance of each nutrient varies dependent on the environment (Smith 1983). For example, in nitrogen-fixing cyanobacteria such as *C. raciborskii* and *A. ovalisporum*, phosphate becomes the controlling nutrient (Burford and O'Donohue 2006; Padisák 1997; Harris and Baxter 1996; Branco and Senna 1994; Reynolds 1993a; Tóth and Padisák 1986). In freshwater environments dissolved inorganic phosphorus is available as orthophosphate.The availability of phosphorus may explain why *Aphanizomenon* responds strongly to fresh flushes of new nutrients (Degerholm *et al.* 2006). In Lake Kinneret, Israel, *A. ovalisporum* acquired its nitrogen nutrition from NH_4 and nitrate (NO_3) (Berman 2001; Berman 1997). Nitrogen, phosphate and sulphate have been identified as the significant limiting nutrients in the production of CYN in *A. ovalisporum* (Basci *et al.* 2006).

Like nutrients, soluble metals can affect the growth of phytoplankton. For example, biologically available iron in Lake Kinneret was shown to have impact on the *A. ovalisporum* population. (Parparova and Yacobi 1998). Iron is an essential micronutrient involved in several key metabolic pathways in phytoplankton, and its availability directly affects algal growth and competitiveness. The study in Lake Kinneret showed that cyanoprokaryotes were more sensitive to biologically available iron levels than cultures of *Peridium* and diatoms (Parparova and Yacobi 1998).

The available nutrients and metals play important roles in the population assemblages of cyanoprokaryotes in freshwater bodies which in turn can affect both human and animal health.

2.3 Cyanoprokaryotes and public health

Cyanoprokaryotes are one group of organisms included as phytoplankton and are regarded as some of the earliest inhabitants on earth (Schopf and Packer 1987). These microscopic photosynthetic organisms occur globally and in all types of natural water systems. Early fossils from the Cambrian era suggested that cyanoprokaryotes played a role in bringing about the early oxygenation of the earth's atmosphere (McCarthy and Orchard 2007; Lee 1999). Some cyanoprokaryote species are capable of producing a range of potent toxins known as cyanotoxins (Carmichael *et al.* 1988), which can become a potential health hazard in drinking water supplies. The best known and most extensively studied of these toxins responsible for human injury are microcystins and cylindrospermopsins (Falconer and Humpage 2006). *Microcystis aeruginosa* has the potential to form blooms and thick surface scums as well as to produce the hepatotoxin microcystin, which can be a human, livestock and native animal health hazard (Codd *et al.* 1999). Human illness has also been attributed to the presence of cyanoprokaryote toxins in drinking water. Internationally, the first documented human deaths from these toxins occurred in February 1996, in a renal dialysis clinic in Caruaru, Brazil, where 52 patients died following intravenous exposure to a combination of microcystins and cylindrospermopsins passing through clinic filters (Carmichael 2001; Carmichael *et al.* 2001; Jochimsen 1998). Neurotoxicity caused by Australian cyanoprokaryotes has been associated to date with *A. circinalis* which has been shown to produce paralytic shellfish poison (PSP) (Negri and Jones 1995; Baker and Humpage 1994).

The number of cyanoprokaryote species that have been identified as potentially toxic and the diversity of toxic compounds produced by cyanoprokaryotes continue to expand both in Australia and internationally. Recently, for example, Bernard *et al.* (2011) reported the toxicity of a strain of a *Limnothrix*/*Geitlerinema* cyanoprokaryote (strain AC0243). This strain was found to have toxic effects, although it did not produce any of the known

cyanotoxins (Humpage *et al.* 2012). *Limnothrix* strain AC0243 was acutely toxic to mice, affecting the liver, kidneys and gastrointestinal tract (Humpage *et al.* 2012). These results suggest that *Limnothrix* AC0243 is capable of producing a novel water-soluble toxin. This new toxic metabolite, Limnothrixin is pH and temperature stable and highly soluble in water (Whan 2012), and could therefore pose a public health risk for drinking water supplies. The chemical properties of this toxin are currently under investigation and the producing organism, *Limnothrix* strain ACO243, is also under scrutiny. Laboratory trials have shown the species to be able to grow at temperatures between 7ºC and 55ºC; with increased growth above 25ºC and optimal growth at 35ºC (Daniels 2012 unpublished data). Daniels found that *Limnothrix* (strain ACO243) was highly adaptable with the potential to grow in pipelines, hot springs and estuaries (Daniels 2012 unpublished data).

2.4 Cylindrospermopsin

Cylindrospermopsin is a cytotoxic protein-synthesis inhibitor that is produced by a small number of freshwater cyanoprokarotes: *C. raciborskii* (Ohtani *et al.* 1992); *Umezakia natans* (Harada *et al.* 1994); *A. ovalisporum* (Shaw *et al.* 1999; Banker *et al.* 1997); *Anabaena bergii* (Fergusson and Saint 2000); *Raphidiopsis curvata* (Li *et al.* 2001); *Aphanizomenon flos-aquae* (Preuβel *et al.* 2006); *Anabaena lapponica* (Spoof *et al.* 2006), *Lyngyba wollei* (Seifert *et al.* 2007), *Oscillatoria* sp. (Mazmouz, *et al.* 2010), *Aphanizomenon aphanizomenoides* (now *Sphaerospermopsis aphanizomenoides* (Zapomělová *et al.* 2009)) (Bittencourt-Oliveira *et al.* 2011) and *Raphidiopsis mediterranea* (McGregor *et al.* 2011). This toxin has been found to be toxic by both ingestion and intraperitoneal injection (Shaw *et al.* 2000; Seawright *et al.* 1999; Ohtani *et al.* 1992) because it affects every cell in the body, especially rapidly growing cells due to its ability to inhibit protein synthesis (Metcalf *et al.* 2004; Froscio *et al.* 2003; Terao *et al.* 1994). Cylindrospermopsin is toxic to liver and kidney

tissue, covalently modifies DNA and RNA, and is a possible carcinogen (Shen *et al.* 2002; Falconer 2001; Humpage *et al.* 2000).

Cylindrospermopsin has been linked with livestock deaths (Saker *et al.* 1999). On Palm Island (Australia) in 1979, 148 people with symptoms of hepatoenteritis linked to CYN were hospitalised (Bourke *et al.* 1983; Blyth 1980). In Australia, this well-known serious case of human poisoning occurred in tropical north Queensland where over 100 children were hospitalised with symptoms of gastroenteritis. This illness was attributable to the CYN, which was found in the drinking water supply after dosing with copper sulphate to control a severe algal bloom (Hawkins 1986; Hawkins 1985; Bourke *et al.* 1983). The patients involved suffered severe hepatoenteritis and renal tubular damage with many requiring intravenous therapy (Hawkins *et al.* 1985).The organism responsible for this particular episode was *C. raciborskii* (Hawkins *et al.* 1985). This species was historically a tropical cyanoprokaryote occurring in a wide range of water bodies worldwide (Padisák 1997). However, it is now being detected in temperate regions globally, including Australia, Europe, North and South America and New Zealand (Barone *et al.* 2010; Messineo *et al.* 2010; Kling 2009; Hamilton *et al.* 2005; Griffiths and Saker 2003; Neilan *et al.* 2003; Stirling and Quilliam 2001). The increasing global distribution of CYN-producers, especially *C. raciborskii*, poses problems for water managers worldwide.

A naturally occurring variant or analog of CYN is deoxy-CYN (Falconer and Humpage 2006; Norris *et al.* 1999). Isolation and identification of CYN and deoxy-CYN from a Thailand strain of *C. raciborskii* showed that the deoxy-CYN was present at about one tenth of the CYN concentration, but the toxicity of deoxy-CYN was not investigated (Li *et al.* 2001). Earlier research by Norris *et al.* (1999) suggested that deoxy-CYN did not contribute significantly to the toxicity of *C. raciborskii* and a study of both CYN and deoxy-CYN from *Raphidiopsis curvata* in China also supported that conclusion (Li *et al.* 2001). However, other trials showing protein-synthesis inhibition have challenged this (Looper *et al.* 2005;

Terao *et al.* 1994). Recent study of the production of CYN and deoxy-CYN by the benthic *L. wollei* showed that deoxy-CYN was the dominant compound produced, being some ten to 300 times higher in concentration than CYN (Seifert *et al.* 2007). Those results suggested that the toxicity of CYN and deoxy-CYN should be considered equally and that more research is needed to establish toxicity of deoxy-CYN (Seifert *et al.* 2007). Deoxy-CYN has also been extracted, concentrated, purified and quantified from field-harvested *L. wollei* (Stewart *et al.* 2012). In that study, there were no signs of acute toxicity in mice injected with this toxin and the tissue histology was unremarkable, although CYN dosed mice in the same experiment displayed changes typical of acute cylindrospermopsin intoxication (Stewart *et al.* 2012).

Wide ranging monitoring of the toxin content in drinking water reservoirs contaminated with blooms of *C. raciborskii* has been carried out in Queensland, Australia (McGregor and Fabbro 2000). Cylindrospermopsin has also been detected in a number of regions around the world such as Florida (Burns *et al.* 2000), Germany (Fastner *et al.* 2003), Northwest Germany (Fastner *et al.* 2007) and Brazil (Carmichael 2001). The presence of cyanoprokaryote toxins such as CYN in many different water supplies highlights serious health concerns and the need for appropriate treatment of water for drinking (Falconer and Humpage 2006).

Significant research has been undertaken on CYN since the Palm Island incident. A study of CYN production in laboratory cultures of *C. raciborskii* showed that 20ºC was the optimum temperature for CYN concentrations, whereas the temperature for optimum growth of the filaments was 30ºC (Griffiths and Saker 2003). Studies with *C. raciborskii* indicated that 90% of the toxin remains within the cells during the early phase of active growth, whereas, as the bloom ages, extracellular CYN can increase up to 50% (Saker and Griffiths 2000). A study by Chiswell *et al.* (1999), found that 70–98% of toxins from a bloom of an ageing *C.*

raciborskii population was dissolved in the water column. This extracellular toxin poses management issues as it remains in the water column as the cell count decreases.

A thick brown surface scum was observed in one study with a CYN-producing *A. ovalisporum* bloom in newly constructed lakes in Hervey Bay, Queensland (Shaw *et al.* 1999). McGregor and Fabbro (2000) reported a scum in Cania Dam (also in Queensland) in 1998 when *C. raciborskii* cell concentrations exceeded 600,000 cells mL^{-1}. This was unusual since *C. raciborskii* rarely forms scums, and there is no taste or odour connected with toxin production (McGregor and Fabbro 2000). This increases the potential risk to human health and animals when water is affected by CYN, since blooms and toxin production may go undetected. This is particularly the case if managers don't fully appreciate the diversity of forms and the difficulty in distinguishing toxin-producing blooms.

2.5 Degradation of CYN

Details of the degradation or biodegradation of CYN and deoxy-CYN through a vertical depth profile in a stratified water body have not yet been described in the scientific literature. Degradation of the toxins at depth, with low light and low oxygen concentrations, may be slower than in the photic zone and possibly allow toxin accrual. Chiswell *et al.* (1999) found that CYN is stable in the dark and is degraded by sunlight in natural waters, but that the degree of degradation is affected by both turbidity and the depth of the photic zone. Changes in pH and boiling of the toxin did not influence degradation (Chiswell *et al.* 1999). Studies on the role of microbial activity in accelerating degradation are few, with previous work being focused on microcystin rather than CYN, such as the research by Lemes *et al.* (2007) where microcystin was found to be degraded by a bacterium from the genus *Burkholderia* (Lemes *et al.* 2007).

Cylindrospermopsin produced by *A. ovalisporum* was not degraded by co-occurring natural bacteria during a 40 day trial (Wormer *et al.* 2008). In contrast, biodegradation of CYN was investigated in Australia and it was found that the toxin was biodegraded in water supplies that had a history of *C. raciborskii* blooms (Smith *et al.* 2008). There was a lag period prior to the start of biodegradation, and repeated exposure to CYN (from consecutive blooms) resulted in a considerable decrease in the lag period. The initial concentration of CYN influenced biodegradation with a near linear relationship ($R^2 = 0.9549$) existing between the biodegradation rate and the initial CYN degradation. This infers that if CYN is an inducer, then a minimum concentration of CYN would be required to activate the genes involved in biodegradation (Smith *et al.* 2008). Smith *et al.* (2008) suggested that the observed lag periods may be due to the time required for the degrading organisms to reach a specific concentration, and hence produce sufficient quantities of the enzymes required to degrade CYN. The optimum temperature for biodegradation was found to be between 25°C and 30°C. These authors also found that copper-based algaecides were detrimental, not only because they lyse the cyanoprokaryote cells and release extra quantities of metabolites including toxins into the surrounding water, but also because they inhibit the biodegradation of these metabolites, most likely due to inactivation of the degrading organisms or their enzymes (Smith *et al.* 2008).

2.6 Methods of CYN detection

In the early phases of experimentation to understand the mode of action of CYN, toxin analyses for CYN from *C. raciborskii* were performed using mouse bioassay. Pure cultures of the cyanoprokaryote were grown under laboratory conditions and the extract injected into three-month old white mice and the toxicity of this extract at various concentrations assessed by autopsy (Hawkins *et al.* 1985). However, the use of live animals for research has been a contentious issue for many years. This, coupled with the fact that mouse bioassay is time

consuming and therefore expensive, has favoured the development of a faster and more economical method of CYN detection and measurement. Chemical analysis using High Pressure Liquid Chromatography (HPLC) was originally pioneered by Ohtani *et al.* (1992) and has been further refined (Banker *et al.* 1997; Hawkins *et al.* 1997).

Chemical analysis was further improved using HPLC/electrospray ionisation mass spectrometry (Eaglesham *et al.* 1999). This made the preparation simpler, with a reduction in time and cost, and enabled rapid analysis with excellent sensitivity. This is currently the method of choice for water management facilities; however, there are several alternative methods available. The use of HPLC with photodiode array detection has been developed for quick and inexpensive detection of CYN in bloom samples dominated by *C. raciborskii* (Welker *et al.* 2002a). Further refinement of this method using HPLC photodiode-array detection has allowed for improved concentration and detection capabilities (Metcalf *et al.* 2002).

Enzyme-linked immunosorbent assay (ELISA) uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid or wet sample. ELISA kits provide a low cost, semi-quantitative analytical method of measuring microcystins, the most common toxin released by cyanobacteria (Carmichael and An 1999), as well as saxitoxin and CYN (Abraxis LLC, Warminster, PA, USA).

2.7 Polymerase Chain Reaction (PCR) technique

Traditional morphological taxonomy methods are unable to differentiate between toxic and non-toxic strains of cyanoprokaryotes. Consequently, the identification of a potentially toxin-producing species generally requires chemical or immune-based, enzyme-linked methods to detect the presence of toxin (Welker *et al.* 2002a; Eaglesham *et al.* 1999). An example of these techniques is Matrix-assisted laser desorption/ionisation (MALDI-TOF) mass spectrometry (Welker *et al.* 2002b) which is an ionisation technique used to analyse

fragile biomolecules such as DNA, proteins and peptides as they can fragment under more conventional ionisation methods. Use of these types of techniques involves considerable sample preparation and specialised instrumentation, resulting in high costs and long turn-around times due to both transport and handling.

Molecular technologies have been used to identify toxin producing genes in environmental samples. Polymerase chain reaction (PCR) methods have been used mainly for qualitative testing; identifying the presence or absence of particular species or toxin-producers (Monis *et al.* 2012). Cyanoprokaryotes produce a chemically diverse array of peptides and polyketides called secondary metabolites when normal growth ceases (Carmichael, 1992). Cyanotoxins such as CYN are examples of harmful secondary metabolites. Polyketide synthase (PKS) and peptide synthase (PS) are enzymes involved in biosynthesis of secondary metabolites (Schembri *et al*. 2001). Schembri *et al*. (2001) demonstrated a potential correlation between the presence of the *pks* and *ps* genes and the ability of *C. raciborskii* to produce CYN. These genes were also reported from CYN-producing *A. ovalisporum* from Israel (Shalev-Alon *et al.* 2002) and also from both *C. raciborskii* and *S. aphanizomenoides* from Brazil (Bittencourt-Oliveira *et al.* 2011) as well as from *C. raciborskii* from reservoirs on Kinmen Island, Taiwan (Marbun *et al.* 2012) and Vela Lake, Portugal (Moreira *et al.* 2011). Mihali *et al.* (2008) characterised the full gene cluster responsible for CYN production in *C. raciborskii*, determining that the enzyme family PKS (AoaC) is derived from the gene *cyrC*.

Using conventional gel PCR, Fergusson and Saint (2003) developed a multiplex PCR assay combining the *pks* and *ps* genes with the species-specific *C. raciborskii rpoC1* (chloroplast RNA polymerase) gene assay developed by Wilson *et al.* (2000). Fergusson and Saint (2003) screened 39 isolates representing three species known to potentially produce CYN: *C. raciborskii*, *A. ovalisporum* and *Anabaena bergii*. They found that if *pks/ps* genes were

present, they produced toxin. Conversely if they were shown to be negative for *pks/ps* gene sequences, they were isolates known not to produce toxin.

The monitoring of environmental blooms using conventional PCR in Australia was demonstrated by Baker *et al.* (2002) for the detection of toxigenic blooms of *Microcystis* and *Anabaena* strains during the summer of 2000 to 2001. Using their assay, they were able to record changes in toxicity over the course of the bloom in a more effective way than microscopy and mouse bioassay and therefore able to enhance the management of a significant public health hazard.

In Spain, simultaneous detection of microcystins and CYN in mixed populations of cyanobacteria was described by Barón-Sola *et al.* (2012). These authors designed various primer sets using *mcy* and *aoa* gene sequences related with microcystin and CYN synthesis respectively and indicated that their method could be applied to environmental samples, allowing a rapid, economical and simple way to detect the presence simultaneously of CYN and microcystin producing cyanobacteria.

Real-time PCR provides a faster and more economical method than conventional PCR (Rinta-Kanto *et al.* 2005; Kurmayer and Kutzenberger 2003; Higuchi *et al.* 1992), as it has the capability to detect and enumerate low gene copies in a closed tube format (Pearson and Neilan 2008). Rasmussen *et al.* (2008) developed a quantitative real-time PCR method using TaqMan probes to detect the *rpoC1* and *pks* genes. In field samples, this assay showed a good correlation between quantification of the species-specific *rpoC1* gene PCR assay for *C. raciborskii* with microscopy-based cell counts.

Field samples from Vela Lake in Portugal were analysed using real-time PCR methodology to quantify the variation of specific genetic markers with primers previously described

characterizing total cyanobacteria (16S rRNA), *C. raciborskii* (*rpoC1*), and cylindrospermopsin synthetase gene (*pks*) (Moreira *et al.* 2011). These authors were able to obtain a higher sensitivity limit of only a few cells mL^{-1} compared to that of 1,000 cell mL^{-1} for *C. raciborskii* previously described by Rasmussen *et al* (2008). They believed this was due to the higher annealing conditions of 55 °C rather than with the 45 °C as reported by Rasmussen *et al* (2008). Moreira *et al.* (2011) found that real-time PCR proved to be a valuable tool in monitoring *C. raciborskii* population distribution and seasonal dynamics at Vela Lake and validated it as a tool to be used in an ecological approach and toxicity evaluation for *C. raciborskii* and cyanobacteria in any given environment.

Orr *et al.* (2010) found significant correlation between the presence of intracellular CYN's and the presence of the *cyrC* gene described by Mihali *et al*. (2008), on samples from three subtropical Australian reservoirs, which would suggest that the *cyrC* gene could be used to screen bloom material. In contrast to previous work, Orr *et al.* (2010), found no correlation between the *rpoC1* gene and *C. raciborskii* cell counts. They also found that real-time PCR was not an effective method for quantitating the gene cell counts of the 16S rRNA gene as an alternative to total cell counts (Orr *et al.* 2010).

Importantly, using cultures, Monis *et al.* (2012) found that *A. circinalis, C. raciborskii* and *M. aeruginosa* always contained multiple copies of the genome. The number of genomes per cell was affected by growth cycles, with the highest number of genomes per cell observed in late log phase of growth/early stationary phase (Monis *et al.* 2012). This suggests that the number of genomes per cell can vary depending on the growth status of the cells. This is only for a narrow window in the growth cycle but does pose a question over the capacity for DNA-based molecular methods to completely replace the conventional cell count method of determining relative toxicity. Environmental populations are more likely to be comprised of cells at various stages in the growth cycle as opposed to controlled laboratory cultures (Monis *et al*., 2012) and therefore more prone to growth cycle variations.

Al-Tebrineh *et al.* (2012a) describe a multiplex assay to detect four gene sequences using fluorescent Taqman probes in a real-time format. The single tube reaction was developed for the detection and quantification of the cyanotoxin biosynthesis genes for the four toxin classes: microcystin (*mcy*), nodularin (*nda*), cylindrospermopsin (*cyr*), and saxitoxin (*sxt*). The development of this assay suggests that there is potential for the rapid assessment of complex bloom samples as it could be used for continuous high-throughput and cost effective monitoring of drinking and recreational water supplies. The same research team were able to use this assay in environmental samples from the Murray River, Australia, involving a cyanobacterial bloom of *A. circinalis, Microcystis flos-aquae* and *C. raciborskii* (Al-Tebrineh *et al.* 2012b). By using this multiplex real-time format, it was possible to determine the bloom's potential toxicity, and water management authorities had access to a rapid risk assessment protocol. This allowed for early implementation of control strategies to avoid human and livestock exposure to the problematic cyanobacteria in one of Australia's major river systems (Al-Tebrineh *et al.* 2012b).

Researchers elsewhere have had similar success in using real-time PCR to monitor toxigenic algae blooms. Marbun *et al.* (2012) performed on-site testing of reservoir samples from Kinmen Island in Taiwan, being able to quantify *C. raciborskii* and detect cylindrospermopsin-producers within 2 h after sampling with a detection limit at about 1,000 cells mL^{-1}. They used the same genetic determinants, namely the *rpoC1* gene for *C. raciborskii* detection and *pks* for CYN producer detection used in the present study.

An alternative to probe based detection using real-time equipment is melt-curve analysis, involving the use of fluorescent double-stranded DNA-specific intercalating dyes first described by Higuchi et al. (1992). A relatively small number of dyes have been used, with SYBR Green I being the most popular (Wittwer *et al*.1997). Monis *et al.* (2005) describe the use of SYTO 9 in preference to SYBR Green I, because STYO9 has better reproducibility and lower inhibition which would make SYTO9 useful in a diagnostic context.

2.8 ANZECC guidelines

The Australian and New Zealand Environment Conservation Council (ANZECC) published the revised Australian and New Zealand Guidelines for Fresh and Marine Water Quality in 2000. They are referred to as the ANZECC guidelines and provide a framework for water quality in Australian and New Zealand rivers, lakes, estuaries and marine waters. The ANZECC guidelines provide trigger values for a variety of physical and chemical parameters based on the region, water body type, ecosystem condition and water quality objectives (ANZECC, 2000). These guideline trigger values may indicate at what point a water body may be susceptible to algal blooms.

2.9 NHMRC drinking water guidelines (cyanoprokaryotes)

Monitoring programs are generally based on the microscopic detection of cyanoprokaryotes in both drinking and recreational water supplies. These cell counts and biovolume measurements are more economical and quicker than toxin analyses. Toxin testing is usually implemented once a trigger or guideline cell count/biovolume is reached. There are a number of different toxins produced by a variety of cyanoprokaryotes in Australia and these are listed in the Australian Drinking Water Guidelines published by the National Health and Medical Research Council (NHMRC 2004) (Table 2.1).

Table 2.1. List of known cyanotoxin producing cyanoprokaryotes in Australian waters, from the Australian Drinking Water Guidelines (NHMRC, 2004).

Cyanoprokaryote	Toxin
Cylindrospermopsis raciborskii *Aphanizomenon ovalisporum* *Aphanizomenon flos-aquae* *Raphidiopsis curvata* *Umezakia natans*	Cylindrospermopsins (*Cylindrospermopsis raciborskii* is the dominant toxin producer in Australia).
Microcystis *Anabaena* *Planktothrix (Oscillatoria)* *Nostoc* *Anabaenopsis* *Radiocystis*	Microcystins (*Microcystis* spp., particularly *Microcystis aeruginosa* are the dominant toxin producers in Australia).
Nodularia spumigena	Nodularins
Anabaena *Lyngbya* *Oscillatoria* *Cylindrospermopsis* *Cylindrospermum* *Aphanizomenon*	Saxitoxins, anatoxin-a and anatoxin-a (s). (*Anabaena circinalis* is the dominant toxin producer in Australia).

The World Health Organization (WHO) Guidelines also set international standards for drinking water quality regulation (WHO, 1998). The international guidelines for cyanobacterial toxins are presently under review by the WHO; however, an accepted standard is not currently in place for most potentially toxic species, including CYN producers, and the guidelines for microcystin are accepted instead as an interim reference guide for other taxa (WHO, 1998). The WHO microcystin standard is 1.0 µg L^{-1} (WHO, 1998), and is restricted to the single compound microcystin-LR which is the most widespread variant in geographic terms (Nicholson and Burch 2001). Toxicologically, microcystin-LR is the best characterised of this group of hepatotoxins, of which there are more than 75 structural types (Nicholson and Burch 2001).

The National (Australian) Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Waters (NPMC Monitoring Protocol) recommends an Alert Levels Framework that describes a monitoring and management action plan to be implemented when a potentially toxic cyanoprokaryote bloom is detected in drinking water supplies (Jones *et al.* 2002). This protocol involves cell counts combined with toxin analyses. The 2002 protocol was updated to include biovolume measurements (Newcombe *et al.* 2010). The revised protocol is based on cell concentrations of *M. aeruginosa* plus biovolumes of other cyanoprokaryotes detected. The NPMC Monitoring Protocol has been incorporated into the Australian Drinking Water Guidelines (NHMRC 2004).

2.10 NHMRC recreational water guidelines (cyanoprokaryotes)

As well as drinking water supplies, recreational water bodies are also a potential health hazard because freshwater cyanoprokaryotes under bloom conditions are capable of producing potent toxins that cause specific damage to hepatic or central nervous systems. This usually occurs dermally, orally by ingestion of recreational water, and possibly by inhalation of aerosols (Stewart *et al.* 2006).

The WHO suggested a three level alert guideline for recreational water (WHO 2003). The Australian Recreational Water Guidelines recommend a similar alert framework based on the cell concentration and biovolume of *M. aeruginosa* (NHMRC 2008). In summary, these guidelines state that an Australian freshwater body being used for recreational purposes should not contain ≥ 10 µg L^{-1} total microcystins, $\geq 50{,}000$ cells mL^{-1} toxic *M. aeruginosa*, or a biovolume ≥ 4.0 mm^3 L^{-1} total of all cyanoprokaryotes where a dominant known toxin-producer is present. If there is no known toxin-producer present, a biovolume ≥ 10 mm^3 L^{-1} of total cyanoprokaryotes should be used. There should not be any cyanoprokaryote scums visible.

2.11 *Cylindrospermopsis raciborskii* – CYN-producer

Cylindrospermopsis raciborskii occurs in a wide range of water bodies worldwide (Padisák 1997). In tropical Australia, *C. raciborskii* produces high cell concentrations in waters with high pH (≥ 8.1), warm surface water temperatures (28–32°C), a stable water column and long retention times (McGregor and Fabbro 2000; Boland and Griffiths 1996; Branco and Senna 1994). *Cylindrospermopsis raciborskii* often forms maximum cell concentrations below the surface (Fabbro and Duivenvoorden 1996), which contrasts with the scum formation and surface cell accumulation normally associated with other cyanoprokaryotes such as *Microcystis* spp. (White *et al.* 2003) and *A. circinalis* (Bormans *et al.*1997).

Due to the fact that *C. raciborskii* does not often form surface scums and that CYN is readily released from the cells, simply removing the intact filaments from the water will not necessarily remove the toxins.

Cylindrospermopsis raciborskii has generally been recorded in warmer tropical and subtropical environments but it appears to be spreading into cooler areas (Sinha *et al.* 2012; Saker and Griffiths 2000; Padisák 1997). Allelopathy, where one organism produces biochemicals that may influence the growth, survival, and reproduction of other organisms, has been suggested as a reason for the dominance and spread of *C. raciborskii* with long lasting blooms in cooler zones (Figueredo *et al.* 2007). Figueredo *et al.* suggested that this ecological dominance might be explained by antagonistic interaction with other phytoplankton species due to production of allelopathic metabolites which showed inhibitory effects on the other phytoplankton photosynthetic activities. These authors also indicated that this potential allelopathic advantage could also explain the geographic expansion of this species at midlatitudes (Figueredo *et al.* 2007). Also, global warming is increasing human risk by allowing the spread of traditionally tropical species into cooler regions (O'Neil *et al.* 2012; Paerl *et al.* 2011; Paerl and Huisman 2009; Wiedner *et al.* 2007; Codd *et al.* 2005;

Briand *et al.* 2004). A detailed investigation of the roles that eutrophication and climate change may be having on harmful cyanobacteria blooms has been reported (O'Neil *et al.* 2012). O'Neil *et al.* reviewed the relationships between eutrophication, climate change and representative cyanobacterial genera from freshwater, estuarine and marine ecosystems. These authors found that while the interactive effects of future eutrophication and climate change on harmful cyanobacterial blooms are complex, much of the current research suggests these conditions are likely to enhance the size and frequency of such blooms. A more species specific investigation involving climate change has addressed the increased incidence of *C. raciborskii* in temperate zones worldwide (Sinha *et al.* 2012). Sinha *et al.* found that the occurrence and domination of *C. raciborskii*, particularly in temperate regions, has increased and that this expansion has been assisted by a number of changing conditions in these environments. Sinha *et al.* suggest the geographical expansion of both the organism and toxin production can be attributed to conditions such as eutrophication and climate change. With the development of greater awareness both in Australia and globally regarding the health hazards of cyanoprokaryote toxins, more extensive monitoring is being implemented and hence more species, including *C. raciborskii,* are being detected.

2.12 *Aphanizomenon ovalisporum* – CYN-producer

Aphanizonemon ovalisporum was first identified as a CYN producer in Lake Kinneret, Israel (Banker *et al.* 1997). Blooms have occurred in conjunction with surface temperatures between 26ºC and 30ºC, high pH (≥ 8.7), low light, high phosphorus availability (Hadas *et al.* 2002), high dissolved organic nitrogen availability (Berman 2001; Berman 1997), high concentrations of biologically available iron (Parparova and Yacobi 1998) and low dissolved selenium (Nishri *et al.* 1999). The first documentation of *A. ovalisporum* in Australia was from newly constructed lakes at Hervey Bay, Queensland and reported a toxic bloom with an accompanying thick brown surface scum (Shaw *et al.* 1999). The conditions in the lakes

were slightly saline with a moderate level of hardness and a high level of nutrients (Shaw *et al.* 1999). *Aphanizomenon ovalisporum* has also been detected in algal blooms in Italy (Bazzichelli and Abdelahad 1994), Greece (Gkelis *et al.* 2005) and Spain (Quesada *et al.* 2005).

2.13 Phytoplankton studies in the Tweed Catchment.

Historical data from routine monitoring indicate that cyanoprokaryote blooms have occurred in Clarrie Hall Dam and the Tweed River at Bray Park (unpublished data) but there have been no reports in the literature of phytoplankton studies and toxic algal blooms in the Tweed Shire. In 2010 an investigation to examine the source of elevated manganese levels within the reticulated water distribution system in the Tweed Shire was conducted by Hunter Water Australia (Hunter Water, 2011). The source of the elevated manganese concentrations was traced to Clarrie Hall Dam and consequently downstream via the Tweed River to the uptake point for processing at Bray Park. Hunter Water (2011) reviewed historical data recorded from routine monitoring of the catchment between 1997 and 2011 and assessed the efficiency of the mechanical mixer installed in the dam in 2002 and its effect on manganese concentrations as well as nutrient levels and cyanoprokarote concentrations.

A study in nearby south eastern Queensland was carried out on seven subtropical reservoirs (Burford *et al.* 2007). This study suggested that the watershed (urban/agricultural land use and forestry buffer zones) as well as the reservoir characteristics including volume and depth, have an effect on the type of algal blooms that may develop (Burford *et al.* 2007). The dominance and management of *C. raciborskii* in North Pine Dam, a subtropical reservoir in south eastern Queensland was also reported (Burford and Davis 2011; Burford *et al.* 2006; Burford and O'Donohue 2006; Antenucci *et al.* 2005). Earlier, McGregor and Fabbro (2000) conducted an extensive study of 47 reservoirs and weir pools throughout Queensland,

including several water bodies located in the subtropical south eastern corner of the state. This research concentrated on *C. raciborskii* and found that its dominance was due to a variety of factors including high pH, high temperature, long residence time and a thermally stratified water column. They also found that cell concentrations of approximately 20,000 cells mL^{-1} generally produced toxin concentrations of 1.0 µg L^{-1} and recommended that this cell count be used as a trigger point at which toxin monitoring be commenced. A study was also carried out on 26 freshwater coastal dune lakes in South-east Queensland, including those on Fraser Island, Moreton Island, and North Stradbroke Island and also in the Cooloola sand mass (Bowling 1988). The optical properties, nutrients and phytoplankton concentrations were studied. However, these lakes were acidic and low in nutrients and conductivity (Bowling 1988). Consequently, although they are situated in a similar climatic region to the water bodies of the Tweed Catchment, they have vastly different chemical and physical properties.

Lake Ainsworth, a coastal dune lake situated at Lennox Head, near Ballina on the far north coast of New South Wales, was the subject of a study involving the diatom record of a sediment core to track the marine history of the now freshwater lake (Tibby *et al.* 2008). The diatom record was compared with local knowledge of how and when the lake was joined to the ocean (Tibby *et al.* 2008). Lake Ainsworth is a popular recreational lake and has a history of potentially toxic cyanoprokaryote blooms (*A. circinalis*) in the summer months (Plumb, 2009. personal communication). This lake is in a similar climatic region to the Tweed Shire, but has very different natural properties to the water bodies of the Tweed Catchment. A sixteen year study of the phytoplankton biomass in North Pine Dam near Brisbane indicated that this subtropical reservoir is characterised by strong variability in rainfall with intermittent rain storms causing partial turnovers and large outflows which strongly affect the phytoplankton assemblage (Harris and Baxter 1996). The subtropical climate, combined with a highly variable rainfall pattern and large outflows after heavy rain, is similar to that experienced in the Tweed Shire. However, the Clarrie Hall Dam in the

Tweed Shire is considerably smaller and cooler than the North Pine Dam and partial turnovers are not common. Hence it would be expected that different phytoplankton assemblage and succession patterns would be found.

3 MATERIALS and METHODS

3.1 Sampling Sites

Three sampling sites were selected for this study: Cobaki Village Lake; the Clarrie Hall Dam; and the Tweed River at Bray Park (Table 3.1). The sampling commenced on 5th January 2007 and finished on 22nd August 2008.

Table 3.1. Key characteristics of the three Tweed Shire monitoring sites used in this study.

	Cobaki Village Lake	**Clarrie Hall Dam**	**Tweed River at Bray Park (Pump station/weir)**
Latitude	28 18º S	28 34º S	28 34º S
Longitude	153 49º E	153 22º E	153 38º E
Elevation (m)	4	120	8
Surface area (ha)	2.6	340	35.5
Max depth (m)	19	20	4
Average depth (m)	17	20	4
Construction date	1994	1982	1964
Pre-development state	Coastal scrubland	Creek	River
Storage capacity (ML)	Est. 2,000	15,500	838.5
Percent retained	100 100 Approximate only. Data not recorded	2007 av. 98.4 2008 av. 86.2	30 0 Approximate only. Data not recorded
Mixing type	Monomictic	Monomictic	Flowing

3.1.1 Cobaki Village Lake

Cobaki Village Lake (Cobaki Lake) is a small, stratified, man-made freshwater lake with an average depth of 17 m (Figure 3.1), constructed in 1994 as a focal point for a retirement village. The lake is self-contained, approximately 2.6 ha in area, with no input from creeks, springs, or rivers. The only surface water to enter the lake is from rainwater and storm water runoff from the retirement village and surrounding parkland. At times of high rainfall, the excess water is released into Cobaki Creek via a series of one way locks. Cobaki Creek is a small estuarine waterway that feeds into Terranora Inlet, which in turn is part of the main Tweed River waterway. Cobaki Lake is stocked with *Bidyanus bidyanus* (silver perch) and *Macquaria novemaculeata* (Australian bass) and is used for recreational purposes, including swimming, as well as a source of water to irrigate a community vegetable garden and individual gardens throughout the village. The lake bottom slopes down towards the centre. Monthly depth profiles were taken from the centre of the lake and details of the sampling program carried out at this site are outlined in Table 3.2. At times when cyanoprokaryote populations reach bloom proportions, weekly algal samples were taken from sites around the perimeter of the lake. These samples were taken at approximately 0.5 m depth and provided an indication of the algal populations present in the lake. When there were no obvious cyanoprokaryote blooms present in the lake these sites were sampled monthly (concurrently with the depth profiles) (Table 3.2).

Figure 3.1. Cobaki Lake showing the position of the sampling sites (Tweed Shire Council, 2007).

Table 3.2. Overview of the sampling program implemented at Cobaki Lake.

Sampling sites	Lake Centre vertical depth profiles Site 1. SE corner pump station 0.5 m grab sample Site 2. Western side 0.5 m grab sample Site 3. NW corner jetty 0.5 m grab sample Site 5. Eastern side 0.5 m grab sample Site 7. NE corner 0.5 m grab sample
Sampling frequency	Monthly for 20 months Weekly 0.5 m grab samples during blooms (> 5,000 cells mL^{-1})
Phytoplankton	Algal ID and enumeration of all samples
Field data	pH, conductivity (K_{25}), DO, temp, turbidity, Secchi depth
ICP mineral scan	K, Ca, Mg, Na
ICP metal scan	Mo, Se, Si, Mn, Fe, Al
FIA nutrients	TN, TP, NO_x, NH_4N, SRP
Cyanotoxin by HPLC-MS/MS	CYN, deoxy-CYN, Microcystin, Saxitoxin
PCR genetics	*pks* assay, *rpoC1* assay, melt curve

3.1.2 Clarrie Hall Dam

Clarrie Hall Dam (Figure 3.2) was built on Doon Doon Creek, a tributary of the Tweed River, and is the sole drinking water storage facility for the Tweed Shire. When required, water is released from the intake tower through the wet and dry tunnel and cone valve and flows into the Tweed River before travelling to the Bray Park Weir approximately 15 km downstream and then to the river mouth at Tweed Heads. Water is extracted upstream of the Bray Park Weir and treated at the Water Treatment Plant prior to distribution to the reticulated water network. The area of the dam itself is 340 ha at full capacity. Tweed Shire Council owns 926 ha of forested

land (native regrowth) in the catchment area and a State-owned forest also adjoins the dam, with both of these acting as buffer zones for the waterway. To improve water quality, and to deter the formation of algal blooms in the summer months, a destratification unit (mixer) was installed in Clarrie Hall Dam in late January 2002. The aim of the mixer was to eliminate different temperature and oxygen layers within the dam. This destratification system used model SMDI-5 02 (Surface Mounted Destratification Impellers) with a 5 m diameter rotating impeller and 10 m draft tube assembly mounted on a floating platform. It was powered by a 4 kW electric motor. The floating platform was anchored in the dam. The unit circulated up to 10,000 L s^{-1} water. Figure 3.2 is an aerial photograph of the Clarrie Hall Dam showing the sampling sites and the location of the mixer. The intake tower was the drawdown point of the dam, where water was released from the dam into the river. The detailed sampling program is outlined in Table 3.3. Monitoring was carried out monthly; however, this reverted to weekly if cyanoprokaryote counts exceeded 5,000 cells mL-1, indicating that a bloom was imminent, and daily if counts exceeded 50,000 cells mL^{-1} (Table 3.3). Depth profiles were taken each month at site X, which is situated between the spillway and the mixer.

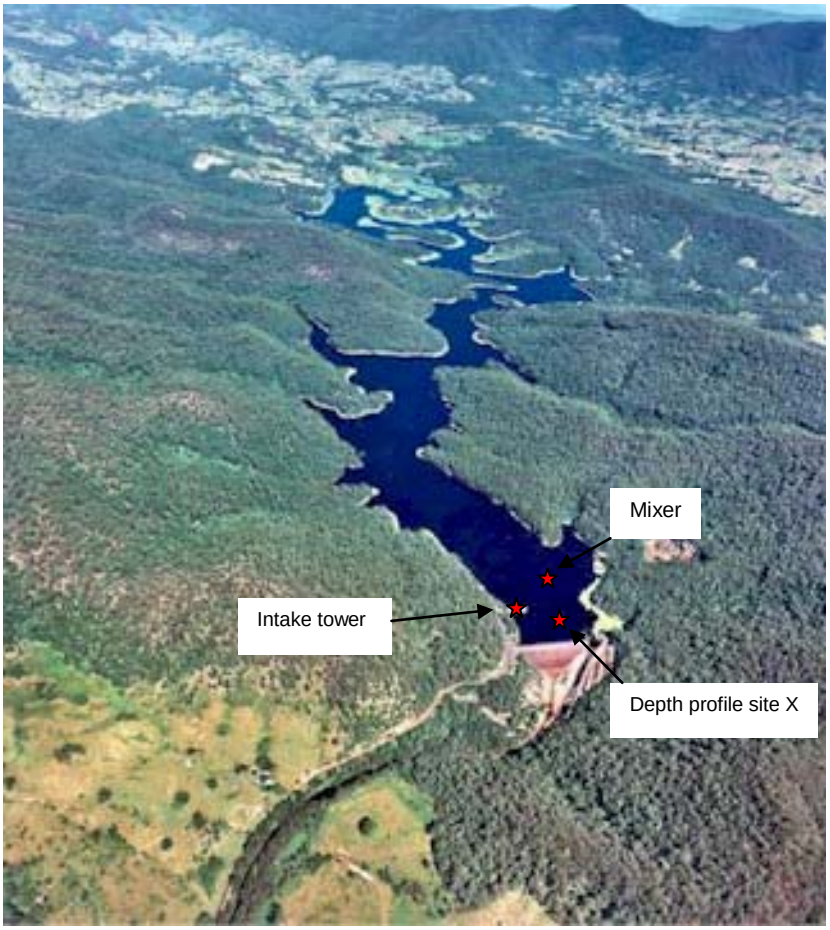


Figure 3.2. Clarrie Hall Dam showing sampling points and mixer location (Tweed Shire Council, 2007).

Table 3.3. Overview of the sampling program implemented at Clarrie Hall Dam.

Sampling sites	Site X (between spillway and mixer) vertical depth profiles Intake Tower – 0.5 m grab samples
Sampling frequency	Monthly for 20 months (all sites) Weekly 0.5 m grab samples during blooms (> 5,000 cells mL^{-1}) Daily 0.5m grab samples during blooms (> 50,000 cells mL^{-1})
Phytoplankton	Algal ID and enumeration of all samples
Field data	pH, conductivity (K_{25}), DO, temp, turbidity
ICP mineral scan	K, Ca, Mg, Na
ICP metal scan	Mo, Se, Si, Mn, Fe, Al
FIA nutrients	TN, TP, NO_x, NH_4N, SRP
Cyanotoxin by HPLC-MS/MS	Microcystin, Saxitoxin, CYN, deoxy-CYN

3.1.3 Tweed River Bray Park Pump Station and Weir

The Tweed River at Bray Park pump station is the drinking water source for the Tweed Shire. The Bray Park water treatment plant is situated approximately 3 km south west of Murwillumbah in northern NSW. Slightly further downstream from the Bray Park pump station (approximately 500 m) is the Bray Park Weir (Figure 3.3). The river at the pump station is normally flowing with an average depth of 4 m in the middle of the channel. In times of hot dry weather and low flow, this part of the river can become impounded and is susceptible to the development of cyanoprokaryote blooms (Tweed Shire Council historical data). Samples were collected monthly from the Bray Park pump station pontoon, including surface and depth samples (at 0.5 m and 4.0 m, respectively). From this point, water is pumped to the water treatment plant for processing and distribution throughout the Tweed Shire reticulation system.

Monitoring was carried out monthly; however, if cyanoprokaryote counts exceeded 5,000 cells mL^{-1}, indicating that a bloom was imminent, weekly monitoring was conducted and if counts exceeded 50,000 cells mL^{-1}, daily monitoring was instigated (Table 3.4).

Figure 3.3. Tweed River, showing location of Bray Park Weir and the Bray Park pump station (Tweed Shire Council, 2007).

Table 3.4. Overview of the sampling program implemented at the Tweed River Bray Park pump station.

Sampling sites	Bray Park pump station pontoon vertical depth profiles Bray Park pump station pontoon 0.5 m grab samples
Sampling frequency	Monthly for 20 months Weekly 0.5 m grab samples during blooms (> 5,000 cells mL^{-1}) Daily 0.5m grab samples during blooms (> 50,000 cells mL^{-1})
Phytoplankton	Algal ID and enumeration of all samples
Field data	pH, conductivity (K_{25}), DO, temp, turbidity
ICP mineral scan	K, Ca, Mg, Na
ICP metal scan	Mo, Se, Si, Mn, Fe, Al
FIA nutrients	TN, TP, NO_x, NH_4N, SRP
Cyanotoxin by HPLC-MS/MS	Saxitoxin

3.2 Methods

Sampling was conducted according to Australian Standard for Water Quality Sampling (AS/NZS5667.1998). All analyses, except cyanotoxin testing, were carried out at the Tweed Laboratory Centre. This facility has NATA accreditation according to ISO/IEC 17025 with Chemical NATA accreditation No. 12754 and Biological NATA accreditation No. 13538. The microbiology laboratory is a containment level 2 facility (under Australian Standard 2243.3-1991). Analyses were carried out using accredited methods such as the relevant Standard Methods APHA, US EPA Guidelines and Australian Standards (refer to summary in Table 3.5). The Flow Injection Analyses (FIA) of the nutrient samples and the inductively coupled plasma-

optical emission spectroscopy (ICP-EOS) metal analyses were performed by experienced technicians with NATA signatory status. The field sampling, algal analyses, species culturing and isolation, and some of the PCR analyses were undertaken by the author, who is a NATA signatory in phycology, bacteriology and field sampling. Extra species culture and isolation (for confirmation and publication) was performed at Queensland Health Forensic and Scientific Services (QHFSS) in Brisbane. Additional PCR analyses (for confirmation and publication purposes) were performed at South Australia Water in Adelaide.

Samples for cyanotoxin analysis were sent to Geoff Eaglesham at QHFSS in Brisbane. Toxin detection is a highly specialised field, and it was deemed necessary for quality assurance purposes that such testing should be carried out in a suitably certified laboratory using internationally approved methodology. The QHFSS laboratory utilises high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) as described in Eaglesham *et al.* (1999).

Table 3.5. Methods summary for analyses undertaken for this project.

Analysis type/parameters measured	**References**	**Equipment/instrumentation**
Phytoplankton identification, enumeration, culture, isolation and PCR		
Algae identification and enumeration	Standard Methods for the Examination of Water and Wastewater (2005) 21st Edition. APHA, AWWA, WEF (Eaton *et al.* 2005) (Hötzel and Croome 1998) (Baker and Fabbro 1999) (Anderson 2005) (Rasmussen *et al.* 2008; Pearson and Neilan 2008)	Sedgewick Rafter Cell; Nikon Eclipse E400 Phase Contrast Microscope.
Cell count (cells mL^{-1}) and biovolume (mm^3 L^{-1})		Eyepiece micrometer; Lugol's preservative.
A. ovalisporum, *C. raciborskii* culture and isolation		Olympus SZ40 inverted microscope; eyelash brush; 12 hr light (25ºC)/18 hr dark (18ºC) chamber.
Chemical field analysis		
pH	Standard Methods (2005) No. 4500 – HB (LoD 0.1)	Auto-titrator TIM870. TPS Data Logger with glass pH electrode.
Specific conductance (K_{25}) (µS cm^{-1})	Standard Methods (2005) No. 2510B (LoD 1.0 µS cm^{-1})	Auto titrator TIM870. TPS Data Logger with EC probe.
Turbidity (NTU)	Standard Methods (2005) No. 2130B (LoD 0.1 NTU)	Eutech Turbidimeter TN-100.
Dissolved Oxygen (mg L^{-1})	Standard Methods (2005) No. 4500 O.C. (LoD 0.1 mg L^{-1})	YSI model 50B DO meter. YSI probe 5905.
Soluble metal scan and nutrient analyses		
Metal scan-ICP (µg mL^{1}) Silica and Selenium -AA (mg L^{-1})	In-house method with reference to Standard Methods (2005) No. 3120 (LoD 10 µg $mL^{-1}$for all metals except silica and selenium). Silica and selenium Standard Methods (2005) No. 3111 (Silica LoD = 0.1 mg L^{-1}) (Selenium L LoD = 0.1 mg L^{-1})	ICP-OES. Inductively Coupled Plasma-Optical Emission Spectrometry. Atomic Absorption Spectrometry.
Oxides of Nitrogen NOx (μg^{-1}) Ammonium nitrogen NH_4N (µg mL^{-1}) Soluble Reactive Phosphorus SRP (µg mL^{-1}) Total Phosphorus TP (µg mL^{-1}) Total Nitrogen TN (µg mL^{-1})	Standard methods (2005) No. 4500 (LoD 50 µg mL^{-1})	Lachat Flow Injection Analyser (FIA).

