**Division of Science and Engineering**
**Department of Environmental Biology**

# Freshwater Cyanoprokaryota Blooms in the Swan Coastal Plain Wetlands: Ecology, Taxonomy and Toxicology

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**Declaration**

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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Date: 16th January 2009

# Abstract

Relatively little published information on cyanoprokaryote (blue-green algal) blooms in the freshwater wetlands in Western Australia is available. There has been little research on the urban lakes and rivers, examining the relationship between environmental conditions and toxin-producing blooms. In this project the ecology, morphology and toxicity of cyanoprokaryota blooms in 27 metropolitan lakes and sumplands, as well as three major rivers, from 2000 to 2003, on the Swan Coastal Plain (SCP) in the southwest of Western Australia were investigated.

A total of 24 species were identified and described, of which nine species had not been previously documented in the area. This included the potentially toxic species *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum* and *Anabaena bergii* var. *limnetica*. An illustrated guide to the common bloom-forming species was generated using conventional taxonomic criteria.

*Microcystis flos-aquae* and *Microcystis aeruginosa* were the dominant bloom-forming cyanoprokaryotes, widespread in their distribution. *Anabaena circinalis, A. bergii* var. *limnetica* and *Anabaenopsis elenkinii* were the common filamentous species. *Anabaena circinalis* was common to certain freshwater sites, while A. *bergii* var. *limnetica* and *A. elenkinii* occurred in salinity ranging from fresh (< 1ppt) to hyposaline (3-10 ppt). Sites with similar species assemblages were identified using two-way indicator species analysis and clustering analysis. From this, a distinct distribution pattern emerged, which was defined by the main genera observed in the lakes – *Microcystis, Anabaena*, *Aphanizomenon* and *Anabaenopsis*.

The spatial and temporal distribution of the common bloom-forming cyanoprokaryote species was examined in conjunction with spring-summer physico-chemical data using principal component analysis. It was found that pH, water temperature and electrical conductivity/salinity accounted for variations among the lakes, with electrical conductivity the variable explaining the greatest variation. Lakes located on the coast, or further inland at the base of the Darling Scarp, were

more hyposaline to saline, and the remaining lakes were fresh. Although the SCP lakes form consanguineous groups based on geochemistry and hydrology, no similarities among them were found in terms of water quality.

The relationship between nitrogen (N) and phosphorus (P) concentrations (total and dissolved inorganic) and cyanoprokaryote community structure (N-fixing versus non-N-fixing species) was investigated in five selected lakes; Yangebup Lake, Bibra Lake, Blue Gum Lake, Tomato Lake and Emu Lake. The lakes ranged from mesotrophic to eutrophic and supported spring-summer blooms containing multiple species. Overall an inverse relationship between cyanoprokaryote abundance and total ambient nutrient concentrations at the time of the blooms was evident. No transition in dominance in the community was observed in Yangebup Lake, Emu Lake and Bibra Lake, as they were dominated by non-heterocytic species (*M. aeruginosa* and *M. flos-aquae*) throughout spring and summer. For Yangebup Lake and Bibra Lake, the abundance of non-heterocytic species decreased concomitantly with decreasing dissolved inorganic N. In contrast, heterocytic species (*A. circinalis*) dominated the spring community in Tomato Lake, and summer community in Blue Gum Lake, when N and P concentrations were at their highest.

The presence of microcystins in *Microcystis* dominated blooms was examined using high performance chromatography. A total of 32 natural bloom samples, representing 13 lakes, were analysed for microcystin variants; -LR, -RR and -YR. Twenty-eight samples proved to be toxic with the highest total microcystin concentration from 1645 to 8428.6 $\mu g\ L^{-1}$, the lowest concentrations were less than 10 $\mu g\ L^{-1}$ with some below the detection limit. *Microcystis aeruginosa* and *M. flos-aquae* were associated with these microcystin-containing blooms, although *M. flos-aquae* appear to be less toxic. The presence of *Nodularia spumigena* in the Lake Yangebup was associated with high concentrations of nodularin (1664 $\mu g\ L^{-1}$).

Employing enzyme-linked immunosorbent assay for microcystins and the brine shrimp (*Artemia*) bioassays allowed a greater number of lake samples to be analysed and provided a rapid assessment of toxicity. The three methods for cyanotoxin

detection verified Yangebup Lake, Herdsman Lake, Hyde Park, Jackadder Lake and Emu Lake as highly toxic sites. Low toxicity was demonstrated in samples from Lake Goollelal, Lake Joondalup, Lake Claremont, Blue Gum Lake and North Lake. These results provided the first evidence of cyanotoxin producing blooms in urban lakes of the SCP.

A comparative study on cyanoprokaryota blooms in Swan River estuary, upper Canning River and upper Serpentine River found that these sites, although hyposaline to saline, contained species that were common in the freshwater lakes. Sampling the river systems showed *M. aeruginosa*, *M. flos-aquae*, *A. circinalis*, *A. elenkinii* and *Planktothrix planctonica* to be cosmopolitan in distribution, present in SCP wetlands of varied salinity. Similarities between the upper Canning River and lakes in environmental conditions and species assemblage were demonstrated using multivariate analyses.

Toxin analysis of bloom samples from the Swan River and upper Canning River revealed microcystin concentrations were less than that of the surrounding lakes (1.05-124.16 $\mu g\ L^{-1}$). Similarly, nodularin concentrations were higher in Yangebup Lake than the upper Serpentine River. However, the dominance of *Anabaena* in Canning River samples, and the highly toxic result from the *Artemia* bioassay suggests microcystin is not the predominant cyanotoxin in this wetland.