Cyanotoxins		
Cylindrospermopsin & deoxy-CYN	Eaglesham *et al.* (1999) and Norris *et al.* (1999) (LoD 0.2 µg mL^{-1})	HPLC using Electrospray ionisation and tandem mass spectrometry.
Saxitoxin	Lawrence *et al*. (2005) (LoD 2 µg mL^{-1})	HPLC-fluorescence – peroxide oxidation
Microcystin	QHFSS method (LoD 0.5 µg mL^{-1})	HPLC – solid phase extraction – diode array detection

3.3 Sampling

Sampling was performed according to the Australian Standard 5667-Water Quality Sampling AS/NZS 5667, 1998 between January 5th 2007 and August 22nd 2008. The samples for chemical analyses were collected in polyethylene bottles. Samples for dissolved nutrients were filtered on site through a 0.45 µm cellulose acetate filter (Billerica, MA, USA) into 10 mL polyethylene tubes. Total nutrient samples were sub-sampled directly into 10 mL polyethylene tubes. The nutrient tubes were frozen on return to the laboratory and the samples for metal analysis were acidified with nitric acid (concentrated "Aristar" nitric acid) on collection. All samples collected were transported on ice in insulated cooler boxes for analysis, compliant with ISO/IEC 17025. A TPS 90-FL Field Data Logger (TPS, Brisbane, Australia) was used to record data for both vertical depth profiles and individual grab samples (various sites, at a depth of 0.5 m). Vertical depth profile data readings were logged and samples collected at 1 m intervals using a Van Dorn sampler (manufactured in accordance with APHA design requirements) (Eaton *et al*. 2005). The data logger was calibrated before each field trip and checked on return to the laboratory. Secchi disc depth readings were recorded according to Australian Standard 5667. The euphotic depth (Z_{eu}) was calculated using a formula based on Secchi depth (Kirk 1994). Rainfall data were obtained from Bureau of Meteorology Stations: station number 040717 at Coolangatta Airport for Cobaki Lake, and station number 058158 at Murwillumbah (Bray Park) for the Bray Park Weir and Clarrie Hall Dam sites.

3.4 Chemical Analyses

3.4.1 Nutrient analyses

Soluble nutrient samples were filtered on site, and non-filtered (total) nutrient samples were digested under pressure in an autoclave for 30 min at 15 psi and 120ºC using potassium

persulphate and then analysed for total nitrogen (TN) and total phosphorus (TP). Nutrient samples were analysed with a Lachat Flow Injection Analyzer Quickchem 8000 (Lachat, Milwaukee, WI, USA) (Eaton *et al*., 2005; Lachat Quickchem method 31-115-01-3B "Determination of Phosphate in Brackish Water or seawater"). The Certified Reference Material (CRM) for TP and TN were supplied by supplied by Graham B Jackson (Aust) Pty Ltd, Dandenong, Australia and produced by ERA, a Waters Company (Arvada, CO, USA; an accredited reference material producer). The soluble nutrients CRM were also supplied by Graham B Jackson (Aust) Pty Ltd, Dandenong, Australia and were produced by Sigma-Aldrich RT Corporation (Laramie, WY, USA; another accredited reference material producer).

3.4.1.1 Total Phosphorus (TP)

During digestion, organic and polyphosphates were oxidised or hydrolysed to the orthophosphate ion. The digested samples were run through the FIA analyser where the orthophosphate ion reacted with ammonium molybdate and antimony potassium tartrate under acidic conditions to form an antimony phosphomolybdate complex, which was then reduced with ascorbic acid to form an intensely blue coloured complex with absorbance at 880 nm. This was performed at a slightly elevated temperature (37ºC) to ensure completeness of the reaction before passage through the detector area. The absorbance is proportional to the concentration of orthophosphate in the sample.

3.4.1.2 Total Nitrogen (TN)

During digestion, the various species of nitrogen compounds (excluding N_2 gas) were oxidised to nitrate. The digested samples were then run through the FIA analyser. Here the nitrate was quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column at a pH of 8. The nitrite (reduced nitrite + original nitrite) was then

determined by diazotisation with sulphanilamide under acidic conditions to form a diazonium ion which was coupled to N-(1-Naphthyl) Ethylenediamine Dihydrochloride (NEDD), forming a pink dye with absorbance at 520 nm.

3.4.1.3 Soluble Reactive Phosphorus (SRP)

The filtered samples were run through the FIA analyser where the orthophosphate ions react with ammonium molybdate and antimony potassium tartrate under acidic conditions to form an antimony phosphomolybdate complex which is reduced with ascorbic acid to form an intensely blue complex with absorbance at 880 nm.

3.4.1.4 Ammonium (NH_4N)

This analysis technique is based on the Bertholot reaction. Ammonium reacts in alkaline solution with hypochlorite to form monochloramine at a pH between 8.0 and 11.5 which, in the presence of phenol, catalytic amounts of nitroprusside and excess hypochlorite, forms indophenol blue with absorbance at 630 nm.

3.4.1.5 Nitrate (NO_3N), Nitrite NO_2N) and NO_x (soluble nitrogen)

Nitrate was quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column at pH 8.0. The nitrite (reduced nitrite + original nitrite) was then determined by diazotisation with sulphanilamide under acidic conditions to form a diazonium ion which was coupled with N-(1-Naphthyl) Ethylenediamine Dihydrochloride (NEDD), forming a pink dye with absorbance at 520 nm. For nitrite analysis alone, the cadmium column was taken off line. The oxidized nitrogen (NO_x) was the sum of nitrate plus nitrite.

3.4.2 Metals Analyses

Samples for soluble metal detection were analysed using a GBC Integra XL ICP (ICP-OES) (GBC Scientific Equipment Pty Ltd, Dandenong, Australia) using inductively coupled plasma-optical emission spectroscopy (ICP-EOS) Method No 3120 "Metals by Plasma Emission Spectroscopy" (Eaton *et al.* 2005). Samples for soluble metals analyses were filtered on site using 0.45 µm mixed cellulose ester syringe filters (Billerica, MA, USA) and then acidified using 1% HNO_3. The filtrate was then analysed by ICP-OES. For every ten samples analysed, a blank, control and duplicate were analysed. The duplicate analysis had to conform to the acceptance criteria outlined in the method. The control was a CRM which fell within the 95 percentile confidence value. The CRMs were supplied with an accompanying Certificate of Analysis, lot number and expiry date.

3.4.2.1 Soluble metals suite (ICP-OES)

The GBC Integra XL ICP Spectrometer consists of a flowing stream of argon gas ionised by a free running solid state radio frequency oscillating at 40 MHZ. A sample aerosol is generated using a high precision concentric nebuliser. The aerosol flows into the plasma torch through an injector tube located in the centre of the torch. Temperatures in the plasma range from 2,000ºK to 10,000ºK, but the emission lines of most importance are in the region of the plasma from 6,000ºK to 8,000ºK– called the normal analytical zone. Because this results in almost complete dissociation of molecules, significant reduction in chemical interferences is achieved. The high temperature of the plasma excites atomic emission efficiently. Ionisation of a high percentage of atoms produces ionic emission spectra. The light emitted from the ICP is focused and collected through a purge window onto a computer controlled mirror. This mirror allows the adjustment of viewing height. The signals then pass through a variable width entrance slit which separates the emission lines into orders and

spectra (Eaton *et al.* 2005). All optics are housed in a temperature controlled argon-purged compartment. Spectra in the ultra violet wavelength range (167–375 nm) and the visible range (375–782 nm) are each focused onto separate argon-purged segmented array-charge coupled detectors. These solid state devices allow the measurement of up to 5,000 analytical lines simultaneously, giving the ability to monitor several lines per element. Instrumentation, analysis and signal processing are all controlled via PC based Integra XL5 software.

3.4.2.2 Soluble selenium by Atomic Absorption Spectrometry (AAS)

Samples were analysed for soluble selenium with a Varian Spectra AA600 and GTA100 Furnace (Varian, Mulgrave, Victoria, Australia) using Method No 3111 – "Metals by Atomic Absorption Spectroscopy" (Eaton *et al.* 2005). A discrete sample volume was dispensed into the graphite sample tube on an inserted platform. The determinations were made by heating the sample in three or more stages. Firstly, a low current heats the tube to dry the sample. The second stage destroyed organic matter and volatilised other matrix components at an intermediate temperature. Finally, a high current heated the tube to incandescence and atomised the selenium. Additional stages were frequently added to aid in drying and charring, and to clean and cool the tube between samples. The resultant ground-state atomic vapour absorbed mono-chromatic radiation from the source. A photoelectric detector measured the intensity of transmitted radiation. The inverse of the transmittance was related logarithmically to the absorbance, which is directly proportional to the number density of vaporised ground-state atoms (The Beer-Lambert Law) over a limited concentration range. Electro-thermal atomisation determinations may be subject to significant interferences from molecular absorption as well as chemical and matrix effects. Chemical modifiers generally modify relative volatilities of matrix and metal by enhancing matrix removal and isolating the metal. For selenium determinations, a 2% nickel nitrate modifier was used. During each set of analyses, a blank, external control and duplicate were analysed at the beginning and at the end of each batch. The control was a CRM and was within 95% confidence levels.

3.5 Cyanoprokaryote identification and enumeration

Species-level identification and enumeration of phytoplankton were carried out on Lugol's preserved samples using procedures outlined in Standard Methods for the Examination of Water and Wastewater (Eaton *et al.* 2005), the Phytoplankton Methods Manual for Australian Rivers (Hötzel and Croome 1998) and the Guide to the identification of common blue-green algae (cyanoprokaryotes) in Australian Freshwaters (Baker and Fabbro 1999). Lugol's solution is an acidified iodine solution achieved when 20 g of potassium iodide and 10 g of iodine crystals are dissolved in 200 mL of distilled water containing 20 mL of glacial acetic acid. Samples for algal identification and enumeration were preserved on site with 0.5 mL acidified Lugol's solution per 100 mL of sample and kept in the dark at room temperature in brown polyethylene terephthalate (PET) 50 mL bottles. Samples were analysed within seven days of collection. Identification and enumeration were performed using a glass Sedgwick-Rafter counting chamber (Pyser SGI S50 microlitre, Graticules Ltd, Kent, United Kingdom) and phase contrast microscopy (Nikon Eclipse E400, Nikon Corporation, Tokyo, Japan) with 10x, 20x and 40x long working distance objectives. The glass Sedgwick-Rafter counting chamber holds 1 mL of liquid, is 50 mm long, 20 mm wide and 1 mm deep, and the base is marked with a grid of one thousand 1 mm squares. A cover glass traps liquid to the correct depth. The volume of the Sedgwick-rafter cells is calibrated annually.

Enumeration of individual species was carried out with the aid of a hand-held counter. A minimum of three and a maximum of 200 squares on the Sedgwick-Rafter cell were examined for every sample. Except where 200 squares had already been examined, 50 units (colony or trichome) were counted for each potentially toxic species of cyanoprokaryote. A maximum of 40 squares were examined using 400x magnification to ensure minute

phytoplankton were identified. To enumerate the number of cells within a unit 400x magnification was used. Where picoplankton was concerned, a maximum of 40 squares was examined even if 50 units had not been reached. According to Hötzel and Croome (1998), the 50 unit count achieves ± 20% precision.

The cyanoprokaryote cell dimensions were measured using an ocular micrometer at 400x magnification. The calibrated eyepiece micrometer was used to measure cell size for both identification and for the calculation of cell biovolume. The algal counts were expressed as cells mL^{-1} and biovolume as $mm^3\ L^{-1}$ (Eaton *et al.* 2005; Hötzel and Croome 1999). A minimum of 30 and a maximum of 100 cells were measured per sample.

3.6 Cyanobacterial cell biovolume

The biovolume of specific cyanoprokaryote cells in a sample was calculated by measuring the individual cell dimensions using a calibrated eyepiece micrometer in the phase contrast microscope. A representative number of cells was measured in each sample (a minimum of 30 and a maximum of 100 cells were measured as recommended by Hötzel and Croome (1999)). A computer program based on the "BIOVOL 2.1 program" developed by David Kirschtel, Michigan State University, USA, was used and this involved three basic geometric shapes, namely the sphere, the cylinder and the ellipsoid, and required the measurement of the diameter and/or the length of each cyanoprokaryote cell. Measurements were appropriate for the closest geometric shape and were made to the nearest 0.5 µm. These measurements were used in the "BIOVOL" computer program to calculate an average cell biovolume for each species. The calculated biovolume was multiplied by the cells per mL count which gave the species-specific biovolume in the sample and was expressed as $mm^3\ L^{-1}$ (Eaton *et al.* 2005; Hötzel and Croome 1999).

3.7 Morphology of *A. ovalisporum* and *C. raciborskii*

A study of the morphological changes of both *A. ovalisporum* and *C. raciborskii* cells from Cobaki Lake was carried out during a toxin (CYN) producing bloom. A phase-contrast microscope (Nikon Eclipse E400; Nikon Corporation, Tokyo, Japan), 40x long objective and eye piece micrometer were used. This study included observations of the appearance and dimensions of akinetes, heterocytes, filaments and vegetative cells over time and depth. A minimum of 30 trichomes were examined per sample with a minimum of 30 individual specialised cells measured per sample; the exact number depending on cell concentration. The average cell dimensions were calculated for each sample. The dimensions measured included length and width of trichomes, vegetative cells, akinetes and heterocytes. The prevalence of the specialised cells on each trichome was also determined. Here, prevalence was measured by counting and measuring the number of trichomes, the number and size of vegetative cells and also the number and size of specialised cells (both akinetes and heterocytes).

3.8 Cyanotoxin analyses

All cyanotoxin analyses were carried out at Queensland Health Forensic and Scientific Services in Brisbane. An ultra-sonic probe was used to lyse the cells for all three toxin determination methods. Samples for CYN determination were analysed using a HPLC-MS/MS method, modified from Eaglesham *et al.* (1999). The instrumentation used was an AB/Sciex API4000Q mass spectrometer equipped with an electrospray (TurboV) interface coupled to a Shimadzu Prominence HPLC system (Shimadzu Corp., Kyoto, Japan). Samples for saxitoxin (paralytic shellfish poisons) determination were analysed by HPLC Fluorescence using a Shimadzu LC10AD HPLC system (Shimadzu Corp., Kyoto, Japan) based on the peroxide oxidation work of Lawrence *et al.* (2005). Samples for microcystin

analyses were pre-concentrated using an online solid phase extraction technique and analysed using HPLC with diode array detection. The microcystins were determined using a Gilson GX271 Liquid Handler (Gilson SAS., Villiers-le-Bel, France) directly coupled to a Shimadzu LC20AD HPLC system.

3.9 Cyanoprokaryote culture and isolation

Strains of both *C. raciborskii* and *A. ovalisporum* were isolated from Cobaki Lake environmental samples using an Olympus SZ40 inverted microscope (Olympus Australia, Codleigh, Victoria, Australia) and an eyelash brush (Anderson 2005). The isolates were grown under 12 hour light (25º C) / dark (18º C) cycle in ASM-1 medium (Gorham *et al.* 1964) using tissue culture vessels (Iwaki, Tokyo, Japan, 75 cm^2, canted neck, vented). Queensland Health, Forensic and Scientific Services in Brisbane also cultured and isolated *A. ovalisporum* from Cobaki Lake. Multi-trichome isolates were grown by QHFSS for two strains of *A. ovalisporum*, SEC A and SEC B. Single trichomes were picked from a thinly-poured McBride Listeria Agar (MLA) (1%) with an eyelash brush, and transferred into 3 mL well plates (ProSciTech Pty Ltd, Kirwan, Australia) filled with 1.5 mL of MLA liquid medium (Bolch and Blackburn 1996). The cultures were grown in a Binder growth chamber, (12 hr light (24^{o}C) /dark (18^{o}C) cycle; light intensity 10–30 μmol m^{-2} s^{-1}).

Uncontaminated isolates of the SEC B strain were observed in five wells, the contents of which were then transferred to five tissue culture vessels (Iwaki, 75cm^2, canted neck, vented). SEC B cultures continued to grow, with no apparent visual algal contamination. No growth for SEC A was noted in the well plates after five weeks. SEC A was re-isolated again using the method described above. After seven weeks, SEC A demonstrated positive growth, with green algal contamination present in several wells. Using an eyelash brush, several individual trichomes were placed on MLA agar and MLA agarose (0.5%) plates. Using an

inoculation loop, the culture was also streaked onto MLA agar and MLA agarose using the 16-streak method. Successful growth of SEC A was observed on the MLA agar plates after three weeks; these were transferred again by inoculation loop onto MLA agar plates to check for any further contamination. After seven weeks of growth without any apparent visible algal contamination, multiple trichomes were selected with an eyelash brush from one of the MLA agar plates and transferred into four 75cm^2 tissue culture vessels containing MLA liquid medium. The cultures grew vigorously, without contamination. Later, 24 single genetic strain isolates for both SEC A and SEC B were successfully generated from a single vessel of each strain by transferring single trichomes into individual well plates with an eyelash brush. After three weeks, five isolates were selected and transferred into five 75cm^2 tissue culture vessels containing MLA liquid medium and maintained in the growth chamber under the same conditions as described above.

3.10 Real-Time PCR Analyses

3.10.1 Real-time PCR (Tweed Laboratory Centre)

A duplex probe assay and a melt curve assay were conducted using the real-time PCR instrument Corbett Rotor-Gene 6000 (Corbett Research, Sydney, Australia) on laboratory-isolated strains of *A. ovalisporum* and *C. raciborskii*.

DNA was extracted from 1,000 µL subsamples which were taken from well mixed samples, centrifuged for 10 min at 11,000 g min^{-1} and then the supernatant removed with a fine-tipped pipette leaving the pellet and a minimum of moisture. Then 200 µL of '*Instagene Matrix*' (Bio-Rad Laboratories Pty., Ltd, Gladesville, NSW, Australia) was added to this pellet and the tube heated at 56ºC in a dry heat block for 20 min. After heating, the tube was vortexed for 10 s and returned to the heat block at 100ºC for 8 min and then vortexed again for 10 s.

The tube was then centrifuged for 10 min at 14,500 g min^{-1} and the resulting supernatant was frozen and used for PCR analysis.

3.10.1.1 Duplex TaqMan probe analysis

The duplex TaqMan probe-based method was used based on that described by Rasmussen *et al.* (2008) utilising PCR amplification of *pks* genes, and ultimately CYN-producing strains (*A. ovalisporum* and *C. raciborskii*), and the *rpoC1* gene specific to the species *C. raciborskii*. After extraction, 2 µL of DNA supernatant was added to 23 µL of reaction mix (Table 3.6) and analysed in the Corbett Rotor-gene 6000. The primer pair *pks*-F2 *pks*-R1 and the *pks* TaqMan probe are modifications determined by Barbara Sendall from QHFSS (Barbara Sendall, QHFSS, unpublished data) (Table 3.7). Cycling conditions were also modified: hold of 3min at 95ºC, followed by 40 cycles of 10 sec at 95ºC and 60 sec at 60ºC. The amplified PCR products were visualised graphically and numerically using the Roto-Gene 6000 software (version Roto-Gene 1.7.75). Two TaqMan probes were detected by fluorescence using the excitation, emission and gain as follows: 6-carboxyfluorescein (FAM) for *pks* probe (470 nm/510 nm/6) and 5-carboxyrhodamine (ROX) for *rpoC1* probe (585 nm/610 nm/8). Results were presented using cycle threshold (Ct) values. These values are defined as the PCR cycle number at which the gain in fluorescence generated by the accumulating exceeds baseline fluorescence (Jung *et al.* 2000). The Ct is proportional to the number of template (target gene) copies present in the sample (Gibson *et al*. 1996). Using the Ct Calculation function within the Rotorgene program, the fluorescence threshold was set at 0.1, above which data was recorded. Control cultures included CYN positive control *C. raciborskii* isolate Cy 1209 from QFHSS and negative control *C. raciborskii* isolate Cy 1184 from QFHSS. Ultrapure water that had been through the extraction process represented a no template control.

Table 3.6. Individual reaction-mix components for probe and melt curve analyses.

Reagent	**Dual probe** individual reaction-mix concentration (µL)	**Melt curve** individual reaction-mix concentration (µL)	**Supplier/Reference**
10x PlatinumTaq PCR Buffer	2.5	2.5	Invitrogen Corp
10mM dNTPs	0.4	0.4	Bio-Rad
25mM $MgCl_2$	3.0	3.0	Invitrogen Corp
Primer: cyl2 @ 6.25µM	0.5	-	Geneworks. Ref: Wilson *et al*. (2000)
Primer: cyl4 @ 6.25µM	0.5	-	Geneworks. Ref: Wilson *et al*. (2000)
Primer: *pks*-F2 @ 6.25µM	1.0	1.0	Geneworks. Ref: Barbara Sendall (QHFSS) unpublished data 12/8/08
Primer: *pks*-R1 @ 6.25µM	1.0	1.0	Sigma. Ref: Barbara Sendall (QHFSS) unpublished data 12/8/08
Probe: *rpoC1* @ 25µM	0.2	-	Geneworks. Ref: Rasmussen *et al.* (2008)
Probe: *pks*-QHSS @ 25µM	0.2	-	Geneworks. Ref: Barbara Sendall (QHFSS) unpublished data 12/8/08
SYTO 9	-	1.0	Invitrogen Corp
PlatinumTaq @ 5U/µL	0.2	0.2	Invitrogen Corp
MQ Water (18 megaohm)	13.5	13.9	n/a
Total Volume	23.0	23.0	

Table 3.7. Primer and probe sequences for probe and melt curve analyses for CYN gene (*pks*) and *C. raciborskii* gene (*rpoC1*).

Primer and Probes	Primer and probe sequence
Primer: cyl2 @ 6.25µM	5'GGCATTCCTAGTTAT ATTGCCATACTA3'
Primer: cyl4 @ 6.25µM	5'GCCCGTTTTTGTCCC TTTGCTGC3'
Primer: *pks*-F2 @ 6.25µM	5'GATCGAAAACAG GAGTCGGA3'
Primer: *pks*-R1 @ 6.25µM	5'CTCTGACAGGCTTGA TGAAC3'
Probe: *rpoC1* @ 25µM	ROX 5'TCCTGGTAA TGCTGACACACTCG3'BHQ2
Probe: *pks*-QHSS @ 25µM	FAM 5'TGCCGGCAG CAACACTCACATCAGT3'BHQ1

3.10.1.2 Melt curve analysis

Melt curve analysis assay was used to analyse the laboratory cultured strains of *A. ovalisporum* (Strains A and B from QHFSS) from Cobaki Lake. These samples were run using the reaction mix and fluorescent dye dimethylsulfoxide (SYTO 9) components given in Table 3.6. SYTO9 was shown by Monis *et al.*(2005) to be a better dye than the more widely used SYBR Green I dye. Only the CYN primers (*pks*-F2 and *pks*-R1) (Table 3.7) were used in the reaction. Instrumentation and cycling conditions were as for the probe assay above followed by a melt step consisting of: a 90 s pre-melt, ramping from 70ºC–99ºC steps with a 2 s hold after each step and auto gain optimisation set to 70. The melt data were analysed using conventional melt curve analysis on the Rotorgene software with plots of the negative first derivative of fluorescence vs temperature (-dF/dT) against temperature. The threshold value above which data capture occurred was set at ≤ 0.5 dF/dT. Control cultures included CYN positive control *C. raciborskii* isolate Cy 1209 from QFHSS and negative control *C. raciborskii* isolate Cy 1184 from QFHSS. Ultrapure water that had been through the extraction process represented a no template control.

3.10.2 Real-time PCR (South Australia Water)

Real-time PCR analyses were carried out by South Australia Water on environmental water column depth profiles from Cobaki Lake sampled monthly, at every metre, between January and June 2007. These depth profiles were analysed by South Australia Water using the duplexTaqMan probe-based assay developed by Rasmussen *et al*. (2008) for the detection of the *rpoC1* and *pks.*

The thermocycling protocol was performed using a Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Sydney, Australia). TaqMan probes were detected by fluorescence using the excitation, emission and gains as follows: 6-carboxyfluorescein-PKS and 6-carboxyfluorescein-CYAN 328R (470 nm/510 nm/6); CAL Fluor Orange 560-ANZm-P (530 nm/555 nm/10) and Cyanine 5-RPOC1 (625 nm/665 nm/7). They then quantitated the dynamic properties of the assay using an absolute DNA standard from purified PCR amplicons and prepared standard curves from the PCR product that had been purified using a DNA cleanup spin column, quantified using a high precision UV spectrophotometer, then diluted to create a set of standards ranging in log-fold concentrations from 10 to 1 - 10^8 copies per reaction. South Australia Water expressed their results as gene copies per reaction.

3.11 Presentation of physical and chemical data

Physical and chemical data collected during depth profiling at the three Tweed Shire sites are presented using contour plots (Golden Software, Inc. 1993-2002 SURFER® 8 contouring and surface mapping program). The contour plots are based on Julian dates and are compiled using the "Triangulation with Linear Interpolation Gridding method" (SURFER® 8). The contour plots were further refined by using either Grid/Spline Smooth or Grid/Filter function depending on the individual graphs involved (Kari Dickenson, personal communication with

Technical Support, Golden Software, Inc. 2010). The depth profile data presented include temperature, pH, dissolved oxygen, conductivity, soluble manganese, total nitrogen and soluble iron.

The physical and chemical data relating to surface water parameters are presented using simple line graphs (SigmaPlot 2002 version 8.02).

3.12 Statistical analyses

Multivariate analyses were carried out using the PRIMER V6 statistical software (Plymouth Marine Laboratory, Plymouth, United Kingdom). The environmental data for the three sites were initially assessed in a series of Draftsman's Plots to determine which variables required transformation and which type of transformation was suitable. The log-transformed data were normalised and a resemblance matrix (between sample similarities) developed using Euclidian distance, and a Principal Components Analysis (PCA) ordination was produced. In a similar way, the biological data were square-root transformed and a resemblance matrix of between-sample similarities was produced using the Bray-Curtis coefficient. A non-metric Multidimensional Scaling (MDS) ordination was plotted from this. A factor labelled 'SITE' was introduced to the analysis with the aim of comparing the three sites (D-Clarrie Hall Dam; R-The Tweed River at Bray Park and C-Cobaki Lake). A second factor labelled 'YEAR' was also introduced with the aim of comparing the two sampling seasons (Dry 2007 '7' and Wet 2008 '8'). The PRIMER V6 BEST analysis was run on the log-transformed normalised environmental data to determine the best match between the multivariate among-sample patterns of the environmental variables and biological data. The permutation feature of BEST using 999 permutations was used with a significance level of 0.001 producing a sample statistic (Rho). This analysis indicated which variables best explained the biological data. The PRIMER ANOSIM analysis was run on a Euclidean distance matrix from log transformed, normalised environmental data and on Bray Curtis resemblance matrix from

square root transformed biological data. The resulting Analysis of Similarities produced a global R statistic which established if there were statistically significant differences between the data sets. The groupings or dissimilarities between groups are shown pictorially using cluster dendrograms, cluster overlays on MDS plots and bubble plots superimposed on the environmental PCA ordinations. The PRIMER V6 SIMPER analysis showed the similarity percentages or species/division contributions at each sampling site. The analysis was carried out on the Bray-Curtis similarity matrix on both the algal and algal division assemblages. Statistical correlation analyses were performed using SPSS Inc. PASW Statistics 17. A significant relationship was defined as $p < 0.05$ as recommended by McKillup (2007).

3.13 ANZECC Guidelines

The physical and chemical data recorded from samples collected at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Village Lake are discussed in relation to the relevant ANZECC guidelines (ANZECC 2000). Using the descriptions outlined in the ANZECC guidelines, Clarrie Hall Dam and Cobaki Village Lake are classified as *Freshwater Lakes and Reservoirs*, and the Tweed River at Bray Park is classified as a *Lowland River* due to its altitude below 150 m. All three sites are also categorised as *slightly to moderately disturbed systems* (Hunter Water 2011; ANZECC 2000). The ANZECC parameter guideline trigger values are the protection criteria for an *Aquatic Ecosystem* (defined in Table 3.3.2 and Table 3.4.1 of the ANZECC guidelines). The ANZECC guideline trigger values for soluble iron concentration are the protection criteria for *Recreation and Aesthetics* (defined in Table 2.3 and p. 5-5 of the ANZECC guidelines). There was no trigger value for soluble silica concentration in the ANZECC guidelines; therefore, the Australian Drinking Water guideline (NHMRC 2004) for soluble silica was adopted.

The ANZECC guidelines can be modified into regional, local or site-specific guidelines involving factors including variability of the ecosystem, geology of the district, rainfall and development (ANZECC 2000). The report by Hunter Water (2011) examined the Tweed catchment water quality between 1997 and 2011 and recommended some adjustments to the ANZECC trigger values in regard to the Tweed catchment system.

3.14 The Australian Drinking Water Guidelines

The phytoplankton data recorded from samples collected at Clarrie Hall Dam and the Tweed River at Bray Park are discussed in relation to the Australian Drinking Water Guidelines (NHMRC 2004). These guidelines present an alert levels framework for cyanoprokaryote concentrations in Australian drinking water supplies (Table 3.8). As well as this general alert levels framework, threshold concentrations of known toxin producing cyanoprokaryotes have also been published in the Australian Drinking Water Guidelines (NHMRC 2004) (Table 3.9).

Table 3.8. Protocol for alert levels of cyanoprokaryotes in Australian Drinking Water Guidelines (NHMRC 2004).

Alert Level	**Criteria (cells mL^{-1})**
Detection Level Low Alert	$\geq$ 500 and < 2,000 cells mL^{-1} *Microcystis aeruginosa* or Total biovolume of all cyanoprokaryotes $\geq$ 0.05 and < 0.2 $mm^3 L^{-1}$
Alert Level 1 Medium Alert	$\geq$ 2,000 and < 6,500 cells mL^{-1} *Microcystis aeruginosa* or *Anabaena circinalis* or Total biovolume of all cyanoprokaryotes $\geq$ 0.2 and < 0.6 $mm^3 L^{-1}$
Alert Level 2 High Alert	$\geq$ 6,500 cells mL^{-1} *Microcystis aeruginosa* or *Anabaena circinalis* or Total biovolume of all cyanoprokaryotes $\geq$ 0.6 $mm^3 L^{-1}$
Alert Level 3 Very High Alert	$\geq$ 65,000 cells mL^{-1} *Microcystis aeruginosa* or *Anabaena circinalis* or Total biovolume of all cyanoprokaryotes > 6 $mm^3 L^{-1}$

Table 3.9. Threshold concentrations for known toxic cyanoprokaryotes published in the Australian Drinking Water Guidelines (NHMRC 2004).

Species	Notification Alert Level 1 (Cells mL $^{-1}$)	Notification Alert Level 1 Biovolume (mm^3 L^{-1})	Alert Alert Level 2 (Cells mL^{-1})	Notification Alert Level 2 Biovolume (mm^3 L^{-1})	Notification Alert Level 2 Toxin concentration (µg L^{-1})
Microcystis aeruginosa	2,000	0.20	6,500	0.60	1.3 microcystin-LR
Anabaena circinalis	6,000	1.50	20,000	5.00	3.0 STX-eq
Cylindrospermopsis raciborskii	4,500	0.18	15,000	0.60	1.0 CYN
Nodularia spumigena	12,000	2.70	40,000	9.10	1.3 nodularin

The Australian Guideline for microcystin-LR is 1.3 µg L^{-1}. A *M. aeruginosa* cell count of approximately 6,500 cells mL^{-1} (biovolume 0.6 mm^3 L^{-1}) would be equivalent to the guideline of 1.3 µg L^{-1} microcystin–LR; however, at this cell concentration toxin determination would be required for health risk assessment. It has been suggested that The Australian Drinking Water Guidelines for CYN be set at 1.0 µg L^{-1} (Humpage and Falconer 2003; Shaw *et al.* 2000). Toxin concentrations at that level have been associated with cell counts of *C. raciborskii* of between 15,000 and 20,000 cells mL^{-1} (biovolume 0.6–0.8 mm^3 L^{-1}) (McGregor and Fabbro 2000).

There is currently no Australian guideline value set for concentrations of saxitoxins in drinking water supplies. Cell densities exceeding 20,000 cells mL^{-1} (biovolume 5 mm^3 L^{-1}) of *A. circinalis* are generally used as a guide for health assessment. Drinking water with cell densities at this concentration would most likely have taste and odour problems and would be unpalatable.

3.15 The Australian Recreational Water Guidelines (NHMRC, 2008)

The phytoplankton data recorded from samples collected at Cobaki Lake are discussed in relation to the Australian Recreational Water Guidelines (NHMRC 2008). These guidelines recommend a three tier alert framework based on the cell concentration and biovolume of *M. aeruginosa* (Table 3.10).

Table 3.10. Protocol for alert levels of cyanoprokaryotes in Australian recreational waters (NHMRC, 2008).

Alert Level	**Criteria (cells mL^{-1})**
GREEN Surveillance mode	≥ 500 and < 5,000 cells mL^{-1} *Microcystis aeruginosa* or Total biovolume of all cyanoprokaryotes > 0.04 and < 0.4 $mm^3 L^{-1}$
AMBER Alert mode	≥ 5,000 and < 50,000 cells mL^{-1} *Microcystis aeruginosa* or Total biovolume of all cyanoprokaryotes ≥ 0.4 and < 4 $mm^3 L^{-1}$ (where a known toxin producer is dominant in the total biovolume) or Total biovolume of all cyanoprokaryotes ≥ 0.4 and < 10 $mm^3 L^{-1}$ (where a known toxin producer is not present)
RED Action mode	Level 1 guideline Total microcystins ≥ 10 μg L^{-1} or ≥ 50,000 cells mL^{-1} toxic *Microcystis aeruginosa* or Total biovolume of all cyanoprokaryotes ≥ 4 $mm^3 L^{-1}$ (where a known toxin producer is dominant in the total biovolume) or Level 2 guideline Total biovolume of all cyanoprokaryotes ≥ 10 $mm^3 L^{-1}$ (where a known toxin producer is not present) or Cyanoprokaryote scums are consistently present

4 PHYSICAL and CHEMICAL DATA

4.1 Introduction

Phytoplankton populations in still (lentic) water bodies are generally determined by the same chemical and physical parameters that govern riverine populations, including temperature, light and nutrient concentrations (Reynolds 1993a). It is self-evident that lakes, reservoirs and rivers each differ in terms of their physical geography; so too, it would be expected that their phytoplankton assemblages would vary accordingly. For example, in rivers, successful species have the ability to survive turbid flow and light fluctuations and would thus be dependent on fast growth rates (Reynolds *et al.* 1994). In contrast, many still water bodies experience seasonal thermal stratification during which nutrient concentrations in the epilimnion may be reduced to limiting concentrations as a result of algal uptake (Reynolds 1998; Reynolds 1980). Phytoplankton are sensitive to changes in nutrients, responding quickly when levels increase (Paerl *et al.* 2007). They are responsive to environmental changes and sensitive to diverse environmental stressors and this, coupled with their short life cycle, makes them good indicators of water quality as well as excellent indicators of ecological change (Paerl *et al.* 2007; Hötzel and Croome 1998).

Climate and latitude are natural determinants of phytoplankton assemblages. In addition to these, anthropic impacts may result in further modifications to phytoplankton assemblages, species dominance and/or successional sequences (Reynolds 1993b; Reynolds 1989; Reynolds 1984). Anthropic impacts include the construction of small and large dams, riverine regulation (introduction of weirs or other structures) or use of management tools (for example, pumping and mixing devices) (Reynolds *et al.* 1984). The hydrological changes imposed on rivers can result in sections of water within a river that are incompletely mixed with the bulk of the flow, and which might travel at different velocity, hence having variable retention times (Reynolds 1988). These types of hydrological changes, coupled with anthropic eutrophication, can produce increased residence times resulting in severe

environmental issues such as those experienced in the Murray, Lower Goulburn and Murrumbidgee rivers in Australia (Hötzel and Croome 1998; Baker 1996; Bowling and Baker 1996; Hötzel and Croome 1994; Bowling 1992). In these situations, where sections of water experience increased temperatures and low flow periods, conditions can develop conducive to the growth of troublesome cyanoprokaryotes similar to those experienced in still, stratified water bodies.

Australia offers a unique hydrological environment often impacted by the extremes of flood and drought. Australia has a comparatively high coefficient of variation in river flow; the mean peak annual floods of Australian streams are much higher than elsewhere globally (Lake *et al.* 1985). For this reason, understanding changes in phytoplankton populations in Australian streams is particularly useful in helping water managers predict, and respond to, extreme weather events. Bormans and Condie (1998) have already demonstrated the ability to predict stratification dynamics and algal speciation at the temperate Maude weir pool on the Murrumbridgee River, Australia. Here, under low flow conditions, a decline in *Melosira* accompanied the development of stratification. The appearance of *A. circinalis* was facilitated by limited vertical mixing which conferred a competitive advantage to the cyanoprokaryote. In this context, the linkages between dam and weir constructions on otherwise natural systems, the retention time, and the subsequent development of particular phytoplankton populations are very important. Human intervention in the form of physical manipulation of a weir pool (artificial destratification) has also been shown to significantly reduce the frequency and severity of cyanoprokaryote blooms (Webster *et al*. 1996).

Slow moving rivers which are impounded, or have low flow, typically experience some natural periods of eutrophication; however, dominance of cyanoprokaryotes during summer months is becoming more common (Chorus and Salas 1997). For example, a study of the phytoplankton dynamics in two tropical rivers in southeast Brazil revealed that both rivers became eutrophic due to the increased human population of the region (Soares *et al.* 2007).

In that case, the presence of a reservoir and sewage inflow were both important in moderating the phytoplankton community structure, ultimately resulting in dominance by a toxin-producing population of *C. raciborskii*.

Cyanoprokaryotes respond more strongly to rising temperatures than do green algae and diatoms, resulting in higher growth rates and peak abundances of cyanoprokaryotes in warmer temperatures (Lisette *et al.* 2007). However, Lisette *et al.* (2007) found that the spring sequence of succession from diatoms to green algae to cyanoprokaryotes was not affected by different climate scenarios. This has also been shown in Central Queensland, Australia (Fabbro and Duivenvoorden 2000). The dominance of *C. raciborskii* in water bodies has been studied worldwide; for example, in Brazil (Branco and Senna 1994), and in Hungary (Tóth and Padisák 1986), as well as in Australia at the North Pine Dam (Harris and Baxter 1996) and the Fitzroy River impoundment (Fabbro 1999). The Fitzroy River catchment experienced extreme weather patterns as is typical of tropical monsoonal conditions. The dominance of *C. raciborskii* in dry years with hot summers was documented.

The Tweed Shire is located in eastern Australia approximately 750 km south of the Fitzroy River and has a subtropical climate characterised by summer months of December, January and February. The autumn months of March, April and May are the most troublesome time with respect to cyanoprokaryote blooms. As a result of human modification, each site in this study varied in size and retention time, yet all were exposed to a similar climatic regime. Detailed analyses of the physical and chemical properties of the three different water bodies assist in understanding the site-based variations of phytoplankton species, populations and assemblages. This information can then be compared with the Australian and New Zealand Guidelines for Fresh and Marine Water Quality Guidelines (ANZECC 2000), the Australian Drinking Water Guidelines (NHMRC 2004) and the Australian Recreational Water Guidelines (NHMRC 2008).

4.2 Results

The three sampling sites were, as expected, significantly different with respect to environmental conditions. An ANOSIM analysis of key environmental parameters (discussed more fully below) produced a global R statistic of 0.802. Differences in water quality parameters were also recorded in the dry (2007) and wet (2008) seasons.

4.2.1 Rainfall

In 2008, rainfall in the Tweed Shire at Murwillumbah was more than double that of 2007 (1,518.0 mm compared to 706.2 mm), and included a localised flood event in January (Figure 4.1). At Cobaki Lake, the total annual rainfall was 846.0 mm (2007) and 1,220.0 mm (2008). However, statistically, significant differences were not revealed in respect to the seasonal rainfall pattern recorded in the two sampling years (ANOSIM Global R statistic of -0.017).

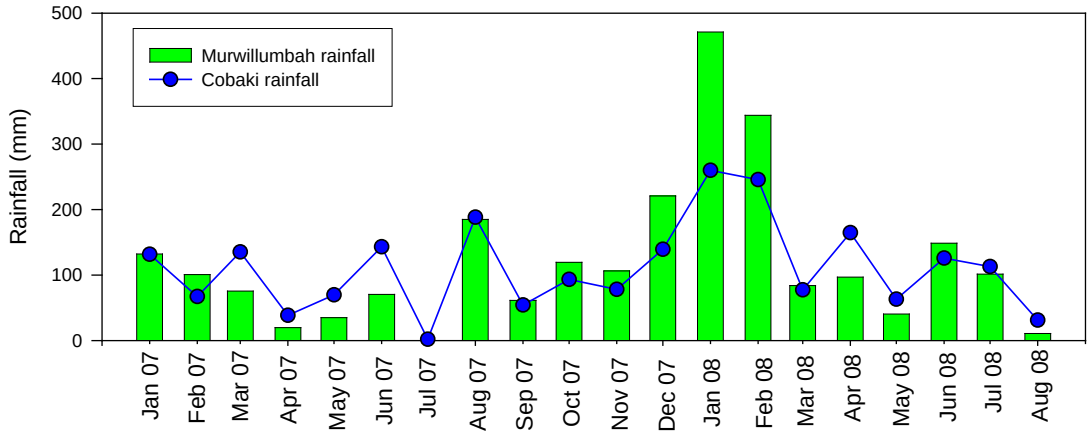


Figure 4.1. Rainfall (mm) recorded at the Australian Bureau of Meteorology Murwillumbah (Bray Park) Observation Station 058158 and Coolangatta Observation Station 040717 (for Cobaki Lake) during the sampling period January 2007 to August 2008.

4.2.2 Temperature

Clarrie Hall Dam and Cobaki Lake generally followed a monomictic pattern during 2007 and 2008 (Figures 4.2; 4.3). The temperature profiles suggest that the mixer installed in Clarrie Hall Dam in 2002 was not effective in preventing thermal stratification from occurring during 2007. However increased mixing was observed in 2008, although this may have been influenced by the increased rainfall during the summer months. Thermal profiles of Cobaki Lake indicate that holomixis was incomplete in 2008 as there was a partially mixed zone in the hypolimnion. The Tweed River at the Bray Park pump station was not stratified but clearly showed seasonality.The surface water temperatures at the three sites decreased slightly between the two sampling years (2007 and 2008) (Figure 4.2). Significant differences in the surface water temperature were not detected at any of the three sites (ANOSIM Global R of -0.023) (Table 4.1) or between the two sampling years (ANOSIM Global R of 0.004) (Table 4.1). Summary statistics of the surface water temperatures are presented in table 4.2.

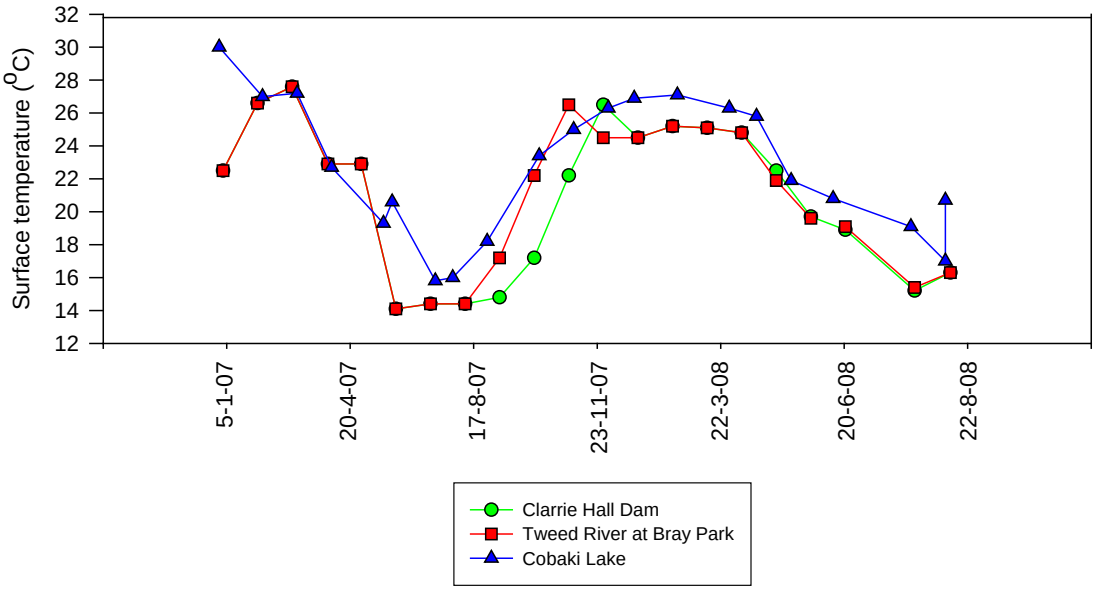


Figure 4.2. Surface temperatures (ºC) recorded at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake at monthly intervals between January 2007 and August 2008.

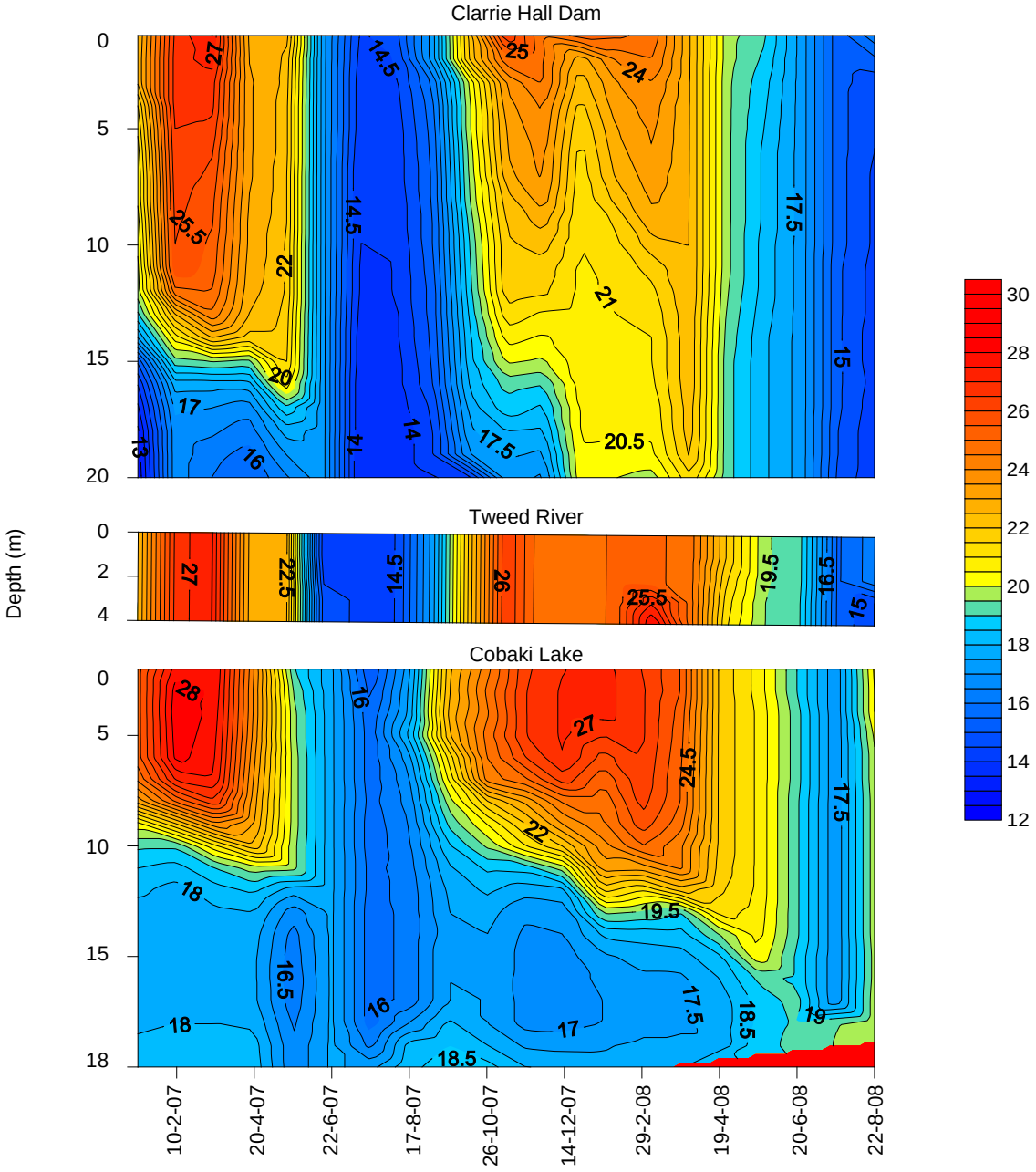


Figure 4.3. Isotherms (ºC) recorded monthly at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake between January 2007 and August 2008.

Table 4.1. ANOSIM (Analysis of Similarities) table showing the Global R statistic involving two sets of comparisons (SITE and YEAR) and multiple environmental variables.

	ANOSIM Global R statistic (amongst 3 Sites) Cobaki, CHDam, Tweed River	**ANOSIM Global R statistic (between 2 years) 2007/2008**
All environmental variables	0.802	-0.017
Dissolved oxygen (mg L^{-1})	0.021	0.079
Conductivity (K_{25}) (µS cm^{-1})	0.854	-0.021
pH (pH units)	0.354	0.004
Temperature (ºC)	-0.023	0.004
Turbidity (NTU)	0.063	0.011
Interpretation: If the global R statistic is close to 1.0, this indicates a complete separation of groups. If the global R statistic is close to 0, this indicates very little or no separation of the groups.		

Table 4.2. Summary statistics of surface water temperatures at Clarrie Hall Dam, Tweed River at Bray Park and Cobaki Lake recorded at monthly intervals between January 2007 and August 2008.

Surface water (Temperature)	**Clarrie Hall Dam (ºC)**	**Tweed River (ºC)**	**Cobaki Lake (ºC)**
Mean	20.9	21.3	23.5
Median	22.5	22.5	24.6
Minimum	14.1	14.1	15.8
Maximum	27.6	27.6	30.0

4.2.3 pH

Clarrie Hall Dam was slightly acidic (pH 5.8–6.5) during the sampling period, with a slightly alkaline (pH 8.0) hypolimnion developing during periods of stratification (Figures 4.4 and 4.5). The Tweed River was generally neutral to slightly alkaline. During the autumn and winter of 2007 (during decreased rainfall), the pH of the river became slightly alkaline (7.7–8.0), however in 2008 the pH returned to neutral (7.0–7.3). Clarrie Hall Dam and the Tweed River surface water pH also reduced slightly with increased rainfall in 2008 (Figure 4.4). Cobaki Lake featured the opposite trend to Clarrie Hall Dam with pH stratification evident involving an alkaline epilimnion (pH 7.5–9.8) and a slightly acidic hypolimnion (6.0–6.5). The pH of the surface water of Cobaki Lake increased considerably during the summer period between October 2007 and March 2008, and this was a different pattern to the pH at both Clarrie Hall Dam and the Tweed River (Figures 4.4; 4.5; Table 4.3). This difference in patterns demonstrated at Cobaki Lake was reflected in the statistics (ANOSIM Global R statistic of 0.354) (Table 4.1). Surface samples collected at Cobaki Lake were the only ones which exceeded the ANZECC trigger values for pH (Figure 4.4). Significant differences in pH were not detected between the 2007 and 2008 sampling years (ANOSIM Global R of 0.004) (Table 4.1).

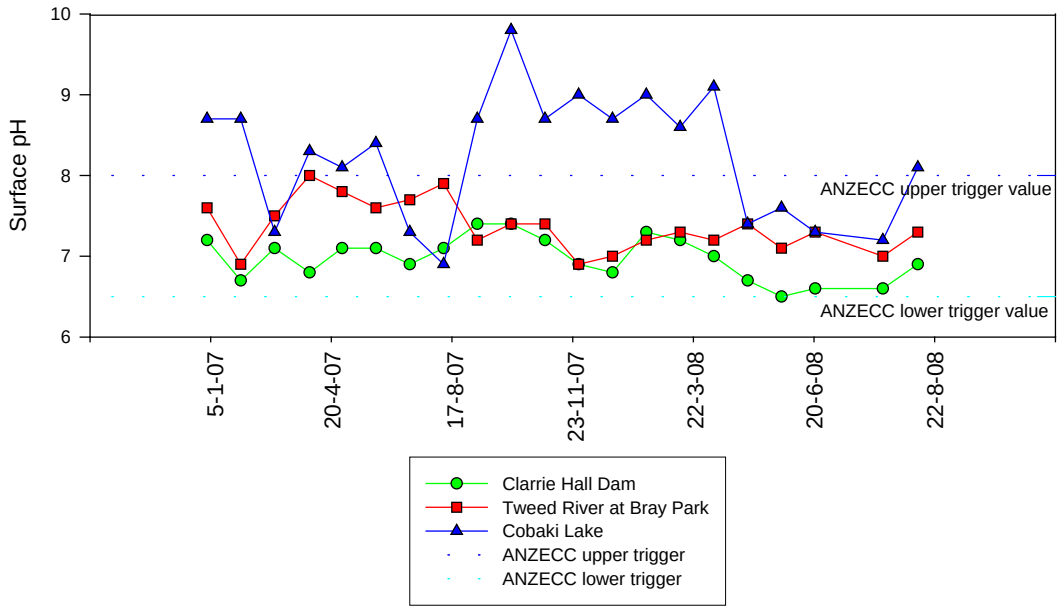


Figure 4.4. Surface pH recorded monthly at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake between January 2007 and August 2008.

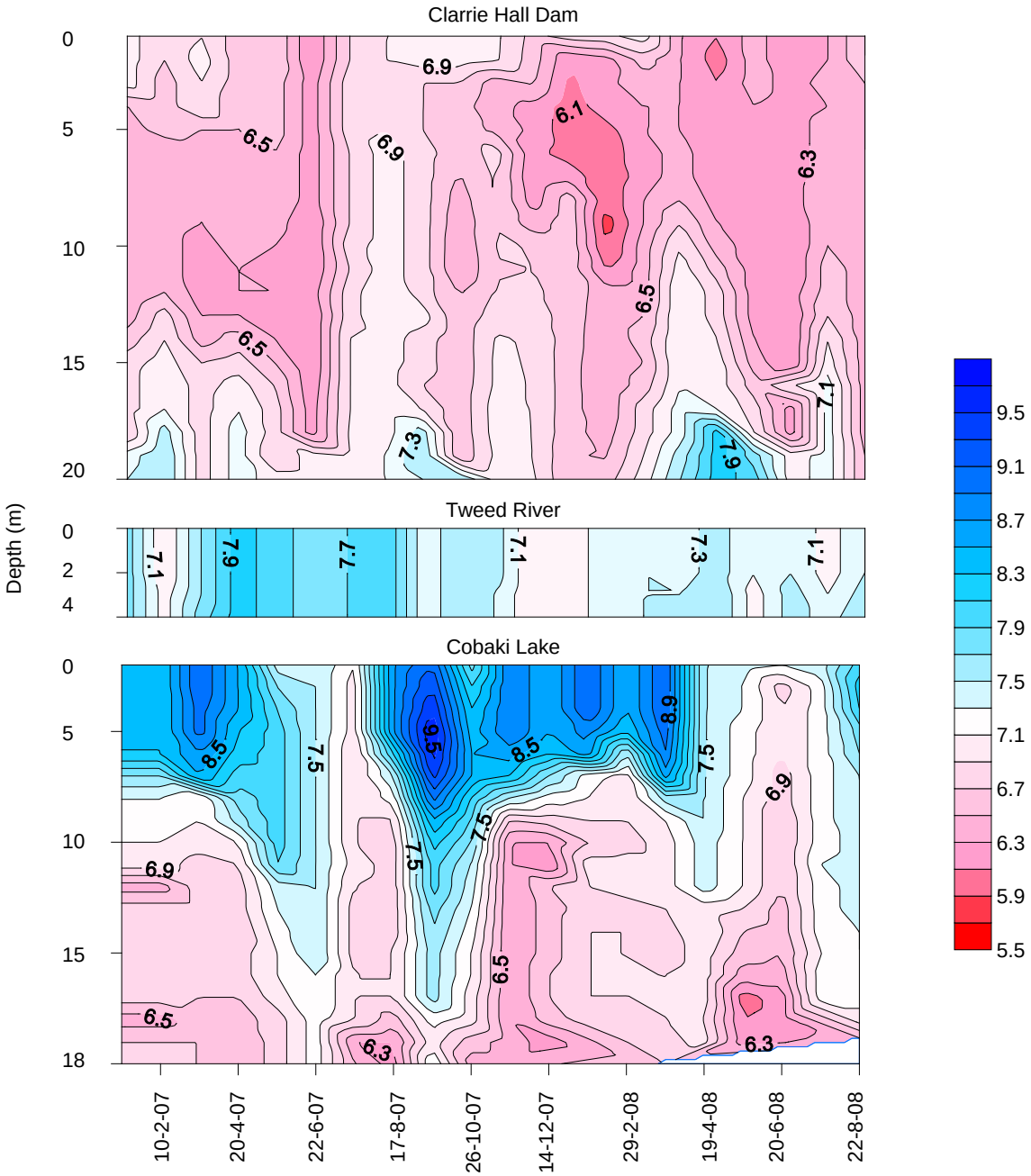


Figure 4.5. Isopleths of pH recorded monthly at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake between January 2007 and August 2008.

Table 4.3. Summary statistics of surface water pH recorded at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake at monthly intervals between January 2007 and August 2008.

Surface water	**Clarrie Hall Dam (pH)**	**Tweed River (pH)**	**Cobaki Lake (pH)**
Mean	6.9	7.4	8.3
Median	7.0	7.3	8.6
Minimum	6.5	6.9	6.6
Maximum	7.2	7.9	9.8

4.2.4 Dissolved oxygen concentrations

Clarrie Hall Dam and Cobaki Lake had anoxic hypolimnia indicative of stratified water bodies, whereas the Tweed River did not (Figure 4.7). Cobaki Lake was strongly stratified during the sampling period, with the surface layer delimited by a thermocline and oxycline between 4 and 8 m. The dissolved oxygen concentration in the surface layer fluctuated during the sampling period, with spikes occurring at winter mixing (Clarrie Hall Dam and Cobaki Lake) (Figure 4.6). In Clarrie Hall Dam, the isopleths (Figure 4.7) illustrate the mixing of an anoxic large volume hypolimnion into the surface waters in April, 2008. There was an unusual spike (> 10.0 mg L^{-1}) in Cobaki Lake surface water in January 2008 followed by a sudden decrease (5.3 mg L^{-1}) in February of the same year (Figure 4.6; Table 4.4). A localised flood event that occurred in January 2008 may account for this fluctuation. All sites exceeded the ANZECC trigger values at various times (Figure 4.6). Despite these fluctuations, there was no significant difference overall between the dissoloved oxygen concentrations at the sites (ANOSIM Global R of 0.021) (Table 4.1) or between the sampling years (ANOSIM Global R of 0.079) (Table 4.1).

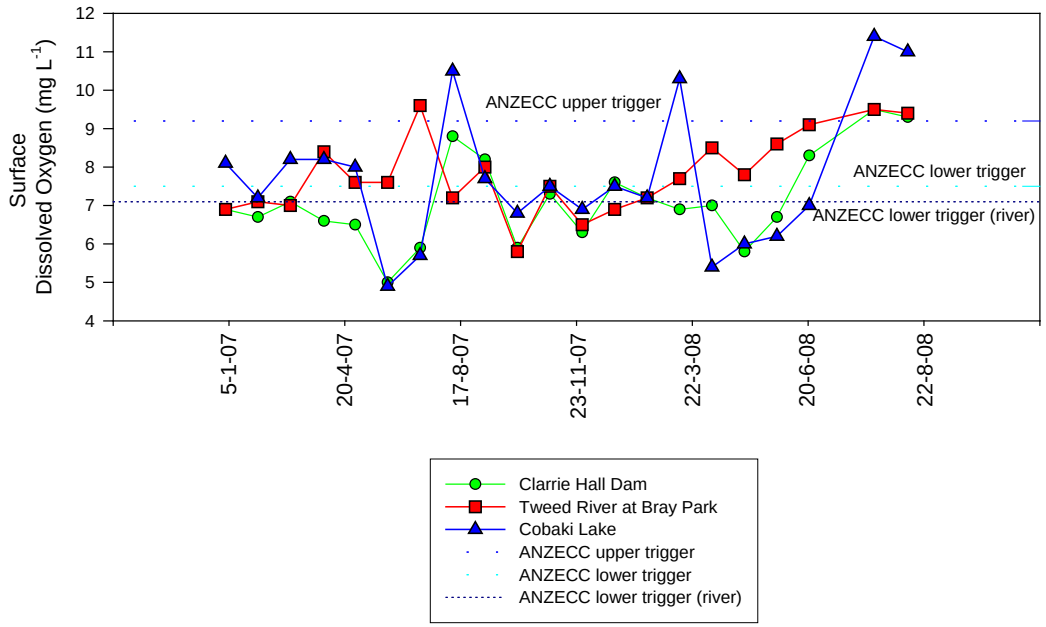


Figure 4.6. Dissolved oxygen concentration (mg L^{-1}) in surface water recorded monthly at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake between January 2007 and August 2008.

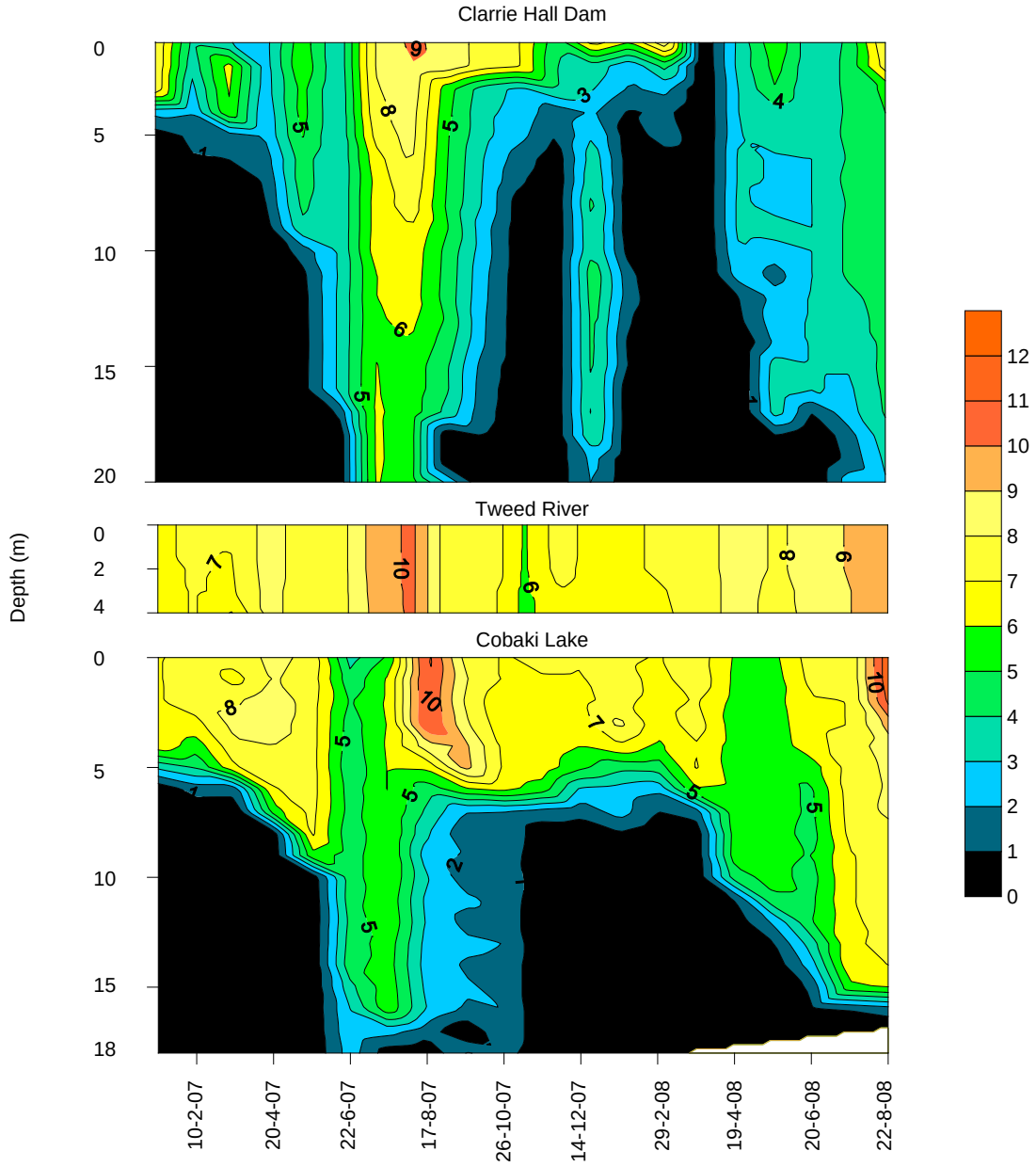


Figure 4.7. Isopleths of dissolved oxygen (DO) concentration (mg L^{-1}) recorded monthly at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake between January 2007and August 2008.

Table 4.4. Summary statistics of dissolved oxygen concentrations in surface water recorded monthly at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake between January 2007 and August 2008.

Surface water (DO)	**Clarrie Hall Dam (mg L^{-1})**	**Tweed River (mg L^{-})**	**Cobaki Lake (mg L^{-1})**
Mean	7.1	7.8	7.1
Median	6.9	7.6	7.1
Minimum	5.0	5.8	4.3
Maximum	9.0	10.0	11.4

4.2.5 Specific conductance (conductivity) (K_{25})

The conductivity (K_{25}) at the three sampling sites was significantly different, two being fresh water (< 800 µS cm^{-1}) and the third involving a brackish/saline hypolimnion (Figures 4.8; 4.9) (ANOSIM Global R of 0.854) (Table 4.1). Some general ranges for Australian conductivity are: freshwater rivers (0-800 µS cm^{-1}), brackish water (1,600–4,800 µS cm^{-1}), saline water (> 4,800 µS cm^{-1}) and sea water (51,500 µS cm^{-1}) (Eaton *et al.* 2005; Waterwatch 2002). Cobaki was the only one of the three sites that featured a halocline. The Tweed River showed a slight decrease in conductivity (K_{25}) with the increased rainfall in January 2008 and localised flood event, dropping from a mean profile value of 177 µS cm^{-1} to a minimum of 127 µS cm^{-1} (Table 4.5). At Clarrie Hall Dam, a reduction in the conductivity (K_{25}) was also recorded during the same severe weather event (decrease from a mean profile value of 118 µS cm^{-1} to a minimum of 88 µS cm^{-1} (Table 4.5). Cobaki Lake showed strong conductivity stratification during the sampling period. The lower anoxic layers were delineated by a halocline between 15 and 17 m. The conductivity (K_{25}) recorded in Cobaki Lake in the surface layers ranged from 325 to 582 µS cm^{-1} (Table 4.5), whereas it peaked below the halocline at 26,700 µS cm^{-1}. This brackish concentration in the anoxic layers of this lake suggests a salt intrusion possibly due to intermixing or seepage from the estuarine Cobaki Creek, which was located within 18.0 m of Cobaki Lake. The ANZECC guidelines were exceeded by the Cobaki Lake samples throughout the sampling period. The conductivity (K_{25}) of the surface water at the three sites did not vary significantly between 2007 and 2008 (ANOSIM Global R of -0.021) (Table 4.1).

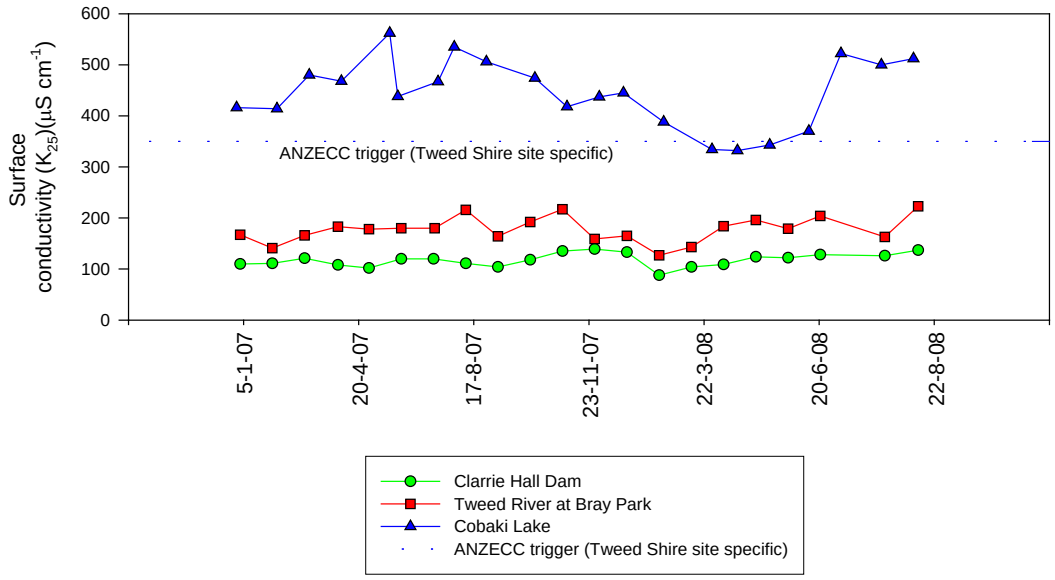


Figure 4.8. Conductivity (K_{25}) recorded at monthly intervals in surface water at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake between January 2007 and August 2008.

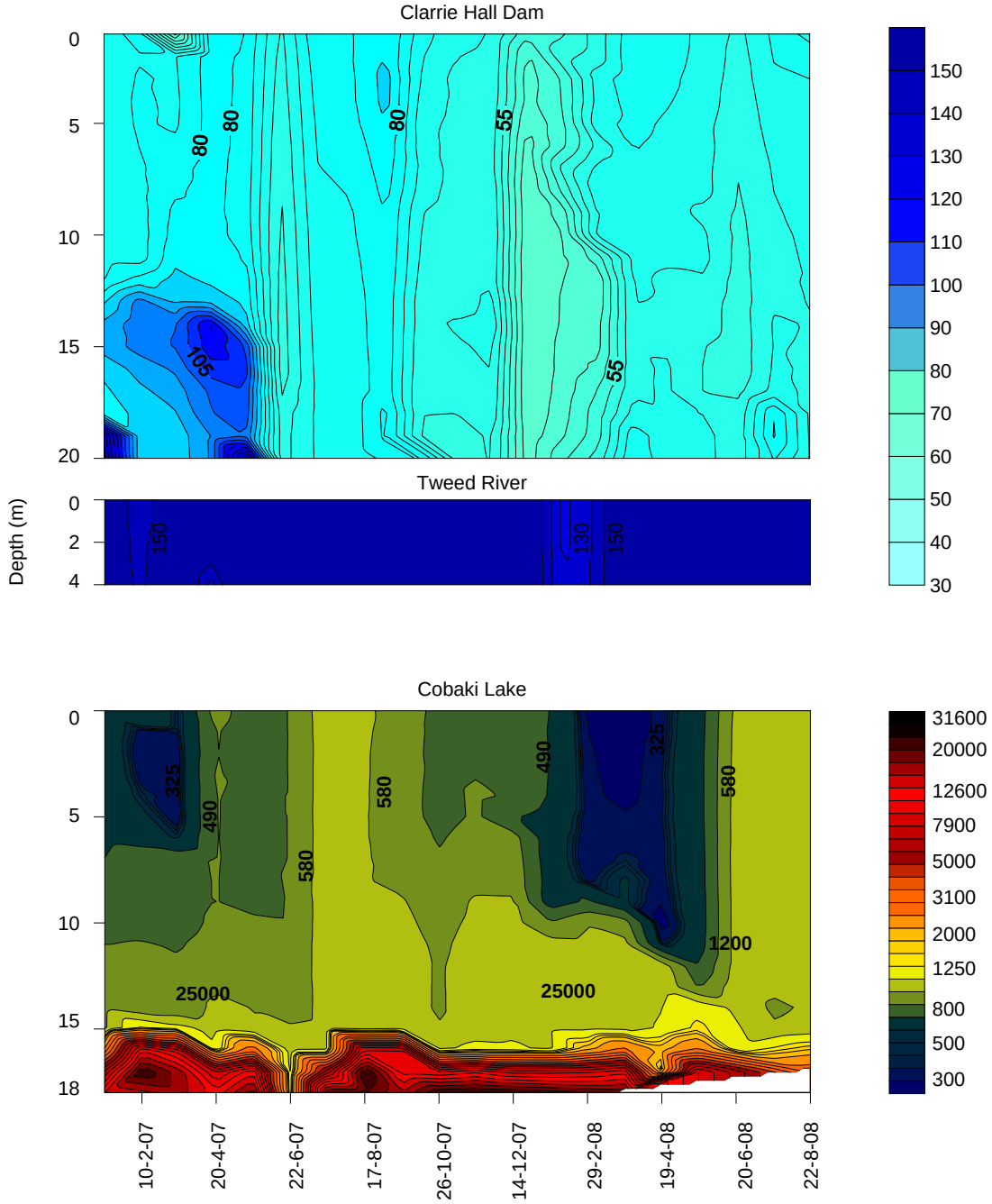


Figure 4.9. Isopleths of conductivity (K_{25}) (µS cm^{-1}) recorded monthly at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake monthly between January 2007 and August 2008.

Table 4.5. Summary statistics of conductivity (K_{25}) recorded monthly in surface water at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake between January 2007 and August 2008.

Surface water (K_{25})	Clarrie Hall Dam (µS cm^{-1})	Tweed River (µS cm^{-1})	Cobaki Lake (µS cm^{-1})
Mean	118	177	433
Median	120	179	437
Minimum	88	127	325
Maximum	139	223	582

4.2.6 Turbidity

Turbidity did not vary significantly between the three sampling sites. The median turbidity at Clarrie Hall Dam was 3.0 NTU; at the Tweed River it was 3.2 NTU; and at Cobaki Lake it was 2.3 NTU (Table 4.6). There was a spike in turbidity in Clarrie Hall Dam with the winter turnover in July 2007 when it increased to 12 NTU (Figure 4.10). A smaller spike (8 NTU) accompanied the January 2008 flood event. There was no spike in the winter of 2008 perhaps due to the increased rainfall throughout the year. The Tweed River at Bray Park spiked between November 2007 and March 2008, with a peak in January 2008 (16 NTU) coinciding with the increased rainfall and higher flow rates experienced during this time. Another spike in turbidity (25 NTU) also occurred in the river in July 2008. A similar spike was not recorded in either Clarrie Hall Dam or at Cobaki Lake at this time; the riverine spike could possibly have been caused by a localised storm event which is common at this time of year. Electrical storms occur frequently in the Tweed Shire and are usually quite random and localised and produce sudden downfalls of rain and gusts of wind, all of which could affect the turbidity of the Tweed River at Bray Park. The turbidity recorded in the surface water did not vary significantly between the three sites (ANOSIM Global R of 0.063) (Table 4.1) and, despite the difference in rainfall, there was no significant difference in surface water turbidity between 2007 and 2008 (ANOSIM Global R of 0.011, Table 4.1). The ANZECC guideline trigger value was only exceeded once, in the Tweed River during July 2008.

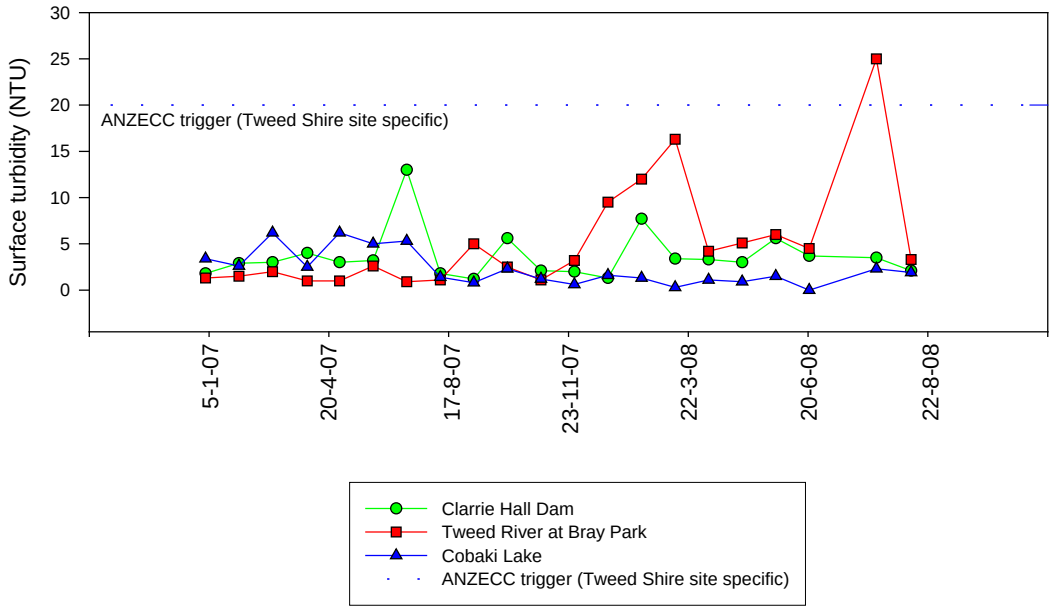


Figure 4.10. Turbidity recorded monthly in surface water at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake between January 2007 and August 2008.

Table 4.6. Summary statistics of surface water turbidity at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded at monthly intervals between January 2007 and August 2008.

Surface water (Turbidity)	**Clarrie Hall Dam (NTU)**	**Tweed River (NTU)**	**Cobaki Lake (NTU)**
Mean	3.6	5.1	2.6
Median	3.0	3.2	2.3
Minimum	1.2	0.9	0.0
Maximum	12	25	6.2

4.2.7 Nutrients

4.2.7.1 Soluble nutrients

Dissolved inorganic nitrogen species spiked at winter mixing in both Clarrie Hall Dam and Cobaki Lake in 2007 (Figure 4.11; Table 4.7). These two water bodies were stratified with nutrients from the hypolimnion being uplifted to the surface layers when mixing occurred. In 2008 this trend was less defined. The NOx concentration in Cobaki Lake increased from 0 to 50 µg L^{-1} in the winter of 2008 and Clarrie Hall Dam did not feature any nitrogen spikes at all. Samples from the Tweed River featured a 0 to 50 µg L^{-1} increase in the concentration of oxidised nitrogen in August 2008; it is likely that this accompanied the (storm-induced) turbidity spike. The surface NH_4N spiked in Clarrie Hall Dam in autumn 2008 (April/May) but not when it mixed in winter (Figure 4.11; Table 4.8). Cobaki Lake did not spike at all in 2008 (Figure 4.11; Table 4.8).

Despite mixing in each of the two stratified water bodies in the winter of 2008, the concentrations of available nitrogen species in the surface water did not produce high peaks as they did during the winter mixing in the drier year of 2007. This may be related to the increased rainfall which occurred in the shire in 2008. The soluble reactive phosphorus (SRP) concentrations in Clarrie Hall Dam remained constant throughout the sampling period except for a spike in August 2008 when the dam mixed (Figure 4.12; Table 4.9). There was no spike recorded during the winter mix of 2007. The Tweed River showed a slight increase in SRP concentration in January 2008 which coincided with the flood event. Cobaki Lake showed increases in SRP concentration in both October and December 2007 but none in 2008. An examination of the ANZECC guideline trigger values (Figures 4.11; 4.12) show that dissolved inorganic nutrient concentrations in the surface waters of Cobaki Lake were consistently equal to or less than these trigger values except when mixing occurred in 2007, whereas Clarrie Hall Dam and the Tweed River at Bray Park were generally equal-to or greater than these trigger values.

These dissolved inorganic nutrient results generally demonstrate that in the drier year (2007) when strong stratification was present, the winter mixing showed marked spikes in nutrient loading in the surface water, whereas in the high rainfall year (2008), these spikes in dissolved inorganic nutrients were reduced. The dissolved inorganic nutrient concentrations recorded in the surface water indicated some difference between the sites (ANOSIM Global R of 0.468) (Table 4.10) but no significant difference between the years (ANOSIM Global R of -0.005) (Table 4.10).

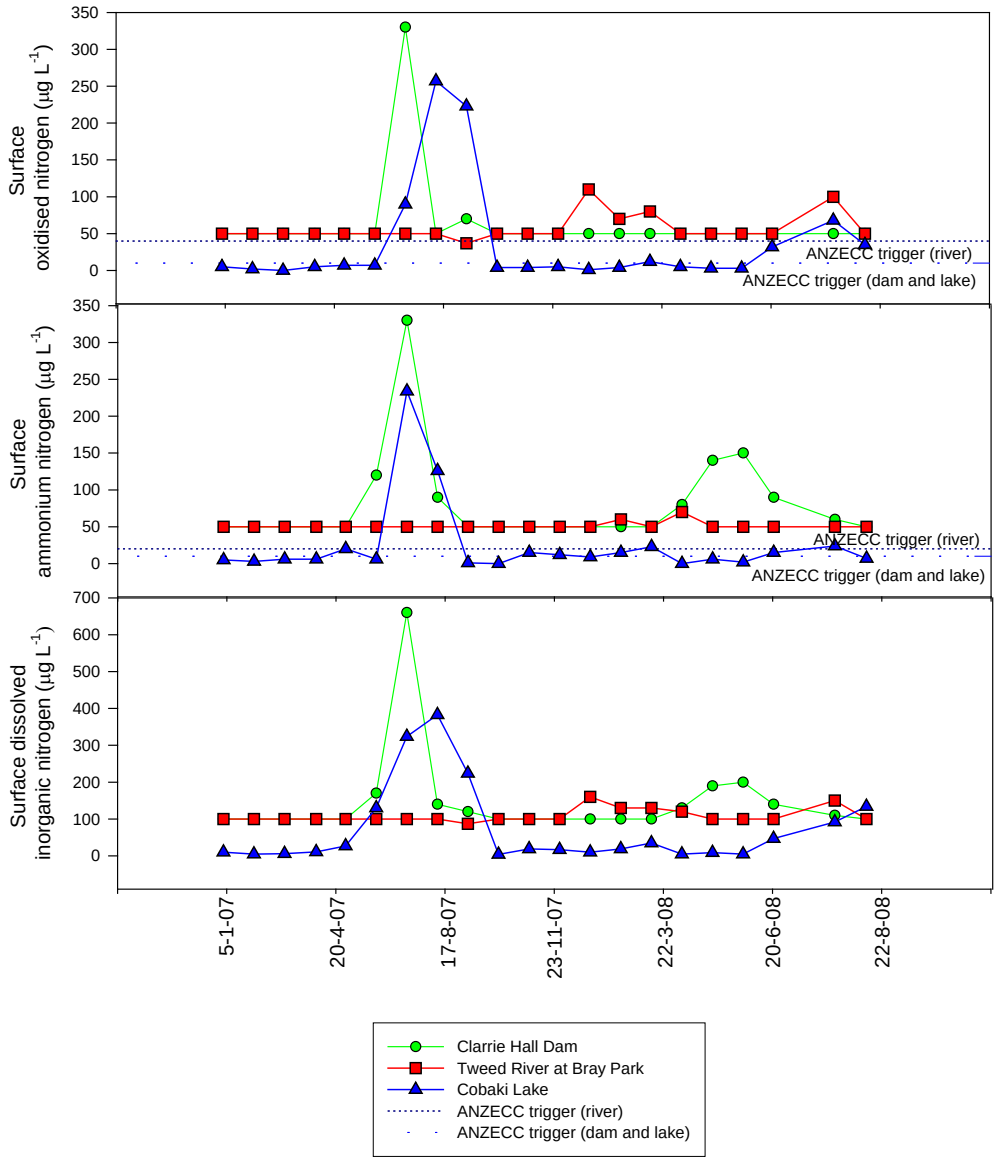


Figure 4.11. Concentrations of the oxidised nitrogen (NO_x), ammonium nitrogen (NH_4N) and dissolved inorganic nitrogen (DIN) in samples of surface water from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

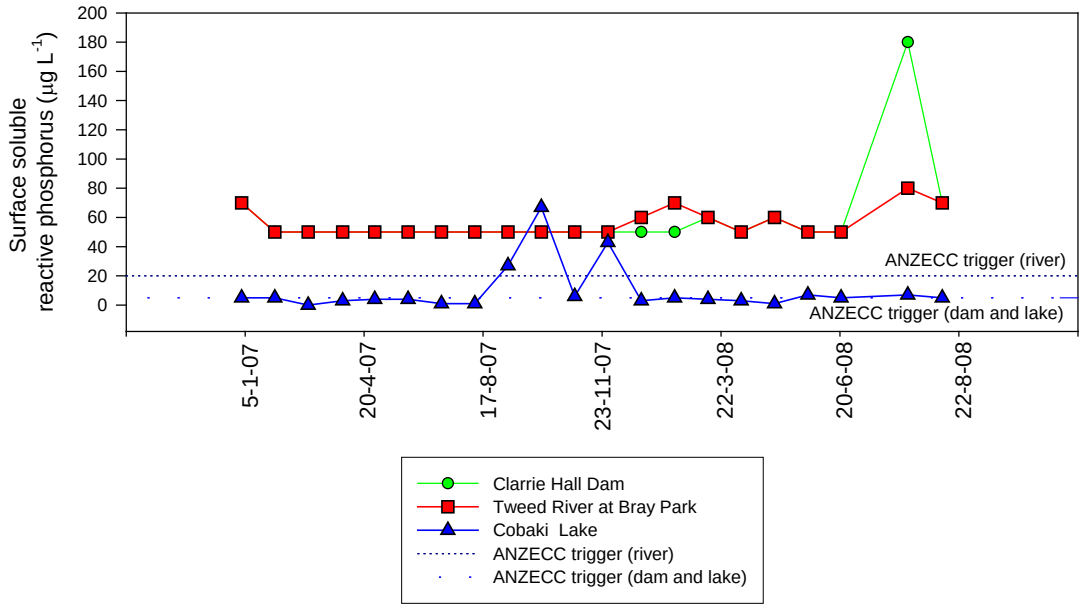


Figure 4.12. Concentrations of soluble reactive phosphorus (SRP) in samples of surface water at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

Table 4.7. Summary statistics of concentrations of oxidised nitrogen (NO_x) in samples of surface water at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

Surface water (NO_x)	**Clarrie Hall Dam (µg L^{-1})**	**Tweed River (µg L^{-1})**	**Cobaki Lake (µg L^{-1})**
Mean	64	57	30 (< LoD)
Median	50	50	5 (< LoD)
Minimum	50	37 (< LoD)	0 (< LoD)
Maximum	330	110	257

Table 4.8. Summary statistics of concentrations of amonium nitrogen (NH_4N) in samples of surface water from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

Surface water (NH_4N)	**Clarrie Hall Dam (µg L^{-1})**	**Tweed River (µg L^{-1})**	**Cobaki Lake (µg L^{-1})**
Mean	81	51	34 (< LoD)
Median	50	50	6 (< LoD)
Minimum	50	50	0 (< LoD)
Maximum	330	70	234

Table 4.9. Summary statistics of concentrations of soluble reactive phosphorus (SRP) in samples of surface water from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

Surface water (SRP)	**Clarrie Hall Dam (µg L^{-1})**	**Tweed River (µg L^{-1})**	**Cobaki Lake (µg L^{-1})**
Mean	58	55	8 (< LoD)
Median	50	50	5 (< LoD)
Minimum	50	50	0 (< LoD)
Maximum	180	80	67

Table 4.10. ANOSIM (Analysis of Similarities) data showing the Global R statistic involving two sets of comparisons (SITE and YEAR) for soluble nutrient concentrations at Clarrie Hall Dam (D), the Tweed River at Bray Park (R) and Cobaki Lake (C) recorded at monthly intervals between January 2007 and August 2008.

Variable	ANOSIM R Statistic (SITE)	ANOSIM R Statistic (YEAR)
Soluble nutrients (NO_x, + NH_4N + SRP) ($\mu g\ L^{-1}$)	Global 0.468 C, D 0.694 C, R 0.777 D, R 0.019	Global -0.005
Oxidised nitrogen (NO_x) ($\mu g\ L^{-1}$)	Global 0.398 C, D 0.597 C, R 0.654 D, R 0.002	Global -0.006
Amonium nitrogen (NH_4N) ($\mu g\ L^{-1}$)	Global 0.465 C, D 0.672 C, R 0.841 D, R 0.032	Global -0.012
Dissolved inorganic nitrogen (DIN) ($\mu g\ L^{-1}$)	Global 0.346 C, D 0.489 C, R 0.567 D, R -0.006	Global -0.003
Soluble reactive phosphorus (SRP) ($\mu g\ L^{-1}$)	Global 0.485 C, D 0.753 C, R 0.818 D, R -0.008	Global -0.009

4.2.7.2 Total nutrients

Total nitrogen concentrations in the depth profiles of Clarrie Hall Dam (median 540 $\mu g\ L^{-1}$) (Table 4.11) and the Tweed River at Bray Park (median 420 $\mu g\ L^{-1}$) were low compared with those of Cobaki Lake (median 574 $\mu g\ L^{-1}$). This appears to be due to the strong stratification of Cobaki Lake with a maximum TN concentration in the hypolimnion of 93,076 $\mu g\ L^{-1}$ (Figure 4.13).

Cobaki Lake TP concentration (median 11 µg L^{-1} which is < LoD) was low compared with both Clarrie Hall Dam (median 50 µg L^{-1}) and the Tweed River at Bray Park (median 60 µg L^{-1}) (Table 4.12).

Comparison of total nutrient concentrations across the three sites revealed little difference between each location (ANOSIM Global R of 0.214) (Table 4.13) and no significant difference between the years (ANOSIM Global R of 0.039) (Table 4.13). The total phosphorus concentrations at Cobaki Lake were lower than those of the Clarrie Hall Dam and the Tweed River (Table 4.13). The slightly stronger dissimilarity is indicated by the R statistics of 0.511 (Cobaki Lake and Clarrie Hall Dam comparison) and 0.571 (Cobaki Lake and the Tweed River comparison).

Table 4.11. Summary statistics of concentrations of total nitrogen (TN) in depth profile samples from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

TN (Depth profiles)	**Clarrie Hall Dam (µg L^{-1})**	**Tweed River (µg $L^{-1)}$**	**Cobaki Lake (µg L^{-1})**
Mean	561	464	3,061
Median	540	420	574
Minimum	1 (< LoD)	260	263
Maximum	1,520	1,140	93,076

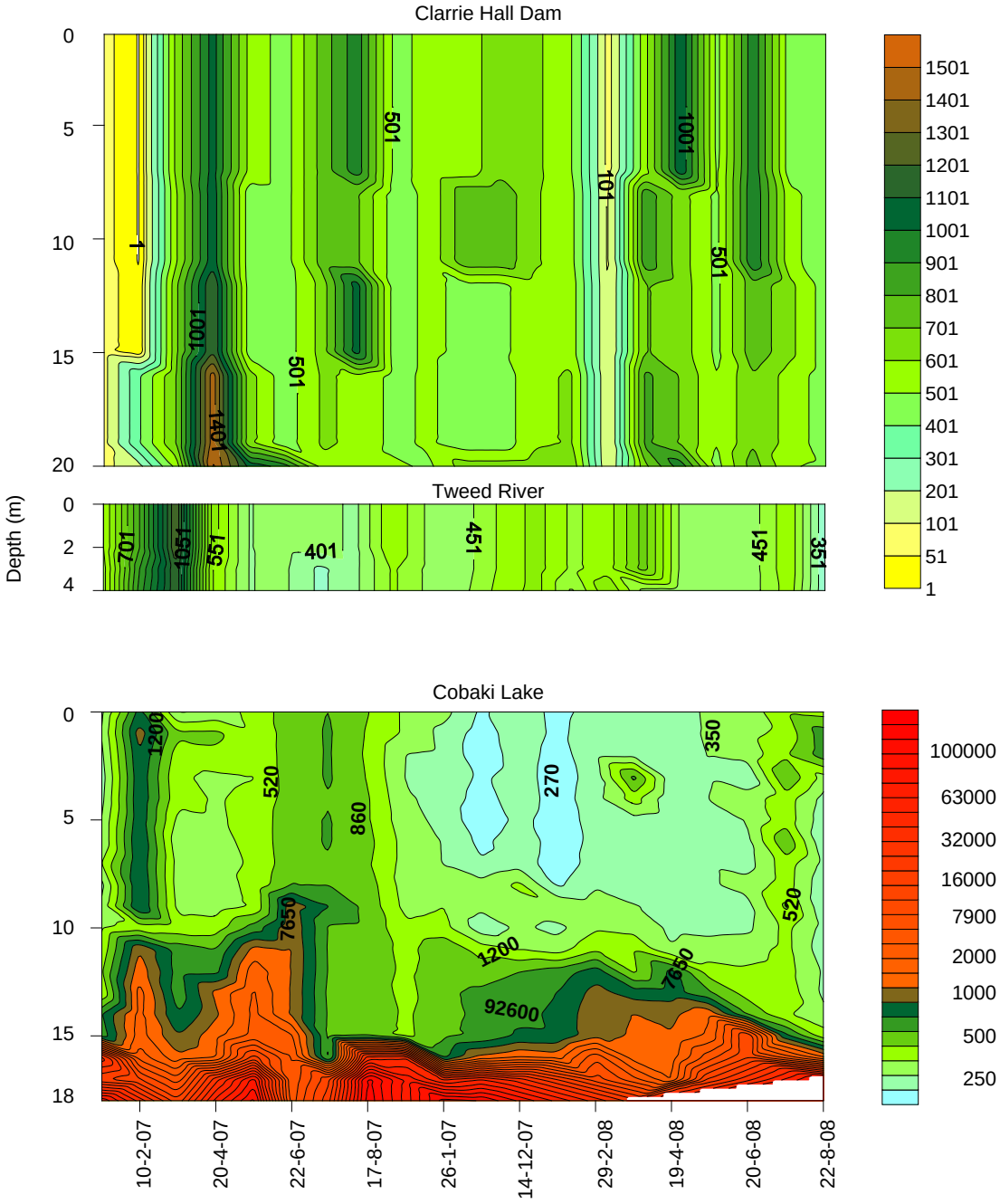


Figure 4.13. Isopleths of concentrations of total nitrogen (TN) (µg L^{-1}) in samples from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

Table 4.12. Summary statistics of concentrations of total phosphorus (TP) (µg L^{-1}) in depth profile samples from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly between January 2007 and August 2008.

TP (Surface water)	**Clarrie Hall Dam (µg L^{-1})**	**Tweed River (µg L^{-1})**	**Cobaki Lake (µg L^{-1})**
Mean	65	69	21 (< LoD)
Median	50	60	11 (< LoD)
Minimum	50	50	3 (< LoD)
Maximum	180	110	93

Table 4.13. ANOSIM (Analysis of Similarities) showing the Global R statistic involving two sets of comparisons (SITE and YEAR) for total nutrient concentrations at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded at monthly intervals between January 2007 and August 2008.

Variable	**ANOSIM R Statistic (SITE)**	**ANOSIM R Statistic (YEAR)**
Total nutrients (TN + TP) (µg L^{-1})	Global 0.214 C, D 0.288 C, R 0.268 D, R 0.090	Global 0.039
Total nitrogen (TN) (µg L^{-1})	Global 0.113 C, D 0.182 C, R 0.076 D, R 0.079	Global 0.051
Total phosphorus (TP) (µg L^{-1})	Global 0.385 C, D 0.511 C, R 0.571 D, R 0.067	Global -0.001

4.2.8 Soluble metals

4.2.8.1 Soluble metals (surface water)

4.2.8.1.1 Silica (soluble)

The highest concentrations of soluble silica in the surface water were recorded from the Tweed River (maximum 26.0 mg L^{-1}) (Figure 4.14; Table 4.14), the higher concentrations occurring in the summer of 2008, the year of increased rainfall and localised flooding. Clarrie Hall Dam followed a similar trend although lower concentrations were detected (maximum 23.0 mg L^{-1}). Cobaki Lake was quite different with very low concentrations of soluble silica throughout (maximum 1.5 mg L^{-1}). These trends indicate that a significant difference in soluble silica concentration exists between water samples collected from the three sites (ANOSIM Global R of 0.790); however, there was no significant difference between the sampling years (ANOSIM Global R of 0.072). The soluble silica concentrations at all sites were below the NHMRC (2004) guideline (Figure 4.14).

4.2.8.1.2 Iron (soluble)

The highest concentrations of soluble iron were recorded in the surface water samples from Clarrie Hall Dam, which reached a maximum of 1,170 µg L^{-1} in late autumn 2008 and a slightly lower spike (700 µg L^{-1}) with winter mixing in August 2008 (Figure 4.14; Table 4.15). The soluble iron concentrations were lower in the drier year of 2007 showing a number of smaller spikes leading up to the winter mixing but no dramatically increased spike occurred at mixing. The soluble iron concentrations were below the limit of detection during the summer months. The surface water soluble iron concentrations in samples from the Tweed River at Bray Park were low (10 µg L^{-1}) throughout 2007 and the early months of 2008, spiking in autumn (March, April and May) and low again in June with a smaller spike at winter mixing in August (680 µg L^{-1}). In Cobaki Lake, the soluble iron concentrations in surface water remained consistently low throughout the sampling period and showed little to no variation. The soluble iron concentrations were mostly below the limit of detection; however a maximum of 77 µg L^{-1} was recorded during winter mixing in both years. The soluble iron concentrations in samples from Cobaki Lake and the Tweed River at Bray Park were below the ANZECC trigger guideline for total iron throughout 2007; however, the

Tweed River exceeded this guideline after heavy rain in 2008 (Figure 4.14). Soluble iron concentrations in samples from Clarrie Hall Dam frequently spiked above the ANZECC total iron trigger value throughout the sampling period reaching a maximum after heavy rain in 2008 (Figure 4.14). The variations in soluble iron concentrations between the sites (ANOSIM Global R of 0.310) and between the years (ANOSIM Global R of 0.054) were not significant.

4.2.8.1.3 Manganese (soluble)

Clarrie Hall Dam featured similar trends with the soluble manganese concentrations in surface water samples as it had with the soluble iron concentrations, reaching a maximum of 220 µg L^{-1} in late autumn 2008 and with a similar spike (220 µg L^{-1}) during winter mixing in August 2008 (Figure 4.14; Table 4.16). The soluble manganese concentrations were lower in the drier year of 2007, showing a number of smaller spikes leading up to the winter mixing, but showed no increased spike at this time. The soluble manganese concentrations were below the limit of detection during the summer months. The soluble manganese concentrations in surface water samples from the Tweed River at Bray Park remained constant throughout, with an increase in concentration in August 2008 (10 to 30 µg L^{-1}). Soluble manganese concentrations in surface water samples from Cobaki Lake remained consistently low throughout the sampling period and showed little variation. The soluble manganese concentrations were mostly below the limit of detection, however a maximum of 46 µg L^{-1} was recorded during the winter overturn in both years. The available manganese concentrations in samples from all sites were below the ANZECC trigger guidelines for total manganese (Figure 4.14). The variations in soluble manganese concentrations between the sites (ANOSIM Global R of 0.230) and between the years (ANOSIM Global R of - 0.016) were not significant.

4.2.8.1.4 Aluminium (soluble)

Soluble aluminium concentrations in samples of surface water from Clarrie Hall Dam were consistently low throughout most of the sampling period (Figure 4.14; Table 4.17). Spikes

were recorded in the autumn of both 2007 (25 µg L^{-1}) and 2008 (62 µg L^{-1}) with no detectable spike at winter mixing. Samples of surface water taken from the Tweed River at Bray Park recorded different soluble aluminium concentrations compared to samples of surface water taken from Clarrie Hall Dam. The aluminium concentrations were low throughout early 2007 (10 µg L^{-1}), spiking at winter mixing (Clarrie Hall Dam) in August (25 µg L^{-1}). A pattern of increase and decrease preceeded winter mixing (Clarrie Hall Dam) in 2008 when the dam soluble aluminium concentration peaked at 120 µg L^{-1}. The aluminium concentrations from surface water samples taken from Cobaki Lake varied from the other two water bodies, spiking in March 2007 (97 µg L^{-1}) and decreasing to below the limit of detection for the remainder of the sampling period. Apart from a couple of spikes, the concentrations of soluble aluminium in samples from all sites were below the ANZECC trigger value (Figure 4.14). The variations in soluble aluminium concentrations between the sites (ANOSIM Global R of 0.210) and between the years (ANOSIM Global R of 0.061) were not significant.