This study has produced an overview of the distribution and morphology of cyanotoxin-producing cyanoprokaryotes in the SCP wetlands. The data presented provide the basis for further cyanoprokaryote research in Western Australia, in particular the molecular characterisation of bloom-forming toxic species.

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## Scope and structure of thesis

The ecology, taxonomy and toxicity of freshwater cyanoprokaryota blooms in the wetlands of the Swan Coastal Plain (SCP), Western Australia, have been investigated. The results of this research project are presented in this thesis to provide a clear picture of bloom ecology in both the rivers and freshwater lakes of the SCP, with individual chapters focusing on environmental conditions, taxonomy and toxicity. Each chapter is presented with an introduction, methods, results and discussion sections.

The thesis is divided into ten chapters and seven appendices. Chapter 1 provides an introduction to the study with relevant literature survey and defines the research objectives. The literature survey provides background information on the global and national occurrence of freshwater cyanoprokaryota blooms, associated toxin production and the impact on human health. In Chapter 2 the study lakes, featuring location, water quality and geochemical characteristics to assist with interpreting spatial distribution of blooms are described. In Chapter 3, the physico-chemical data from each study lake for the three years of fieldwork and multivariate statistical analysis to separate and define sites based on environmental conditions are presented. This information is then utilized in chapters 4 and 6 to explain the distribution of species.

Chapter 4 specifically concentrates on cyanoprokaryotic blooms in the urban lakes of the SCP, focusing on the spatial and temporal distribution of the major bloom-forming species. The community structure and water quality conditions associated with the distribution of the common species are examined. As a comparison to the lake systems, and to produce an overview of the Perth region, *Microcystis* and *Anabaena* blooms in the Swan-Canning Estuary is the subject of Chapter 5. The *Nodularia spumigena* blooms that occur in the upper Serpentine River and adjacent lakes have been included in this chapter.

The effects of nitrogen and phosphorus concentrations on the succession and abundance of nitrogen fixing and non-nitrogen fixing species of cyanoprokaryotes are treated in Chapter 6. The morphology and taxonomy of the cyanoprokaryota species with descriptions, light micrographs and distributional data are presented in Chapter 7.

Chapter 8 documents the occurrence and diversity of microcystins, both intracellular and extracellular, in bloom samples collected from both the lakes and rivers. The samples were analysed using High Performance Chromatography (HPLC). Chapter 9 then evaluates the use of immunoassays and invertebrate bioassays as an alternative to HPLC for screening bloom samples for cyanotoxins. Within this chapter, the presence of saxitoxins and nodularin, in addition to microcystins, are also dealt with. The thesis is concluded with a synthesis of the findings from previous chapters and recommendations for future research and management in Chapter 10.

Appendix A provides photographs of the urban lakes sampled in this research project, Appendix B presents the environmental data, and Appendix C presents the cell counts, collected from each lake. Appendix D gives the constituents of the BG-11 medium used in Chapter 7. Appendix E shows the morphological measurements that assisted with species identification, and Appendix F summarises the morphological features of the order Nostocales. Finally, Appendix G presents the conference presentations and referred journal publications generated from this thesis.

# Chapter 1: General Introduction

## 1.1 Background

Over the past decade an increased occurrence of toxic phytoplankton blooms throughout the world has invoked the need for a better understanding of the conditions supporting algal growth and bloom development (Bartram *et al.* 1999; Oliver and Ganf 2000; Falconer 2005; Cronberg and Annadotter 2006). Blooms cause fatal effects to biota through secondary metabolites (cyanotoxins) that either damage the liver and gastrointestinal tract or paralyse muscles resulting in respiratory failure. Therefore, understanding the ecology of the toxic species is essential for their control and assists with managing their occurrence in Australian waters (Steffensen *et al.* 1999).

Nuisance cyanoprokaryota blooms have occurred in the past in some of Australia's most important waterways (Huber 1980; Bowling and Baker 1996; Fabbro and Duivenvoorden 1996; Atkins *et al.* 2001). The first report of toxic algal blooms dates back to 1878 when stock deaths were linked to a *Nodularia spumigena* bloom in Lake Alexandrina, South Australia (Francis 1878 cited in Codd *et al*., 1994). However, despite the early evidence of toxic cyanoprokaryotes and the many advances in research in Australia, there is still much that remains unexplored about freshwater blooms in Western Australia.

The two most common toxic species in temperate Australia, *Anabaena circinalis* and *Microcystis aeruginosa*, have been reported in several wetlands of the Swan Coastal Plain (SCP) (Gordon *et al*. 1981; Davis *et al.* 1993; Hosja and Deeley 1994), but there has been very little published information about their distribution, morphology and toxicity (John and Kemp 2006). It is important to identify all toxic species that occur in Western Australia and the factors that promote their growth. The identification and quantification of cyanoprokaryota is essential for the development of an effective early warning system to combat potentially toxic blooms (Lawton *et al.* 1999). The outcomes of this study would improve identification of toxic species

in the southwest wetlands and reduce the need for costly toxin analysis, which is not currently available in Western Australia.

### *1.1.1 Cyanoprokaryota blooms*

Cyanoprokaryotes (cyanobacteria) are highly differentiated bacteria that are capable of producing dense water blooms in freshwater and estuarine environments. These blooms result from the redistribution and rapid accumulation of buoyant planktonic species (Fay 1983). They can contain one or two species, but are defined by the dominant phytoplankton type, such as *Microcystis* bloom (Oliver and Ganf 2000). In temperate regions, which include the SCP, cyanoprokaryota blooms commonly dominate the spring and summer phytoplankton (Schmidt and Rosich 1993; Vincent 1995; John and Kemp 2006). The factors favouring this bloom formation are calm conditions with minimal vertical mixing, reduced turbidity, warm water temperatures, high pH, and high ammonia and phosphorus concentrations (Schmidt and Rosich 1993; Bowling and Baker 1996; Atkins *et al.* 2001; Thompson *et al.* 2003; Rahman *et al.* 2005). However, when conditions are adverse, there are morphological features that enable cyanoprokaryotes to exploit their environment or undergo dormancy.