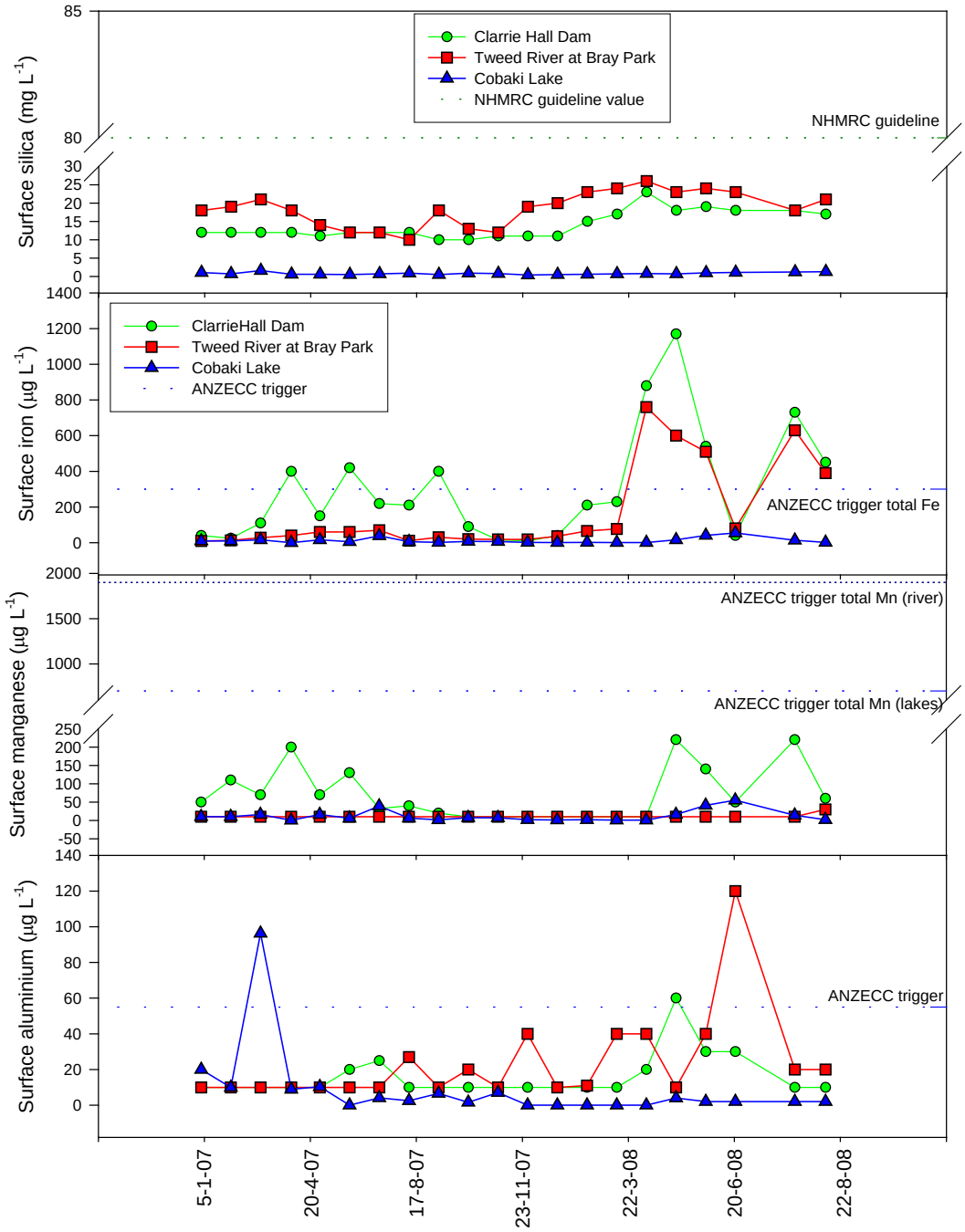


Figure 4.14. Concentrations of soluble silicon (Si), soluble iron (Fe), soluble manganese (Mn) and soluble aluminium (Al) in samples of surface water from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

Table 4.14. Summary statistics of concentrations of soluble silicon (Si) in samples of surface water from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

Surface water (Si)	**Clarrie Hall Dam (mg L^{-1})**	**Tweed River (mg L^{-1})**	**Cobaki Lake (mg L^{-1})**
Mean	13.9	18.5	0.8
Median	12.0	19.0	0.8
Minimum	9.6	9.7	0.4
Maximum	23.0	26.0	1.6

Table 4.15. Summary statistics of concentrations of soluble iron (Fe) in samples of surface water from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

Surface water (Fe)	**Clarrie Hall Dam (µg L^{-1})**	**Tweed River (µg L^{-1})**	**Cobaki Lake (µg L^{-1})**
Mean	304	168	9 (< LoD)
Median	210	60	7 (< LoD)
Minimum	10	10	0 (< LoD)
Maximum	1,170	760	77

Table 4.16. Summary statistics of concentrations of soluble manganese (Mn) in samples of surface water from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded at monthly intervals from January 2007 to August 2008.

Surface water (Mn)	**Clarrie Hall Dam (µg L^{-1})**	**Tweed River (µg L^{-1})**	**Cobaki Lake (µg L^{-1})**
Mean	71	11	23
Median	50	10	9 (< LoD)
Minimum	10	10	0 (< LoD)
Maximum	220	30	46

Table 4.17. Summary statistics of concentrations of soluble aluminium (Al) in samples of surface water from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

Surface water (Al)	Clarrie Hall Dam (µg L^{-1})	Tweed River (µg L^{-1})	Cobaki Lake (µg L^{-1})
Mean	16	23	19
Median	10	10	7 (< LoD)
Minimum	10	10	0 (< LoD)
Maximum	62	120	97

4.2.8.2 Soluble Metals (at depth)

4.2.8.2.1 Iron (soluble)

The water column depth profile of dissolved iron concentrations in samples taken at Clarrie Hall Dam showed an increased concentration of soluble iron (maximum 9,240 µg L^{-1}) below 15 m in the summer and autumn of 2007 (Figure 4.15), a trend which did not occur during the wetter year (2008). When the dam mixed in the winter of 2007, the iron concentration decreased to 30 µg L^{-1} throughout the water column. In late 2007 and early 2008, soluble iron concentrations increased below 12 m (2,000 µg L^{-1}) but reduced again throughout the water column (250 µg L^{-1}) in January 2008. This decrease coincided with a localised flood event in the Shire. In March 2008 the hypolimnetic concentrations increased again (2,000 µg L^{-1}). At mixing in August 2008 the soluble iron concentrations throughout the water column decreased to 250 µg L^{-1}. The soluble iron concentrations in samples from the Tweed River at Bray Park were quite different to Clarrie Hall Dam with a minimum of 10 µg L^{-1} and a maximum of 760 µg L^{-1}. These concentrations were low in the surface water for the first three months of 2007 and through the whole water column in April 2007. There was another pocket of low soluble iron levels in the surface water in July 2007 and from October 2007 to February 2008 (late autumn and summer). There was some stratification in the Tweed River in 2007 in relation to soluble iron concentrations, and higher concentrations of soluble iron were recorded below 2 m. The soluble iron concentration increased gradually after the flooding rainfall in January 2008 and remained distributed throughout the water column for the remainder of the sampling period. Samples taken from Cobaki Lake indicated low

soluble iron concentrations (not detectable) above 10 m throughout both years of sampling, but some very high concentrations were recorded in hypolimnetic samples below 15 m with a maximum concentration of 57,100 µg L^{-1}.

The soluble iron depth profiles are presented using contour plots (Figure 4.14). This figure indicates the stratification of Cobaki Lake and Clarrie Hall Dam and the increased concentrations of soluble iron in the hypolimnion. Summary statistics of depth profile water soluble iron (Fe) concentrations (µg L^{-1}) are presented in Table 4.25.

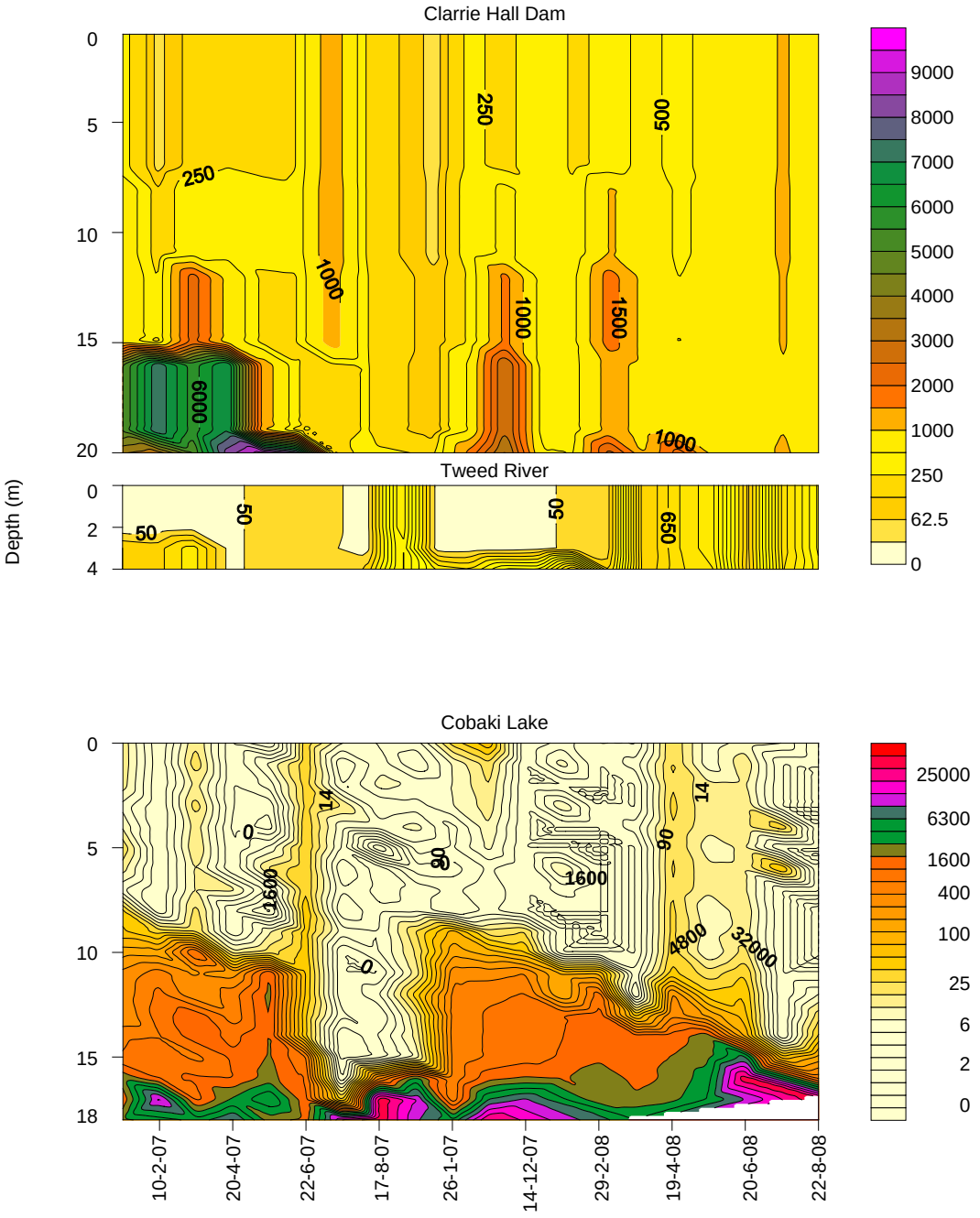


Figure 4.15. Isopleths of concentrations of soluble iron (Fe) (µg L^{-1}) in samples from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly between January 2007 and August 2008.

4.2.8.2.2 Manganese (soluble)

Depth profile samples taken from Clarrie Hall Dam showed that the dam had stronger stratification in 2007 than in 2008 with regard to soluble manganese concentrations (Figure 4.16). This could be related to the difference in rainfall for the two years. The minimum concentration recorded was 30 µg L^{-1} and the maximum was 4,240 µg L^{-1} in the hypolimnion in November 2007. There was only partial mixing in the winter of 2007 and complete mixing in the winter of 2008. Low concentrations were evident in January 2008 when the district experienced an extreme rain event with localised flooding. Samples taken from the Tweed River at Bray Park indicated low concentrations of soluble manganese (< 25 µg L^{-1}) throughout the sampling period and throughout the water column. A slight increase in concentration (760 µg L^{-1}) occurred at depth (4 m) in late January 2008 and could have been carried down-stream from Clarrie Hall Dam during the extreme rain event that occurred during this period. Samples taken from Cobaki Lake showed strong soluble manganese stratification throughout the sampling period, mostly below 8 m, with extremely high concentrations below 16 m (maximum 73,200 µg L^{-1}). Spikes occurred in June of both years with partial mixing occurring in the winter of 2007 and 2008.

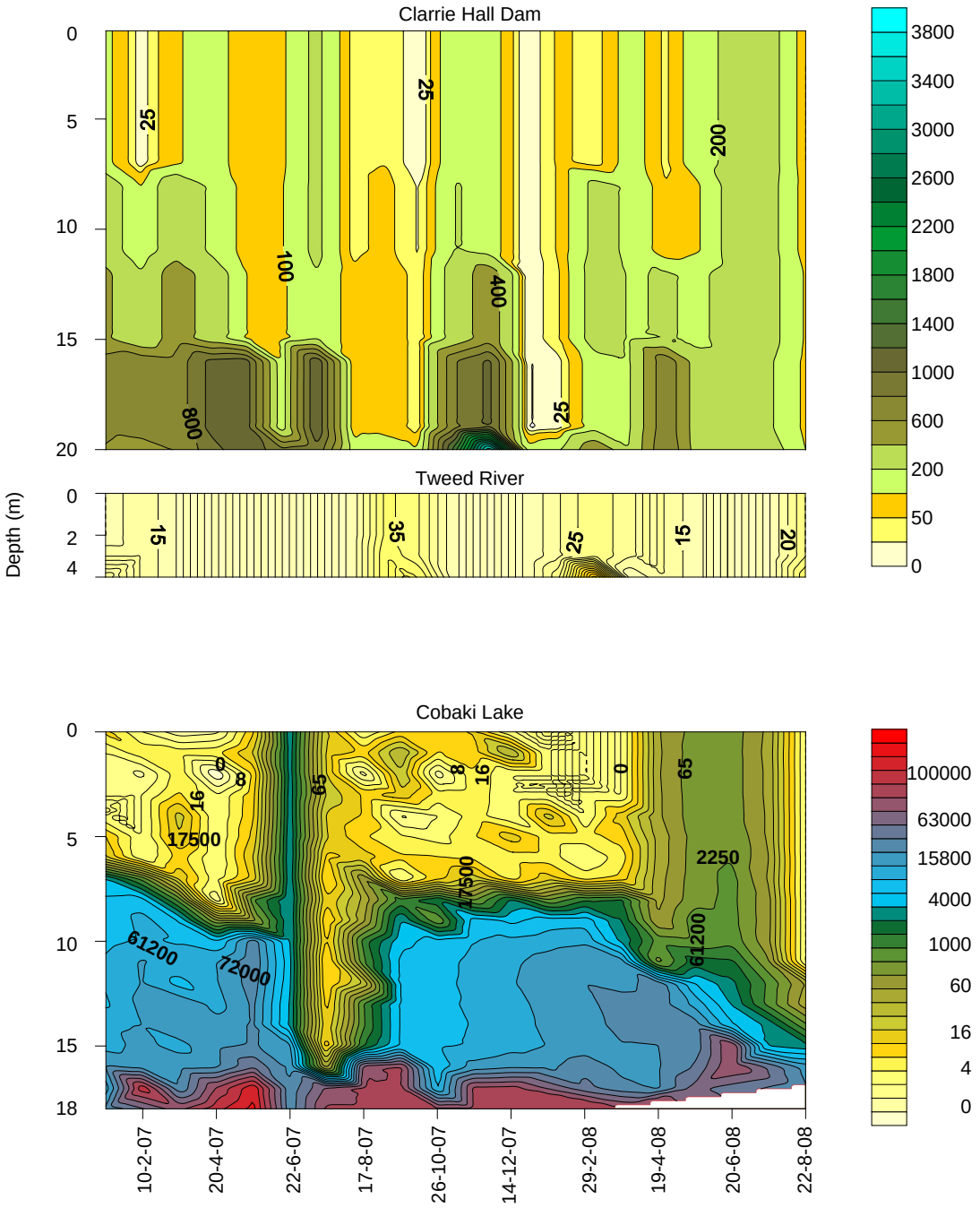


Figure 4.16. Isopleths of concentrations of soluble manganese (Mn) (µg L^{-1}) in samples from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake recorded monthly from January 2007 to August 2008.

4.2.9 ANZECC Guidelines

Comparisons of water quality parameter median values at the Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake to ANZECC trigger values, are presented in Tables 4.18 and 4.19. The data presented in these tables are from water samples collected between January 2007 and August 2008.

Table 4.18. Comparison of water quality parameter median values at the Clarrie Hall Dam and Cobaki Lake to ANZECC (2000) trigger values. Parameters exceeding trigger values are indicated in red. The data were recorded monthly from January 2007 to August 2008.

Parameter	Unit	ANZECC trigger values *Freshwater Lakes and Reservoirs*	Clarrie Hall Dam	Cobaki Lake
Conductivity (K_{25})	µS cm $^{-1}$	20–30 [1]* 350	120	437
Turbidity	NTU	1–20 [1]* 20	3.0	2.3
pH		6.5–8.0	7.0	8.6
Total phosphorus (TP)	µg L^{-1}	10	50	11
Soluble reactive phosphorus (SRP)	µg L^{-1}	5	50	5
Total nitrogen (TN)	µg L^{-1}	350	540	574
Oxidised nitrogen (NO_x)	µg L^{-1}	10	50	5
Ammonium (NH_4N)	µg L^{-1}	10	50	6
Dissolved oxygen (DO)	% sat	90–110	83	88
Soluble manganese (Mn)	µg L^{-1}	n/a total Mn = 700	50	9
Soluble iron (Fe)	µg L^{-1}	n/a total Fe = 300	210	7
Soluble aluminium (Al)	µg L^{-1}	55	10	7
Soluble silica (Si) [2]**	mg L^{-1}	n/a NHMRC (2004) = 80	12	0.8

[1] * Site-specific adjustment with reference to the ANZECC guidelines for upland rivers.

[2] ** There was no trigger value for Si in ANZECC guidelines; therefore the Australian Drinking Water Guideline (NHMRC, 2004) is indicated.

Table 4.19. Comparison of water quality parameter median values at the Tweed River at Bray Park to ANZECC trigger values. Parameters exceeding trigger values are indicated in red. The data were recorded monthly between January 2007 and August 2008.

Parameter	**Unit**	**ANZECC trigger values** ***Lowland River***	**Tweed River at Bray Park**
Conductivity (K_{25})	µS cm $^{-1}$	2200	179
Turbidity	NTU	6–50	3.2
pH		6.5–8.0	7.3
Total phosphorus (TP)	µg L^{-1}	50	60
Soluble reactive phosphorus (SRP)	µg L^{-1}	20	50
Total nitrogen (TN)	µg L^{-1}	500	420
Oxidised nitrogen (NO_x)	µg L^{-1}	40	50
Ammonium (NH_4N)	µg L^{-1}	20	50
Dissolved oxygen (DO)	% sat	85–110	91
Soluble manganese (Mn)	µg L^{-1}	n/a total Mn = 1900	10
Soluble iron (Fe)	µg L^{-1}	n/a total Fe = 300	60
Soluble aluminium (Al)	µg L^{-1}	55	10
Soluble silica (Si) [3]*	mg L^{-1}	n/a NHMRC (2004) = 80	19

[3] * There was no trigger value for Si in ANZECC guidelines; therefore the Australian Drinking Water Guideline (NHMRC, 2004) is indicated.

4.3 Discussion

4.3.1 Clarrie Hall Dam

Clarrie Hall Dam is surrounded by a vegetation buffer zone, with very little development threatening the quality of the dam water. Upstream from the dam is mostly low-impact agricultural land use, which to date has had minimal impact on the water quality flowing into the dam. Regular monitoring of the catchment ensures that possible problems are identified quickly and processes put into place to prevent significant impact of the inbound water quality.

Hunter Water (2011) recommended some adjustments to the ANZECC trigger values in regard to the Tweed catchment system. It stated that conductivity (K_{25}) values of < 125 µS cm^{-1} and < 30 µS cm^{-1} do not indicate poor quality water within Clarrie Hall Dam. Rather, the 'upland river' (altitude > 150 m) (ANZECC 2000) trigger value of 350 µS cm^{-1} for conductivity (K_{25}) would be a more appropriate local environmental level for Clarrie Hall Dam conditions. Turbidity was also assessed in Hunter Water (2011) and it was recommended that the low range turbidity trigger (1 NTU) be removed since < 6 NTU typically indicates high quality water for the Tweed River catchment area.

The median values of the key water quality parameters at Clarrie Hall Dam were within the ANZECC trigger values for 'freshwater lakes and reservoirs' for conductivity (K_{25}), turbidity, pH, and concentrations of soluble manganese, soluble iron, soluble aluminium and soluble silica (NHMRC 2004). The median nutrient concentrations (both soluble and total) exceeded the ANZECC trigger values for 'freshwater lakes and reservoirs'. The median dissolved oxygen concentration was below the ANZECC trigger levels. The nutrient concentrations above the ANZECC trigger values in Clarrie Hall Dam suggest that, under favourable weather conditions, this water body could sustain the growth of cyanoprokaryote

blooms including those of potentially toxin-producing species. This would compromise the quality of this water storage.

Clarrie Hall Dam is stratified, particularly in the summer months, despite the installation of a mechanical mixer in 2002. When thermal stratification occurs in a water body, two separate masses of water (the epilimnion and hypolimnion) develop with transitional layer (the thermocline) between them. Cyanoprokaryotes are able to regulate their buoyancy and move between these layers so that they can obtain nutrients from the hypolimnion and optimal light conditions in the epilimnion. This gives them an advantage over other phytoplankton (ANZECC 2000; Oliver and Ganf 2000; Ganf and Oliver 1982).

It is often difficult to reduce nutrient levels in the natural environment; however, mechanical mixing can be used to prevent thermal stratification and decrease the likelihood of anoxia in the hypolimnion and release of nutrients from the sediments, at the same time inhibiting the ability of cyanoprokaryotes to control their depth and access to sunlight (Burford *et al.* 2007). While the mechanical mixer in Clarrie Hall Dam appears to be inadequate in terms of preventing stratification, as noted in Hunter Water (2011), it has been sufficient to maintain the presence of nitrogen in the surface water layer, as well as deterring the occurrence of dinitrogen-fixing nostocaleans. Consequently, in 2007 and 2008, Clarrie Hall Dam featured a short nutrient-rich water column (exceeding the ANZECC trigger levels) with a eutrophic epilimnion. This kind of environment can be expected to decrease the likelihood of blooms of problematic genera.

Mixing modifies the chemistry of the hypolimnion, thus avoiding or reducing the development of anoxia. Anoxic conditions in and near sediments caused by microbial or chemical activity may result in the release of phosphorus from the sediments (Burford *et al.* 2012; Burford *et al.* 2006; ANZECC 2000; Nealson *et al.* 1988). In a well-mixed water body

(that is: oxygenated conditions) the phosphorus-rich sediments are sealed by an oxidised surface layer that involves an iron-phosphorus complex (ANZECC 2000). Given that phytoplankton, including cyanoprokaryotes, require phosphorus as a food source, destratification of the dam and consequent reduction in the available phosphorus could assist with the management of cyanoprokaryote populations. This is particularly important given that both the total phosphorus and available phosphorus concentrations in Clarrie Hall Dam exceeded the ANZECC trigger values.

Hunter Water (2011) also mentioned that, without sufficient oxygen in the lower depths of Clarrie Hall Dam, manganese oxides can be reduced resulting in the release of soluble manganese (Zaw and Chiswell 1999; Nealson *et al.* 1988; Balzer 1982; Hoffman and Eisenreich 1981). Winter dam mixing can result in the lower layers of poorer quality water mixing throughout the entire water column. Soluble manganese concentrations in Clarrie Hall Dam did spike during 2007 and 2008; however, there is no trigger value for soluble manganese in the ANZECC guidelines, only a total manganese trigger value of 700 µg L^{-1}. The Australian Drinking Water guidelines (NHMRC 2004) recommend an aesthetic trigger value of 100 µg L^{-1} for manganese but this can be problematic in terms of taste, staining and discolouration in drinking water. Similar to manganese, high concentrations of iron can also be mixed throughout the water column when lower layers of poorer quality water are uplifted at turnover. The Australian Drinking Water Guidelines (NHMRC 2004) has an aesthetic trigger value of 300 µg L^{-1} iron for drinking water at which taste is affected. High concentrations of iron can also result in water discolouration and cause staining of laundry and plumbing fittings and blockages in irrigation systems. The ANZECC guidelines have a trigger value of 300 µg L^{-1} total iron but do not have a trigger value for soluble iron. Clarrie Hall Dam median soluble iron concentration was 210 µg L^{-1}, however, like manganese, spikes of higher iron concentrations occurred during the cooler months and lowered the overall quality of the water at these times. The lack of mixing in Clarrie Hall Dam may have contributed to the result where the ANZECC trigger values were exceeded.

It seems that the combination of catchment management and monitoring approaches are important in preventing the occurrence of toxic algal blooms. For example, in the neighbouring shire of the Gold Coast, Burford *et al*. (2007) reported that the phytoplankton assemblage in subtropical reservoirs with the lowest percentage forest cover in the catchment were dominated by cyanoprokarotes and had the highest frequency and magnitude of toxic species. This contrasts with the characteristics of Clarrie Hall Dam which features a high percentage of forest cover in the catchment and low cyanoprokaryotic populations. Catchment management, together with destratification of the water body, appears to be the key to controlling nutrient concentrations in Clarrie Hall Dam. Nutrient management would also assist in cyanoprokaryote bloom management as well as water quality in terms of manganese and iron concentrations.

4.3.2 The Tweed River at Bray Park

The key water quality parameters in the Tweed River at Bray Park which had median values less than the ANZECC trigger values for 'Lowland rivers' were conductivity (K_{25}), turbidity, pH, soluble manganese, iron, aluminium and silica (NHMRC, 2004). The nutrient concentration median values (both soluble and total), except for total nitrogen, were, however, greater than the the ANZECC trigger values for lowland rivers. The dissolved oxygen concentration median value was greater than the ANZECC data range trigger levels. The nutrient concentration levels which were above the ANZECC trigger values in the Tweed River at Bray Park indicate that, under favourable weather conditions, this water body could sustain the growth of cyanoprokaryote blooms that would compromise the water quality.

In a typical lotic ecosystem diatoms are the major taxonomic group, but share a lower fraction in tropical rivers which tend to contain desmids instead (Reynolds *et al.* 1994; Rojo *et al.* 1994; Reynolds *et al.* 1991). However, when times of low river flow (due to natural

conditions or human impact) are combined with factors such as increased temperature, stratification, or increased retention time, conditions similar to those in lakes and reservoirs occur, including differing phytoplankton assemblages. Paerl *et al.* (2007) used phytoplankton to characterise community responses to human (eutrophication) and climatic (hydrologic) perturbations in the Neuse River, North Carolina, USA. High flow following hurricanes favoured dominance by the fast growing chlorophytes and cryptophytes, whereas diatoms tended to dominate under moderate flow. On the other hand dinoflagellates and cyanoprokaryotes increased in dominance when low flow prevailed. Similar sequences have been observed in Australian rivers such as the Fitzroy (Fabbro and Duivenvoorden 2000), the Murray-Darling (Hötzel and Croome 1994) and the Darling-Barwon (Bowling 1992), and these illustrate a continuum between lotic and lacustrine environments. Fabbro and Duivenvoorden (2000) described the cosmopolitan nature of the phytoplankton, and how specific genera (and species) were suited to a particular niche irrespective of how that niche is attained. This is also the case in the Tweed River at Bray Park, where a naturally flowing river forms a riverine impoundment at the weir, and flushing of the impoundment is minimal during periods of low river flow. Stratification was not detected; however, the habitat changes favoured potentially toxic cyanoprokaryotes during times of low flow and, as the weir is the uptake point for water supply, careful management of this site is required.

4.3.3 Cobaki Lake

The key water quality parameters in Cobaki Lake which had median values less than the ANZECC trigger values for 'freshwater lakes and reservoirs', were turbidity, soluble manganese, iron, aluminium and silica (NHMRC, 2004). The median dissolved oxygen concentration was below the ANZECC trigger levels. The median concentrations of total nitrogen and total phosphorus were greater than the ANZECC trigger values for 'freshwater lakes and reservoirs'. In contrast, the dissolved inorganic nutrient concentrations of NO_x and NH_4N fell below the ANZECC trigger values, with SRP concentration equalling the trigger value in Cobaki Lake. The conductivity (K_{25}) and pH in Cobaki Lake also exceeded the ANZECC trigger values for 'freshwater lakes and reservoirs'. These high concentrations

combined with strong stratification and low soluble nutrient concentration predispose Cobaki Lake to the growth of nostocaleans such as *C. raciborskii*. It is also consistent with patterns recorded in Brazil (Branco and Senna 1994), where the dominance of *C. raciborskii* was linked with nutrient depletion in the epilimnion as a result of thermal stratification during the wet season, as well as with high pH and water temperatures. The rise in *C. raciborskii* cell concentrations parallels the decrease in soluble nutrients in the surface layer (Fabbro *et al.* 2001; Fabbro and Duivenvoorden 1996; Branco and Senna 1994).

The strong halocline observed in Cobaki Lake could be related to a saline intrusion. Studies of groundwater have been undertaken on the Pimpama coastal plain in southern Moreton Bay, about 50 km north of Cobaki Lake, in subtropical eastern Australia (Harbison and Cox 2002; Preda and Cox 2000). Harbison and Cox (2002) noted that a common feature of groundwater bodies in coastal settings is elevated salinity. This groundwater may also be enriched with iron and manganese due to the weathering of basement rocks, and is highly reduced and oxygen deficient. Cobaki Lake is in a similar geographic zone and climate and, despite the soluble iron and manganese concentrations in the surface waters being below the ANZECC trigger levels, the concentrations of both elements in the hypolimnion were elevated. Research into groundwater and salinity issues were also undertaken in Tuan State Forest, a coastal pine forest situated in southern Queensland, about 300 km north of Brisbane with a subtropical climate typical of south-east Queensland and north-east New South Wales (Wang *et al.* 2008). This research identified localised salinity occurring in the groundwater and affecting pine tree growth.

4.3.4 General discussion

The impacts of water column dynamics and temperature on the chemistry and algal assemblages are particularly obvious in the three water bodies investigated in this study. The water body with the most stable stratification, Cobaki Lake, was also that with the highest

temperatures. This lake had no natural flushing or inflow (except for rain and groundwater), with excess water in times of high rainfall being released from the lake into a natural semi-saline creek via a system of one-way valves. Cobaki Lake exhibited both thermal and chemical stratification accompanied by an anoxic hypolimnion. The habitat created by Cobaki Lake was ideal for a number of potentially toxic cyanoprokaryote species. In terms of phytoplankton succession, this lake closely followed that of many water bodies in the arid tropics with dominance of *C. raciborskii* (Everson *et al.* 2009). The dominance of *C. raciborskii* in this developed catchment contrasts to the situation in the Clarrie Hall Dam with the mixer and the Tweed River at Bray Park, although all three were exposed to the same climatic conditions. Cobaki Lake also stands as an example of what could happen in subtropical areas when in-flow is minimised or non-existent, temperatures increase, and where there is intrusion of saline groundwater. It is also representative of what might be expected in relation to the construction of artificial dams and lakes in coastal and near-shore environments, particularly in areas of similar climatic regime to the Tweed Shire.

Stratification and increased retention time, combined with increased temperature and nutrients, appear to be the dominating factors in phytoplankton successions in lakes and reservoirs (Fabbro and Duivenvoorden 2000; Reynolds *et al.* 2000; Reynolds *et al.* 1994; Reynolds *et al.* 1991). Rainfall changes impact on runoff and river discharge in turn affects the retention time (Jones and Elliott 2007). The number and duration of cyanoprokaryote blooms has been investigated since 2007 in New South Wales, to evaluate if climatic conditions have impacted on bloom occurrence (Bowling 2012). This investigation found that red alerts increased during three years of drought, and decreased during the following two years of higher rainfall. Coastal regions showed less variation between the years; however, in general, higher rainfall in catchment areas meant higher flows in rivers and lower cyanobacterial populations. In subtropical Brazil, the study of Faxinal Reservoir (a warm, monomictic, meso-eutrophic water body) found that the mixing regime was the main determinant of the seasonal dynamics of the phytoplankton community and that the nutrient

dynamics were driven by this. The dominant phytoplanktonic functional groups in the Brazilian reservoir showed a close relationship with the relative water-column stability, and also, as a consequence of the mixing regime, with nutrient availability (Becker *et al.* 2009).

The correlation between temperature and the dominance of various Australian cyanoprokaryote species is well established (Griffiths and Saker 2003; Fabbro and Duivenvoorden 2000). In addition, the links between various species and global warming have also been highlighted (Barone *et al.* 2010; Figueredo *et al.* 2007; Wiedner *et al.* 2007; Briand *et al.* 2004). In tropical regions, the combination of stratification and higher temperatures is often accompanied by dominance of cyanoprokaryotes, particularly of *C. raciborskii* (Fabbro and Duivenvoorden 2000; Boland and Griffiths 1996; Hawkins and Griffiths 1993). Cobaki Lake exhibited a unique set of physical and chemical parameters with a strongly stratified profile combined with a saline anoxic hypolimnion. The ramifications of these unique physical and chemical properties become clear when the cyanoprokaryote assemblages and cyanotoxin depth profiles are examined in the following chapters.

5 PHYTOPLANKTON ASSEMBLAGES

5.1 Introduction

The term phytoplankton refers to all suspended microalgae in a water body, across a range of taxonomic groups, including the cyanoprokaryotes or blue green algae (Hötzel and Croome 1999). There are two principal reasons why phytoplankton are of interest to water scientists and the managers of water infrastructure: one is their ability to provide insights about ecological health of an aquatic system; the second is the potential for human and ecological health risks due to the production of potent toxins.

Phytoplankton are excellent indicators of ecological change. They are generally easy to detect, identify and quantify; they represent a large share of primary production and they are sensitive to diverse environmental stressors. Each of these characteristics means that phytoplankton are important contributors to the assessment of riverine, lake and/or reservoir health (Paerl *et al.* 2007; Hötzel and Croome 1999). From a conservation perspective, the importance of monitoring phytoplankton populations is to better understand and manage the ecological functioning of both standing and running freshwaters.

The supply of quality drinking water is one of many important operational areas for local government utilities. The potential risks posed by toxin-producing phytoplankton blooms are therefore of critical importance to water managers. For example, *C. raciborskii* is a toxin-producing organism that is traditionally a tropical species and occurs in a wide range of water bodies worldwide (Padisák 1997; Branco and Senna 1994). This species has also been extensively reported in tropical Australia (McGregor and Fabbro 2000; Boland and Griffiths 1996). Toxic *A. ovalisporum* has also been detected worldwide, including in Israel (Hadas *et al.* 2002; Pollingher *et al.* 1998), Italy (Bazzichelli and Abdelahad 1994), Greece (Gkelis *et al.* 2005), Spain (Quesada *et al.* 2005) and Australia (Shaw *et al.* 1999).

The number of potentially toxic cyanoprokaryote species in Australia and overseas is constantly increasing, causing concern for water supply managers. Toxicity of a strain of *Limnothrix* detected in Central Queensland dams and identified as *Limnothrix* AC0243 has recently been reported by Bernard *et al.* (2011). Humpage *et al.* (2012) found that *Limnothrix* AC0243 is capable of producing a novel water-soluble toxin ("Limnothrixin"). The genus *Limnothrix* occurs in a range of freshwater habitats as does *Geitlerinema*. The concern is that both genera are morphologically and genetically identical (Bernard *et al.* 2011) and may present a health risk in drinking water supplies.

Global climate change may result in subtropical winters, such as those experienced in the Tweed Shire, becoming warmer. Recent reports worldwide have also linked increased temperatures with an increased global distribution of *C. raciborskii* (Sinha *et al.* 2012; O'Neil *et al.* 2012; Barone *et al.* 2010; Messineo *et al.* 2010; Kling 2009). Mehnert *et al.* (2010) identified *C. raciborskii* as one of a number of invasive species and *A. ovalisporum* as a potentially invasive species. This suggests that global temperature changes may trigger a shift in dominance to favour invasive nostocalean species. This is a concern and presents a serious management issue for water authorities in cooler climatic zones because laboratory studies have shown that maximal toxin production by *C. raciborskii* occurs at cooler temperatures (Saker and Griffiths 2000).

Harris and Baxter (1996) undertook an extensive study of North Pine Dam, 164 km north of the Tweed Shire in south-east Queensland. The North Pine Dam is larger and cooler than the Clarrie Hall Dam, thus supporting a different phytoplankton assemblage and succession to those experienced in the Tweed Shire. The river systems in this south-eastern corner of Queensland have also been monitored regularly (Hötzel and Croome 1996; Hötzel and Croome 1994; Mackay *et al.* 1988; Shiel *et al.* 1982). Despite the frequent monitoring in Queensland, there has not been an in-depth study of the phytoplankton assemblage of the Tweed Shire. The Tweed system is unique since the storage facility (Clarrie Hall Dam) is 20

km upstream from the draw off point for water treatment (Bray Park Weir). The Tweed is also a critical system since it is the juncture between subtropical and tropical habitats and thus is a likely location at which the 'creep' of invasive species will be recognised. The Tweed is a good example of this kind of habitat and, prior to this study, was undescribed. This project therefore aimed to provide new insights into the phytoplankton trends of the Tweed, and in doing so, develop globally applicable information for management of anthropically modified systems in sub-tropical climates. Two sampling seasons (2007 and 2008) were compared to reveal the differences in phytoplankton assemblages recorded between a dry and wet season. Then the interaction between the distribution of phytoplankton and the physico-chemical parameters was explored. Finally, the phytoplankton assemblages, and in particular the potentially toxic species of cyanoprokaryotes, are discussed in relation to the NHMRC Drinking Water Guidelines (NHMRC 2004) and NHMRC Recreational Water Guidelines (NHMRC 2008).

5.2 Results

The three sampling sites at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake had, as expected, significantly different algal assemblages. Clarrie Hall Dam was dominated by the division Chlorophyta (50%), with 28% Cyanoprokaryota and 19% Bacillariophyta. In contrast, the Tweed River at Bray Park was dominated by Cyanoprokaryota (47 %) with 35 % Bacillariophyta and 18 % Cryptophyta. Cobaki Lake was dominated by Cyanoprokaryota (94 %) (Table 5.2).

5.2.1 Algal species

A variety of taxa were identified from the vertical depth profile sampling undertaken from January 2007 to August 2008 (Table 5.1). Cyanoprokaryota and Chlorophyta dominated the assemblage and Dinophyta, Bacillariophyta and Cryptophyta were also detected.

5.2.1.1 Temporal speciation changes

A clear variation across time was evident in the dominant algal divisions recorded from the three sites (Figure 5.1). Clarrie Hall Dam samples were dominated by chlorophytes with some cyanoprokaryotes between October 2007 and March 2008. Low concentrations of cyanoprokaryotes were recorded from the Tweed River at Bray Park during the dry year (2007) with diatoms and cryptophytes dominating in the wet year (2008). Cobaki Lake samples were dominated by cyanoprokaryotes throughout, with higher concentrations recorded in the dry year (2007).

Samples taken from Clarrie Hall Dam contained low concentrations of toxic species during 2007 with higher concentrations of *A. ovalisporum, A. circinalis* and *G. amphibium* recorded in 2008, which triggered drinking water alerts (Figure 5.2). Samples taken from the Tweed River at Bray Park contained low concentrations of *A. circinalis* below both drinking water and recreational water guidelines in the dry year (2007) with no potentially toxic species recorded during 2008 (Table 5.2). Samples taken from Cobaki Lake indicated the dominance of *A. ovalsiporum* and *C. raciborskii* in the first half of 2007 followed by lower concentrations of *M. aeruginosa* and *A. circinalis* in the later part of 2007 and also 2008 (Figure 5.2). The high concentrations of both cells and toxin recorded in Cobaki Lake resulted in the lake being closed for recreational use between January and June 2007.

Table 5.1. Species list of phytoplankton assemblages in samples from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake Between January 2007 and August 2008. + present; - absent.

Division	Family	Species	Dam	River	Cobaki (Lake)
Cyanoprokaryota	Nostocaceae	*Anabaena bergii*	-	-	+
	Nostocaceae	*Anabaena circinalis*	+	+	+
	Nostocaceae	*Aphanizomenon gracile*	+	-	-
	Nostocaceae	*Aphanizomenon ovalisporum*	-	-	+
	Nostocaceae	*Cylindrospermopsis raciborskii*	-	-	+
	Merismopediaceae	*Aphanocapsa delicatissima*	+	-	+
	Merismopediaceae	*Aphanocapsa holsatica*	+	-	+
	Synechococcaceae	*Cyanogranis imperfectum*	-	-	+
	Synechococcaceae	*Cyanogranis libera*	-	-	+
	Chroococcaceae	*Chroococcus minimus*	+	-	-
	Synechococcaceae	*Gloeothece* c.f. *subtilis*	-	-	+
	Microcystaceae	*Microcystis aeruginosa*	-	-	+
	Microcystaceae	*Microcystis panniformis*	-	-	+
	Synechococcaceae	*Myxobaktron plankticus*	-	-	+
	Phormidiaceae	*Arthrospira maxima*	+	-	-
	Pseudanabaenaceae	*Geitlerinema amphibium*	+	-	+
	Oscillatoriaceae	*Oscillatoria princeps*	+	+	-
	Phormidiaceae	*Phormidium ambiguum*	+	+	-
	Pseudanabaenaceae	*Pseudanabaena limnetica*	+	-	+
	Pseudanabaenaceae	*Spirulina latissima*	+	-	-

Table 5.1 (Continued)

Division	Family	Species	Dam	River	Cobaki (Lake)
Dinophyta	Peridinaceae	*Ceratium* spp.	+	-	+
	Peridinaceae	*Peridinium* spp.	+	-	-
Bacillariophyta	Acanthocerataceae	*Acanthoceras* spp.	+	-	-
	Aulacoseiraceae	*Aulacoseira* spp.	+	-	-
	Stephanodisceae	*Cyclotella* spp.	+	-	-
	Fragilariaceae	*Fragillaria* spp.	+	-	-
	Fragilariaceae	Pennate Diatoms	+	+	+
	Rhizosoleniacea	*Urosolenia* spp.	+	-	-
Chlorophyta	Chlamydomonadaceae	*Chlamydomonas* spp.	-	-	+
	Desmidiaceae	*Staurastrum* spp.	+	-	-
	Desmidiaceae	*Closterium* spp.	+	-	+
	Desmidiaceae	*Cosmarium* spp.	+	-	-
	Siphonocladaceae	*Botryococcus* spp.	+	-	-
	Siphonocladaceae	*Dictyosphaerium* spp.	+	-	-
	Oocystaceae	*Oocystis* spp.	+	-	-
	Palmellaceae	*Pseudosphaerocystis* spp.	+	-	-
	Scenedesmaceae	*Scenedesmus* spp.	+	-	-
	Volvocaceae	*Volvox* spp.	+	-	-
Cryptophyta	Cryptomonadaceae	*Cryptomonas* spp.	-	+	+
	Dinobryaceae	*Dinobryon* spp.	+	-	-

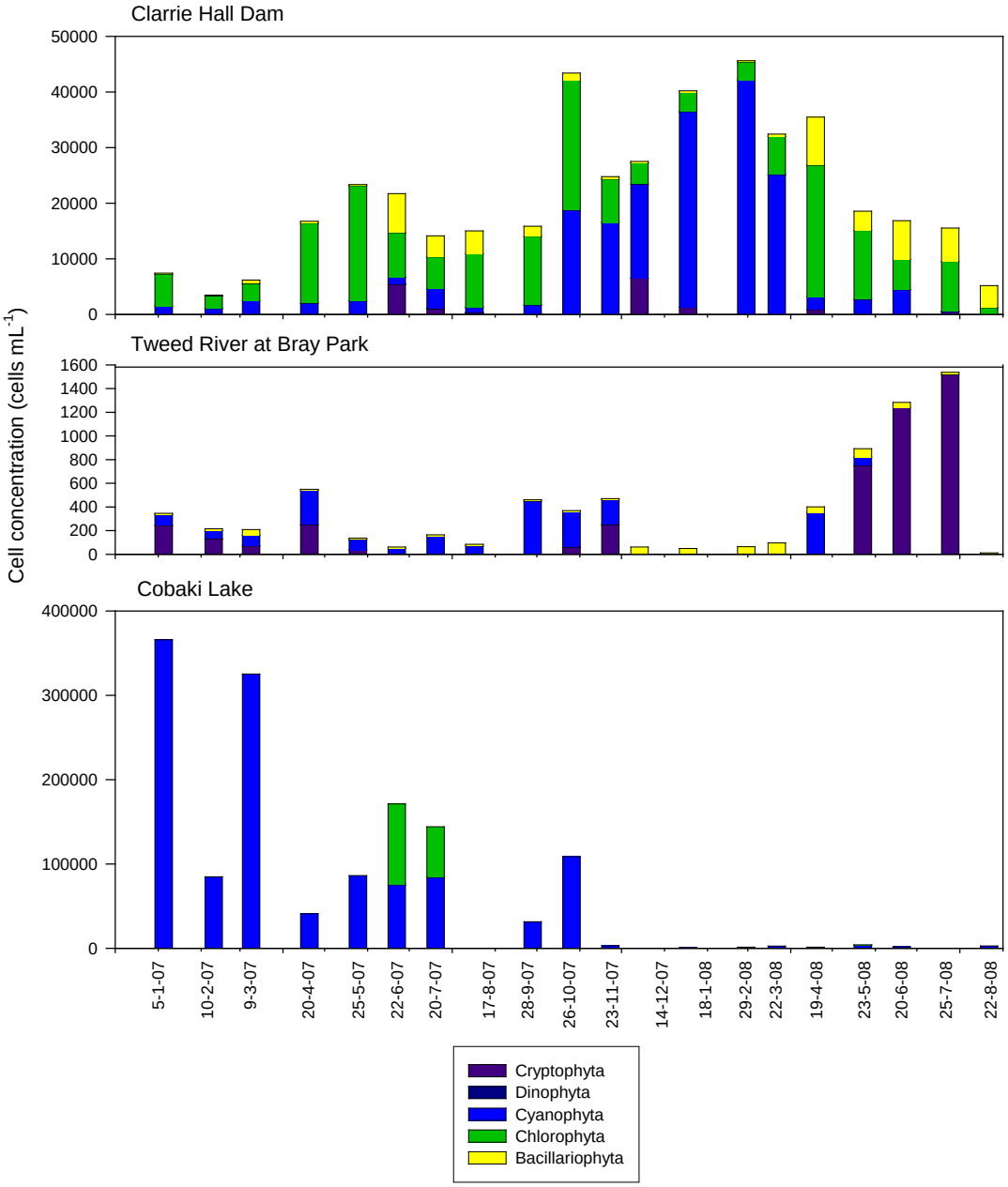


Figure 5.1. Time series plots of the main algal divisions detected in samples from 0.5 m at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake at monthly intervals between January 2007 and August 2008.

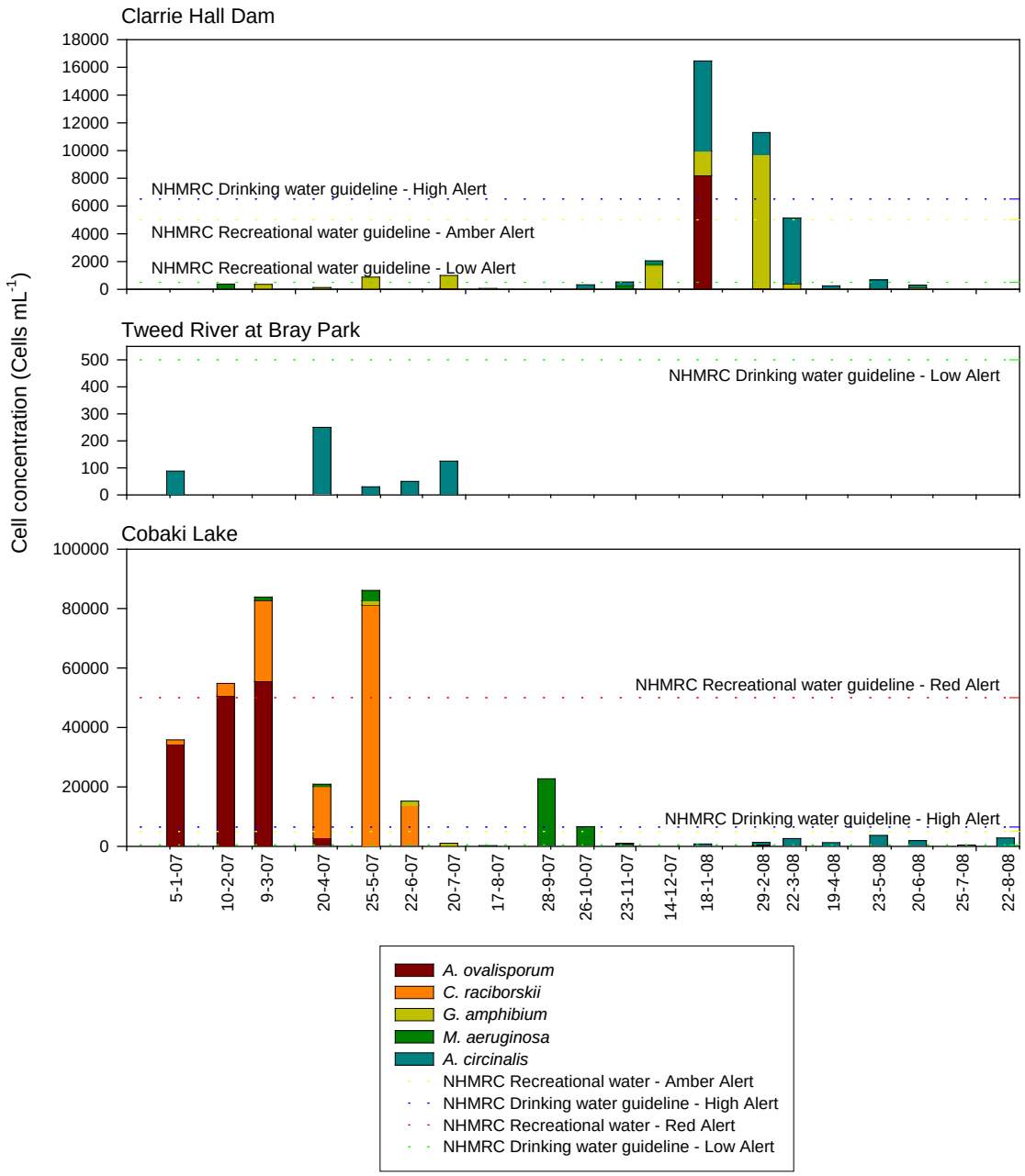


Figure 5.2. Time series plots of potentially toxic cyanoprokaryote species detected in samples from 0.5 m at Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake at monthly intervals between January 2007 and August 2008.