Species from the order *Nostocales* form akinetes and heterocytes in response to unfavourable environmental conditions. Akinetes are reproductive spores that reside in the sediment during winter conditions forming the inoculum for spring growth. Conditions resulting in akinete formation include the depletion of nutrients, reduced light intensity or water temperature, or the extreme conditions faced when a bloom 'scums' at the water surface. Heterocytes are nitrogen-fixing cells that form under nitrogen-depleted conditions providing a competitive advantage when nitrogen is limiting (Fay 1983). Colonial cyanoprokaryotes also overwinter on the benthos and can survive without light or oxygen for several years (Reynolds 2006). It is this sediment population that provides the inoculum (spore-bank) to support seasonal blooms and allow for the rapid onset of nuisance blooms (Huber 1984; Baker 1999).

Currently concerns relating to the bloom-forming species centre on the production of potentially lethal toxins, termed cyanotoxins. By producing cyanotoxins, as well as aesthetic problems (odours and taste), blooms are recognised as a serious threat to humans through their occurrence in recreational water bodies and drinking water supplies. In Australia, these toxic water blooms have been a long-standing problem due to the production of the cyanotoxins: microcystin (MC), nodularin (NOD), saxitoxins (STX) and cylindrospermopsin (Falconer 2001).

### *1.1.2 Cyanotoxins*

Cyanotoxins are synthesised via a number of different metabolic pathways yielding: cyclic peptides (MCs, NODs), alkaloids (anatoxin, STX and cylindrospermopsin) and lipopolysaccharides. These cyanotoxins are classified according to their effects on the organs or systems in higher animals, and are known as hepatotoxins, neurotoxins and endotoxins. Hepatotoxins, for example, target the liver causing haemorrhaging. Neurotoxins, in contrast, interfere with the functioning of the nervous system by blocking neuronal ion channels, anti-actylcholinesterase activity, or by preventing signal transmission across the neuromuscular junction (Carmichael 1994).

Hepatotoxins are water-soluble and are unable to penetrate directly into the lipid membrane of the cell, therefore they require membrane transporters. Entry into the hepatocytes occurs via the bile-acid transport system (Codd *et al.* 1999a). Subsequently structural damage to the liver, results in haemorrhaging, and can lead to fatal circulatory shock, or interfere with the normal functioning of the liver (Carmichael 1994). Toxicology studies using mice showed no change in the heart, kidney, adrenal glands, brain, stomach and small and large intestines, yet the liver showed hepatocyte disintegration, structural disruption and extensive haemorrhaging (Falconer *et al.* 1981). Trials where MCs from *M. aeruginosa* were administered to pigs showed only the liver exhibited injury, while no damage was observed in the lung or the kidney (Falconer *et al.* 1994). These results confirm that MC is a hepatotoxin.

Microcystins, the cyclic heptapeptide toxins, are the most commonly found and studied hepatotoxin produced by *Microcystis, Oscillatoria*, *Anabaena, Anabaenopsis* and *Nostoc* (Carmichael 1992; Sivonen and Jones 1999). In Australia MCs are produced mainly by species of *Microcystis*. Hepatotoxity has been associated with Australian populations of *Anabaena*, however the presence of MCs in this genus is yet to be confirmed (Steffensen *et al.* 1999). The first MC was isolated from *M. aeruginosa* strain NRC-1 by Bishop in 1959. By the 1980s MC was fully characterised and given the name microcystin (Carmichael 1992). Microcystins produced by *M. aeruginosa*, are the most common cyanotoxin found in drinking water. Nodularins are cyclic pentapeptides, with six known variants (Codd *et al.* 1999a). These toxins are produced by *Nodularia spumigena*, and because of its high tolerance to salinity, NOD is a problem in hyposaline and marine environments. Both MC and NOD are enzyme inhibitors, impacting on the structure of cytoskeleton filaments (MacKintosh *et al.* 1990; Camichael 1994).

In recent years research has focused on the occurrence and toxicity of the newest hepatotoxin, cylindrospermopsin (CYN). Cylindrospermopsin is a distinct alkaloid hepatotoxin produced by *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum* (Hawkins *et al.* 1997; Shaw *et al.* 1999). The genes responsible for CYN production have also been identified in isolates of *Anabaena bergii* (Schembri *et al.* 2001). Cylindrospermopsin toxicity is not just restricted to the liver, but also to the kidney and gastrointestinal tissue (Falconer 2005). In addition, *Cylindrospermopsis raciborskii* can produce paralytic shellfish poisons similar to those from *A. circinalis* (Lagos *et al.* 1999).

Anatoxin-a, anatoxin-a(s) and STXs are known neurotoxins associated with cyanoprokaryota. Anatoxin-a has been isolated from strains of *Anabaena, Aphanizomenon*, *Cylindrospermum, Microcystis*, *Oscillatoria*, and *Phormidium* (Codd *et al.* 1999a; Sivonen and Jones 1999). Clinical trials using calves, rats, mice and ducks proved anatoxin-a can mimic the neurotransmitter acetylcholine, but it cannot be degraded by acetylcholinesterase, thereby causing persistent postsynaptic depolarising neuromuscular blockage (Carmichael *et al.* 1975). Overstimulated

muscles are soon fatigued, which can lead to paralysis. When respiratory muscles are affected, the victim suffers from convulsions and dies from suffocation (Carmichael *et al.* 1975). Anatoxin-a (s) is an acetylcholinesterase inhibitor and differs in toxicity to anatoxin-a by a defined symptom of hypersalivation (s = salivation) (Carmichael 1992).