5.2.2 Phytoplankton assemblages - site comparison

The algal assemblage (proportions based upon cell concentrations) within Clarrie Hall Dam was dominated by the division Chlorophyta, making 50% contribution, with Cyanoprokaryota contributing 28% and Bacillariophyta 18% (Table 5.2; Figure 5.3). *Aphanocapsa holsatica*, a species which is not yet known to produce toxins, was the most abundant cyanoprokaryote at 9% (Table 5.3; Figure 5.4). The Tweed River at Bray Park had a different assemblage, with Bacillariophyta contributing 47%, Cyanoprokaryota 35% (Table 5.2; Figures 5.5, 5.4) and Cryptophyta 18% (Table 5.2; Figure 5.6). The dominant cyanoprokaryote in the river assemblage was *Phormidium ambiguum* which contributed 9% (Table 5.3; Figure 5.4). Cobaki Lake was dominated by Cyanoprokaryota (94%) (Table 5.2; Figure 5.4), of which 86% was represented by four potentially toxic species (Table 5.3; Figure 5.4).

Table 5.2. Simper analysis of similarity percentages and division contributions in samples from Cobaki Lake, Clarrie Hall Dam and the Tweed River at Bray Park taken monthly between January 2007 and August 2008.

Division	**Contribution %**
Clarrie Hall Dam	
Chlorophyta	49.88
Cyanoprokaryota	28.31
Bacillariophyta	18.58
Tweed River at Bray Park	
Bacillariophyta	46.98
Cyanoprokaryota	35.47
Cryptophyta	17.55
Cobaki Lake	
Cyanoprokaryota	94.38

Table 5.3. Simper analysis of similarity percentages and species contributions in samples from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake taken monthly between January 2007 and August 2008.

Species	Contribution %
Clarrie Hall Dam	
Scenedesmus sp.	17.21
Closterium sp.	14.26
Aulacoseira sp.	9.42
Aphanocapsa holsatica	9.04
Aphanocapsa delicatissima	8.06
Pennate diatoms spp.	5.58
Pseudosphaerocystis	5.21
Cosmarium sp.	4.16
Oocystis sp.	2.98
Acanthoceras sp.	2.92
Geitlerinema amphibium	2.52
Pseudanabaena limnetica	2.44
Chroococcus minimus	2.04
Botryococcus sp.	1.76
Dictyosphaerium sp.	1.71
Oscillatoria princeps	1.57
Tweed River at Bray Park	
Pennate diatoms spp.	58.40
Cryptomonas sp.	21.87
Phormidium ambiguum	9.58
Oscillatoria princeps	5.60
Cobaki Lake	
Anabaena circinalis	56.69
Microcystis aeruginosa	13.42
Cylindrospermopsis raciborskii	9.67
Aphanizomenon ovalisporum	6.29
Cryptomonas sp.	3.07
Closterium sp.	2.99

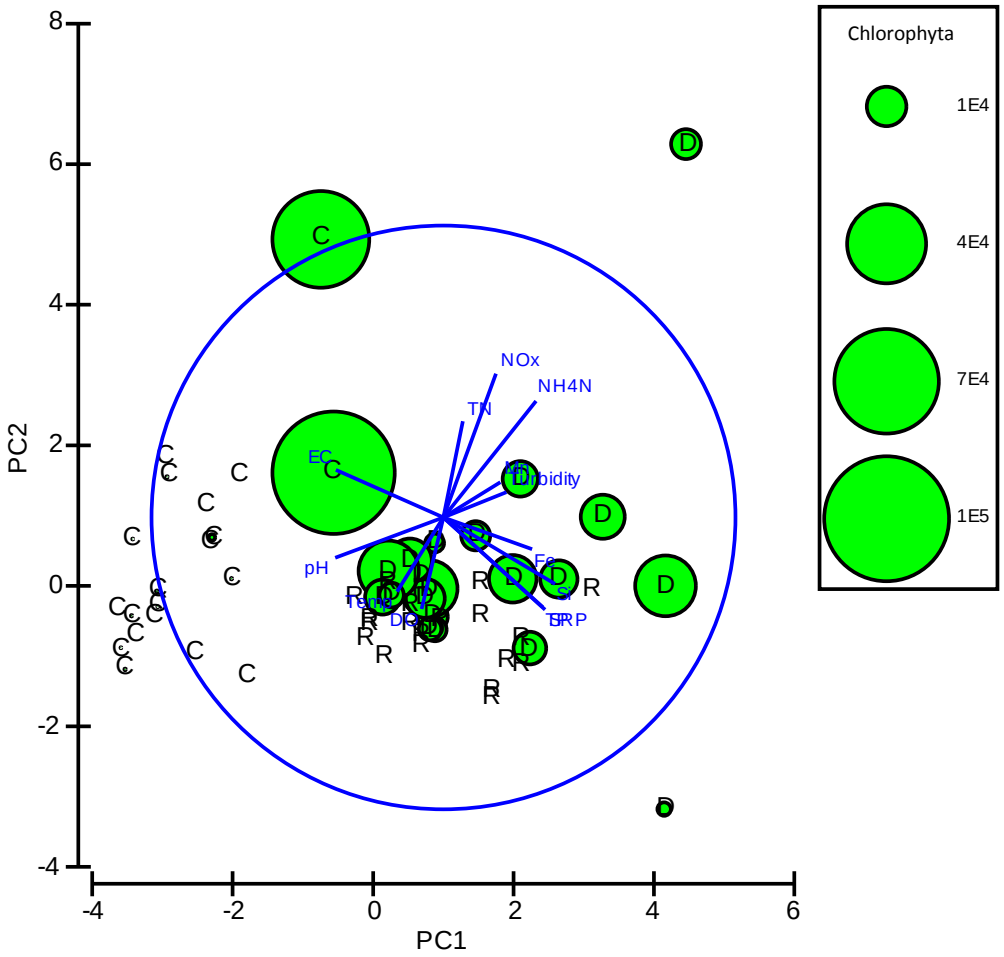


Figure 5.3. Chlorophyta bubble plots superimposed on PCA ordinations of environmental data from samples taken from Clarrie Hall Dam (D), the Tweed River at Bray Park (R) and at Cobaki Lake (C) collected monthly between January 2007 and August 2008.

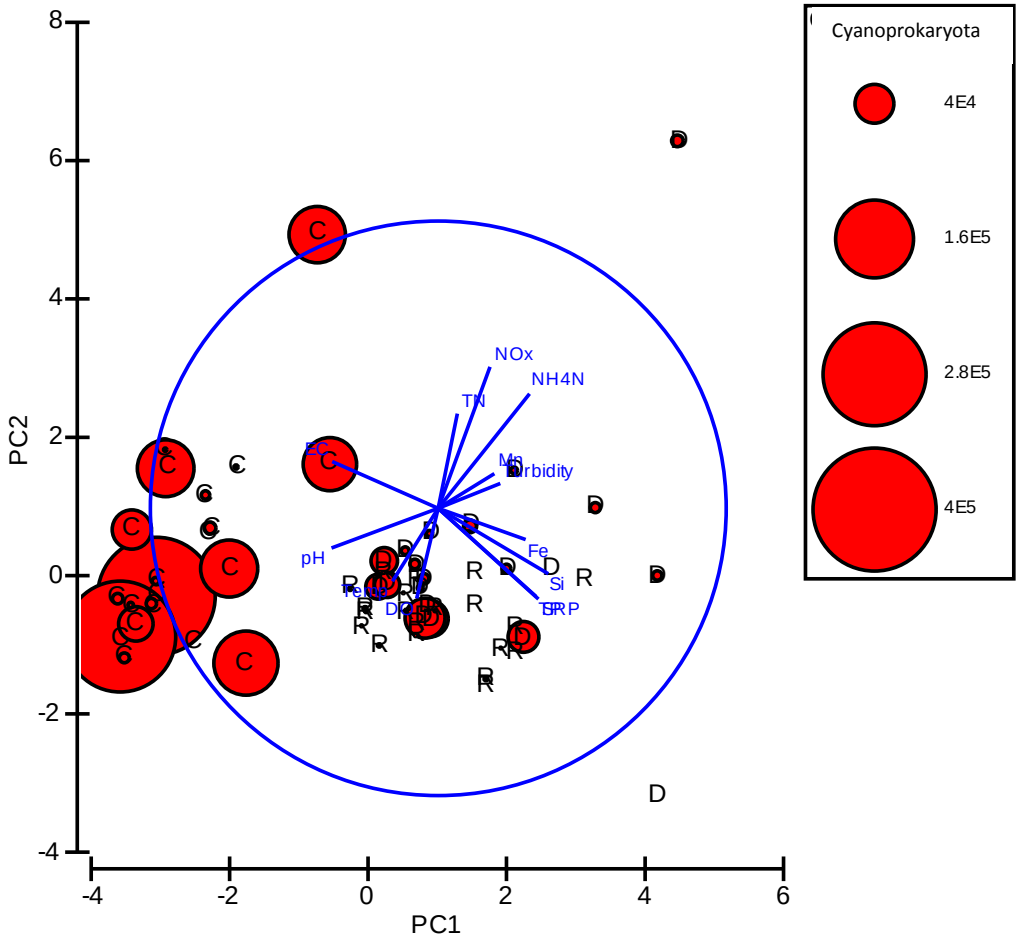


Figure 5.4. Cyanopropokaryota division bubble plots superimposed on PCA ordinations of environmental data from samples taken from Clarrie Hall Dam (D), the Tweed River at Bray Park (R) and at Cobaki Lake (C) collected monthly between January 2007 and August 2008.

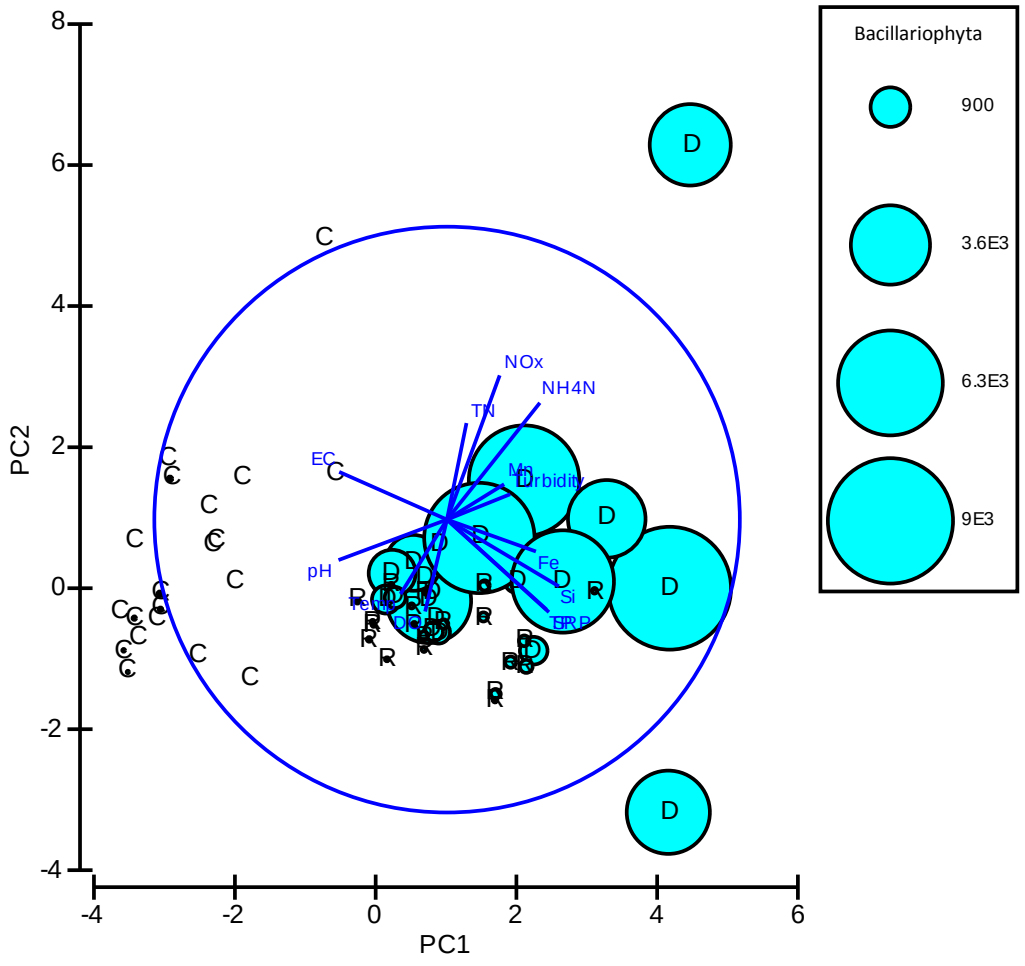


Figure 5.5. Bacillariophyta bubble plots superimposed on PCA ordinations of environmental data from samples taken from Clarrie Hall Dam (D), the Tweed River at Bray Park (R) and at Cobaki Lake (C) collected monthly between January 2007 and August 2008.

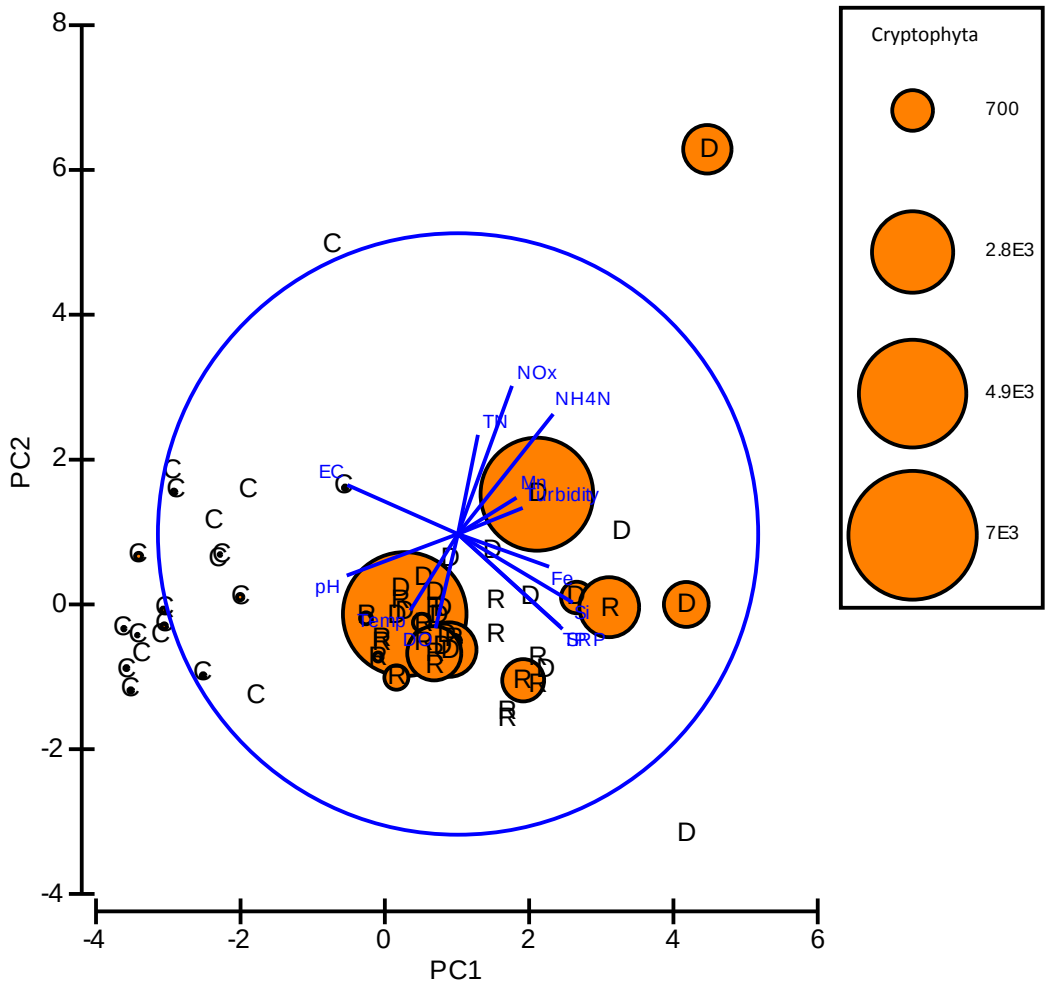


Figure 5.6. Cryptophyta bubble plots superimposed on PCA ordinations of environmental data from samples taken from Clarrie Hall Dam (D), the Tweed River at Bray Park (R) and at Cobaki Lake (C) collected monthly between January 2007 and August 2008.

5.2.3 Phytoplankton assemblages. Inter-annual comparison

The dominant groups of phytoplankton in Clarrie Hall Dam showed little variation between the dry and wet years, with Chlorophyta dominating in both cases (Table 5.4). There was, however, some variation in the cyanoprokaryote assemblage, although small chroococcales were present in both years (*A. holsatica* and *A. delicatissima*) (Table 5.5). In the dry year (2007), small percentages of oscillatoriales were present (*Phormidium limnetica*, *Oscillatoria princeps* and *G. amphibium*) whereas in the wet year (2008) a small percentage of the nostocalean species *A. circinalis* was present. The river at Bray Park was dominated by cyanoprokaryotes in the dry year with Bacillariophyta and Cryptophyta also present (Table 5.4). In contrast, bacillariophytes dominated in the wet year. The cyanoprokaryotes present in the dry year were *P. ambiguum* and *A. circinalis* (Table 5.5). Cobaki Lake showed little variation between the two years with cyanoprokaryotes dominant throughout, however the cyanoprokaryote contributions at species level revealed considerable variation between the years. The dry year was dominated by *C. raciborskii*, *M. aeruginosa* and *A. ovalisporum.* In contrast, the wet year was strongly dominated by *A. circinalis.*

Table 5.4. Division contributions (Simper analysis) of phytoplankton in monthly samples taken from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake during 2007 and 2008.

Division	Contribution %	Contribution %
Clarrie Hall Dam	**2007**	**2008**
Chlorophyta	54.26	42.62
Cyanoprokaryota	27.97	33.22
Bacillariophyta	15.34	20.90
Tweed River at Bray Park	**2007**	**2008**
Cyanoprokaryota	39.17	0
Bacillariophyta	35.90	71.67
Cryptophyta	23.93	18.40
Cobaki Lake	**2007**	**2008**
Cyanoprokaryota	97.26	92.64

Table 5.5. Species contributions (Simper analysis) of phytoplankton in samples taken monthly from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake during 2007 and 2008.

Species	Contribution %	Contribution %
Clarrie Hall Dam	**2007**	**2008**
Scenedesmus spp.	19.24	14.11
Closterium spp.	16.01	11.66
Aulacoseira spp.	8.12	9.58
Pseudosphaerocystis spp.	7.32	3.63
Aphanocapsa holsatica	6.58	12.97
Aphanocapsa delicatissima	5.89	11.54
Oocystis spp.	4.86	0
Cosmarium spp.	4.43	3.34
Pennate diatoms spp.	3.90	7.36
Pseudanabaena limnetica	3.90	0
Oscillatoria princeps	3.25	0
Acanthoceras spp.	2.24	3.43
Staurastrum spp.	2.17	0
Dictyosphaerium spp.	1.84	0
Geitlerinema amphibium	1.65	3.20
Anabaena circinalis	0	4.46
Chroococcus minimus	0	2.77
Botryococcus spp.	0	2.14
Tweed River at Bray Park	**2007**	**2008**
Pennate diatoms spp.	35.90	77.63
Cryptomonas spp.	23.93	17.19
Phormidium ambiguum	18.48	0
Anabaena circinalis	16.88	0
Cobaki Lake	**2007**	**2008**
Cylindrospermopsis raciborskii	37.61	0
Microcystis aeruginosa	21.35	2.80
Aphanizomenon ovalisporum	16.71	0
Cyanogranis libera	5.38	0
Chlamydomonas spp.	5.38	0
Microcystis panniformis	4.35	0
Anabaena circinalis	0	87.05
Cryptomonas spp.	0	2.86

5.2.4 Influence of environmental parameters

5.2.4.1 Principal Component Analysis (PCA)

The linkage between the phytoplankton assemblages and the physical and chemical factors (refer to Chapter 4) was assessed using multivariate analyses (PRIMER V6). The Principal Components Analysis (PCA) ordination eigenvalues showed that a 2-d PCA gave a good description of the environmental structure in high dimensional space, with PC1 and PC2 accounting for 62.3% of the variability (Table 5.6; Figure 5.7). The first principal component was highly positively correlated with conductivity (K_{25}) and pH, whereas it was highly negatively correlated with TP, NH_3N, NO_x, dissolved inorganic nitrogen (DIN), SRP, soluble iron and soluble silicon (Table 5.7). The second principal component was highly positively correlated with oxidised nitrogen and dissolved inorganic nitrogen, whereas it was highly negatively correlated with temperature (Table 5.7).

Table 5.6. Table of eigenvalues and percentage variation of environmental data for samples taken from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake.

Principal Component	Eigenvalues	% Variation	Cum.% Variation
PC 1	8.04	47.3	47.3
PC 2	2.55	15.0	62.3
PC 3	1.49	8.8	71.1
PC 4	1.22	7.2	78.2
PC 5	1.04	6.1	84.3

Table 5.7. Principal components of the PCA analysis of samples from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake sampled monthly between January 2007 and August 2008.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5
Dissolved oxygen	0.052	-0.155	-0.690	0.046	-0.079
Conductivity (K_{25})	0.307	0.082	-0.064	-0.134	0.151
pH	0.289	-0.222	-0.204	0.093	-0.016
Temperature	0.108	-0.294	0.273	0.420	-0.383
Turbidity	-0.194	-0.013	-0.162	0.362	0.438
Total nitrogen	-0.095	0.246	0.250	0.586	-0.067
Total phosphorus	-0.289	-0.228	0.089	-0.102	-0.026
Ammonium nitrogen	-0.302	0.126	0.029	-0.025	-0.178
Oxidised nitrogen	-0.254	0.315	-0.224	-0.007	-0.305
Dissolved inorganic nitrogen	-0.269	0.329	-0.140	-0.085	-0.246
Soluble reactive phosphorus	-0.301	-0.224	-0.002	-0.043	-0.240
Soluble iron	-0.296	-0.082	-0.085	0.002	0.289
Soluble manganese	-0.212	0.123	0.376	-0.211	0.393
Soluble silicon	-0.330	-0.079	-0.087	0.078	-0.155
Soluble aluminium	-0.219	-0.050	-0.252	0.353	0.349

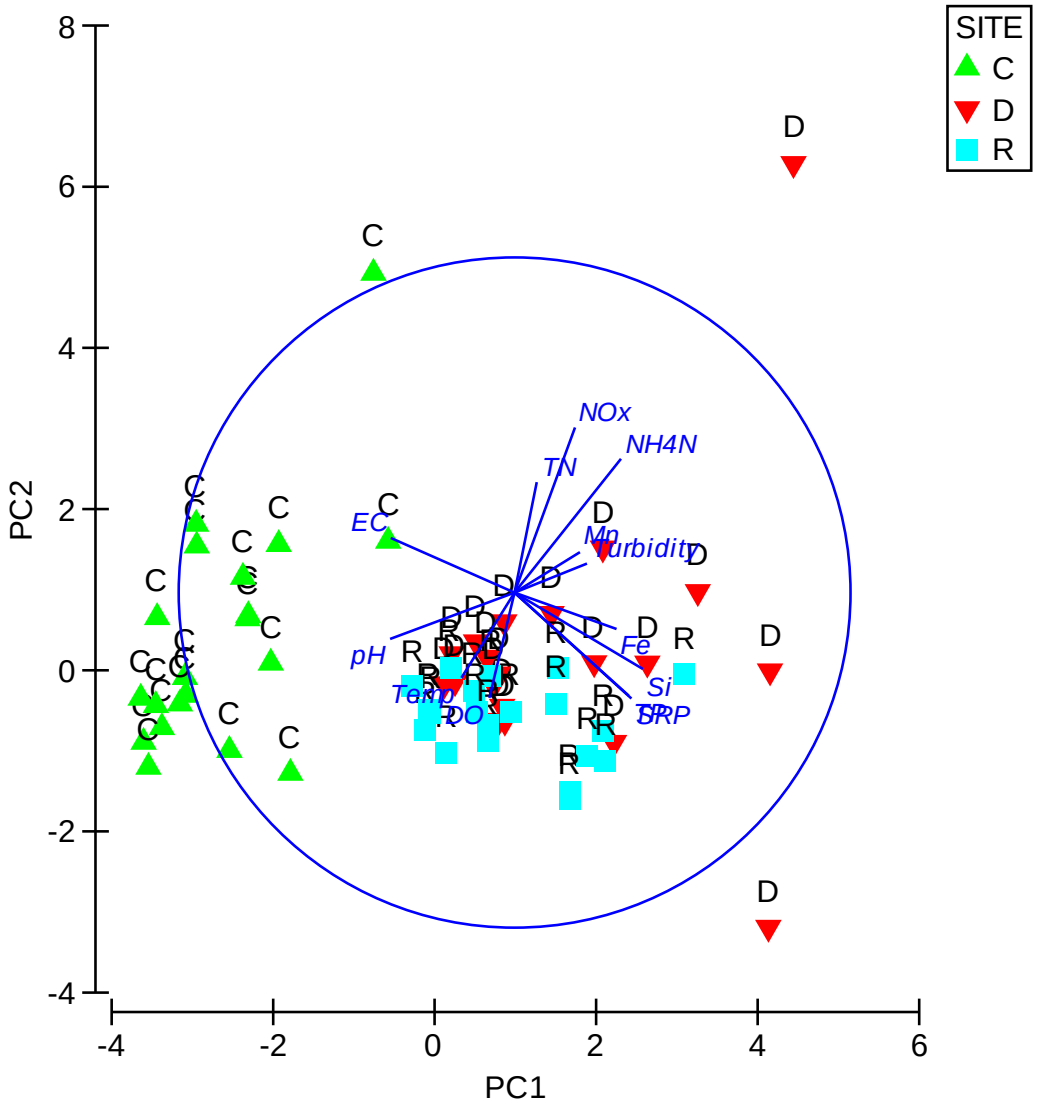


Figure 5.7. Principal component analysis PC1 and PC2 ordinations for environmental samples taken from Clarrie Hall Dam (D), the Tweed River at Bray Park (R) and Cobaki Lake (C) between January 2007 and August 2008.

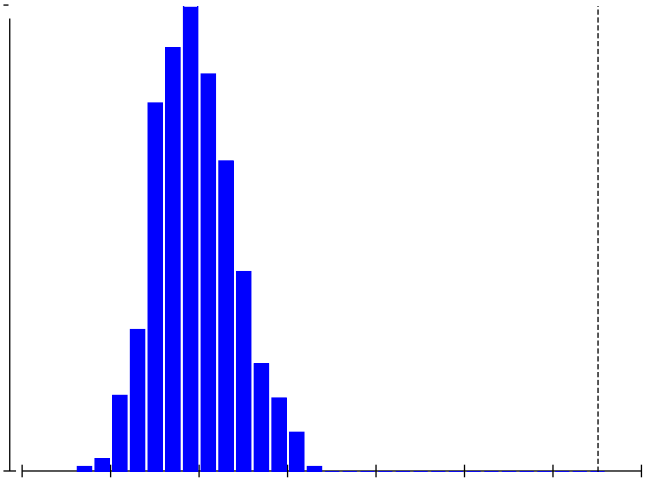
5.2.4.2 BEST analysis (Which variables best explained the biological data)

The BEST analysis (Table 5.8; Figure 5.8) has shown that the algae (and to a slightly lesser extent the algal assemblage variability across the three sites) were associated with three dominating environmental variables: conductivity (K_{25}), NO_x and soluble silicon concentrations. These are included in the PCA ordinations shown in Table 5.7 with the addition of TP, NH_4N, DIN and SRP concentrations.

Table 5.8. BEST correlation statistic (Rho) and the environmental variables associated with this statistic. The PCA ordination results are included in the table for comparison. The data were collected from monthly samples from Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake between January 2007 and August 2008.

Biological Data	**BEST Correlation statistic (Rho)**	**BEST Selection of statistically significant variables**	**PCA components responsible for 62.3% of variability in PC1 & PC2**
Algae	0.551	Conductivity (K_{25}) Oxidised nitrogen Soluble silicon	
Algal Divisions	0.335	Conductivity (K_{25}) Oxidised nitrogen Soluble silicon	
All biological data			Conductivity (K_{25}) Oxidised nitrogen Soluble silicon Ammonium nitrogen Dissolved inorganic nitrogen Soluble reactive phosphorus Total phosphorus Temperature

(A)



(B)

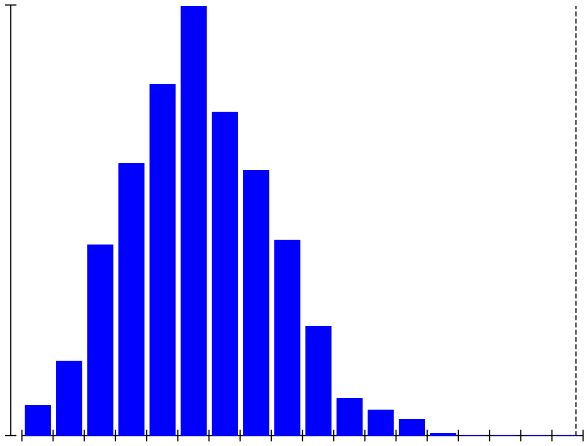


Figure 5.8. Histograms of the BEST correlations between the environmental variables (A) and the two biological assemblages (algae and algal divisions) (B). The test statistic Rho is shown as a dotted vertical line to the far right in each graph representing algal assemblages (A) and algal divisions (B). 999 permutations, $\alpha = 0.001$.

5.2.4.3 Multidimensional Scaling

The samples taken and analysed from each site were compared in relation to environmental parameters and algal assemblages at the levels of division and species (Multidimensional Scaling (MDS) ordination) (Figure 5.9). Comparing the environmental variables, Cobaki Lake is significantly different to Clarrie Hall Dam and the Tweed River at Bray Park, which are grouped together (Table 5.9). The algal species and algal division distribution shows that each site is significantly different from the others. Environmentally Clarrie Hall Dam and the Tweed River at Bray Park are clumped together, interestingly however; the algal species and algal divisions at each of the three sites are quite different.

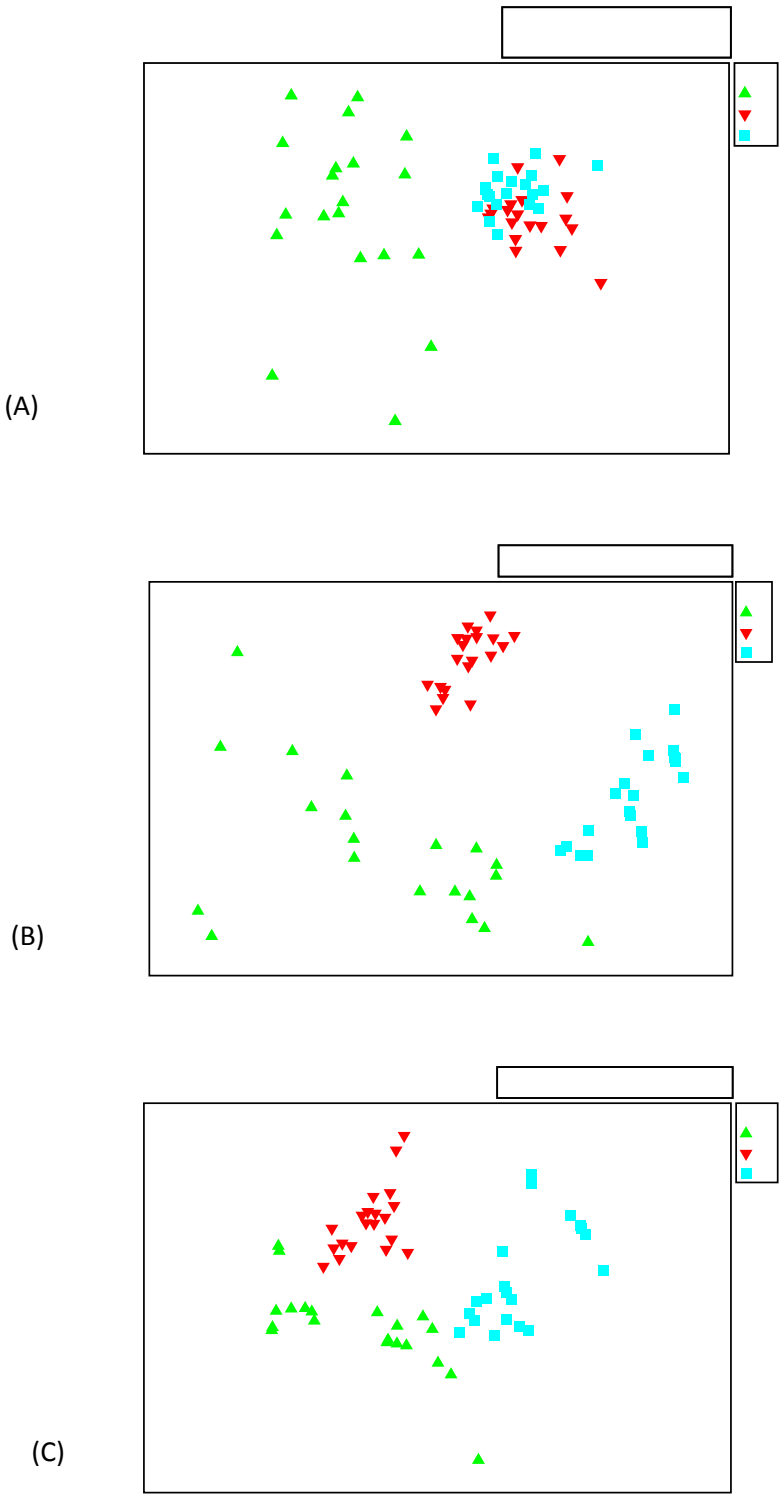


Figure 5.9. MDS scatter plots of the environmental data (A) and the biological data including algae (B) and algal divisions (C) at Cobaki Lake (C), Clarrie Hall Dam (D) and the Tweed River at Bray Park (R) sampled monthly between January 2007 and August 2008.

5.2.4.4 ANOSIM (Analysis of Similarities) – site comparison

The PCA and MDS analyses indicated that the three sites are statistically different. The ANOSIM analysis studies the three components involved in more detail: environmental variables; algal assemblages; and algal division assemblages; and shows where the higher similarity or dissimilarity is revealed (Table 5.9; Figure 5.10). Table 5.9 shows that, environmentally, the three sites were statistically significantly different (Global R = 0.802). Cobaki Lake and Clarrie Hall Dam were completely separated (R = 1.000), as were Cobaki Lake and the Tweed River at Bray Park (R = 1.000). On the other hand, the Tweed River and Clarrie Hall Dam were not significantly different (R = 0.270). The algal species and algal division ANOSIMS produced a high R statistic globally as well as between individual sites, and established that there were significant differences between all sites (Table 5.9).

Table 5.9. ANOSIM data taken from monthly samples from Cobaki Lake (C), Clarrie Hall Dam (D) and the Tweed River at Bray Park (R) between January 2007 and August 2008.

Data	Data development	ANOSIM Global R Statistic		Conclusion
Environmental	Log transformed	Global	0.802	
	Normalised	C, D:	1.000	
	Euclidean Distance	C, R:	1.000	
		D, R:	0.270	
Algae	Square-root transformed	Global:	0.720	The high R statistic between all 3 sites establishes that there are significant differences between all sites.
	Bray-Curtis coefficient	C, D:	0.599	
	Resemblance matrix	C, R:	0.629	
		D, R:	0.996	
Algal Divisions	Square-root transformed	Global:	0.671	
	Bray-Curtis coefficient	C, D:	0.527	
	Resemblance matrix	C, R:	0.609	
		D, R:	0.922	

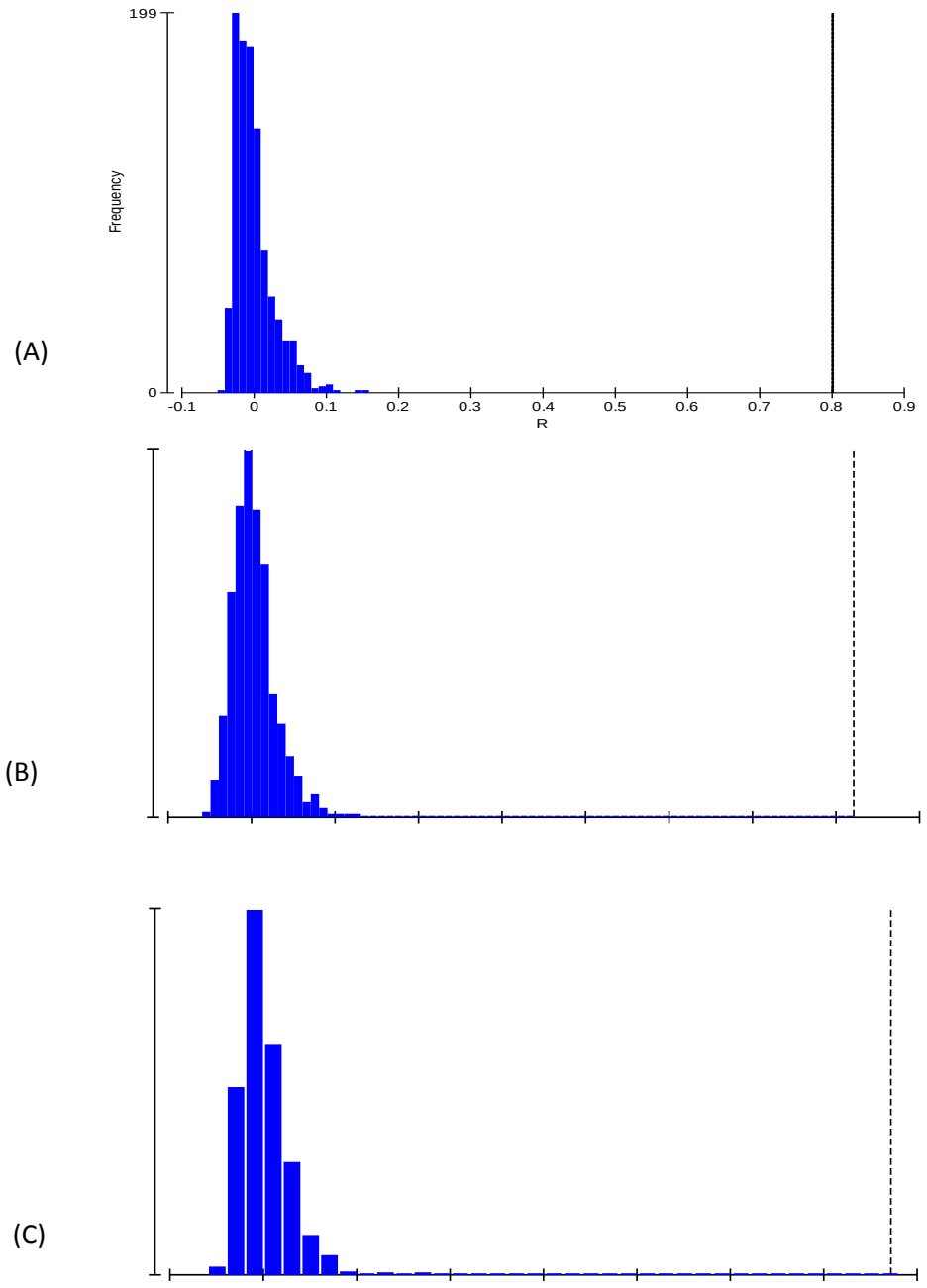


Figure 5.10. ANOSIM histograms (Site comparison) of the null hypothesis distributions of the test statistic showing real ρ as a dotted vertical line to the right in each graph using 999 permutations, α = 0.001. Histogram (A) represents environmental data, (B) algae, and (C) algal divisions.

5.2.4.5 Cluster dendrograms and cluster overlays

The data analyses indicated that the environmental variables were globally dissimilar and the algae species and the algal division assemblages showed complete separation of groups between all sites. These dissimilarities can be shown pictorially using cluster dendrograms (Figures 5.11; 5.12; 5.13) and cluster overlays (Figure 5.14). The cluster dendrograms indicated that the environmental variables for the Tweed River and Clarrie Hall Dam were clustered together and quite distant from Cobaki Lake (Figure 5.11). The algal assemblages (Figure 5.12) were clustered differently for each site, as were the algal divisions (Figure 5.13). The similarity linkages between the algal and algal division assemblages were different but showed some connection at higher levels whereas the environmental linkages were similar for the Tweed River and Clarrie Hall Dam but clearly separate from Cobaki Lake. The cluster overlays (Figure 5.14) reinforced the linkages shown in the cluster dendrograms, indicating that the environmental variables for Clarrie Hall Dam and the Tweed River were clustered together and quite separate to Cobaki Lake (Figure 5.14). They also showed that the algal assemblages were clustered differently for each site (Figure 5.14) and that the algal divisions were clustered differently for each site but not as clearly as the algal assemblages (Figure 5.14).

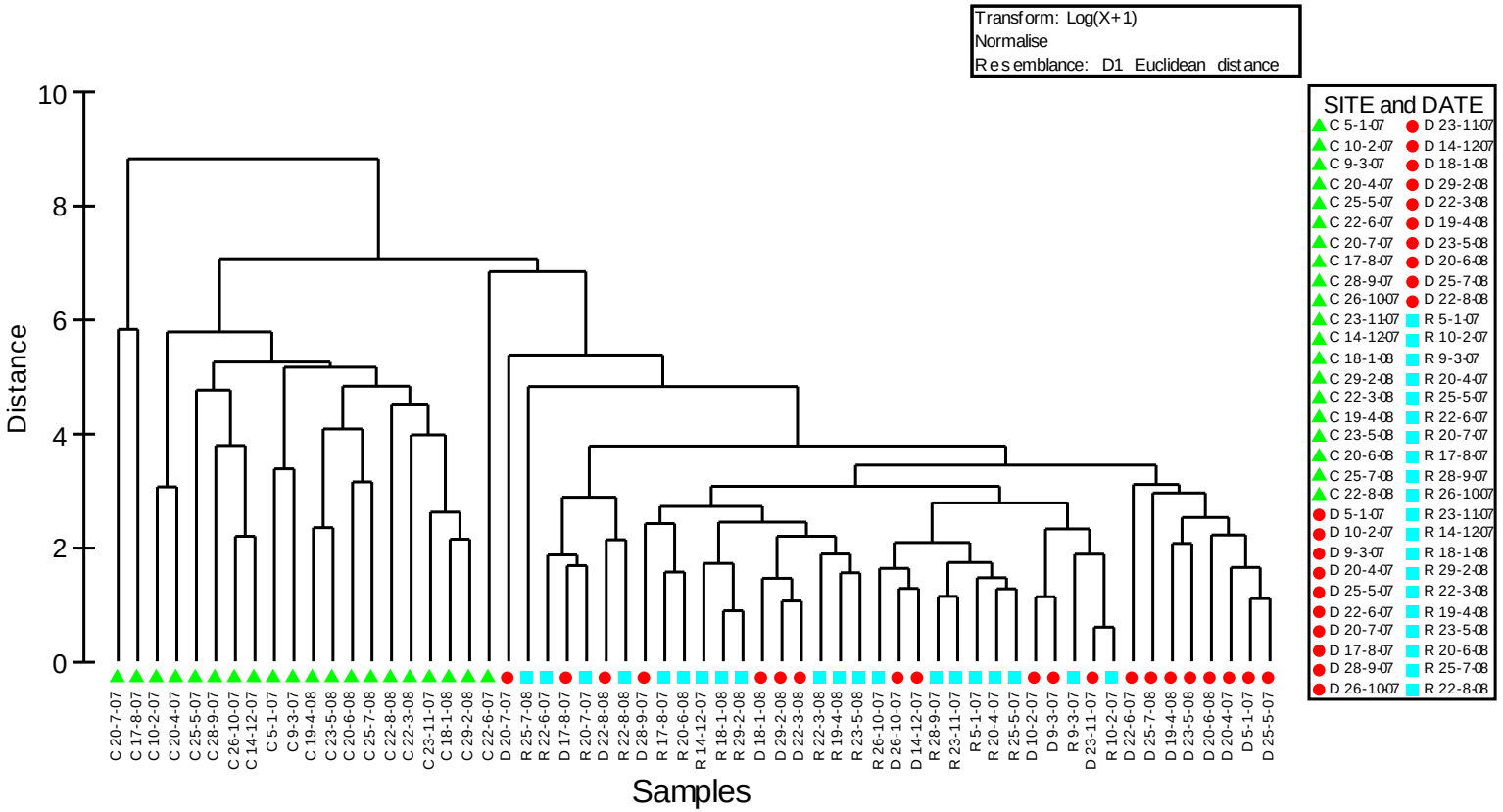


Figure 5.11. Environmental variables dendrograms based on hierarchical agglomerative clustering of samples from Clarrie Hall Dam (D), the Tweed River at Bray Park (R) and Cobaki Lake (C). The samples were collected monthly between January 2007 and August 2008.

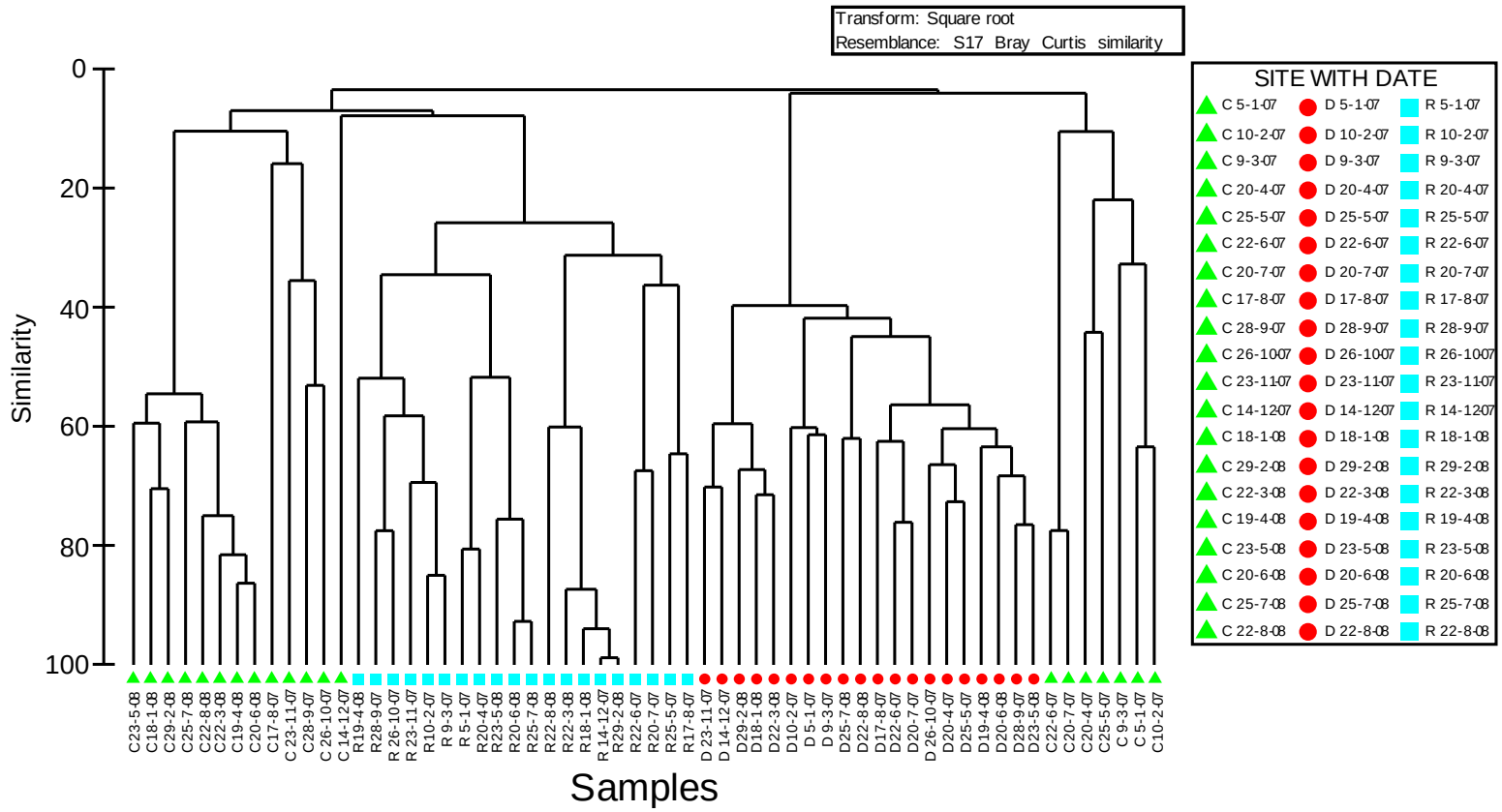


Figure 5.12. Algal species dendrograms based on hierarchical agglomerative clustering of samples from Clarrie Hall Dam (D), the Tweed River at Bray Park (R) and Cobaki Lake (C). The samples were collected monthly between January 2007 and August 2008.