Saxitoxin, also known as a paralytic shellfish poison, is the most widely known neurotoxin of this group due to the association with marine dinoflagellate blooms (red tides). This toxin acts as a sodium-channel blocking alkaloid, which inhibit nerve impulse transmission (Humpage *et al*. 1994). Large amounts can accumulate in the digestive tracts and soft tissue of shellfish, which is a risk when eaten. Saxitoxin has been identified in *Anabaena circinalis* (Humpage *et al*. 1994), *Aphanizomenon gracile* (Pereira *et al.* 2004), *Lyngbya wollei* (Onodera *et al.* 1997b), *Planktothrix* (Pomati *et al.* 2000) and *C. raciborskii* (Lagos *et al.* 1999).

Early research into the occurrence of neurotoxic blooms was not as advanced as studies into hepatotoxins (Codd *et al.* 1989) with Himberg (1989) reporting that it was rare for anatoxins to be studied. The limited number of reported studies have been confined to the distribution of anatoxin-a in Canada (Carmichael *et al.* 1975), Denmark (Onodera *et al*. 1997a), Finland (Sivonen *et al.* 1989), Germany (Bumke-Vogt *et al.* 1999), Ireland (James *et al.* 1997), Italy (Viaggiu *et al.* 2004), North Korea (Park *et al.* 1998b), Japan (Park *et al.* 1993) and Kenya (Krienitz *et al.* 2003). Only recently was anatoxin-a reported from New Zealand (Wood *et al.* 2007). Furthermore, in Australia, neurotoxicity has not yet been associated with anatoxin-a, with STX being the only neurotoxin identified in Australian populations of *Anabaena circinalis* (Humpage *et al*. 1994; Beltran and Neilan 2000). In contrast, hepatotoxins are still the most frequently found cyanotoxin worldwide in both fresh and hyposaline waters (Sivonen and Jones 1999; Metcalf and Codd 2004).

The final category of cyanotoxins is the liposaccharide endotoxins and exotoxins. These toxins are a component in the cell walls of cyanoprokaryota and they cause irritant and allergenic responses in humans and animals (Torokne *et al.* 2001).

Contact dermatitis has been associated with a range of cyanoprokaryote species including those of *Anabaena*, *Aphanizomenon*, *Gloeotrichia*, *Microcystis* and *Oscillatoria* (Codd *et al.* 1999a). Of these, *Aphanizomenon* has been shown to produce the most severe allergenic and irritative effect in a study using guinea pigs (Torokne *et al.* 2001).

### *1.1.3 Toxin production*

Cyanoprokaryotes can produce several toxins simultaneously with toxin production being strain specific rather than species specific (Sivonen 1996). For example, species of *Anabaena*, *Microcystis* and *Oscillatoria* can produce the same variant of MC-RR (Sivonen 1996). It is not possible to determine toxicity by the morphology of species alone, as not all strains of the same species produce toxins (Falconer 1989; Lahti *et al.* 1995). Sequencing DNA can provide information on the potential toxicity of a strain, something microscopic examination fails to do. For example, primers designed for *mcy* genes, which are responsible for the production of MC, can discriminate between toxic MC-producing strains and non-toxic strains (Pan *et al.* 2002; Hisbergues *et al.* 2002). The ability of a strain to produce toxins may depend primarily on the possession of such genes and their expression under certain environmental conditions. Toxic and non-toxic strains of *M. aeruginosa* have been shown to possess genes coding for peptide synthetase, however the non-toxic strains are most likely to involve the synthesis of non-toxic peptides (Meissner *et al.* 1996). Neilan *et al.* (1999) later found MC synthetase orthologues in toxic strains of *Microcystis*, as well as, MC producing strains of *Anabaena*, *Oscillatoria* and *Nostoc*. The non-toxic strains contained genes for peptide synthetase but of unknown function (Neilan *et al.* 1999). However, as DNA analysis advances, water research laboratories continue to use phenotypic traits to identify and quantify known toxin-producing cyanoprokaryotes as these resources are more widely available.

The toxin content of a bloom depends on the growth phase and the presence of non-toxic or less-toxic strains within the population (Watanabe and Oishi 1985). Cyanotoxins are usually cell bound (intracellular) and their concentration increases during the logarithmic growth phase, when conditions for growth are favourable

(Sivonen 1996). The concentration of dissolved toxin (extracellular) depends on the age of the bloom and rate of decline. When blooms are young, a large percent of toxin is cell bound, but with ageing 70 to 98 % of the total toxin concentration can be dissolved into the water column (Chiswell *et al.* 1999). For *Microcystis*, however, MCs are entirely intracellular and only released into the water column, when the cells die and the bloom decomposes (Welker *et al.* 2001). As a result, the highest concentration of extracellular toxin usually occurs in areas where scum accumulates. Once released into the water column, the MCs can persist for a few days or a few weeks (Welker *et al.* 2001). Therefore health risks associated with cyanoprokaryota blooms are of great consequence, especially once the bloom has visually disappeared.