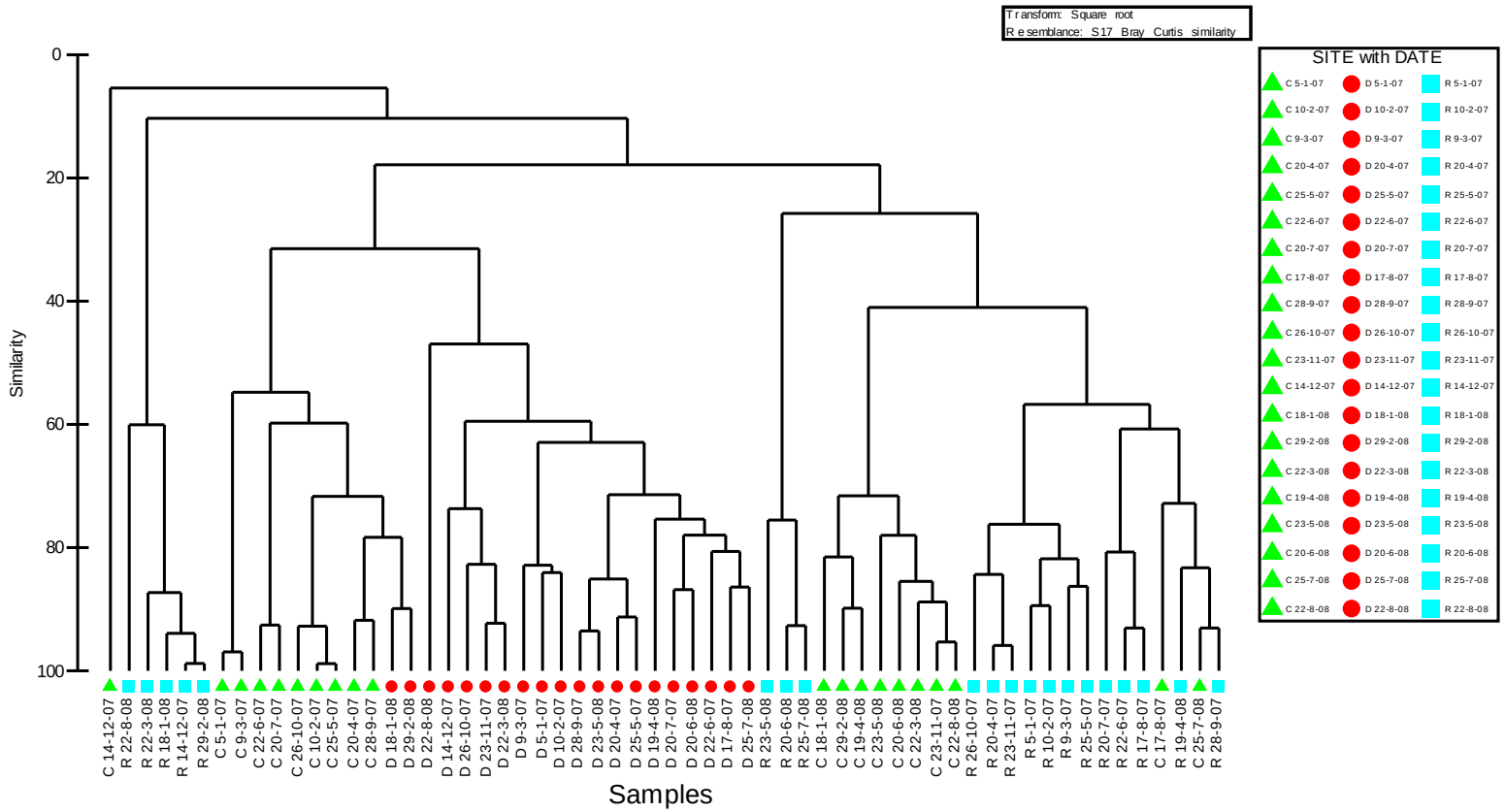
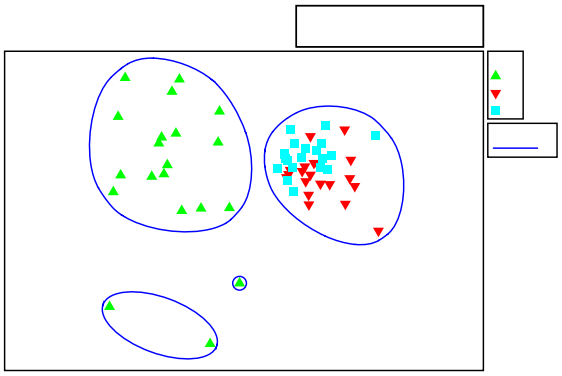
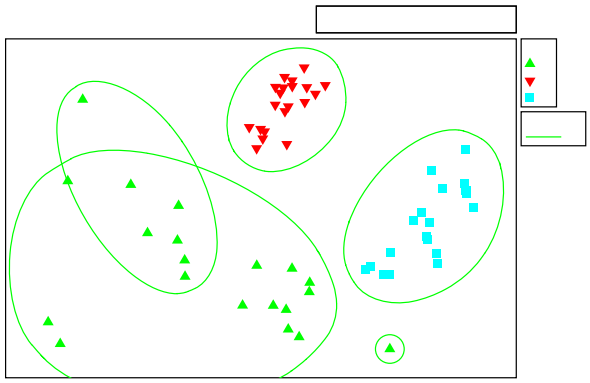


Figure 5.13. Algal division dendrograms based on hierarchical agglomerative clustering of samples from Clarrie Hall Dam (D), the Tweed River at Bray Park (R) and Cobaki Lake (C). The samples were collected monthly between January 2007 and August 2008.

(A)



(B)



(C)

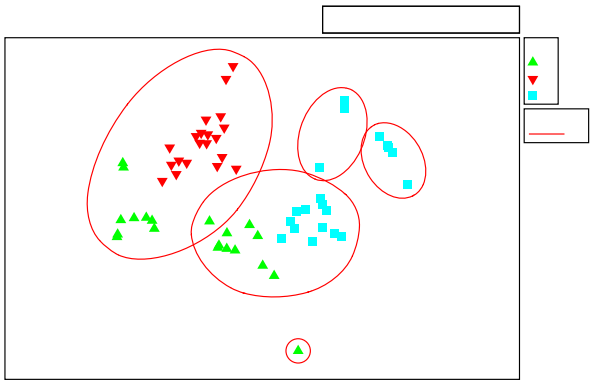


Figure 5.14. Cluster overlays of Clarrie Hall Dam (D), the Tweed River at Bray Park (R) and Cobaki Lake (C) involving environmental variables (Graph A), algal assemblages (Graph B) and algal divisions (Graph C). The samples were collected monthly between January 2007 and August 2008.

5.2.5 Cyanotoxins

Cyanotoxin was not detected in Clarrie Hall Dam despite the fact that samples contained known toxin-producing species such as *A. circinalis*, *M. aeruginosa*, and *A. ovalisporum*. Also, toxin was not detected in Tweed River at Bray Park samples where *A. circinalis* was identified. Similarily, toxin was not detected in Cobaki Lake samples containing *A. circinalis* and *M. aeruginosa*. Toxin was detected, however, in Cobaki Lake samples containing *C. raciborskii* and *A. ovalisporum.* Here the cyanotoxin CYN was detected throughout the water column between January and May 2007, when the lake's water quality was compromised by a successive bloom of these species. In Cobaki Lake, maximum *C. raciborskii* cell concentration occurred in May 2007 with 83,160 cells mL^{-1} occurring in the surface water; concurrently total-CYN in the hypolimnion reached a maximum concentration of 101.4 µg mL^{-1}. The patterns in toxicity detected in the Cobaki Lake samples in 2007 are presented in detail and discussed in this thesis in Chapters 7, 8 and 9.

5.2.6 NHMRC Drinking Water Guidelines and NHMRC Recreational Water Guidelines

Anabaena circinalis was detected in samples from the Tweed River at Bray Park during the first half of 2007 in concentrations below the Australian guideline trigger values for both drinking water and recreational waters (NHMRC 2008; NHMRC 2004). In addition to being a drinking water storage facility, Clarrie Hall Dam is also used for limited recreational use, such as canoeing and fishing. *Anabaena circinalis* was detected in samples taken from Clarrie Hall Dam with cell concentrations reaching a maximum of 6,500 cells mL^{-1} in January 2008. This concentration indicated a drinking water framework alert level 2 (High Alert) and an amber recreational alert level. *Microcystis aeruginosa* was detected in low concentrations (380 cells mL^{-1}) in February 2007 and did not reach alert levels. Cell concentrations of *A. ovalisporum* at 8,200 cell mL^{-1} (biovolume 0.2 mm^3 L^{-1}) in January 2008, and 9,600 cell mL^{-1} (biovolume 0.1 mm^3 L^{-1}) of *G. amphibium* in February 2008,

would also have triggered a drinking water alert level 2 (High Alert) and an amber recreational alert level. In contrast, CYN concentrations in samples from Cobaki Lake were consistently > 1.0 µg L^{-1} between January 2007 and May 2007 with concentrations exceeding 100 µg L^{-1} detected in the hypolimnion. These concentrations forced the closure of the lake for all recreational purposes. In September 2007, the concentration of *M. aeruginosa* (22,750 cells mL^{-1}) triggered a recreational amber alert. In contrast, samples taken in 2008 from Cobaki Lake contained low concentrations of cyanoprokaryotes generally, however *A. circinalis* was detected in August 2008 (2,700 cells mL^{-1}) signifying a recreational green alert.

5.3 Discussion

The phytoplankton assemblages investigated in this study have been found to vary depending on the physical and chemical properties of the raw water in each system. Four potentially toxic species were recorded; *A. circinalis, M. aeruginosa, A. ovalisporum* and *C. raciborskii.* Only two of these species were shown to be toxin-producers, namely *A. ovalisporum* and *C. raciborskii*. Both produced the cyanotoxin CYN, a potent alkaloid cytotoxin which can have adverse effects on livestock, wildlife and humans and as such is a concern worldwide for drinking water management (Shen *et al.* 2002; Falconer 2001; Humpage *et al.* 2000; Shaw *et al.* 2000; Saker *et al.* 1999; Seawright *et al.* 1999; Ohtani *et al.* 1992; Hawkins *et al.* 1985; Bourke *et al.* 1983; Blyth 1980). This study has shown that the species *G. amphibium* was also detected in the Clarrie Hall Dam in both 2007 and 2008 and was morphologically identical to *Limnothrix* AC0243, a recently identified toxin-producer.

The dominance of *C. raciborskii* in Cobaki Lake in the dry year parallels the findings of Fabbro (1999) in which *C. raciborskii* was dominant during hot dry summers in the Fitzroy River impoundment. McGregor and Fabbro (2000) conducted an extensive study of 47 reservoirs and weir pools throughout Queensland including several water bodies located in the subtropical south-eastern corner of the State. They concentrated on the dominance of *C. raciborskii* and found that this dominance was due to a variety of factors including high pH, high temperature, long residence time and a thermally stratified water column. The stratification, available nutrients and metals in Cobaki Lake during the dry year of 2007 is discussed in detail in Everson *et al.* (2011) (see Chapter 8).

Cobaki Lake matched the profile indicated by McGregor and Fabbro (2000) and by Fabbro and Duivenvoorden (2000), where cyanobacteria of the Order Nostocales were found to be

dominant during the dry season in the Fitzroy River impoundment. Drought conditions with low flow and stratification of the water column in rivers and weir pools favour the growth of cyanoprokaryote blooms (Sherman and Jones 1994). Of global interest, a drinking water reservoir located in the semi-arid region of northeast Brazil was studied over two years (1997 and 1998) and, like Cobaki Lake, had environmental conditions such as high temperatures, high pH and annual rain deficit causing a lack of water renewal, all conditions which favoured the dominance of *C. raciborskii* (Bouvy *et al.* 1999).

Further studies have indicated that *C. raciborskii* has low light requirements, near neutral buoyancy, and a wide temperature tolerance providing it with the ability to grow in a wide range of water bodies (Burford and Davis 2011). These authors found that the management of this species was not straight forward. For example, in North Pine Dam in tropical south-east Queensland, destratification was implemented in the 1990's with the purpose of reducing the *C. raciborskii* dominance but had the reverse effect (Burford and Davis 2011). Correlations found that prior to destratification, the dominance of *C. raciborskii* was due its ability to scavange and store low concentrations of phosphorus whereas, after destratification, dominance was linked with adaptation to low light conditions brought about by artificial mixing (Burford *et al.* 2006; Antenucci *et al.* 2005). Therefore, a destratification unit similar to the one installed in Clarrie Hall Dam seems unlikely to be a suitable tool for the management of the dominance of *C. raciborskii* in Cobaki Lake.

Interestingly, in Cobaki Lake, the pH was markedly higher in the dry year, and in both years was higher than that recorded in Clarrie Hall Dam and the Tweed River impoundment at Bray Park (Tweed River site). Earlier studies (Talling 1976; Jackson 1964) found that dense populations of phytoplankton deplete carbon dioxide (CO_2) and raise the pH and that, in most lakes cyanoprokaryotes are dominant only during those periods when the pH is high. Shapiro (1990) discussed in detail the CO_2 / pH association and suggested that cyanoprokaryotes prefer conditions of low CO_2 or high pH, whereas green algae and diatoms

prefer high CO_2 or low pH. Shapiro (1990) suggested that cyanoprokaryotes maintained or increased their dominance when photosynthesis was stimulated by nutrients circulated from the hypolimnion causing pH to increase. If the pH decreased after circulation due to the upwards mixing of CO_2, or increased respiration caused by mixing, then the dominance would shift from cyanoprokaryotes to green algae. The results from Clarrie Hall Dam, which showed that the Chlorophyta was dominant throughout the sampling period, concur with the finding that lower pH waters favour green algal assemblages.

A study by Parparova and Yacobi (1998) of water samples from Lake Kinneret, Israel, found that a reduction of available iron was linked with a decrease of algal photosynthesis. They also found that this trend was species-specific, with the dinophyte genus *Peridinium* being less sensitive to reduced available iron concentrations whereas cyanoprokaryotes and chlorophytes were strongly inhibited. By comparison, Clarrie Hall Dam, despite a large increase in available iron in the wet year, showed little variation in the relative abundance of chlorophytes and cyanoprokaryotes. Similarly, the riverine impoundment also recorded a substantial increase in iron concentrations with no corresponding change in phytoplankton assemblages.

Jones and Elliott (2007) indicated that a change in rainfall could impact upon river discharge and that this, in turn, may determine the retention time of a lake in that river system. They suggested that merely changing the retention time could alter the timing and magnitude of phytoplankton blooms. They theorised that light and temperature are sufficient for population growth, but that increased discharge rates could restrict the bloom by flushing the nutrients out of the system therefore limiting the growth period for the phytoplankton. Therefore, reduced rainfall resulting in longer retention times could allow the spring/summer blooms to start earlier and the autumn blooms to continue longer. A change in the inflow to a lake or reservoir could also change the thermal structure of the water body. This change in thermal structure or stratification could, in turn, influence the magnitude and composition of

the phytoplankton population (Jones and Elliott 2007). These differences in rainfall, and therefore inflow, certainly appear to be the case in two of the three water bodies studied in the Tweed Shire. The riverine impoundment had increased flow in 2008 and featured distinctly different phytoplankton populations than those present in the dry year (2007). Cobaki Lake experienced a similar trend with the cyanoprokaryote species changing from *A. ovalisporum* and *C. raciborskii* in the dry year to only *A. circinalis* in the wet year.

A study of seven subtropical reservoirs in southeast Queensland suggested that watershed patterns and reservoir characteristics, such as water volume and depth, have a substantial effect on the type of algal blooms in reservoirs (Burford *et al.* 2007). These authors found that all reservoirs were dominated by cyanoprokaryotes, and the three reservoirs with the lowest percentage forest cover in their catchment area (about 50%) had the highest frequency and magnitude of toxic species, principally *C. raciborskii*. This trend could be applied to Clarrie Hall Dam with a substantial catchment forest cover and low cyanoprokaryote populations; compared with Cobaki Lake with no catchment forest cover and a cyanoprokaryote population percentage of over 90% in both sampling years, including *C. raciborskii* in the dry year.

The phytoplankton assemblage of the riverine impoundment between the years 2007 (dry) and 2008 (wet) was similar to the trend reported by Harris and Baxter (1996) in North Pine Dam. Diatoms dominated the riverine impoundment in both years; however, in the dry year of 2007, shared the assemblage with cyanobacterial species *A. circinalis* and *P. ambiguum.* With the high inflow of water in 2008, the assemblage became strongly dominated by diatoms, and cyanoprokaryote species were not detected. A substantial increase in both soluble silicon and soluble iron concentrations also occurred in the high inflow year.

An earlier study (Kilham and Kilham 1975) found that the diatom *Aulacoseira* had a high soluble silicon requirement (15–20 mg L^{-1}). Harris and Baxter (1996) noted that the high concentrations of soluble silicon only occurred after strong inflows and coincided with increased growth and abundance of diatoms. Hötzel and Croome (1996) also noted that the high soluble silicon requirements coincided with the frequent occurrence of *Aulacoseira* in Australian water bodies; however, in contrast to Harris and Baxter's study, increased inflow from floods reduced the diatom abundance. Bormans and Webster (1999) found that in the River Murray during times of low flow, *Aulacoseira* concentrations increased until the silica supply was outstripped, whereas in times of high flow, the diatoms had insufficient time to grow before being swept downstream. This agrees with the findings of Hötzel and Croome (1996).

In comparison, Cobaki Lake, although dominated by cyanobacteria, had low soluble silicon and iron concentrations with very few diatoms detected. This concurs with a study by Hawkins and Griffiths (1993) involving Solomon Dam, a small tropical reservoir located on Palm Island, Northern Queensland, where the phytoplankton population, after depletion of the silica in the water, was dominated by cyanoprokaryotes and in particular by *C. raciborskii*. Clarrie Hall Dam, on the other hand, remained populated by chlorophytes through both years despite an increase in soluble silicon and iron.

The influence of nutrients on the variation in algal assemblages is well depicted in the three water bodies from the Tweed Shire. In Cobaki Lake, the available nutrients in the surface waters were low, which is conducive to dominance by cyanoprokaryotes, and particularly species of nostocales. This concurs with the study of the lower Fitzroy River in Rockhampton (Fabbro and Duivenvoorden 2000) where nostocales dominated in low concentrations of oxidised nitrogen and the water was depleted in available CO_2 as a result of high pH and a stratified water column. In contrast, Clarrie Hall Dam (which was dominated by chlorophytes) and the riverine impoundment at Bray Park (which was

dominated by diatoms) both had higher available surface water nutrient concentrations than Cobaki Lake. It is interesting to note that a study by Descy (1987) demonstrated that chlorophytes and diatoms also dominated in nutrient-rich temperate rivers.

In Cobaki Lake, the wet year was still dominated by cyanoprokaryotes but *C. raciboskii* was replaced by *A. circinalis*, another nostocalean species. The increased inflow, slight drop in pH, and increase in available nutrients and temperature appear to have favoured the growth of *A. circinalis*, similar to the trend observed in the Murray River, Australia (Croome *et al.* 2011; Bormans *et al.* 1997; Hötzel and Croome 1994).

As a result of a sixteen year study of North Pine Dam, Harris and Baxter (1996) noted that the phytoplankton populations were depressed for about 3 months after individual storm events and displayed long-term lag effects. The heavy rainfall of 2008 could thus be a contributing factor as to why *C. raciborskii* did not reappear in Cobaki Lake during the second year of study.

To summarize, in the Tweed system, which occupies a subtropical to temperate niche, the phytoplankton assemblages at each of the three sites were significantly different, despite a common climatic and geographic positioning. The data presented here illustrate that lotic conditions resulted in dominance of cryptophytes and diatoms and that low-flow conditions were associated with cyanoprokaryote dominance. The small, strongly stratified lake with long retention times was shown to favour the domination of cyanprokaryotes, particularly in dry times and as temperatures increased. However, the reservoir dynamics illustrated that, with the aid of partial mixing, cyanoprokaryote dominance can be avoided in some cases.

The physical and chemical properties present in Cobaki Lake have been conducive to the growth of particular species of toxin-producing cyanoprokaryotes. These species produced

high concentrations of toxins distributed throughout the water column, but with maximum concentration in the hypolimnion. This toxin distribution through the water column in Cobaki Lake has not been described previously. Details of this work are presented in this thesis in Chapters 7 and 8 in their originally published style and format.

6 COBAKI LAKE OVERVIEW

Cobaki Lake had a broad phytoplankton assemblage which was dominated by cyanoprokaryotes, some of which produced high concentrations of CYN, a cytotoxic cyanotoxin and protein synthesis inhibitor. This small lake provided an abundance of data typical of a stratified water body and the resulting research findings are of global interest and have been published in two internationally peer-reviewed papers. Chapters 7 and 8 are comprised of these publications; these are reproduced *verbatim* and the Journal's style and format of each paper is retained as published. Supplementary information is provided in sections 6.1 and 6.2. Chapter 9 describes the practical application of gene based methodologies (real-time PCR) in a field based CYN producing mixed population of *A. ovalisporum* and *C. raciborskii* from Cobaki Lake.

Supplementary information – Chapter 7

The first publication "Distribution of the cyanobacterial toxins cylindrospermopsin and deoxycylindrospermopsin in a stratified lake in North-Eastern New South Wales, Australia" was published in Marine and Freshwater Research (Volume 60 (1) January, 2009, pp. 25-33) and describes the vertical water column distribution of the cyanoprokaryote toxins CYN and deoxy-CYN in Cobaki Lake. Cobaki Lake contained the cyanoprokaryotes *A. ovalisporum* and *C. raciborskii* (Figures 6.1; 6.2).

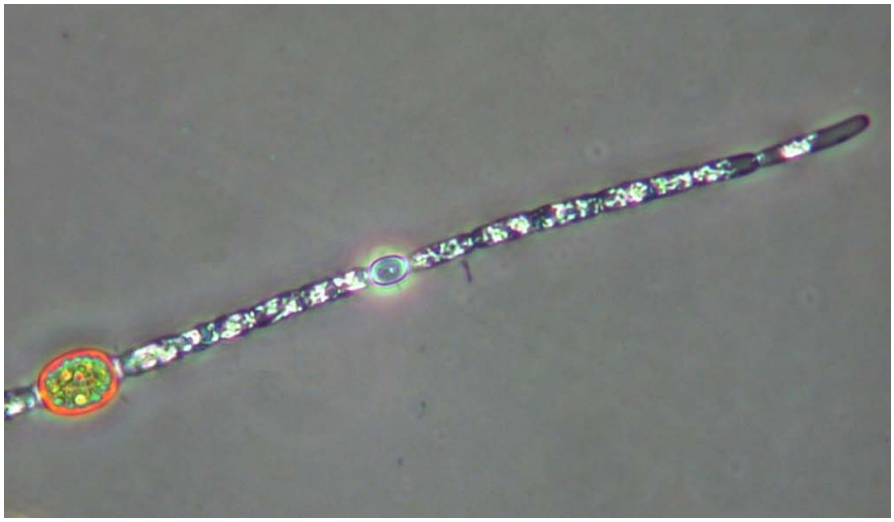


Figure 6.1. *Aphanizomenon ovalisporum*, a potent heptatoxin producer which bloomed in Cobaki Lake in the summer of 2007. Photomicrograph 400x magnification (Photomicrograph image © Sally Everson 2007).

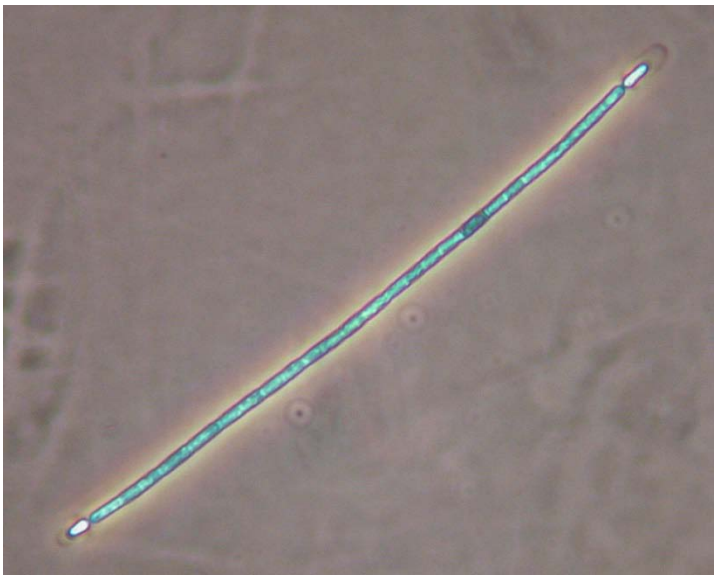


Figure 6.2. *Cylindrospermopsis raciborskii*, a potent heptatoxin producer which bloomed in Cobaki Lake in the late summer and autumn of 2007. Photomicrograph 400x magnification (Photomicrograph image © Sally Everson 2007).

Supplementary information – Chapter 8

The second publication "Extreme differences in akinete, heterocyte and cylindrospermopsin concentrations with depth in a successive bloom involving *Aphanizomenon ovalisporum* (Forti) and *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju" was published in Harmful Algae (Volume 10, January 2011, pp. 265-276) and describes the species-specific responses recorded from *A. ovalisporum* and *C. raciborskii* as they approached an overwintering phase in Cobaki Lake. Each species was examined from samples collected at one metre intervals and analysed with respect to growth of specialized cells (heterocytes and akinetes), presence of cylindrospermopsins and the accompanying seasonal dynamics of the water body. Photomicrographs of filaments of both species depicting the specialised cell formation are presented in Figures 6.3 and 6.4.

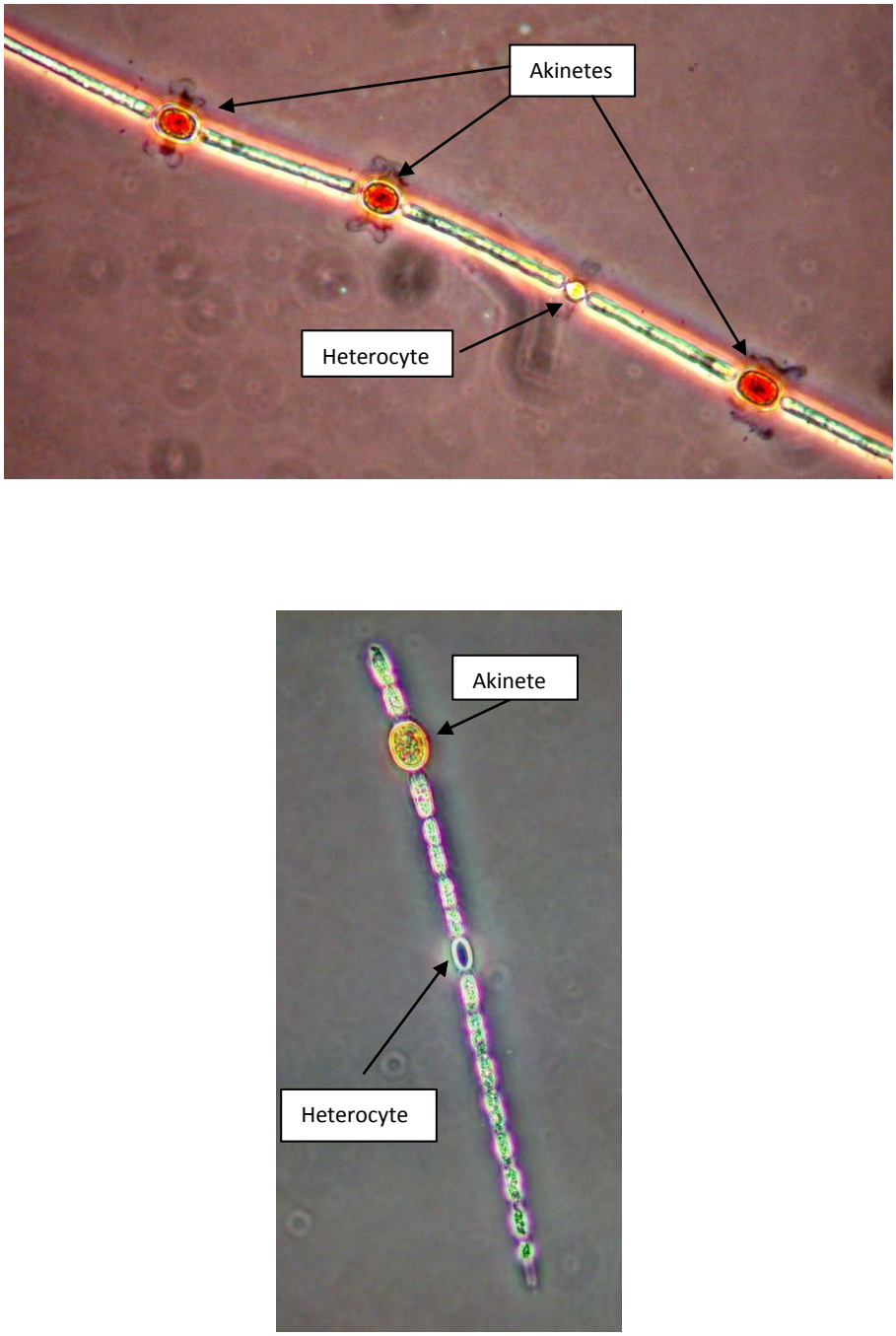


Figure 6.3. Photomicrographs of two filaments of *Aphanizomenon ovalisporum* containing both heterocytes and akinetes. These filaments were collected from Cobaki Lake in March 2007. (Photomicrograph image © Sally Everson 2007).

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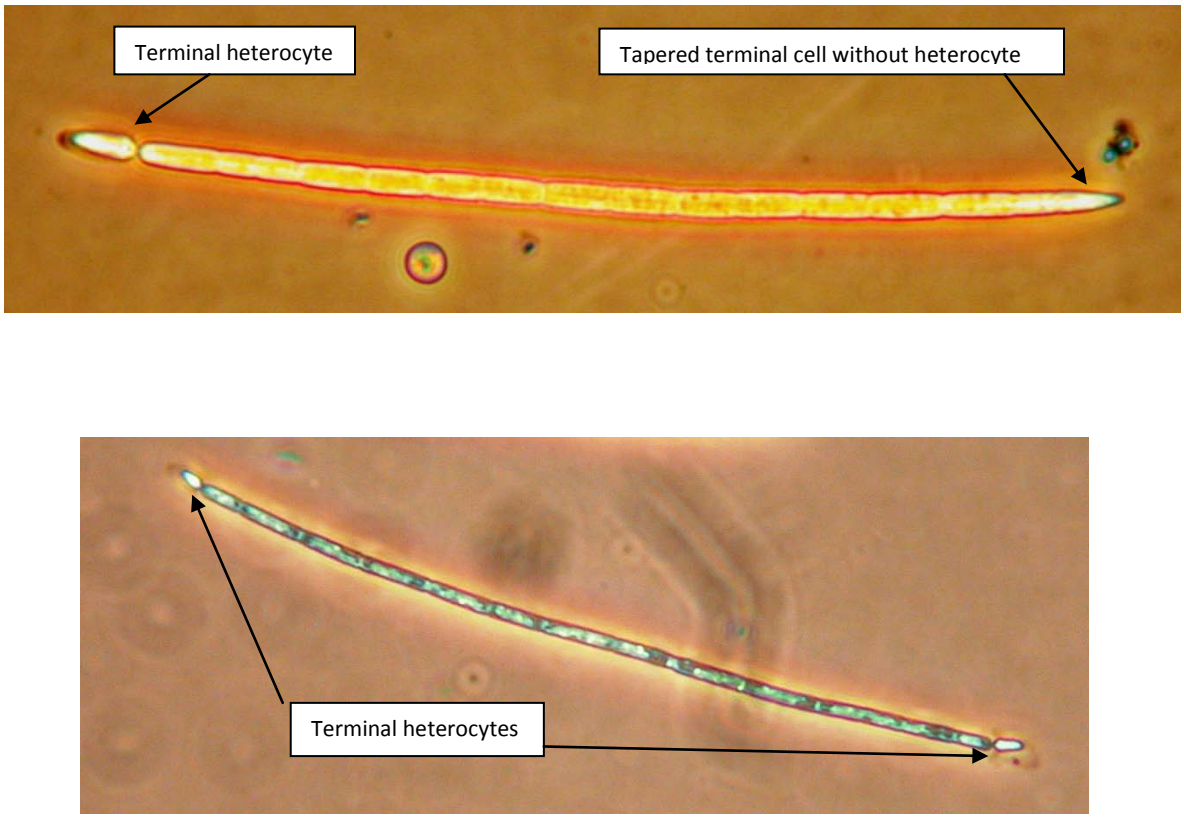

Figure 6.4. Photomicrographs of two filaments of *Cylindrospermopsis raciborskii* containing heterocytes. These filaments were collected from Cobaki Lake in May 2007. (Photomicrograph image © Sally Everson 2007).

7 DISTRIBUTION of CYANOBACTERIAL TOXINS

7.1 Published paper: "Distribution of the cyanobacterial toxins cylindrospermopsin and deoxycylindrospermopsin in a stratified lake in North-Eastern New South Wales, Australia."

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Distribution of the cyanobacterial toxins cylindrospermopsin and deoxycylindrospermopsin in a stratified lake in North-Eastern New South Wales, Australia.

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Cover illustration: Volume 60, issue 1, 2009: A filament of the toxin producing cyanobacteria, Aphanizomenon ovalisporum. Photograph by Sally Everson, Tweed Laboratory Centre (© 2009); used with permission.

Abstract. This paper describes the vertical water column distribution of the cyanobacterial toxins cylindrospermopsin and deoxycylindrospermopsin, detected in a water body containing the cyanobacteria *Aphanizomenon ovalisporum* and *Cylindrospermopsis raciborskii*. The study site was Cobaki Village Lake, a small stratified anthropogenic lake in north-eastern New South Wales, Australia. Water quality analysis indicated that stratification and oxygenation of the water column was significant in both the distribution of the cyanobacterial populations and their associated toxin concentrations. Toxin was distributed throughout the entire water column, but highest concentrations were recorded from the hypolimnion. Maximum toxin concentrations were detected in February 2007 (38.2 µg L^{-1}

CYN and 42.2 µg L^{-1} deoxy-CYN). The relative distribution of CYN and deoxy-CYN paralleled the distribution of NH_3H and NO_X within the water column, with dominance of oxygenated chemical species above 15 m compared with dominance of de-oxygenated chemical species below 15 m. Cyanobacterial cell concentrations were highest in the oxic, warm and low conductivity waters of the epilimnion and cyanobacterial species succession was found to be associated with nutrient and trace metal depletion in this surface layer. These research findings are directly relevant to the management of water supplies affected by toxic blue-green algal blooms, particularly with respect to considered placement of off-take devices to avoid layers of cyanobacterial cell and toxin concentrations.

Additional keywords: *Aphanizomenon ovalisporum, Cylindrospermopsis raciborskii*, cylindrospermopsin, deoxyclindrospermopsin, stratification, blue green algae, cyanobacteria, ecotoxicity.

Introduction

Cylindrospermopsin (CYN) is an alkaloid toxin first isolated from *Cylindrospermopsis raciborskii* (Ohtani *et al.* 1992). The toxin is also produced by *Aphanizomenon ovalisporum* (Banker *et al.* 1997; Shaw *et al.* 1999), *Anabaena bergii* (Fergusson and Saint 2000), *Umezakia natans* (Harada 1994), *Raphidiopsis curvata (*Li *et al. 2001a), Aphanizomenon flos-aquae* (Preussel *et al.* 2006), *Lyngbya wollei* (Seifert *et al.* 2007) and *Anabaena lapponica* (Spoof *et al.* 2006). CYN has been found to be highly toxic following intraperitoneal injection and oral exposure (Ohtani *et al.* 1992; Seawright *et al.* 1999; Shaw *et al.* 2000) with inhibition of protein synthesis an important mechanism with respect to toxicity (Froscio *et al.* 2003). In vitro studies of the toxicity of the analog deoxy-cylindrospermopsin (deoxy-CYN) have demonstrated cytotoxicity and inhibition of protein synthesis similar to that produced by CYN (Neumann *et al.* 2007).

Cylindrospermopsis raciborskii occurs in a wide range of water bodies worldwide (Padisák 1997). In tropical Australia, *C. raciborskii* produces high cell concentrations in waters featuring high pH (8.1), warm surface water temperatures (28 - 32ºC), a stable water column and long retention times (Boland and Griffiths 1996; Branco and Senna 1994; McGregor and Fabbro 2000). *Cylindrospermopsis raciborskii* often forms maxima in cell concentration at depth (Fabbro and Duivenvoorden 1996), which contrasts with the scum formation and surface cell accumulation normally associated with other cyanobacteria such as *Microcystis* (White *et al.* 2003) and *Anabaena circinalis* (Bormans *et al.* 1997).

Aphanizomenon ovalisporum has been detected in algal blooms in Israel (Pollingher *et al.* 1998), Australia (Shaw *et al.* 1999), Italy (Bazzichelli and Abdelahad 1994), Greece (Gkelis 2005) and Spain (Quesada 2005). In Lake Kinneret, Israel, blooms of *A. ovalisporum* have occurred in conjunction with surface temperatures between 26 to 30 ºC, high pH (8.7), low light, high phosphorus availability (Hadas *et al.* 2002), high dissolved organic nitrogen availability (Berman 1997; Berman 2001), high concentrations of biologically available iron (Parparova and Yacobi 1998) and low dissolved selenium (Nishri 1999). In Queensland, Australia, *A. ovalisporum* blooms in constructed lakes at Dundowran, thrived with high concentrations of iron (0.02 – 0.06 mg L^{-1}) and zinc (0.02 – 0.13 mg L^{-1}), conductivity (1650-3550 µS cm^{-1}), and a range of pH 7.7 – 9.5 (Shaw *et al.* 1999).

Despite the existence of several studies examining the environmental conditions present during the increase and decrease of *Cylindrospermopsis* and *Aphanizomenon* cell concentrations, to date, no reports have been made regarding how such conditions may

impact upon the relative proportions of CYN and deoxy-CYN produced and their positioning within the water column. The aim of this research, therefore, is to investigate the vertical water column distribution of the cyanobacterial toxins CYN and deoxy-CYN, produced by *A. ovalisporum* and *C. raciborskii* and the accompanying water quality.

Fig. 1. Map of Australia showing the location of Cobaki Village Lake.

Materials and methods

Study Site

Studies were undertaken on a small manmade lake at Cobaki, Tweed Heads (Fig. 1). Tweed Heads is situated on the border between New South Wales and Queensland on Australia's eastern coastline (153°49E, 28°18S). It has a subtropical climate with an average annual rainfall of approximately 1682 mm, mostly falling in the warmer months of January, February and March. The winter is dry with the cooler months of August and September generally having the lowest average monthly rainfall of approximately 60 mm. Cobaki Village Lake (Cobaki Lake) is a private lake constructed in 1994 as a focal point for a retirement village. The lake is self contained, approximately 2.6 hectares in area and 18 m deep, with no input from creeks, springs, or rivers. The only surface water to enter the lake is from rainwater and storm water runoff from the retirement village and surrounding parkland. At times of high rainfall the excess water is released into Cobaki Creek via a series of one way locks. Cobaki Creek, adjacent to Cobaki Lake, is a small estuarine waterway that feeds into Terranora Inlet, which in turn is part of the main Tweed River waterway.

Sampling

Vertical water column depth profiles of water temperature (°C), dissolved oxygen (mg L^{-1}), conductivity (µS cm^{-1}), pH and Secchi depth (m) were recorded between 5 January and 9 March, 2007. Monthly sampling was carried out from a boat in the centre of the lake using a TPS 90-FL Field Data Logger. Water samples were collected at one-meter intervals using a Van Dorn sampler (manufactured in accordance with design in APHA (Eaton *et al.* 2005)). The samples were placed on ice in eskies and returned to the laboratory for analysis, compliant with ISO/IEC 17025. Rainfall was recorded at the Bureau of Meteorology station number 040717 at Coolangatta Airport (approximately three kilometres from the study site).

Cyanobacterial Identification

Identification and enumeration of cyanobacteria to species level was conducted using Standard Methods for the Examination of Water and Wastewater (Eaton *et al.*2005) and the Phytoplankton Methods Manual (Hötzel and Croome 1999). The algal samples were preserved with Lugol's iodine solution and identified and counted using a Sedgewick rafter counting cell and phase contrast microscope (Nikon Eclipse E400; Nikon, Tokyo, Japan).

Cyanobacterial toxin analysis

Samples for CYN and deoxy-CYN analyses were collected in 500 mL polyethylene bottles, transported chilled in the dark and frozen immediately on return to the laboratory. Toxin determinations were later carried out at Queensland Health Forensic and Scientific Services. Samples were frozen/thawed to lyse all cells and analyzed using a high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) method (modified from) (Eaglesham, 1999). Briefly, CYN and deoxy-CYN were determined by HPLC-MS/MS using an AB/Sciex API4000Q mass spectrometer equipped with an electrospray (TurboV) interface coupled to a Shimadzu Prominence HPLC system (Shimadzu Corp., Kyoto, Japan). Separation was achieved using a 5 micron 150 X 4.6 mm Alltima C_{18} column (Alltech, Australia) run at 40^{o}C, and a flow rate of 0.8 ml min^{-1}with a linear gradient starting at 100% A for 2 minutes, ramped to 40%B in 6 minutes, held for 1 minute and then to 100% A in 0.2 minutes and equilibrated for 4 minutes. (A = 1% acetonitrile /HPLC grade water, B = 95% acetonitrile/ HPLC grade water, both containing 0.1% formic acid).

The mass spectrometer was operated in the positive ion, multiple reaction-monitoring mode using nitrogen as the collision gas. For CYN, retention time 6.8 minutes, the transitions monitored were 416.3 $(M+H)^+$ to 194.2 and 336.2 (collision energies 52 and 34 volts) and for deoxy-CYN, retention time 7.0 minutes, 400.3 $(M+H)^+$ to 194.2 and 320.2 (collision energies 48 and 33 volts). Positive samples were confirmed by both retention time and by comparing transition intensity ratios between the sample and an appropriate concentration standard from the same run. Using a 60 microlitre injection volume the limit of detection for this method is < 0.2 µg L^{-1}and response is linear to at least 500 µg L $^{-1}$.

Nutrients and metal analysis

Nutrients were analysed using a Lachat Flow Injection Analyser Quickchem 8000 (Milwaukee, WI, USA). Before analysis, the samples for dissolved nutrients (ammoniacal nitrogen (NH_3N), oxidized nitrogen (NO_X) and soluble reactive phosphorus (SRP) were filtered through a 0.45µm Millipore mixed cellulose ester syringe filter (Billerica, MA, USA). Non-filtered samples were digested under pressure using potassium persulphate and then analysed for Total Nitrogen (TN) and Total Phosphorus (TP). Methods used were a combination of APHA Standard Methods, section 4500 (Eaton, 2005) and Lachat Quickchem, Australia, instrumental guidelines. The limit of detection for this method is < 1.0 µg L $^{-1}$. Soluble metals were analysed using a GBC Integra XL ICP (Dandenong, Australia). Analyses using inductively coupled plasma-optical emission spectroscopy (ICP-OES) were performed with reference to Standard Methods, section 3120 – Metals by Plasma Emission Spectroscopy (Eaton *et al.* 2005). The limit of detection for this method is < 1.0 µg L^{-1}.

Results

Physical and Chemical Parameters

Cobaki Lake was stratified between 5 January and 9 March, 2007, with three separate layers present. The surface layer was delimited by a thermocline and oxycline between 4 and 8 m, whilst the lower anoxic layers were delineated by a halocline between 15 and 17 m (Fig. 2). Surface temperatures ranged from 25.3 to 28.0 ºC, whereas the hypolimnetic temperatures remained constant at 18.1 ºC. The surface waters were alkaline ranging in pH between 8.3 and 9.0, whilst the hypolimnion was slightly acidic (pH 6.6). The conductivity in the epilimnion ranged between 344 to 397 µS cm^{-1} but peaked at 24290 µS cm^{-1} in the hypolimnion. Rainfall over the three month sampling period increased from 76.8 mm in January, to 84.0 mm in February and to 222.4 mm in March. The increased rainfall and consequent run off from surrounding parklands in March prior to sampling could have resulted in some dilution occurring in the surface layers of the lake (Fig. 3)

The Euphotic depth (z_{eu}) was calculated using a formula based upon Secchi depth (Kirk 1994) (Table 1).

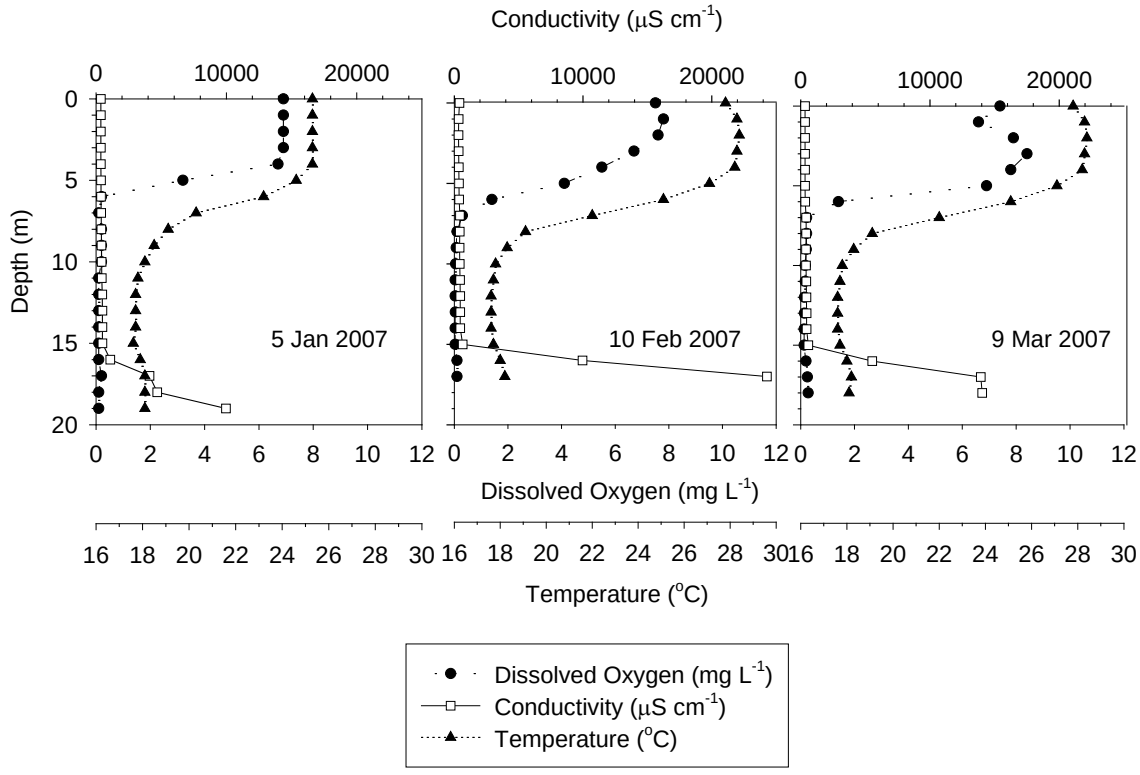

Fig. 2. Vertical water column profiles of dissolved oxygen (mg L^{-1}), conductivity (µS cm^{-1}) and temperature (ºC) recorded in Cobaki Lake between 5 January and 9 March 2007.

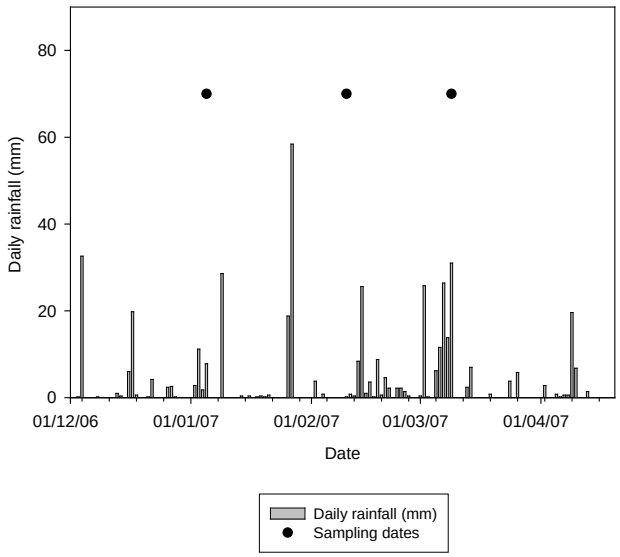

Fig. 3. Daily rainfall recorded at the Bureau of Meteorology station number 040717 at Coolangatta airport between December 2006 and April 2007 with sampling dates indicated.

Table 1. Secchi and euphotic depth from Cobaki Lake at selected dates between January and March 2007.

	January 5 2007	February 10 2007	March 9 2007
Secchi depth (m)	1.2	2.5	2.0
Euphotic depth (m)	3.8	7.9	6.4

Nutrients and metals

Bioavailable nutrients (NH_3N, NOx and SRP) decreased to below detectable limits in the epilimnion over the three month sampling period (Fig. 4). The highest concentrations of metals and cations (in particular iron and potassium) were found in the hypolimnion of the lake (Tables 2 and 3).

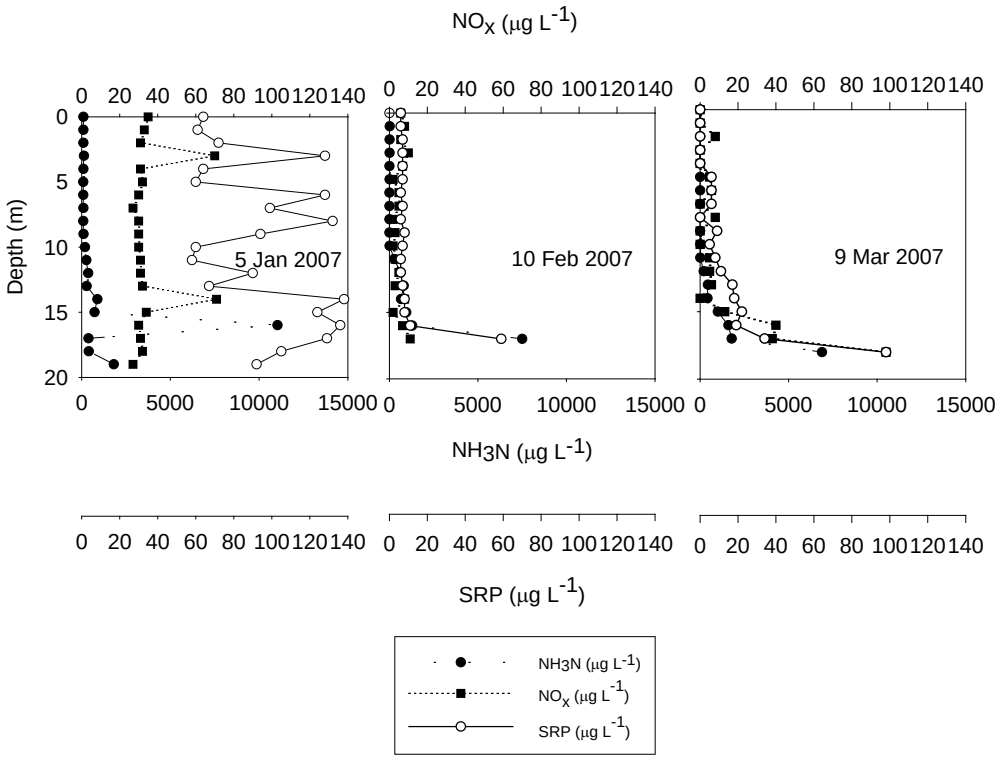

Fig. 4. Vertical water column distribution of oxidised nitrogen (■), ammonium nitrogen (●) and soluble reactive phosphorus (○) in samples taken from Cobaki Lake between 5 January 2007 and 9 March 2007.

Table 2. Epilimnetic and hypolimnetic distribution of soluble iron and potassium concentrations in samples collected from Cobaki Lake at selected dates between January and March 2007.

Sampling Date	Location	Fe (µg L $^{-1}$)	K (µg L $^{-1}$)
5 Jan 2007	Epilimnion	8.8	2200
10 Feb 2007	Epilimnion	0	1579
9 Mar 2007	Epilimnion	0	0
5 Jan 2007	Hypolimnion	3450	16760
10 Feb 2007	Hypolimnion	17400	109000
9 Mar 2007	Hypolimnion	4610	3279

Table 3. Ranges in the concentration of soluble calcium, magnesium and sodium cations determined using samples collected from the epilimnion and hypolimnion of Cobaki Lake between January and March 2007.

Location	Calcium ($\mu g\ L^{-1}$)	Magnesium ($\mu g\ L^{-1}$)	Sodium ($\mu g\ L^{-1}$)
Epilimnion	10900 – 12500	7200 – 8050	55000 – 67500
Hypolimnion	116000 – 550000	98200 – 253000	318000 – 994000

Cyanobacteria

A succession from *Aphanizonemon ovalisporum* to *Cylindrospermopsis raciborskii* occurred in Cobaki Lake during the sampling period. Initially, *Aphanizomenon ovalisporum* was dominant with cell concentrations decreasing from 45 500 cells mL^{-1} in January to 30 000 cells mL^{-1} in March. (Fig. 5) *Aphanizomenon ovalisporum* was most prevalent in the surface layer, although visually identifiable filaments were also recorded at depth (17-18 m). *C. raciborskii* was the second-most numerous cyanobacterium present, gradually increasing in cell concentration during the three month sampling period. The average cell concentration in January was 1200 cells mL^{-1} by February it had doubled to 2500 cells mL^{-1} and by March it had increased to 18 000 cells mL^{-1} (Fig. 5). Both *A. ovalisporum* and *C. raciborskii* were present in the epilimnion with highest cell concentrations occurring at 2 to 3 m depth, whilst cell concentrations decreased below the thermocline at 6 m. At 9 m, a significant decrease in pH occurred; this was concomitant with a noticeable decrease in *C. raciborskii* cell concentrations. In contrast with *A. ovalisporum, C. raciborskii* filaments generally did not appear below 16 m. During the sampling period there was no visible algal scum or discolouration.

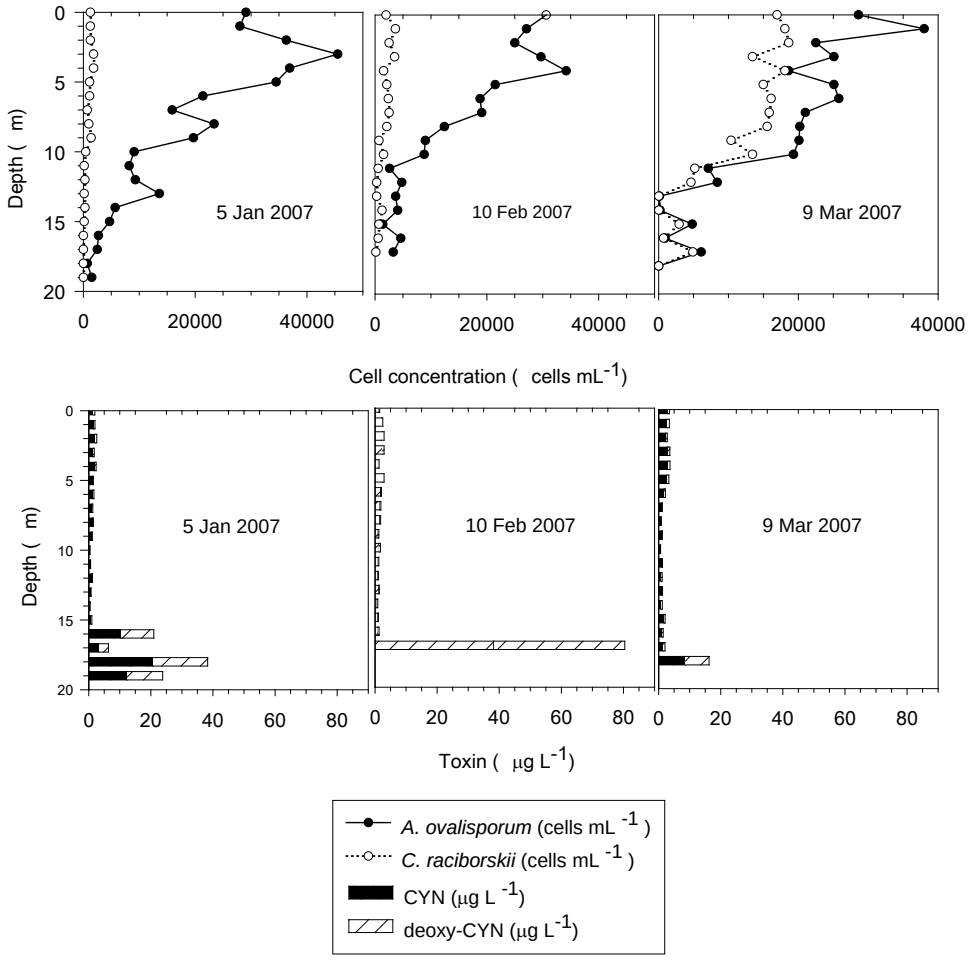

Fig. 5. Vertical water column profile of algal cell concentrations and toxin concentrations recorded at Cobaki Lake between 5 January 2007 and 9 March 2007.

Toxin concentrations

CYN and deoxy-CYN were distributed throughout the entire water column, but highest concentrations were recorded from the hypolimnion (Fig. 5). The maximum toxin concentration detected was in February (38.2 µg L^{-1} CYN and 42.2 µg L^{-1} deoxy-CYN). The relative distribution of CYN and deoxy-CYN paralleled the distribution of NH_3H and NO_X within the water column, particularly in February and March (Fig. 4), with dominance of oxygenated chemical species above 15 m compared with dominance of de-oxygenated chemical species below 15 m. The highest toxin concentrations occurred where the cyanobacterial cell concentrations were lowest. This paper provides key evidence with respect to spatial distribution of toxins and cells for blooms dominated by *A. ovalisporum* and *C. raciborskii*.

Discussion

Cobaki Lake displayed the typical characteristics of a water body stratified as a result of gradients in temperature and conductivity. Increased conductivity with depth may have been due to intermixing or seepage from the estuarine Cobaki Creek, which was 17.3 m distant from the lake.

Three layers were apparent in the lake: the uppermost was oxic (dissolved oxygen concentration ranged between 6.9 and 7.4 mg L^{-1}), warm (25.3 to 28.0 °C) and low in

conductivity (274 to 397 µS cm^{-1}); the middle was anoxic (0.2 to 0.3 mg L^{-1}), temperature 20.3 to 23.9 °C and of low conductivity (299 to 428 µS cm^{-1}) , whilst the third layer was anoxic (dissolved oxygen concentration of 0.1 mg L^{-1}), cool (18.1 °C) and of high conductivity (4970 to 24 290 µS cm^{-1}). The succession of cyanobacterial species corresponds with previous studies indicating that *Cylindrospermopsis raciborskii* produces high cell concentrations in water with high pH (pH > 8.1), warm surface water temperatures (28 – 32°C), and a stable water column (Boland and Griffiths 1996; McGregor and Fabbro 2000). It is also consistent with patterns recorded in Brazil (Branco and Senna 1994), where the dominance of *C. raciborskii* was linked to nutrient depletion in the epilimnion as a result of thermal stratification during the wet season, as well as high pH and water temperatures. The rise in *C. raciborskii* cell concentrations parallels the decrease in nutrients in the surface layer (Branco and Senna 1994; Fabbro *et al.* 2001; Fabbro and Duivenvoorden 1996).

Aphanizomenon ovalisporum typically thrives in surface temperatures between 26 and 30 ºC and high pH (8.7), in addition to high phosphorus availability (Hadas *et al*. 2002) and high concentrations of biologically available iron (Parparova and Yacobi 1998). In Cobaki Lake, the epilimnetic concentration of both SRP and the soluble iron concentrations decreased to low or to undetectable levels during the sampling period. This was reflected in the decrease in the *A. ovalisporum* population.

The maximum toxin concentration was present in the hypolimnion, a region of the water column where there was a surplus of nutrients and trace elements. The dominant form of toxin present at this depth was deoxy-CYN. Interestingly, this coincided with a lack of oxygen and light, both of which are requisites for natural degradation of the toxin. Chiswell *et al.* (1999) found that CYN is stable in the dark and CYN in natural waters is degraded by sunlight, but the degree of degradation is affected by both turbidity and the depth of the photic zone. This could explain the low concentrations of CYN in the photic (upper) layers of the lake. The distribution of oxidized and deoxidized forms of toxin within the water column parallels the distribution of these forms of nitrogen with ammonium nitrogen being the dominant form in the hypolimnion.

There are a number of possible reasons for the high concentrations of toxin being present in the lower layers of the water column. The toxin breakdown rates may be slower in the hypolimnion, thus allowing the toxin to accumulate in this region. Slow breakdown rates may be due to the absence of light and oxygen (as described above). Microbial degradation may be important in contributing to CYN and deoxy-CYN removal. Certain physiochemical conditions that inhibit microbial growth may also indirectly affect biodegradation rates of these toxins. For example, previous studies have shown that temperatures between 25 and 37 °C contribute positively to the microbial breakdown of CYN (Chiswell *et al.* 1999; Smith *et al.* 2008; Nybom *et al.* 2008). The applicability of these results to this research is that reduced water temperature (17-18°C) was recorded at depth within Cobaki Lake; hence, this may be linked with reduced microbial activity and consequently, slower CYN degradation. Increased conductivity and anoxic conditions in the hypolimnion may be further physiochemical conditions in Cobaki Lake which could further inhibit microbial activity associated with CYN degradation.

There is potential for toxins to combine with benthic organic matter and subsequently resisting toxin breakdown. In relation to this benthic organic layer, selective degradation of toxin might be occurring, and as deoxy-CYN is less hydrophilic it might be absorbed to the detritus in the hypolimnion.

Studies have shown that akinetes accumulate in sediment (Barbeiro and Kann 1994; Barbiero and Welsh1992; Hansson and Carpenter 1993; Head *et al.* 1999; Jones 1979; Reynolds 1972). The potential for akinetes to produce high CYN concentrations may be particularly important during bloom senescence, where they are often produced in large numbers. It is conceivable that the large numbers of akinetes that are concentrated in the lower layers of Cobaki Lake could be responsible for increased rates of toxin production, compared with vegetative cells. To date, no laboratory or field studies have demonstrated higher CYN production by akinetes of *C. raciborskii* and *A. ovalisporum.* However, there are some indications this may be possible. For example, akinetes are typically much larger than vegetative cells (in Cobaki Lake the average vegetative cell size for *A. ovalisporum* was 3.75 x 10.0 µm; whereas the average akinetes cell size was 12.5 x 15.0 µm). It has already been shown that cell size provides some indication of likely toxin content, at least for vegetative cells (Hawkins *et al.* 2001; Saker and Neilan 2001). Furthermore, since akinetes and heterocyocytes in *C. raciborskii* begin life vegetatively (Chiswell *et al.* 1999), their genetic capability for toxin production should at least equal that of undifferentiated vegetative cells. This concept clearly requires further investigation.

The possibility that the ratio in which CYN and deoxy-CYN are produced changes with depth should also be considered. The deoxy-CYN at the surface and intermediate layers is approximately one fifth the concentration of CYN, however, in the hypolimnion; almost the entire toxin concentration is represented by deoxy-CYN. Perhaps toxin production by *A. ovalisporum*, especially of the deoxy-CYN molecule, is enhanced in low-light/low dissolved oxygen/higher conductivity environments. In the anoxic hypolimnion, deoxy-CYN cannot oxidize to form CYN so remains in the deoxygenated form. The heavy saline layer at 18 m could be retaining the deoxy-CYN at that depth. This is likely to change with overturn.

The trend indicated from these three depth profiles is that whilst most of the cells are in the upper layers, the toxin is present throughout the water column, but concentrated in the hypolimnion. The data collected in this study have shown that CYN was present above 1.0 µg L^{-1}. CYN usually has no taste or odour when present in a natural water body and therefore is a potential risk for both humans and animals. During this present study, there was no visible algal scum or discolouration. This reinforces the concept that whilst there were no visible signs present, the water quality could still be compromised. Both the algal and toxin profiles in this study have important implications for the management of water utilities. Traditionally, off take pipes are positioned to avoid the highest algal cell concentrations. In addition, most utility managers react not to toxin quantities, but instead to cell concentrations. This management practice reflects not only a gap in the scientific understanding of toxic algal blooms (partly addressed in this study), but also the practical difficulties in toxicity testing. For example, whilst cell counting is generally an economical and speedy process, toxicity testing is more difficult to achieve due to a scarcity of accredited facilities able to perform the analyses, combined with higher costs and more difficult logistics for sampling and testing. This situation, together with the assumption that the toxin remains within the cyanobacterial cells, continues to foster the apparently incorrect practice of using cell counts for management decisions. Studies with *C. raciborskii* indicated that 90% of the toxin remains within the cells during the early phase of active growth, however, as the growth ages, extracellular CYN could be up to 50% (Saker and Griffiths 2000). Another study by Chiswell *et al.* (1999), found that toxin from a bloom of an aging *C. raciborskii* population, 70-98% of CYN was dissolved in the water column. This knowledge combined with the findings presented in this study suggests that where the algal species *C.*

raciborskii and *A. ovalisporum* are concerned, it would be prudent to understand the toxin profile before determining the off take depth.

The work outlined in this present study has wider implications for any water body, regardless of size, prone to CYN producing algal taxa, especially if it is a source for drinking water supplies (human or stock). Collection and analysis of the sediment and hypolimnetic water testing coupled with corresponding physical data could supply further information as to how and why CYN and deoxy-CYN accumulate at depth.

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8 AKINETE, HETEROCYTE and CYN CONCENTRATIONS AT DEPTH.

8.1 Published paper: Extreme differences in akinete, heterocyte and cylindrospermopsin concentrations with depth in a successive bloom involving *Aphanizomenon ovalisporum* (Forti) and *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and **Subba Raju**

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Extreme differences in akinete, heterocyte and cylindrospermopsin concentrations with depth in a successive bloom involving *Aphanizomenon ovalisporum* (Forti) and *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju

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ABSTRACT

This paper describes the species-specific responses recorded from two toxin-producing cyanobacteria *Aphanizomenon ovalisporum* (Forti) and *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju as they approached an overwintering phase in Cobaki Lake (New South Wales, Australia). Each species was examined from samples collected at every metre from a time series of depth profiles and analyzed with respect to growth of specialized cells (heterocytes and akinetes), presence of cylindrospermopsins and the accompanying seasonal dynamics of the water body. Growth and dominance of each species was linked to differing seasonal environmental conditions. Both *A. ovalisporum* and *C. raciborskii* produced specialized cells during the bloom. As the water chemistry changed *A. ovalisporum* produced akinetes before experiencing a rapid decline in cell numbers. In contrast, *C. raciborskii* continued to bloom without producing detectable akinetes. Peak *C. raciborskii* cell concentrations (83,160 cells mL^{-1}) occurred in the late autumn, when surface water temperatures were 19.1 ˚C, and were accompanied by toxin concentrations exceeding 100 μg L^{-1}. These toxin concentrations were highly positively correlated with conductivity, soluble iron, bioavailable nutrient species and heterocyte densities. This is the first field study to provide evidence that *C. raciborskii*, despite being traditionally considered as a tropical species, can be highly toxic in cooler waters especially when accompanied by strong stratification involving an anoxic semi-saline hypolimnion. This has serious implications for both water quality management and human health risks in those subtropical climates where *C. raciborskii* is present.

Keywords: cylindrospermopsin, deoxycylindrospermopsin, blue-green algae, climate change, stratification, akinete, heterocyte, Nostocales, cyanobacteria.

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1. Introduction

Cyanobacteria are photosynthetic organisms commonly found in freshwater, estuarine and marine environments worldwide. Many cyanobacterial species are capable of toxin production and these represent a serious health hazard in both recreational and drinking water supplies (Chorus and Bartram 1999).

Aphanizomenon ovalisporum and *Cylindrospermopsis raciborskii* are two planktonic nostocalean species both capable of producing cylindrospermopsins (CYN) and deoxy-cylindrospermopsin (deoxy-CYN) in their vegetative cells (Banker *et al.* 1997; Ohtani *et al.* 1992; Shaw *et al.* 1999). Both species also produce specialized cells, which influence patterns of survival, reproduction and nutrient assimilation in algal blooms. These specialized cells may be very important in moderating patterns of bloom formation and periodicity (i.e., when a bloom develops, how rapidly it reaches peak cell concentrations and how long the bloom lasts). Heterocytes are specialized cells that develop from vegetative cells. They are commonly reported from field populations of *A. ovalisporum* and *C. raciborskii*, typically when blooms are threatened by nitrogen starvation. In this situation, heterocytes allow nitrogen-fixation to occur in the anaerobic environment created by the thickened cell wall (Komárek and Anagnostidis 1989), thus satisfying the cell's nitrogen requirements for survival. Whilst the physiology of heterocytes is quite well reported, the overall contribution of heterocytes to the toxicity of cyanobacterial blooms remains poorly understood.

Akinetes are another type of specialized cell featured in populations of both *A. ovalisporum* and *C. raciborskii*. These thick-walled resting cells are derived from the enlargement of a vegetative cell and serve as a survival structure for the organism (Komárek and Anagnostidis 1989). Akinetes can survive in unfavourable conditions for long periods, often in sediments (Fabbro and Duivenvoorden 1996; Padisák *et al.* 2003; Wood *et al.* 2008). When suitable conditions for vegetative growth are later resumed, akinetes germinate into vegetative trichomes. This process can result in rapid establishment of blooms.

Studies of the dynamics of specialized cells are important in providing insights into the growth cycles of algal blooms. Unfortunately, the few existing reports on this issue are largely unrelated and in some cases, contradictory. Israeli field studies at Lake Kinneret linked high heterocyte, but low akinete cell numbers with the initial stages of an *A. ovalisporum* bloom. In contrast, the reverse trend was observed as the bloom declined (Hadas *et al.* 1999). Baker (1999) studied akinete development in *Anabaena circinalis* and *Anabaena flos-aquae* in the Murray River (Australia), showing that the ratio of akinetes to vegetative cells was highest at peak cell concentrations. This work suggested that high cell biomass may be an indirect trigger for akinete development. Baker (1999) also established that akinete production was not necessarily linked with sudden changes in physical or chemical water quality.

The stimuli for akinete development sometimes appear to be species-specific. Li *et al.* (1997) reported temperature to be a key environmental factor triggering akinete formation in seven strains of planktonic *Anabaena* spp. These authors report the highest

rates of akinete development were evident at 10°C and 15°C. On the other hand, Moore *et al.* 2005 found that seasonal-water temperature fluctuations were responsible for akinete differentiation in *C. raciborskii.* The successful development of these akinetes was also affected by increased light intensity and available reactive phosphorus concentrations exceeding 70µg L^{-1}. Laboratory trials with *A. circinalis* have also indicated that the production of akinetes would be greater in environments with less blue light, which corresponds with environmental situations involving low flow, stratification, little vertical mixing and turbidity (Thompson *et al.* 2009). Other laboratory trials by Sukenik *et al.* 2007 have shown that mature akinetes of *A. ovalisporum* are not dormant; rather, they maintain a limited level of metabolic activity, including photosynthesis. Sukenik's study also indicated that a population is composed of akinetes from various developmental stages.

Global climate change may result in subtropical winters becoming warmer. Recent reports also associated increased temperatures with an increase in the global distribution of *C. raciborskii* (Barone *et al.* 2010; Kling 2009; Messineo *et al.* 2010). The results of laboratory trials in Germany involving semi-continuous cultures examined the competitiveness of invasive cyanobacteria against native cyanobacteria indicated that at lower temperatures (≤ 10°C) the native species had higher growth rates, while the opposite was true at higher temperatures (≥35°C) while light variances were inconclusive (Mehnert *et al.*2010). The authors indentified *C. raciborskii* as one of a number of invasive species and *A. ovalisporum* as a potentially invasive species. The findings of this study indicate that as temperature increases a shift in dominance would occur in favour of invasive Nostocalean species (Mehnert *et al.* 2010).

It would seem from the above studies, the influence of temperature on akinete formation, and hence the expansion of *C. raciborskii*, is likely to be critical, as akinetes are an important part of its biology. This is a concern since several laboratory studies have shown that maximal toxin production by *C. raciborskii* occurs at cooler temperatures (Saker and Griffiths 2000). This represents a serious management issue for water authorities with operations in subtropical and temperate areas.

2. Aim of Research

The aim of this research is to detail a succession from *A. ovalisporum* to *C. raciborskii* and associated water chemistry dynamics in a strongly stratified water body. It also includes the study of the variation in concentration through depth profiles (at every metre) of the specialized cells and cylindrospermopsins.

3. Materials and Methods

3.1. Study Site

Cobaki Village Lake is a small anthropogenic lake established in 1994. The catchment area borders both New South Wales and Queensland on Australia's eastern coast line (153°49E, 28°18S). A full description of the lake and its study sites is given in Everson *et al.* 2009.

3.2. Sampling

Sampling was conducted in the centre of Cobaki Lake at four-weekly intervals, commencing 5 Jan 2007 and continuing for twenty months. Vertical water column depth profiles were recorded at one metre intervals using a TPS 90-FL Field Data Logger (Brisbane, Australia). Parameters included water temperature (°C), dissolved oxygen (mg L^{-1}), conductivity (µS cm^{-1}), pH, turbidity (NTU) and Secchi depth (m). Water samples were also collected at one-metre intervals using a Van Dorn sampler (manufactured in accordance with APHA design requirements (Eaton *et al.* 2005)). Samples were placed on ice in insulated cooler boxes for return transport to the laboratory for analysis, compliant with ISO/IEC 17025. The euphotic depth (z_{eu}) was calculated using a formula based on Secchi depth (Kirk 1994). Rainfall data were recorded at the Bureau of meteorology station number 040717, located approximately 3 kms from the study site.

3.3. Cyanobacterial identification

Species-level identification and enumeration of cyanobacteria was conducted using Standard Methods for the Examination of Water and Wastewater (Eaton *et al.* 2005), the Phytoplankton Methods Manual (Hötzel and Croome 1994) and the Guide to the identification of common blue-green algae (Cyanoprokaryotes) in Australian Freshwaters (Baker and Fabbro 1999). Samples were preserved on-site using acidified Lugol's iodine solution and analyzed within seven days of collection. Identification and enumeration were performed using a glass Sedgewick rafter counting chamber and phase contrast microscopy (Nikon Eclipse E400; Nikon, Tokyo, Japan). The cell dimensions were measured using an ocular micrometer at 400 x magnification. A minimum of 30 trichomes were examined per sample with a minimum of 30 individual specialized cells measured per sample; the exact number depending on cell concentration. The average cell dimensions were calculated for each sample. The dimensions measured included: length and width of trichomes, vegetative cells, akinetes and heterocytes and the prevalence of the specialized cells on each trichome.

3.4. Cyanobacterial toxin analysis

Cylindrospermopsin (CYN) and deoxycylindrospermopsin (Deoxy-CYN) determinations were carried out at Queensland Health Forensic and Scientific Services (Eaglesham 1999). Samples were freeze-thawed to lyse all cells and analyzed using a high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) method, modified from Eaglesham *et al.* 1999.

3.5. Nutrient and metal analysis

Nutrient concentrations were analyzed by a Lachat Flow Injection Analyser Quickchem 8000 (Milwaukee, WI, USA). Soluble metal scans were performed on a GBC Integra XL ICP (Dandenong, Australia) using inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Eaton *et al.* 2005).

3.6. Correlation Statistics

Correlation analyses were conducted using SPSS Inc PASW Statistics 17. A significant relationship was defined as $p < 0.05$ as recommended by McKillup (2007).

4. Results

4.1. Cyanobacterial populations

The algal populations in the first six months of the sampling period were dominated by *A. ovalisporum* and *C. raciborskii* which were predominantly located in the upper layers of the water column (Fig. 1). Cells were concentrated above 10 m with highest cell concentrations occurring between 2 m and 3 m. Notably, no visible algal scum or discolouration was recorded throughout the sampling period.

Succession from *A. ovalisporum* to *C. raciborskii* cell dominance was evident both spatially and temporally in Cobaki Lake. *A. ovalisporum* dominated the top half of the water column during the first three months of the study, but was below detectable limits by April 2007. Conversely, *C. raciborskii* reached maximum cell concentrations in May (83,160 cells mL^{-1}) before declining in June (14,560 cells mL^{-1}), with only a few short trichomes remaining after the lake overturned. Whilst the blooms of *A. ovalisporum* and *C. raciborskii* occurred in 2007, no such blooms were detectable in the summer of 2008. A population of *Anabaena circinalis* (3,100 cells mL^{-1}) dominated the water body at this time, hence providing interesting comparisons between years.

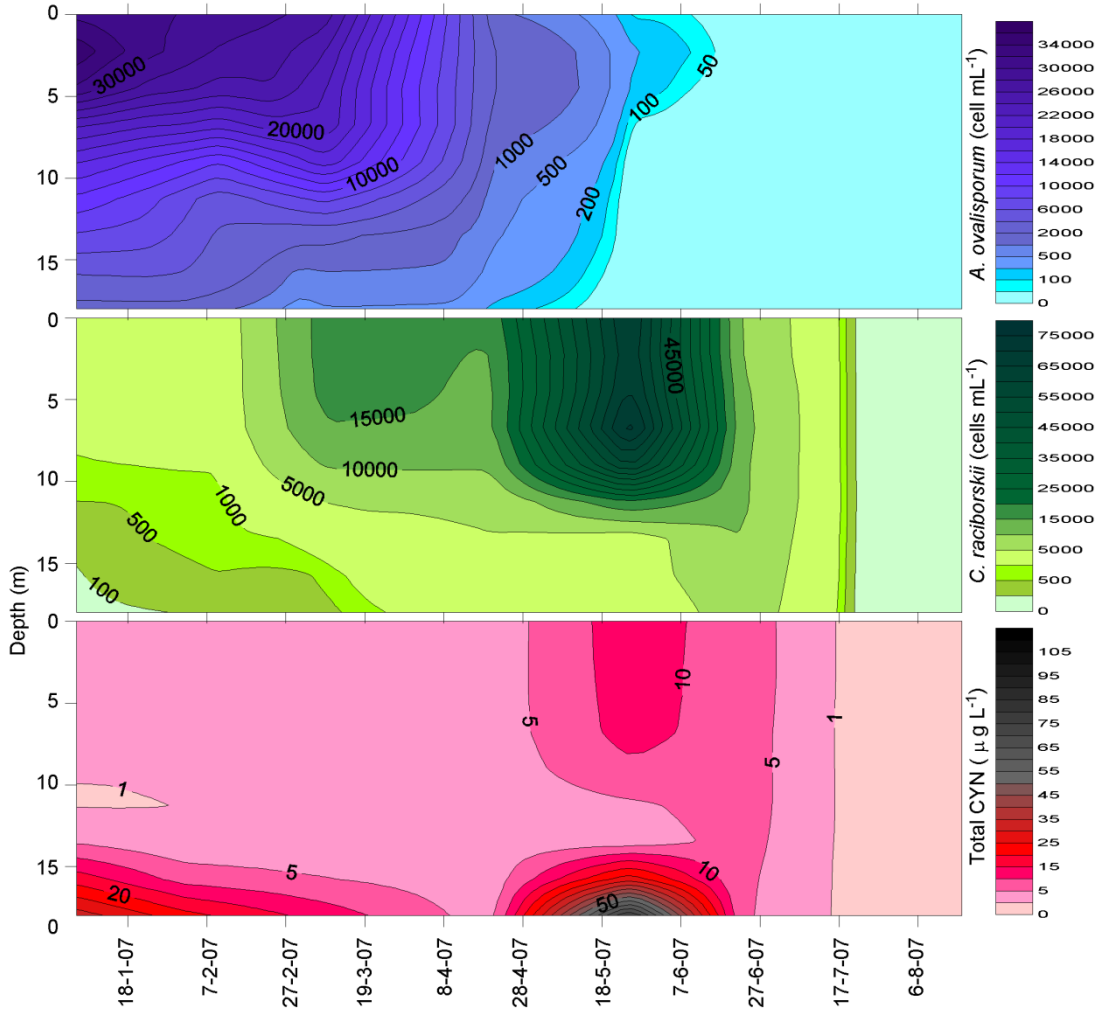

Fig. 1. Cobaki Lake time series depth profiles for eight months from 5 Jan 2007 to 17 Aug 2007 showing *Aphanizomenon ovalisporum* cell concentration (cells mL^{-1}), *Cylindrospermopsis raciborskii* cell concentration (cells mL^{-1}) and total cylindrospermopsins distribution (µg L^{-1}).

4.2. Specialized cells and Filament length

Specialized cells were produced by both *A. ovalisporum* and *C. raciborskii* during the bloom, but these species featured quite different specialized cell dynamics, as well as different cell dimensions (Table 1).

Table 1.

The range of specialized cell and filament dimensions produced by *Aphanizomenon ovalisporum* and *Cylindrospermopsis raciborskii* in Cobaki Lake between 5th January and 22nd June 2007.

	A. ovalisporum	***C. raciborskii***
Vegetative cell size	4.5 – 6.25 (median: **5.0**) µm wide x 9.25 – 11.5 (median: **10.0**) µm long	3.0 – 5.5 (median: **3.75**) µm wide x 9.25– 11.0 (median: **10.0**) µm long
Heterocyte size	3.0 – 5.5 (median: **3.75**) µm wide x 6.0 – 8.25 (median: **7.5**) µm long	3.0 – 5.5 (median: **3.75**) µm wide x 3.0 – 6.75 (median: **5.0**) µm long
Maximum % of trichomes with heterocytes	86%	80%
Akinete size	10.0 – 12.5 x 12.5 – 15.0µm	Not detectable
Maximum % of trichomes with akinetes	32%	Not detectable
Trichome length	252 – 300 µm	102 - 146 µm

4.2.1 A. ovalisporum

Akinetes of *A. ovalisporum* were distributed throughout the water column, to a depth of 17 m, except in January 2007 when concentrations were lower and cells restricted to the surface layers (Fig. 2). Akinetes were formed both singly and in series, and some trichomes contained numerous single akinetes at varying stages of maturity. Akinetes were always distant from heterocytes and not all trichomes produced akinetes.

Maximum akinete concentration for *A. ovalisporum* was recorded in March, 2007, where 32% of trichomes contained akinetes, and akinetes represented up to 0.2% of the total cell number. This was strongly contrasted with April, where neither cells nor akinetes were detectable in the water column. The peak in akinetes during March was also particularly noticeable given that the vegetative cell numbers were declining at that time, and because the bulk of akinetes were located in the hypolimnion.

Heterocytes of *A. ovalisporum* were also recorded in the lake. These were present whenever vegetative cells were recorded, and were observed throughout the entire water column. The highest heterocyte densities were recorded in January and March, at depths greater than 12 m.

There was no statistically significant relationship recorded between trichome length and the prevalence of specialized cells (correlation P = > 0.05), although heterocytes appeared to increase as trichome length decreased. For example, longer trichomes were typically recorded from the surface waters, and at the beginning of the bloom, but this did not coincide with the peak in akinetes or heterocytes (as both these tended to occur at depth).

A. ovalisporum featured long trichomes (averaging 252 µm in length) in the warm oxygenated surface layers, at the beginning of the bloom; however, the longest trichomes were not always recorded from the epilimnion. In February, lengths were almost uniform throughout the water column. In March, the average trichome length increased to 300 µm with some very short trichomes also recorded at depth.

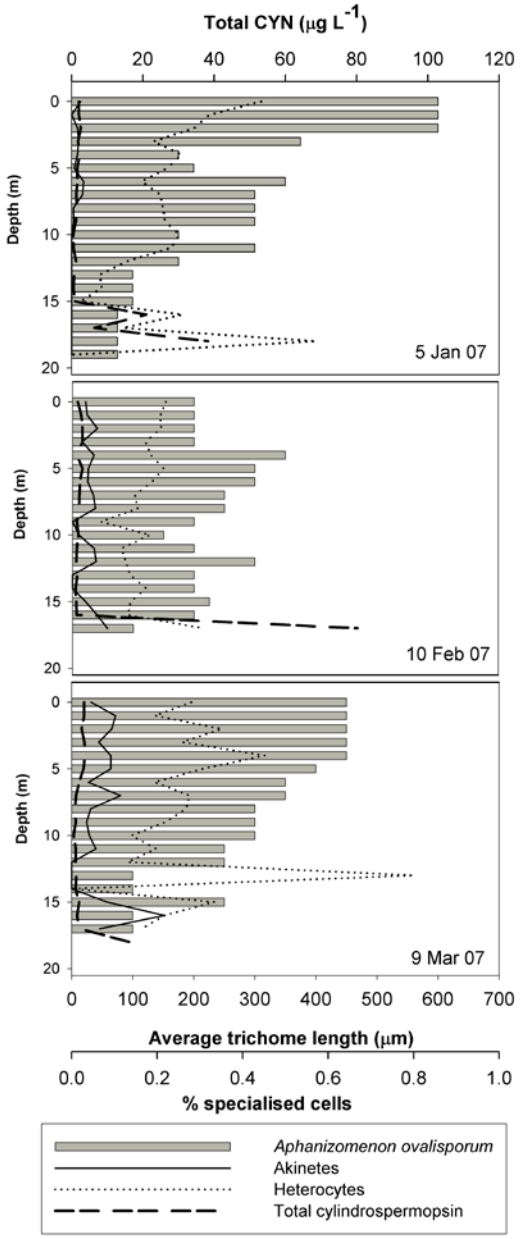

Fig. 2. Depth profiles showing the average trichome length (µm), percentage of specialized cells (akinetes and heterocytes) and total cylindrospermopsins concentration (µg L^{-1}) present in a bloom of *Aphanizomenon ovalisporum* in Cobaki Lake between 5th January 2007 and 9th March 2007.

4.2.2 C. raciborskii

Akinetes were never observed in *C. raciborskii* trichomes in this study. In contrast, the distinctive arrow-shaped terminal heterocytes of this species were present throughout the bloom. The prevalence of heterocytes increased to an average of 80% (0.8) throughout the water column in May when maximum cell concentration was reached (Fig. 4). There was an unexpected spike in the number of heterocytes at 11 m depth in April.

The average trichome length of *C. raciborskii* was shorter overall than that recorded from *A. ovalisporum*, with monthly averages of 105 µm, 139 µm and 146 µm in March, April and May respectively, before decreasing to 102 µm in June. In June there was a change in the morphology of the trichomes which appeared to become irregularly bent with cell content depletion and greater constriction at the cell divisions (Fig. 3).

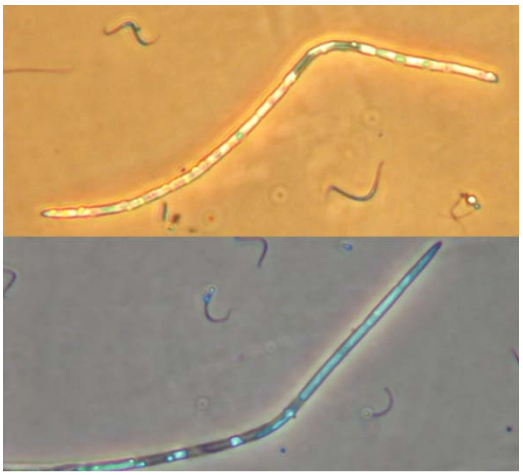

Fig. 3. Photomicrographs (x 400 magnification. Nikon Coolpix E995) showing the extreme morphology noted in trichomes of *Cylindrospermopsis raciborskii* towards the end of the bloom suggesting viral attack. The trichomes are irregularly bent and show the loss of cell contents and greater constriction at the cell divisions. These samples were from Cobaki Lake on 22nd June 2007.

4.3. Concentration of cylindrospermopsins

Cylindrospermopsin analysis revealed that both CYN and deoxy-CYN were distributed throughout the entire water column between January and June 2007. The highest concentrations were recorded from the hypolimnion. The maximum cylindrospermopsin concentration detected was 101.4 µg L^{-1} total CYN, comprised of 52.9 µg L^{-1} CYN and 48.5 µg L^{-1} deoxy-CYN and was recorded below the halocline (Fig. 1).

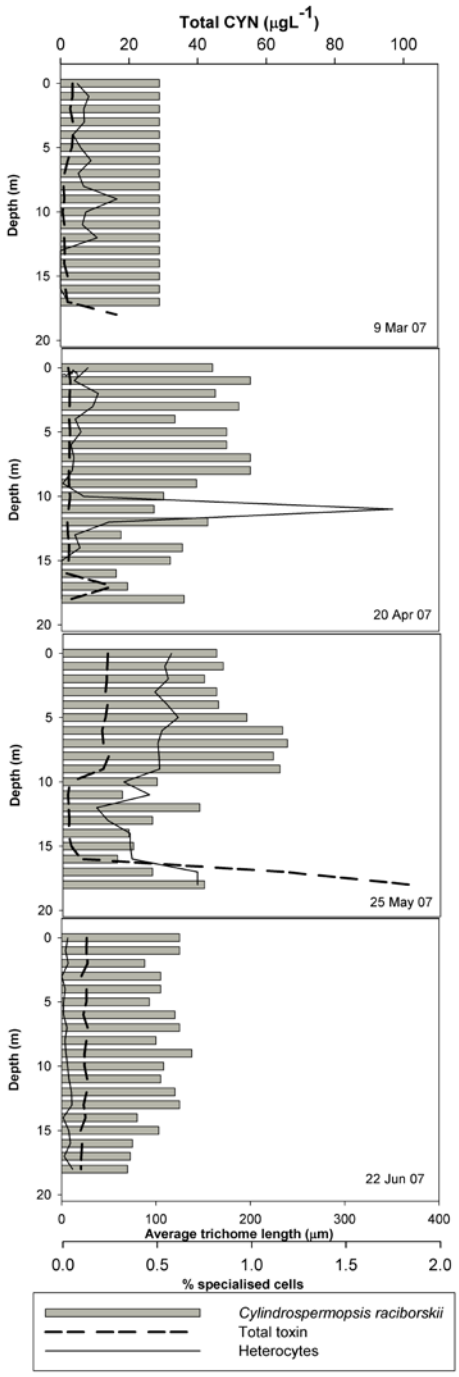

Fig. 4. Depth profiles showing the average trichome length (µm), percentage of specialized cells (heterocytes) and total cylindrospermopsins concentration (µg L^{-1}) present in a bloom of *Cylindrospermopsis raciborskii* in Cobaki Lake between 9th March 2007 and 22nd June 2007.

4.4 Physical and Chemical Parameters

Cobaki Lake was strongly stratified with three separate layers present. The epilimnion was defined by a thermocline (range during bloom between 7-10 m) and an oxycline (range during bloom 5-10 m). The lower anoxic layers were further defined by a halocline (range during bloom between 15-17 m) (Fig. 5).

Isotherms indicated that Cobaki Lake experienced winter overturn in June. There was a spike of cold water at depth in May. The average euphotic depth for the six month bloom period was 5.6 m (Table 2).

The maximum surface water temperature in Cobaki Lake occurred in March, 2007 at 28.9˚C (Fig. 5) and this period corresponded with the highest concentration of *A. ovalisporum* akinetes. In May 2007 the surface water temperature had decreased to 19.1˚C (hypolimnetic temperature was 16.6˚C), which coincided with maximum cell concentration of *C. raciborskii* as well as maximum concentration of total CYN at depth.

Physical and chemical time series plots indicating surface conditions over the 20 month sampling period are shown in Figure 8.6. These graphs clearly indicate seasonal comparisons between 2007 (low rainfall year) and 2008 (high rainfall year). A 30% increase in rainfall in 2008 did not affect the concentration of dissolved oxygen and the surface temperature but it did affect the pH which reached a maximum of 9.1. The conductivity decreased from 500 to 340 µS cm^{-1} with the increase in rainfall.

Table 2.

Secchi and euphotic depth from Cobaki Lake at selected dates between January and August 2007.

	Jan 5	Feb 10	Mar 9	Apr 20	May 25	Jun 22	Jul 20	Aug 17
Secchi depth (m)	1.2	2.5	2.0	3.0	1.0	0.8	1.7	5.0
Euphotic depth (m)	3.8	7.9	6.4	9.6	3.2	2.6	5.4	15.9

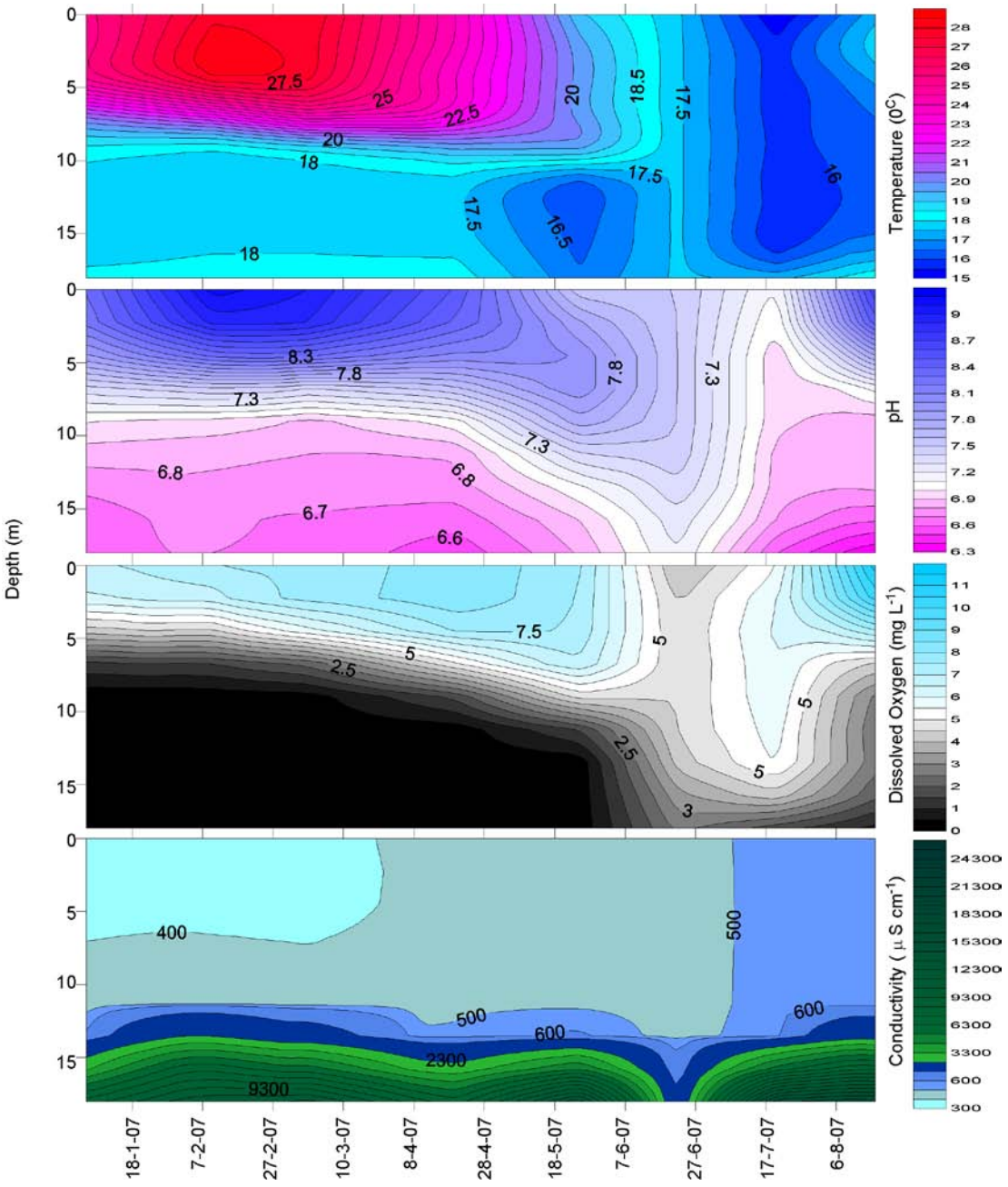

Fig. 5. Cobaki Lake time series depth profiles of physical-chemical conditions for eight months from 5th January 2007 to 17th August 2007 showing temperature (ºC), pH, dissolved oxygen (mg L^{-1}) and conductivity (µS cm^{-1}).

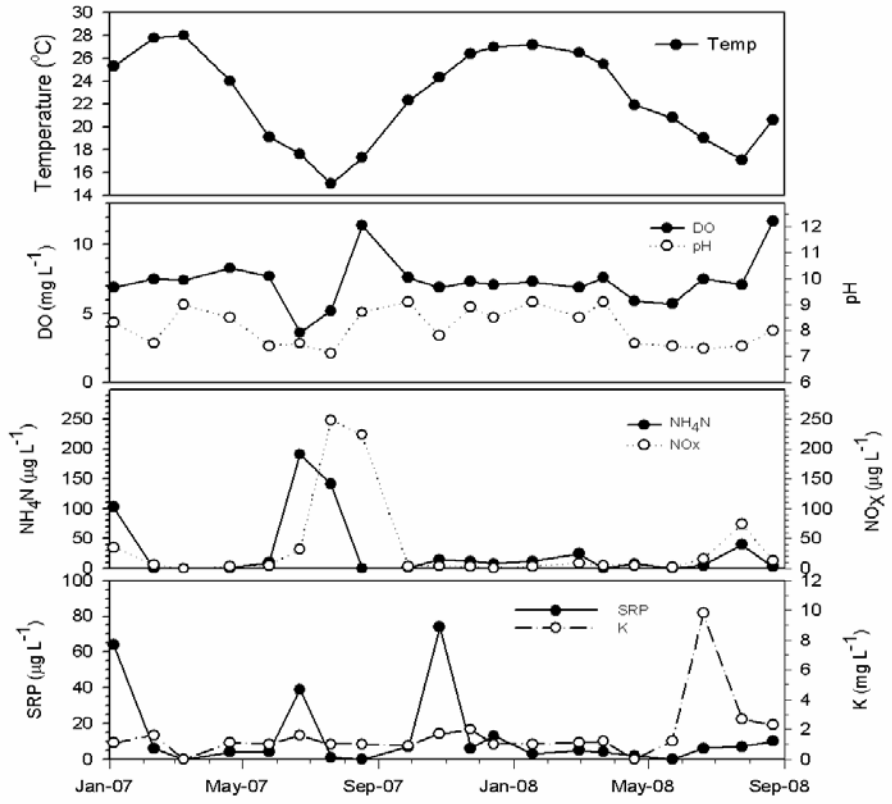

Fig. 6. Surface (0.5 m) water time lines of pH, dissolved oxygen (DO) mg L^{-1}, ammonium nitrogen (NH_4N) µg L^{-1}, oxidised nitrogen (NO_x) µg L^{-1}, soluble reactive phosphate (SRP) µg L^{-1} and potassium (K) mg L^{-1} from Cobaki Lake for 20 months from 5th January 2007 until 22nd August 2008.

4.5 Nutrients and metals

The concentration of bioavailable nutrients including ammonium nitrogen (NH_4N), oxidised nitrogen (NO_x) and soluble reactive phosphorus (SRP) fluctuated in the epilimnion throughout the sampling period (Fig. 7). These bioavailable nutrients decreased to below

detectable limits in March 2007, before gradually increasing in the lead up to winter overturn. In the hypolimnion, the average NH_4N concentration was 23.6 mg L^{-1} with a peak of 68.0 mg L^{-1} in May 2007. Similarly, the average SRP concentration at depth was 0.3 mg L^{-1} with peak concentrations of 0.9 mg L^{-1} occurring in April 2007 and 0.6 mg L^{-1} in May 2007. Flooding occurred in January 2008 and low concentrations of bioavailable nutrients were present in the lake's epilimnion until partial-mixing occurred in July 2008. Figure 6 shows the available nutrient concentrations in the surface water for the 20 month sampling period. With the influx of heavy rain early in the 2008 season, the available nutrients in the lake were diluted and were below detectable limits compared to January 2007 when a concentration of SRP of over 50 µg L^{-1} was detected.

The concentration of soluble trace metals also varied within the lake. In the epilimnion, molybdenum (Mo) and selenium (Se) were relatively unchanged throughout the sampling period with increased concentrations below the halocline (Fig. 8). Soluble iron was present in low concentrations in the surface water (average 5 µg L^{-1}) but increased in concentration through the depth profile with a maximum of 10,900 µg L^{-1} below the halocline (Fig. 9). Soluble potassium (K) concentrations in the epilimnion were relatively unchanged throughout the sampling period (1.5 mg L^{-1}). In March soluble potassium was not detectable in the surface water but was present at depth. Soluble potassium was concentrated below the halocline reaching a maximum concentration of 109 mg L^{-1} (Fig. 8). Like the nutrients, soluble trace metals generally were in higher concentrations in the hypolimnion. Some of these include soluble manganese, iron and calcium (Figs. 8 and 9). For example, the average soluble calcium concentration in the surface water was 13.3 mg L^{-1} whereas in the hypolimnion, the average was 159 mg L^{-1} with a peak of 697 mg L^{-1} occurring in May 2007. Fig. 10 shows the seasonal comparisons with soluble trace metal concentrations between 2007 (low rainfall year) and 2008 (high rainfall year). As recorded with the nutrients, the influx of heavy rain early in 2008 was accompanied by a dilution of the trace metals compared with 2007.