### *1.1.4 Health risks associated with toxic cyanoprokaryota blooms*

Toxic cyanoprokaryota blooms are harmful to humans with most cases of poisoning occurring as a result of recreational activities or consumption of contaminated shellfish. By far, the biggest threat is when toxic species appear in drinking water. Cyanotoxins can enter the body via oral consumption, through inhalation or by skin absorption (Falconer 2001). The poisoning of humans due to the consumption of cyanotoxins has been reported throughout the world, including Australia. However there are a low number of reported cases mainly due to the poisoning symptoms, for example diarrhoea and vomiting, being similar to the symptoms of gastroenteritis or food poisoning. For example, the Palm Island Mystery disease caused by CYN poisoning was initially attributed to the consumption of unripened mangoes (Griffiths and Saker 2003).

It has been shown that MC is toxic to human hepatocytes (Batista *et al.* 2003). Poisoning is also associated with gastroenteritis and inflammation of the digestive tract. In 1988, a severe gastroenteritis epidemic in Brazil resulted in 88 deaths when a water supply containing *Anabaena* and *Microcystis* was used for drinking water (Kuiper-Goodman *et al.* 1999). Later in 1996, up to 131 patients in a dialysis unit in Caruaru, Brazil, developed toxic illness from the use of cyanoprokaryote infested water (Pouria *et al.* 1998, Carmichael *et al.* 2001). Of these, 76 patients died, with 52

cases clearly attributed to cyanotoxin poisoning (Carmichael *et al.* 2001). The patients experienced visual disturbances, nausea, vomiting, muscle weakness and painful liver enlargement. There is also evidence that an increase in liver cancer in specific populations of China were associated with contaminated drinking water. Ueno *et al.* (1996a) demonstrated a correlation between the high incidence of primary liver cancer in Haemin City and the use of pond or river water, containing multiple species capable of producing MC, as a drinking source.

A disease found in outback Australia, known as the "Barcoo spews, Barcoo fever, Barcoo sickness" is characterised by nausea and vomiting (Hayman 1992). Named after the Barcoo River in central Queensland, this illness is the result of *C. raciborskii* poisoning (Hayman 1992). *Cylindrospermopsis raciborskii* is also responsible for the "Palm Island mystery disease", one of Australia's most serious cyanotoxin poisoning cases. In 1979 on Palm Island, Queensland, over 100 people were stricken with hepatoenteritis after consuming water-containing CYN. Typical symptoms were malaise, anorexia, vomiting, dehydration, an enlarged liver followed by kidney malfunction and diarrhoea (Byth 1980; Hawkins *et al.* 1985). In another example, from as early as the 1970s heavy blooms of *M. aeruginosa* were reported in the drinking water supply for the city of Armidale, New South Wales. Examination of the liver function of the local residents, who had consumed water from this reservoir, revealed liver damage similar to alcohol poisoning (Falconer *et al.* 1983). In both of these Australian cases, ill health coincided with the breakdown of blooms by natural processes (Palm Island) or the application of an algicide (Armidale), which further demonstrates the concern for dissolved toxins even after a bloom has dispersed.

During recreational use of a contaminated water-body, poisoning can result from dermal exposure, accidental ingestion and aspiration of cell material. A case review by Stewart *et al.* (2006) found symptoms of contact exposure ranged from mild skin irritations to severe deep blistering, while more serious conditions such as severe headaches, pneumonia, fever, myalgia and gastro-intestinal illness developed from water ingestion. An earlier report described two army recruits requiring

hospitalisation and intensive care after consuming water containing toxic *M. aeruginosa* whilst swimming (Turner *et al.* 1990). In 2003 the first human fatality from accidental ingestion during recreational exposure was reported in the United States when a male teenager died from heart failure two days after swallowing golf course pond water that contained the neurotoxic *A. flos-aquae* (Behm 2003). Algal cells and anatoxin-a were found in his blood and faecal samples (Behm 2003). Newspaper articles provide the only published material describing this event with a comprehensive case report in a scientific or medical journal yet to be presented (Stewart *et al*. 2006).

Dermal toxicity is associated with the lipopolysaccharide component of the cell wall, which can cause allergic reactions such as mouth ulcers, eye and ear irritations, skin rashes and fever (Pilotto *et al.* 1997). Through a series of interviews involving 852 participants that visited water recreation sites in eastern Australia, Pilotto *et al.* (1997) found the incidence of allergic symptoms was associated with the duration of water contact and with cyanoprokaryote cell density. An increase in symptoms occurred concomitantly with an increase in the duration of contact with some participants also experiencing gastro-intestinal symptoms such as vomiting and diarrhoea. Based upon this data, the guideline for safe recreational water body is 20 000 cyanoprokaryote cells per millilitre, which is equivalent to 2 to 4 µg MC per litre if MC-producing cyanoprokaryote are dominant (WHO 2003). Cell densities greater than this have been recorded in the SCP wetlands (Gordon *et al.* 1981; Vincent 1995; Thompson *et al.* 2003).

The cases mentioned above, were all related to the direct consumption of contaminated water. In addition, there is secondary exposure when cyanotoxins are transported through the food chain. For example, Falconer *et al.* (1992) found the edible mussel (*Mytilus edulis*) growing in water containing a *N. spumigena* bloom accumulated toxins in the gut and in the gut contents. In fish, MCs accumulate in the liver, viscera and muscle tissue and can be in concentrations above the recommended limit for human consumption. In crayfish, the main areas MCs accumulate are in the intestine and hepatopancreas. This poses a threat, if these parts are eaten.

Furthermore, the commonly used salad lettuce has been shown to retain MCs after being sprayed with irrigation water that contains *M. aeruginosa* (Codd *et al.* 1999b). This raises questions on the transfer of toxins through the food chain.

Intoxication has also been shown to be fatal to animals that come in contact with bloom material. Australian reports have included *C. raciborskii* causing necrosis of the liver and epicardial hemorrhaging in the heart of cattle in Queensland (Saker *et al.* 1999b), and *A. circinalis* causing paralytic shellfish poisoning and death of sheep in central NSW (Negri *et al.* 1995). Recently, lesser flamingo deaths in Kenyan and Tanzanian lakes have been the result of cyanotoxin poisoning (Ndetei and Muhandiki 2005; Lugomela *et al.* 2006). In Lake Big Momela, Kenya, birds were dying at a rate of between 15 and 50 individuals per day due to toxins from *Arthrospira fusiformis* (Lugomela *et al.* 2006). Other reports detail the deaths of birds, domestic dogs and fish in Danish water-bodies in connection with scums of *Anabaena* (Henriksen and Moestrup 1997; Onodera *et al.* 1997a). Likewise, there have also been reports of rapid neurotoxicosis of domestic dogs after swimming in scum accumulation along shorelines in Scotland (Codd *et al.* 1995).

Surface scums that accumulate along the shoreline pose a greater health threat as they potentially contain the highest concentrations of cyanotoxins (Falconer *et al.* 1999; Welker *et al.* 2001). In the city of Perth, Western Australia, this raises concern regarding the use of the metropolitan wetlands for recreation. In temperate climates, cyanoprokaryota blooms tend to dominate water resources during the summer months, when the demand for recreational water is highest increasing the risk of exposure (Falconer *et al.* 1999). It is important to monitor the Perth wetlands because the public uses them for both primary (swimming, diving and water skiing) and secondary (boating and fishing) forms of recreation. As of yet, there are no documented cases of poisoning associated with cyanotoxins in Western Australia. However, a *Microcystis aeruginosa* bloom in North Lake during spring 1985 was reported to be associated with a number of bird deaths (Bayley *et al.* 1989).

### *1.1.5 Occurrence of toxic cyanoprokaryota blooms*

Toxic cyanoprokaryota blooms have been identified in freshwater lakes, ponds, reservoirs, rivers and estuaries in Europe, North America, South America, New Zealand, Africa and Asia. However, the first scientific account of a toxic cyanoprokaryote bloom originated from Australia. Since the 1878 report in Lake Alexandrina, cyanoprokaryota blooms have been reported in nearly all Australian states and territories, although the main species responsible differ between regions (May 1980). One of the most extensive blooms in Australia, in relation to area, was reported in New South Wales in 1991. Over 1000 kilometres of the Barwon-Darling River system contained a massive bloom of *Anabaena circinalis* that showed moderate to very high toxicity and was implicated in the death of an estimated 10 000 livestock (Bowling and Baker, 1996).

Worldwide, the main genera responsible for producing toxic blooms are numerous, and include *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Microcystis, Nodularia, Nostoc* and *Oscillatoria (Planktothrix)* (Sivonen and Jones 1999). So far there are only six species confirmed as toxic and causing problems in Australian waters; *Microcystis aeruginosa* (Falconer 2001), *Nodularia spumigena* (Jones *et al.* 1994), *Anabaena circinalis* (Humpage *et al.* 1994), *Cylindrospermopsis raciborskii* (Hawkins *et al.* 1997), *Aphanizomenon ovalisporum* (Shaw *et al.* 1999) and *Phormidium* (Baker *et al*. 2001). All except *C. raciborskii* are common in the temperate regions of Australia, including the wetlands of the SCP.

In Western Australia, cyanoprokaryota blooms are a regular occurrence, being reported in the Swan and Canning rivers, the Peel-Harvey estuary, Perth urban lakes and farm dams (Hosja and Deeley 1994). The major species implicated are *Anabaena circinalis, Microcystis aeruginosa* and *Nodularia spumigena* (Davis *et al.* 1993; Hosja and Deeley 1994; John and Kemp 2006). One of the earliest reports of a potentially toxic species in a Perth metropolitan lake dates back to the 1970s when Gordon *et al.* (1981) observed *M. aeruginosa* in three northern metropolitan lakes. *Anabaena* was also prominent in two of these lakes (Gordon *et al.* 1981). At the same time, both species were also causing nuisance odorous blooms in North Lake,

south of the Swan-Canning estuary (Bayley *et al.* 1989). Although these isolated reports date back 30 years, little is documented regarding the taxonomy, distribution pattern, environmental conditions associated with these cyanoprokaryota blooms and the potential threat due to cyanotoxins.

Only a limited number of toxicity tests have been completed on the cyanoprokaryota blooms in Western Australia. For this reason, knowledge of the presence of MC, NOD, STX or CYN in association with *Microcystis*, *Nodularia*, *Anabaena*, *Aphanizomenon* or *Cylindrospermopsis* is poor, yet all five genera are present in many of the SCP wetlands (John and Kemp 2006). Toxicity has been demonstrated by mouse bioassay in *Nodularia* from the Peel Inlet (Falconer *et al.* 1992), and *Anabaena* and *Microcystis* from the Canning River (Hosja and Deeley 1994). Therefore it is crucial that this knowledge is updated to reflect the current situation and extended to include the ecology, taxomomy and toxicology of blooms in the surrounding freshwater lakes.

At the commencement of this research project in the summer of 2000, the Swan-Canning estuary experienced a record bloom of *Microcystis*. Freshwater cyanoprokaryota blooms had been considered extremely rare in this estuary (John 1987). This was the first time a bloom of this magnitude had been recorded in Western Australia. The bloom extended from the Fremantle harbour upstream to the Helena River. At the peak of the bloom, toxin levels reported were 7000 times higher than that of safe standard drinking water and 700 times higher than safe recreational water as stated by the World Health Organisation (Pryer 2000). Due to health concerns related to MCs the estuary was closed for public and commercial use for 12 days. Cyanoprokaryota blooms in major recreational estuaries, such as this bloom in the Swan-Canning estuary, can result in the loss of millions of dollars lost due to the shortfall in tourist income and the expenses of remediation (Falconer 2001).