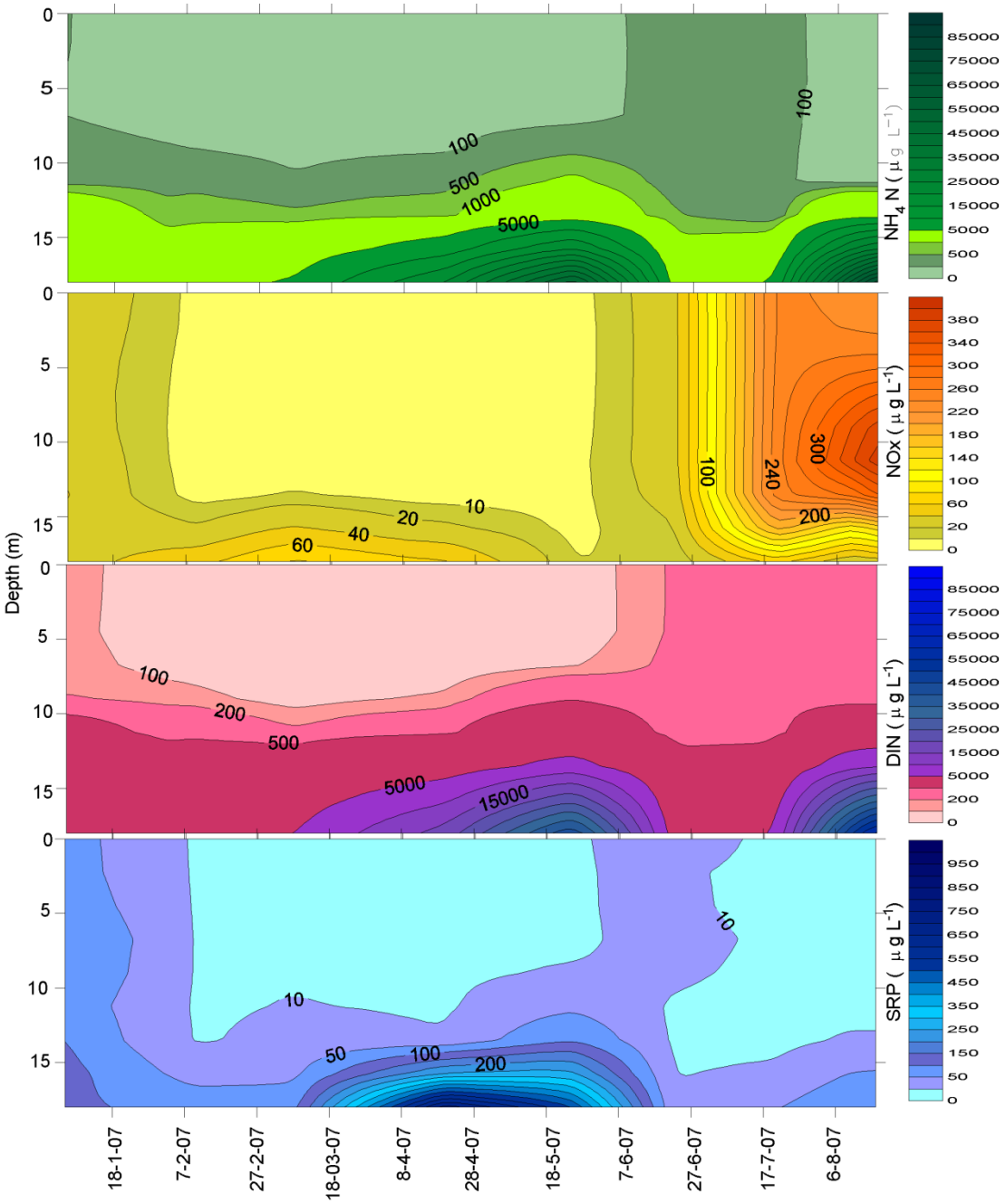

Fig. 7. Cobaki Lake time series depth profiles for nutrient concentrations (µg L^{-1}) for eight months from 5 Jan 2007 to 17 Aug 2007 showing ammonium (NH_4N), oxidized nitrogen (NO_x), dissolved inorganic nitrogen (DIN), and soluble reactive phosphorus (SRP).

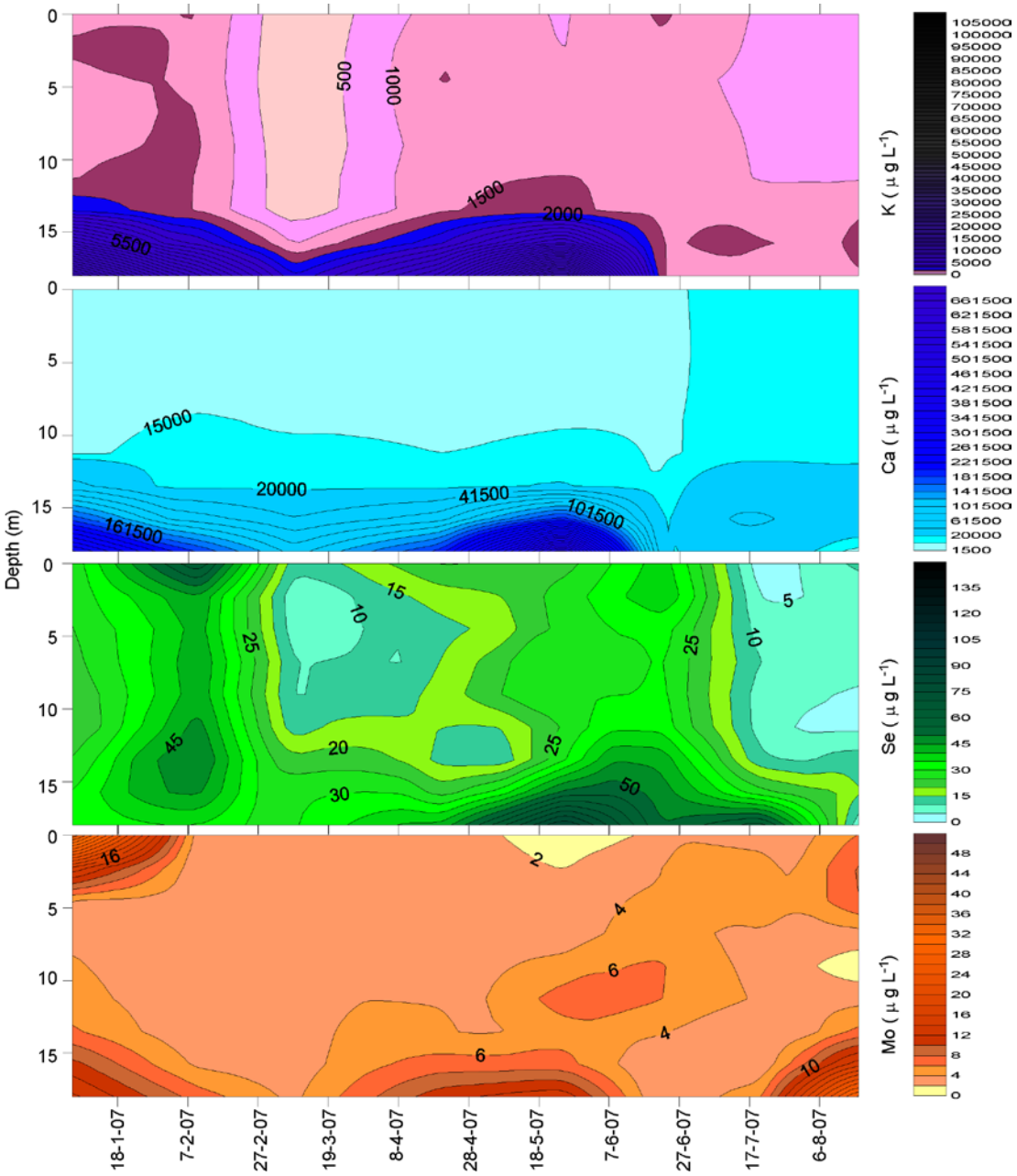

Fig. 8. Cobaki Lake time series depth profiles for trace element concentrations (µg L^{-1}) for eight months from 5 Jan 2007 to 17 Aug 2007 showing potassium (K), calcium (Ca), selenium (Se), and molybdenum (Mo).

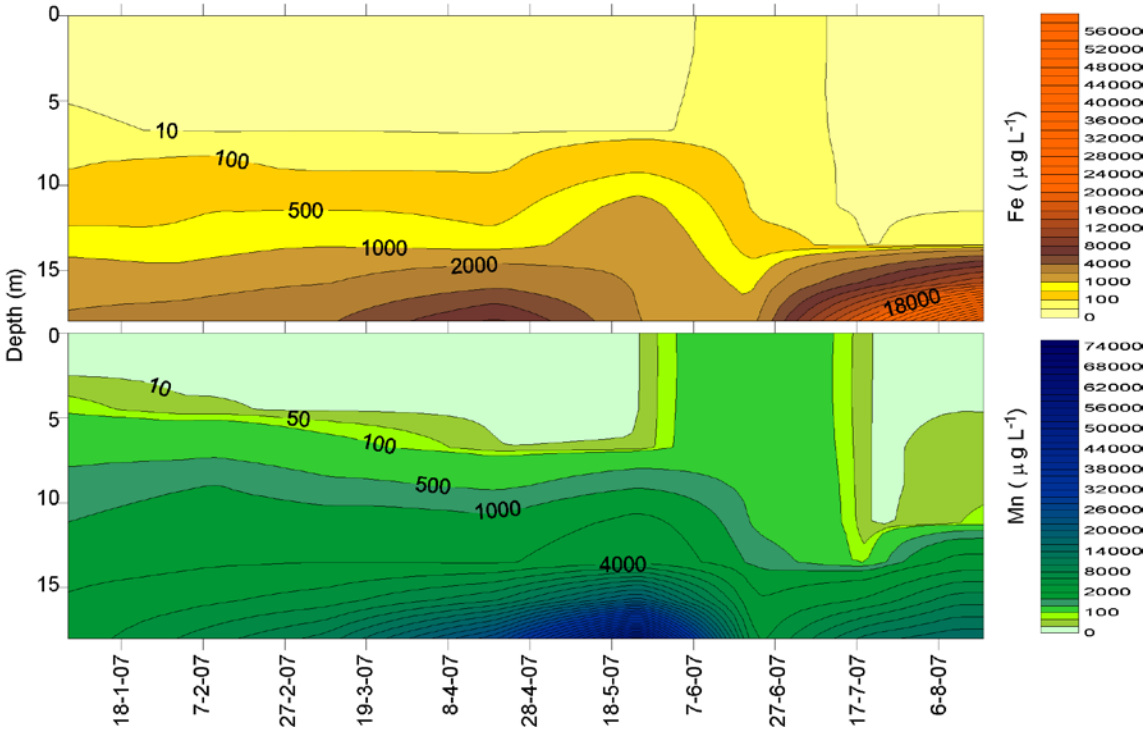

Fig. 9. Cobaki Lake time series depth profiles for trace metal concentrations (µg L^{-1}) for eight months from 5 Jan 2007 to 17 Aug 2007 showing iron (Fe), and manganese (Mn).

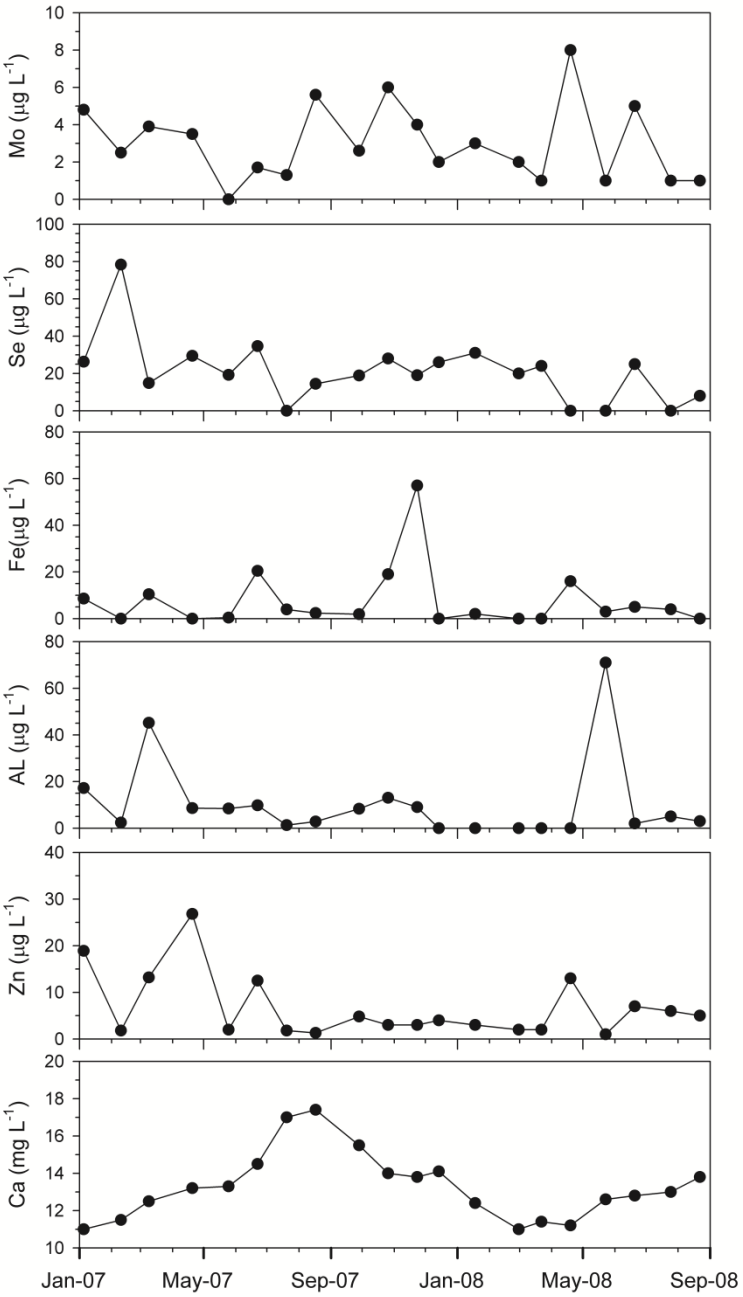

Fig. 10. Surface water (0.5m) trace metal concentrations including molybdenum (Mo) µg L^{-1}, selenium (Se) µg L^{-1}, iron (Fe) µg L^{-1}, aluminium (Al) µg L^{-1}, zinc (Zn) µg L^{-1} and calcium (Ca) mg L^{-1} in Cobaki Lake between 5 Jan 2007 and 22 Aug 2008.

4.6 Correlations between total CYN concentrations and other variables

Statistical analysis of correlations between total CYN concentrations and individual variables including conductivity, soluble iron, biovailable nutrients, cell concentrations, morphological characteristics and specialized cells shows positive correlations between conductivity, soluble iron, bioavailable nutrient species and heterocyte densities (Table 3).

Table 3.

Correlation statistics between total-CYN concentrations and variables including nutrients, soluble iron, specialized cells, filament length, cell count and conductivity in Cobaki Lake between January and June 2007. Correlations that are significant (i.e., p value < 0.05; r value > 0.000) are indicated in bold and shaded squares.

Correlation with Total cylindrospermopsins (µg L^{-1})	Jan 5-1-07	Feb 10-2-07	Mar 9-3-07	Apr 20-4-07	May 25-5-07	Jun 22-6-07
Conductivity (EC) (µS cm^{-1})	**r=0.874 p=0.000**	**r=0.928 p=0.000**	**r=0.623 p=0.004**	r=0.466 p=0.044	**r=0.973 p=0.000**	**r=-0.717 p=0.001**
Soluble Iron (Fe) (µg L^{-1})	**r=0.885 p=0.000**	**r=0.941 p=0.000**	**r=0.779 p=0.000**	r=0.210 p=0.388	**r=0.472 p=0.041**	**r=-0.709 p=0.001**
Ammonium (NH_4N) (µg L^{-1})	r=0.433 p=0.064	**r=0.880 p=0.000**	**r=0.878 p=0.000**	r=0.449 p=0.054	**r=0.966 p=0.000**	**r=-0.711 p=0.001**
Oxidised nitrogen (NOx) (µg L^{-1})	r=-0.126 p=0.607	r=0.540 p=0.017	**r=0.809 p=0.000**	r=0.011 p=0.963	**r=0.600 p=0.007**	**r=0.717 p=0.001**
Dissolved inorganic nitrogen (DIN) (µg L^{-1})	r=0.434 p=0.063	**r=0.883 p=0.000**	**r=0.877 p=0.000**	r=0.448 p=0.054	**r=0.966 p=0.000**	**r=-0.711 p=0.001**
Soluble reactive phosphorus (SRP) (µg L^{-1})	r=0.246 p=0.311	**r=0.775 p=0.000**	**r=0.818 p=0.000**	r=0.454 p=0.051	**r=0.877 p=0.000**	r=-0.033 p=0.893
Aphanizomenon ovalisporum cells (cells mL^{-1})	r=-0.403 p=0.087	r=-0.307 p=0.201	r=0.144 p=0.556	Not detected	Not detected	Not detected
Cylindrospermopsis raciborskii cells (cells mL^{-1})	r=-0.386 p=0.103	r=-0.385 p=0.104	r=-0.170 p=0.487	r=-0.192 p=0.431	r=-0.184 p=0.450	**r=0.500 p=0.029**
Aphanizomenon ovalisporum filament length (µm)	r=-0.344 p=0.150	r=-0.255 p=0.292	r=-0.236 p=0.331	Not detected	Not detected	Not detected
Cylindrospermopsis raciborskii filament length (µm)	**r=-0.820 p=0.000**	r=-0.221 p=0.364	r=0.050 p=0.840	r=-0.300 p=0.212	r=-0.250 p=0.302	**r=0.722 p=0.000**
Aphanizomenon ovalisporum heterocyte density	**r=0.563 p=0.012**	**r=0.626 p=0.004**	r=-0.285 p=0.236	Not detected	Not detected	Not detected
Cylindrospermopsis raciborskii heterocyte density	Not detected	Not detected	r=-0.286 p=0.235	r=-0.158 p=0.517	**r=0.685 p=0.001**	r=-0.081 p=0.741
Aphanizomenon ovalisporum akinete density	r=-0.231 p=0.342	r=0.277 p=0.250	r=0.251 p=0.300	Not detected	Not detected	Not detected

5. Discussion

5.1 Stratification

Cobaki Lake was strongly stratified with peaks in nutrients, trace metals, heterocytes, akinetes and toxin occurring in the anoxic hypolimnion, predominantly below the halocline. This highly anoxic region with elevated salinity and soluble metals including iron could be contributing to the increased concentration of toxin found below the halocline. Iron (soluble) is known to stimulate toxin production in *Microcystis aeruginosa* (Utkilen and Gjolme 1995). Total CYN concentrations in this study are highly correlated with conductivity and soluble iron and hence the conditions in the hypolimnion. A cool temperature spike in May and increase in soluble selenium concentration may indicate an intrusion or seepage from the estuarine Cobaki Creek that runs alongside Cobaki Lake increasing the salinity.

5.2 Akinete formation

Akinete development was quite different between the successive species in this study. Akinete development in *A. ovalisporum* was recorded under conditions of particularly low concentrations of soluble potassium and soluble iron in the surface water, combined with a decrease in water temperatures and bioavailable nutrients. This same water chemistry did not cause *C. raciborskii* to decline and produce akinetes; rather it dominated and grew successfully. This supports the findings of Baker (1999) which suggested akinete production in the field is mediated by a number of inter-related factors that trigger species-specific responses. The sudden disappearance of detectable potassium from the water column in March 2007 coincided with maximum concentration of akinetes in *A. ovalisporum*. A similar trend in water chemistry was repeated in April 2008; however, no cyanobacteria were detected at this time, so any interaction between akinetes and soluble potassium could not be confirmed.

The lack of formation of akinetes in *C. raciborskii* suggested that it might overwinter as vegetative cells rather than akinetes. This trend is consistent with strains from tropical Australia where it persists throughout the year as vegetative cells and rarely produces akinetes (Saker and Griffiths 2001). Kling (2009) also commented on the rarity of tropical *C. raciborskii* populations to form akinetes. Perhaps the water temperature was not low enough for akinete formation in Cobaki Lake or it could be possible that akinete formation may be strain specific.

In tropical and subtropical waters, another method of reproduction has been observed by Alster *et al.* (2010) and Komárek and Hauer (2010). This is the trend for reproduction by fragmentation. Actual fragmentation of the trichomes was not observed in Cobaki Lake; however, as the trichome length decreased from 146 µm in May to 102 µm in

June without the appearance of detectable akinetes an alternative method of preservation (such as fragmentation) is hypothesized. Alternatively, Pollard and Young (2010) found that a cyanophage (a virus which infects cyanobacteria) belonging to the *siphoviridae* family of viruses can affect the abundance and distribution of *C. raciborskii*, the process leaving smaller, but viable, trichome fragments which are more likely to disperse. In Cobaki Lake, the shorter trichome length, extreme morphology and loss of cell contents noted in June may indicate viral activity. If viral attack was involved, then it may have contributed to the non-appearance of trichomes the following season.

Recent field observations in Lake Kinneret (Israel) (Alster *et al.* 2010) involving a non-toxic strain of *C. raciborskii* have revealed a more predictable cycle that was traditionally seen in temperate waters. Maximum cell concentration occurred with maximum temperature (29 - 30 ˚C) and as the temperature decreased below 20 ˚C, the trichomes produced akinetes before disappearing from the water column.

5.3. Heterocyte formation

Both species showed an increase in heterocyte concentration indicating they were fixing their own nitrogen. This trend occurred in the surface water at maximum cell concentrations in both species when the oxidized nitrogen concentration decreased to below 10 µg L^{-1}. This follows a trend previously described by Saker and Neilan (2001). Another trend occurred in Cobaki Lake, where both species produced their highest density per filament of heterocytes at depth below the halocline. In this layer the DIN concentrations were extremely high reaching a maximum concentration in May of 68,015 µg L^{-1} most of which was NH_4N (68,003 µg L^{-1}). It was at this depth and time that the total-CYN reached maximum concentration of 101.4 µg mL^{-1}.

It seems unusual that both species produced their highest densities of heterocytes in water that was so high in DIN.

Laboratory trials in Spain and France have indicated that calcium ions are involved in the early stages of heterocyte differentiation in *Anabaena* sp. strain PCC7120 (Torrecilla *et al.* 2004; Zhang *et al.* 2006). These trials indicated that calcium concentration is critical to heterocyte differentiation. Similar trends were indicated in this field study at Cobaki Lake with higher concentrations of soluble calcium found in the hypolimnion below the halocline.

Cyanobacteria are known for “luxury consumption” of nutrients prior to their use in cellular processes. *Microcystis* is known to store bioavailable nutrients during periods when they are readily available either in the epilimniom or hypolimnion (Oliver and Ganf 2000). It is not inconceivable that prior to a bloom, heterocytes are formed at a depth where necessary soluble nutrients and trace elements are present in excess.

5.4. Filament length

Filament length appears to be a contributing factor with heterocyte density and may contribute to the differences between the species in this area. Different species morphology allowed *A. ovalisporum* to produce as many heterocytes as required, irrespective of filament length at depth whereas *C. raciborskii* was always limited due to its ability to only ever produce two heterocytes per filament (one at each end).

5.5. Species succession

In this study at Cobaki Lake, *C. raciborskii* (traditionally a tropical species) was able to survive and dominate the water column until the lake turned in winter (June). Conversely, *A. ovalisporum* despite not categorized as a tropical species was not detected after the start of autumn (March) and was not able to compete with *C. raciborskii* as it bloomed. This study is consistent with earlier observations showing a worldwide trend for the tropical *C. raciborskii* to flourish in cooler climates as a result of climate change (Briand *et al.* 2004; Figueredo *et al.* 2007; Griffiths and Saker 2003; Padisák 1997; Wiedner *et al.* 2007). The dominance of the invasive *C. raciborskii* over other species including *A. ovalisporum* supports the findings from laboratory trials by Mehnert *et al.* (2010) regarding competitiveness between invasive and native species and temperature variation.

5.6. Seasonal variation

The summer of 2008 that followed this study was a high rainfall year which included a flood event in early January. A similar succession of cyanobacterial assemblages and dominance to that which occurred in the 2007 low rainfall year was not observed. Figure 6.6 depicts the major differences between the two years; showing that in 2007 there is evidence of intrusion of hypolimnetic water into the surface layer providing peaks in bioavailable NH_4N and SRP, whereas in 2008 there is not. This seiching mechanism, which releases dissolved inorganic nutrients from an anoxic hypolimnion to the surface layer at periods of dominance of cyanobacteria such as *Cylindrospermopsis*, has been described by Fabbro and Duivenvoorden (1996) and Bormans *et al.* (2005). It was not possible to compare the onset of vegetative growth in either *A. ovalisporum* (from akinetes) or *C. raciborskii* (overwintering as vegetative cells or a possible victim of a viral attack) at this time. Despite the lack of detectable nutrients, a non-toxin producing population of *Anabaena circinalis* dominated the lake through the summer of 2008, coinciding with the cooler flush of fresh water created by the increased rainfall and consequent flood event.

Soluble selenium (Se) concentrations could also be a contributing factor to the occurrence of the cyanobacterial species across the two different sampling seasons. The higher concentration of available Se in 2007 as opposed to 2008 is consistent with previous research (Abdel-Hamid and Skulberg 1995), in which the cyanobacteria they studied

preferred higher soluble Se levels. The green algae in Abdel-Hamid and Skulberg's study showed stimulated growth at low concentrations of soluble Se.

5.7. Toxin production

The capacity of *C. raciborskii* to flourish in a variety of conditions raises health concerns, both for drinking and recreational supplies. The likelihood that highly toxic blooms will occur in cooler waters denotes a particularly concerning situation as *C. raciborskii* invades temperate environments. Whilst maximum cell concentration of *C. raciborskii* in previous research and published data in laboratory trials occurs at 28 – 30 ˚C; optimum CYN production by this species occurs at lower temperatures (20 ˚C) (Saker and Griffiths 2000). In Cobaki Lake, maximum *C. raciborskii* cell concentration occurred in May 2007 with 83,160 cells mL^{-1} occurring in the surface water, concurrently total-CYN in the hypolimnion reached maximum concentration of 101.4 µg mL^{-1}. The high toxin concentration was accompanied by high DIN and contradicts previous laboratory trials which showed that *C. raciborskii* cultured without fixed nitrogen generally produced more toxin (cell dry-weight basis) than cultures grown with a plentiful supply of nitrogen (Hawkins *et al.* 2001; Saker and Griffiths 2000; Saker *et al.* 1999). However, other variables that accompanied this high toxin concentration which occurred in the anoxic hypolimnion below the halocline were water temperature of 16.6 °C and high concentrations of nutrients, conductivity, soluble metals and trace elements, all of which may have indicated an increase in toxin production or a decrease in toxin degradation. Recent laboratory trials using German isolates by Klitzke *et al.* (2010) indicate that CYN retention in sandy sediments was negligible and that degredation took place mostly in the sediment and not in the water body. These authors also indicate that the availability of dissolved organic matter (DOM) to enhance bacterial growth results in more rapid CYN degredation. On the other hand, further laboratory research from Germany by Grützmacher *et al.* (2010) indicates biodegredation was the dominating process for Microcystin elimination in the sediment, however, under anoxic conditions (conditions generally disadvantageous for microbial activity) degredation rates were substantially lower. This latter research suggests that the high concentrations of cylindrospermopsins at depth recorded in Cobaki Lake could be partially due to lack of microbial activity in the anoxic, dark, semi-saline hypolimnion.

6. Conclusion

The study of Cobaki Lake through depth profiling at every metre provides valuable insight into the complex network of contributing factors that support or deter the growth of toxin producing cyanobacteria and the accumulation of cylindrospermopsins at depth in subtropical water bodies.

Management of water bodies should include consideration of the potential for groundwater intrusions and the characteristics of incoming water as these may impact on the subsequent concentrations of cylindrospermopsins. The intrusion of saline water leads to greater density differences between the layers of a water body and hence more stable stratification.

This is emphasized in this study by the significant correlations found between cylindrospermopsins, high conductivity and soluble iron concentrations in the hypolimnetic water in Cobaki Lake.

A clear delineation was present between the life cycles and the differentiation of specialized cells in the dominant species *A. ovalisporum* and *C. raciborskii*. This work confirms reports by Reynolds *et al*. (2000) that the conditions triggering akinete development are many and varied, with each season, water body and algal species likely to be unique.

This study reinforces the need for the use of water column depth profiles in the monitoring and management of blooms of cylindrospermopsin producing cyanobacteria, particularly those of *C. raciborskii* and supports some conclusions already stated in Everson (2009). The implications for water quality and health risks are quite serious. This bloom did not produce any discolouration or surface scum to indicate water quality deterioration and yet produced high concentrations of toxin at depth. Relying solely on visual inspection of water bodies and surface sampling could result in public health being compromised.

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9 PRACTICAL APPLICATION OF REAL-TIME PCR

9.1 Introduction

In ongoing management of public water facilities, accuracy and speed in delivery of results is critical. Rapid confirmation of toxicity is important when a potentially toxic species of cyanoprokaryote has been identified in a raw water supply. Real-time PCR can be used as a tool in the early warning detection of toxin-producing populations before they become problematic. The aim of this part of the study was to assess the practical application of real-time PCR in a water management situation by applying it to identify which strains of successive blooms of *A. ovalisporum* and *C. raciborskii* in Cobaki Lake in 2007 were capable of producing CYN. Both species were isolated in culture and their DNA analysed using real-time PCR. The traditional cell counts and toxin concentrations were compared with real-time PCR results.

To briefly recap; Schembri *et al*. (2001) demonstrated a potential correlation between the presence of the *pks* and *ps* genes and the ability of *C. raciborskii* to produce CYN. Fergusson and Saint (2003) screened 39 isolates representing three species known to potentially produce CYN: *C. raciborskii, A. ovalisporum* and *Anabaena bergii*. They found that if *pks/ps* genes were present, they produced toxin. Rasmussen *et al.* (2008) developed a quantitative real-time PCR method using TaqMan probes to detect the *rpoC1* and *pks* genes. In field samples, this assay showed good correlation with quantification of the species-specific *rpoC1* gene PCR assay for *C. raciborskii* and microscopy-based cell counts. Marbun *et al.* (2012) performed on-site testing of reservoir samples from Kinmen Island in Taiwan, being able to quantify *C. raciborskii* and detect cylindrospermopsin-producers within 2 h after sampling with a detection limit at about 1,000 cells mL^{-1}. They used the same genetic determinants, namely the *rpoC1* gene for *C. raciborskii* detection and *pks* for CYN producer detection used in the present study.

PCR methods have been used mainly for qualitative testing, identifying the presence or absence of particular species or toxin-producers (Monis *et al.* 2012). This was due to the inconsistencies between gene estimates and direct cell counts observed, especially with environmental samples (Rinta-Kanto *et al.* 2005; Kurmayer and Kutzenberger 2003; Vaitomaa *et al.* 2003). Monis *et al.* (2012) found that cultures of *A. circinalis, C. raciborskii* and *M. aeruginosa* always contained multiple copies of the genome, with the highest number of genomes per cell observed in late log phase of growth; assumedly, environmental populations are more likely to be comprised of cells at various stages in the growth cycle (Monis *et al*., 2012). Many researchers have found real-time PCR suitable for use on environmental samples with detection levels and technique developments ongoing.

In this study a duplex probe assay and a melt curve assay were used to determine whether laboratory-isolated strains of *A. ovalisporum* and *C. raciborskii* from Cobaki Lake have the capacity to produce CYN. A duplex probe assay performed by South Australia Water was also used on environmental depth profile samples to determine the presence of CYN as well as the CYN producer *C. raciborskii*. These environmental depth profile analyses were compared with both the traditional cell counts as well as toxin analysis for CYN by HPLC.

9.2 Results

9.2.1 Laboratory isolates

The samples used in this assay were collected from Cobaki Lake and isolated by QFHSS into single strain cultures. Using the real-time PCR duplex probe assay targeting *pks* (the gene assay associated with cylindrospermopsin (CYN)) and *rpoC1* (the gene associated for *C. raciborskii*), results showed that isolates of both *C. raciborskii* and *A. ovalisporum* species were positive for the *pks* assay which suggests that both species were potential CYN

producers. As expected, *A. ovalisporum* was negative and *C. raciborskii* was positive for the *rpoC1* assay.

When melt curve analysis with only the primers for *pks* were used, *A. ovalisporum* isolates showed a single peak with a melting temperature (Tm) of 83.8ºC confirming the probe assay that both were likely to be CYN producers. Melt curve analysis is a potentially useful alternative for detecting *pks* (CYN) gene if probe based technology was considered uneconomical or not available bearing in mind that the relevant validation for environmental samples would have to be demonstrated.

9.2.2 Environmental depth profile samples

Environmental depth profile samples taken during the cyanoprokaryote bloom in 2007 were analysed using real-time PCR by South Australia Water. Correlation was assessed for the May depth profile because samples from this month showed *C. raciborskii* cell maxima and also total CYN concentration maxima. Correlations between depth (m), total CYN concentration (μg mL^{-1}), *C. raciborskii* cell count (cells mL^{-1} - CYL), *pks* (CYN gene assay gene copies) and *rpoC1* (*C. raciborskii* gene assay gene copies) were assessed (Table 9.1).

Table 9.1. Correlation data on environmental depth profile samples from Cobaki Lake in May 2007 analyzing *C. raciborskii* cell counts (Cyl; cells mL^{-1}), depth (m), CYN concentrations (µg mL^{-1}), *pks* gene copies and *rpoC1* gene copies.

		Depth	**CYN**	***C. raciborskii***	***pks***	***rpoC1***
Depth	Pearson Correlation	1	0.391	**-0.815****	**-0.854****	**-0.743****
	Sig. (2-tailed)		0.108	**0.000**	**0.000**	**0.000**
CYN	Pearson Correlation	0.391	1	-0.229	0.292	-0.234
	Sig. (2-tailed)	0.108		0.360	0.240	0.351
C. raciborskii	Pearson Correlation	-0.815**	-0.229	1	**0.919****	**0.819****
	Sig. (2-tailed)	0.000	0.360		**0.000**	**0.000**
Pks	Pearson Correlation	-0.854**	-0.292	0.919**	1	**0.869****
	Sig. (2-tailed)	0.000	0.240	0.000		**0.000**
rpoC1	Pearson Correlation	-0.743**	-0.234	0.819**	0.869**	1
	Sig. (2-tailed)	0.000	0.351	0.000	0.000	
** Correlation is significant at the 0.01 level (2-tailed)						

Significant positive correlation occurred between the *C. raciborskii* cell counts with both the *C. raciborskii* gene copies (*rpoC1*) and CYN (*pks*) gene copies (Table 9.1). There was a significant negative correlation between depth and both *C. raciborskii* cell counts and *pks* gene copies, which suggests that viable cells were mostly at the surface. The highest concentration of CYN was in the hypolimnion although the *pks* assay did not detect any CYN genes below 10 m. The *C. raciborskii* cell count and *rpoC1* gene numbers below 10 m were both considerably reduced. There was no significant correlation between CYN concentration and depth comparison (Table 9.1) and the CYN concentration of 64.4 µg mL^{-1} at 16 m and 101.4 µg mL^{-1} at 17 m (Everson *et al.* 2011) was also not detected using the real-time PCR *pks* assay.

9.2.3 Environmental surface samples

Real-time PCR analyses using *pks* and *rpoC1* duplex assays were carried out on environmental surface samples from Cobaki Lake during a bloom involving both *A. ovalisporum* and *C. raciborskii* which occurred between January and June 2007. Correlation was assessed between *A. ovalisporum* and *C. raciborskii* cell counts, time (January to June 2007), *pks* and *rpoC1* Ct values and CYN concentration (Table 9.2).

There was a significant positive correlation between time and *rpoC1* Ct values and CYN and a positive correlation (not significant) between time and *C. raciborskii* cell counts. The stongest correlations were between *C. raciborskii* cell counts and *rpoC1* Ct values as well as between CYN and *rpoC1* Ct values. These correlations agree with the bloom dynamics where *A. ovalisporum* started the bloom and was succeeded by *C. raciborskii* which dominated the assemblage with the CYN concentrations followed the same trend, gradually increasing coinciding with *C. raciborskii* cell maxima.

Table 9.2. Correlation data on environmental surface samples from Cobaki Lake between January and June 2007. Correlations were analysed involving *A. ovalisporum* cell counts (Ao) and *C. raciborskii* cell counts (Cyl), time, *pks* Ct values, *rpoC1* Ct values and CYN concentrations.

		A. ovalisporum	***C. raciborskii***	***pks***	***rpoC1***	**Time**	**CYN**
A. ovalisporum	Pearson Correlation	1	-0.374	0.056	-0.589	-0.628	-0.525
	Sig. (2-tailed)		0.287	0.879	0.073	0.052	0.120
C. raciborskii	Pearson Correlation	-0.374	1	0.107	**0.768****	0.517	**0.673***
	Sig. (2-tailed)	0.287		0.769	0.010	0.126	0.033
pks	Pearson Correlation	0.056	0.107	1	0.250	-0.064	0.154
	Sig. (2-tailed)	0.879	0.769		0.486	0.860	0.671
rpoC1	Pearson Correlation	-0.589	0.768**	0.250	1	**0.723***	**0.824****
	Sig. (2-tailed)	0.073	0.010	0.486		0.018	0.003
Time	Pearson Correlation	-0.628	0.517	-0.064	0.723*	1	**0.681***
	Sig. (2-tailed)	0.052	0.126	0.860	0.018		0.030
CYN	Pearson Correlation	-0.525	0.673*	0.154	0.824**	0.681*	1
	Sig. (2-tailed)	0.120	0.033	0.671	0.003	0.030	

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed).

9.3 Discussion

Real-time PCR analyses using the *pks* probe, indicate that both the *A. ovalisporum* and *C. raciborskii* strains isolated from Cobaki Lake between January and June 2007 had the capacity to produce the cyanotoxin CYN. When combined with traditional cell counts and chemical CYN concentration analyses, this suggests that it is highly likely that *A. ovalisporum* and *C. raciborskii* were both producing CYN during this time in Cobaki Lake, although the population possibly included a mixture of toxin and non-toxin producing cells.

The correlation analysis has shown that the real-time PCR *pks* assay can be useful as an early indication of whether a particular water body contains toxin-producing strains/species. The lack of correlation between *pks* gene counts and CYN concentration at depth indicates that PCR is unable to detect extracellular CYN in water when the *C. raciborskii* cells were not detectable. PCR shows gene presence, it cannot show presence or absence of CYN itself, only the gene implicated in the production of the toxin while the DNA is viable, once the DNA is degraded, the PCR will no longer detect it. This is the situation that occurred in Cobaki Lake in 2007 when a high concentration of CYN occurred at depth (Everson et al. 2009). Chiswell *et al*. (1999) reported that in turbid and still water, CYN can persist for long periods. Anoxic conditions either inhibited CYN degradation in the sediments completely or retarded CYN breakdown in contrast to oxic conditions (Klitzke and Fastner 2012). These authors also found that a decrease in temperature slowed down degradation. Cobaki Lake was a strongly stratified water body involving a cool, anoxic hypolimnion which contributed to the lack of degredation of CYN at depth.

Monis et al. (2012) suggested that in the future direct gene counts may provide a rapid indication of bloom behaviour without the need for cell counts or other data; however more extensive DNA-based monitoring data is still required to indicate whether this is feasible. Those authors suggested that molecular analyses in the future will directly assist in the management of water facilities affected by troublesome blooms, especially when the bloom dies, necessitating the removal of toxins or taste and odour compounds released from the dead cells (Monis et al., 2012). These authors emphasised the use of DNA-based methods to predict bloom behaviour and duration. When this is combined with the results of the research from Cobaki Lake, it would seem reasonable to conclude that DNA methods are an excellent complement to the traditional methodology. This study has revealed that molecular assays are a cost saving addition to modern water management and provide a quick and useful identification tool for indicating the presence or absence of a specific toxin gene. This research has identified the capacity of PCR methodology to quickly and easily identify the

presence of potential toxin-producers so that they can then be targeted and managed. It also potentially reduces unnecessary chemical analyses of toxin gene negative samples and the managers of an affected water body could be notified within hours of sampling.

Nonetheless, the replacement of traditional microscopy by direct molecular assays is still in the future. At the time of this research, the PCR analyses did not take into account extracellular CYN at depth where the cells were not detectable but the CYN remained dissolved in the water. If most extracellular toxin is released on cell death, there would be no direct correlation between gene and toxin; there would be a lag, gene first then toxin in the water after the gene is no longer viable. It is important to note that PCR under these circumstances could be an excellent tool in predicting future toxin release and possibly future research could show a relationship that is measurable.

The research from Cobaki Lake has contributed to the data set regarding PCR and the capacity of particular species to produce CYN. The ability for PCR to show presence or absence of the *pks* and *rpoC1*genes is established and we have correlation between *pks* genes and CYN presence. The disconnect between PCR and toxin remaining in the water body is in itself intuitive, it needs to be noted that relying soley on PCR is only half the story. In the case of Cobaki Lake and other strongly stratified water bodies that are prone to CYN producing *C. raciborskii* blooms, PCR could be used test the bloom for *pks* and *rpoC1*gene presence and to predict possible toxin release on the collapse of the bloom.

10 SUMMARY and CONCLUSIONS

10.1 Research overview

This research adds to the existing dataset on freshwater Australian systems and, in particular, to coastal subtropical catchments. This project contributes to the understanding of the hydrological status associated with man-made structures (dams/reservoirs, weirs and artificial lakes) which will in turn contribute to the future development of water supply, treatment and storage in the Tweed Shire. In the broader context, it is worthwhile noting that coastal subtropical catchments are of particular conservation and management value, given the multitude of pressures they are now experiencing. For example, this includes drivers such as population growth, climate change (and its influence on ecology as well as on the availability of water resources), changed hydrological regimes (through man-made interventions), and the likelihood that future toxin management will demand even greater sophistication; for example, the ability to respond to cumulative impacts and toxin mixtures, together with zero tolerance involving improved limits of detection (Waterwatch 2002).

This study aimed to assist with the development of best-practice management approaches in response to the occurrence of potentially toxic and other troublesome species of cyanoprokaryoyes in the Tweed Shire raw water supply. For such management frameworks to be effective, it is critical to first develop an understanding of the relationships between phytoplankton assemblages and prevailing environmental conditions at each site, as well as the influence of seasonal variations. This was achieved by way of a detailed comparison of the Clarrie Hall Dam, the Tweed River at Bray Park and Cobaki Lake sampling locations, during 2007 and 2008. The existing management strategies used for these storages, such as reservoir monitoring and operations, can now be revisited and reassessed by the management team in relation to the research findings.

The study showed a statistically significantly increased presence of cyanoprokaryotes in the dry warmer year, compared with the wet, cooler months of 2008. Changes in the speciation and toxicity patterns within the phytoplankton populations were also highlighted. The appearance of non saxitoxin-producing *A. circinalis* in Clarrie Hall Dam in the wet year did not result in a bloom, despite triggering the NHMRC high alert level for drinking water with a concentration greater than 6,500 cells mL^{-1}. Increased rainfall resulted in reduced retention time with significant outflow of water and associated flushing of Clarrie Hall Dam. This flushing contributed to the lack of formation of cyanoprokaryote blooms in Clarrie Hall Dam. The detection of the *A. circinalis* (non saxitoxin-producing strain) in the riverine impoundment at Bray Park in 2007, at concentrations below the NHMRC alert levels, did cause some concern since this is the raw water uptake point for the treatment plant. Although this strain was not producing toxins, aesthetics, taste and odour issues became management concerns as the cell concentrations increased. The production of high CYN concentrations by a combined bloom of *C. raciborskii* and *A.ovalisporum* occurred in Cobaki Lake in the dry year of 2007. An increase in distribution of these toxin-producing blooms affects both the decisions and the cost of raw water treatment and recreational water management, especially in times of low rainfall in the Tweed Shire.

Weather patterns can be unpredictable; especially in sub-tropical regions such as the Tweed Shire where storm and rain events are common regardless of the season and result in flushing of the system. Conversely, in periods of dry, settled weather patterns involving low rainfall, long retention times can result. These extended retention times, combined with reduced nutrient loads and stratification, encourage the growth of troublesome nostocalean cyanoprokarytotes. Management of water bodies during dry, settled weather patterns would therefore aim at effective control of both retention time and stratification of the water bodies. The unique situation in the Tweed Shire allows the riverine impoundment at Bray Park to be flushed by releasing water from Clarrie Hall Dam. This ability to flush the water across the weir when required has proven to be reasonably successful in the past with regard to

minimising algal blooms and maintaining water source quality. Increased population trends in the district have, however, meant that water supply is precious and the decision to release water from Clarrie Hall Dam in times of low rainfall and dropping dam levels is difficult. The data collected at Cobaki Lake provides delineation of the toxin profiles and specialised cell dynamics of different species of cyanoprokaryote species capable of producing potent toxins. These species can appear when conditions are conducive to their growth and distribution.

Water management in the Tweed Shire should aim to prevent the development of similar conditions to those of Cobaki Lake in the main water supply areas of the catchment such as in the main storage facility (Clarrie Hall Dam) and the uptake point for water treatment (Tweed River at Bray Park). Similarily, prevention of similar conditions that have occurred in Cobaki Lake arising in decorative lakes in future development along the coastal fringe of the shire would be preferable.

One of the key pressures on the Tweed system is that of population growth and sustainable development. Existing demographic data for Australia show that coastal cities are consistently experiencing higher growth levels than the rest of the mainland (BITRE (2011)). The BITRE (2011) report states that the average annual growth for coastal cities was 2.3% over the period 2001–2009 whilst metropolitan areas were 1.6%. Understanding the dynamics of man-made decorative and recreational lakes such as Cobaki Lake will assist in the prevention of future public health issues, especially those involving high concentrations of stable dissolved cyanotoxins such as CYN and deoxy-CYN that are associated with marine or saline ground-water intrusions.

10.2 Summary of new knowledge and management implications

1. This research adds to the existing database on the water quality and phytoplankton assemblages of freshwater Australian systems including coastal, subtropical catchments. It involved monitoring of three different types of water bodies in the Tweed catchment (a storage dam, a riverine impoundment and a small decorative lake) over a two year period including both dry and wet seasons. This is the first substantial study of these water bodies in the Tweed Shire.
2. This study has contributed to the understanding of the hydrological status associated with man-made structures (dams/reservoirs, weirs and artificial lakes) and phytoplankton interactions involving toxin producing cyanoprokaryotes. The conditions in Cobaki Lake in 2007 were conducive to the growth of toxin-producing cyanoprokaryotes; and depth profiling of the toxin concentrations revealed the concentration of extracellular CYN at depth.
3. This research will contribute to the future management of raw water quality, storage and treatment in the Tweed shire. In particular, this research has explored the conditions that were conducive to the growth of toxin-producing cyanoprokaryote populations; future management strategies should be aimed at preventing or modifying the development of these conditions in water storages across the Tweed Shire.
4. The research findings at Cobaki Lake will assist in the design and management of future coastal development involving decorative and recreational lakes. The stratification that occurred in Cobaki Lake in 2007 involving a halocline with saline intrusion resulted in the growth of toxin-producing cyanoprokaryotes. These conditions prevented this lake from being used for the purposes that it was intended, such as for recreation and providing a source of extra water for vegetable gardens and external household use.

5. This study indicates the need to use cell concentration depth profiles as a key tool in monitoring and management of CYN-producing blooms, particularly those of *C. raciborskii*. Relying solely on visual inspection of water bodies and surface sampling could result in public health being compromised. It is likely that this situation is true for other sub-tropical sites.

6. This research demonstrates the need to understand the toxin profile before determining the off-take depth where *C. raciborskii* and *A. ovalsiporum* cell concentrations are concerned. Furthermore, a large amount of CYN may be extracellular and dissolved in the water column. This is not reflected in the cell count and for this reason, total toxin determination, including both intracellular and extracellular concentrations should be considered for health risk assessment.

7. This research has identified the ability of real-time PCR to quickly and easily identify potential toxin-producers so that they can be targeted and managed, while being mindful of the issues raised in this study regarding extracellular CYN at depth.

8. This study has revealed the presence of *Limnothrix* in the Tweed Shire, a species capable of producing a novel and dangerous water-soluble toxin ("Limnothrixin").

10.3 Future Work

This research also illustrated other areas of study that have yet to be addressed. Key amongst these are indicated below;

1. This study has revealed that a gap in knowledge exists with regard to why the toxins, CYN and deoxy-CYN are able to accumulate at depth away from the cyanoprokaryote cells which produced those toxins. If these dynamics can be understood the ability to manage and reduce these issues could be devised.

2. Associated with point 1 is the future collection and analysis of the sediment and hypolimnetic water which, coupled with corresponding physical data, could supply further information on how and why CYN and deoxy-CYN accumulate at depth.

3. There is a need to better understand the dynamics of CYN and deoxy-CYN, especially in hypolimnetic water. To this end, laboratory experiments could be performed to identify decomposition rates/factors with a view to generating ways of reducing deoxy-CYN in field environments (in the same way that destratification reduces cell counts).

4. Consideration and examination of the potential for groundwater intrusions and the characteristics of incoming water are necessary, since these may impact on the subsequent concentrations of cylindrospermopsins. The intrusion of saline water leads to greater differences in density of the layers of a water body and hence more stable stratification. The effect these intrusions have on CYN and deoxy-CYN accumulation may also contribute to understanding this problem.

5. Associated with point 4 is the need to understand more about CYN concentrations in the marine environment and its affect on modern sustainable development in coastal cities.

6. This research has shown that PCR is an excellent methodology, however it is unable to detect extracellular toxin in water bodies. Future research could indicate how PCR is able to predict furture toxin release and more importantly, potential concentrations if they can show a relationship that is measurable.

7. There is a need for wider comparison studies for sub-tropical environments and cooler environments where CYN-producers are starting to appear. This will expand the knowledge and database and assist in understanding the CYN depth profile dynamics in a variety of situations world-wide.

8. The data collection in the Tweed Shire catchment could be expanded to include additional tests and a variety of seasons. This will again expand the knowledge and data base and provide a greater base for comparison.
9. *Limnothrix/Geitlerinema* cell concentrations and accompanying toxin should be studied in greater detail in the Tweed Shire as well as nationally.

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