## 1.2 Research objectives

The primary aim of this research project was to systematically study the ecology, morphology, toxicity and geographical distribution of freshwater cyanoprokaryotes in the wetlands of the SCP. In total, 27 lakes in the Perth region, as well as three river systems were investigated to provide a comprehensive picture of cyanoprokaryota blooms in the southwest of Western Australia.

To date only a few studies have reported blooms in the urban lakes of this region. Research has mainly focused on toxic blooms in the Canning River and upper Swan River (John 1987; Vincent 1995; Atkins *et al.* 2001; Robson and Hamilton 2003; Thompson *et al*. 2003), even though blooms of *Microcystis* and *Anabaena* in the surrounding freshwater lakes have been deemed a problem (Gordon *et al.* 1981; Bayley *et al.* 1989; Schmidt and Rosich 1993; Hosja and Deeley 1994; John and Kemp 2006). Both genera contain species that produce toxic blooms throughout temperate Australia (Baker and Humpage 1994; Bowling 1994; Negri *et al*. 1995; Bowling and Baker 1996). Yet in the southwest of Western Australia, little is known about the frequency of blooms, the distribution of the major bloom-forming species and the potential for blooms to be toxic, and consequently a threat to public health.

In Western Australia, wetlands that are at risk of having toxic algal blooms are not routinely monitored and there is the need for improvements in identification and toxin detection (Hosja and Deeley 1994). It is important to have accurate taxonomically defined spatial and temporal distribution records of harmful species for the management of wetlands (Skulberg *et al.* 1993). Very little information has been published regarding the morphology and taxonomy of Western Australian cyanoprokaryotes because Australian identification guides focus mainly on the eastern states (Baker 1991; Baker 1992; McGregor and Fabbro 2001; Baker and Fabbro 2002; McGregor 2007). There is a need to expand the knowledge to include Western Australia populations due to morphological variability in species of *Anabaena* (Baker 1991), *Microcystis* (McGregor and Fabbro 2001; White *et al.* 2003) and *C. raciborskii* (Saker *et al.* 1999a; McGregor and Fabbro 2000).

Furthermore, the high toxin content detected in the 2000 Swan River *Microcystis* bloom highlighted the need for rapid MC analysis to be available in Western Australia. Toxicity testing by HPLC is a costly and time-consuming process. Alternative screening techniques, such as commercially available enzyme linked immunosorbent assays, can provide a rapid assessment without the need for the costly chemical analyses that are lacking in Western Australia.

The specific objectives of this study were to:

- Identify wetlands with recurring cyanoprokaryota blooms,
- Explore the spatial and temporal distribution of the common bloom-forming species in relation to water quality,
- Investigate the link between the Swan-Canning Estuary and surrounding wetlands in relation to species composition and environmental conditions,
- Investigate whether species composition (nitrogen fixing versus non-nitrogen fixing) is related to inorganic nitrogen and phosphorus concentration in selected lakes,
- Provide the basis of a taxonomic guide for local cyanoprokaryotes,
- Examine the occurrence and diversity of MCs in natural bloom samples in relation to species composition and cell numbers and,
- Evaluate the use of both enzyme linked immunosorbent assays and the Brine Shrimp Lethality Test for screening cyanoprokaryote samples for MCs, NODs and STXs.

# Chapter 2: Study Lakes

## 2.1 Introduction

The Swan Coastal Plain (SCP) (31°30′S, 115°04′E) is a narrow strip of land located on the southwestern coast of Western Australia (Fig. 2.1). It is bordered by the Indian Ocean to the west and the Darling Scarp to the east, and extends approximately 550 km from Geraldton in the north to Dunsborough in the south (Balla 1994). Over 25% of the Swan Coastal Plain (SCP) between Wedge Island and Dunsborough is a wetland (Hill *et al.* 1996a).

The location, drainage and orientation of these wetlands reflect the geological history of the area (Seddon 1972). They are surface expressions of the groundwater, lined up in north-south chain formation more or less parallel with the coastline (Seddon 1972). This is due to their formation within the dune ridges and interdunal depressions between the major dune systems; Quindalup (QDS), Spearwood (SDS) and Bassendean (BDS), also aligned in a north-south direction extending from the coast towards the Darling Scarp (Fig. 2.1). As a result, the SCP wetlands can be grouped into consanguineous suites based on geology, origin, hydrology and surrounding landforms (Semeniuk 1988, cited in Semeniuk 1996).

The term ‘wetland’ is a broad definition that collectively describes ponds, lakes, swamps, estuaries, rivers and their tributaries (Semeniuk 1987). However the focus of this research project are the lakes and sumplands of the SCP, defined as permanently inundated (lake) or seasonally inundated (sumpland) basins of variable size (Semeniuk 1987). By world standards the lakes on the SCP are shallow (mean depth < 2 m) and do not stratify for long periods in summer (Davis *et al.* 1993). The majority of lakes are fresh (< 3 ppt) showing variation in salinity due to seasonal rainfall and evaporative concentration (Semeniuk 1996). Such wetlands are described as poikilohaline, as they show changes in salinity throughout the year but are categorized by the salinity state in which it exists for the majority of the year (Semeniuk 1987). Generally, the freshwater wetlands of the SCP do not exceed 1500

mg $L^{-1}$ or 2475 µS $cm^{-1}$ during winter (Davis *et al.* 1993). The lakes positioned near the coast, the Swan River and the Darling Scarp have higher salinity due to the brackish (1000-5000 mg $L^{-1}$) and saline (> 5000 mg $L^{-1}$) groundwater (Department of Environment 2004a).

The shallow nature of the lakes is a reflection of the area's rainfall pattern. They are surface expressions of superficial aquifers with their water levels under the influence of the water table (Davis *et al.* 1993). Therefore, they experience strong seasonal hydrologic cycles, influenced by Mediterranean-type climate characterized by cool wet winters and hot, dry summers. Most rainfall occurs between May and August but the maximum water level in the lakes is reached in spring (September and October) after the winter rains. Minimum water levels are common in March and April or at the end of summer. As a result of this rainfall pattern, the lakes show changes in inundated area and depth (Balla 1994). In wet years the lakes can reach to a depth of 3 to 4 m and in the dry years around one metre deep (Balla 1994). Of the 41 lakes studied by Davis *et al.* (1993), none exceeded 4.5m in depth. The majority were less than 2 m deep and had large surface area to depth ratio.

In total, 27 lakes were chosen for this project (Fig. 2.2). These lakes can be classified into wetland groups according to their location in relation to the Swan-Canning Estuary, physical characteristics and shared influences (Fig. 2.2) (Arnold 1990). Management categories (high conservation, conservation, open space, resource enhancement and multiple use) have been established identifying the valuable attributes, ecological functions and significance of these wetlands (EPA 1993; Hill and Del Marco 1996). Following is a description of the study lakes based on the before mentioned characteristics. Site photographs are presented in Appendix A. The Swan River estuary, upper Canning River and upper Serpentine River were also sampled and described in Chapter 5.

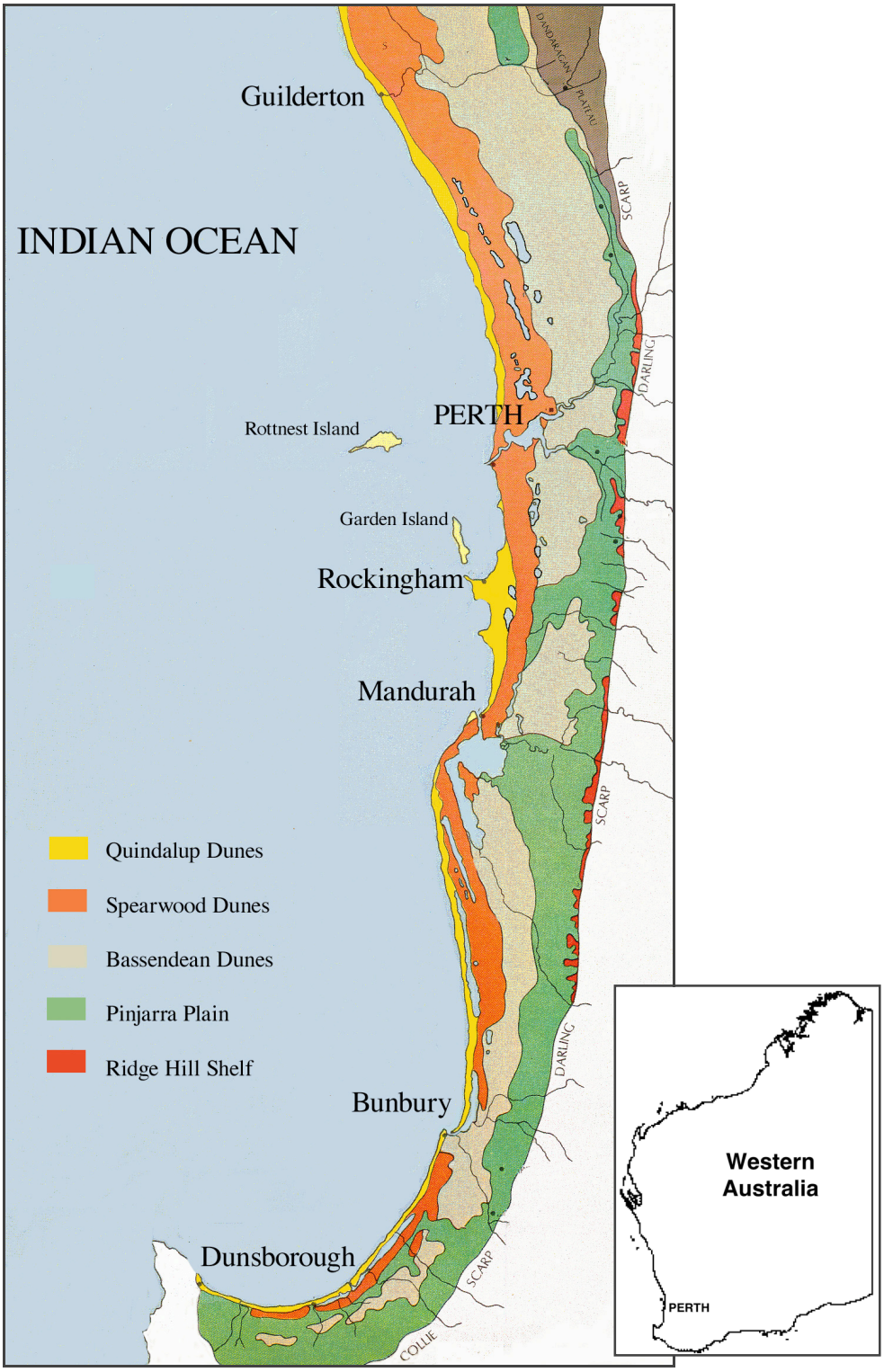



**Figure 2.1.**The location of the wetlands within the depressions of the dune systems of the Swan Coastal Plain, on the southwestern coast of Western Australia. Scale 1:2500000 (Source: Balla 1994, redrawn from Seddon 1972).

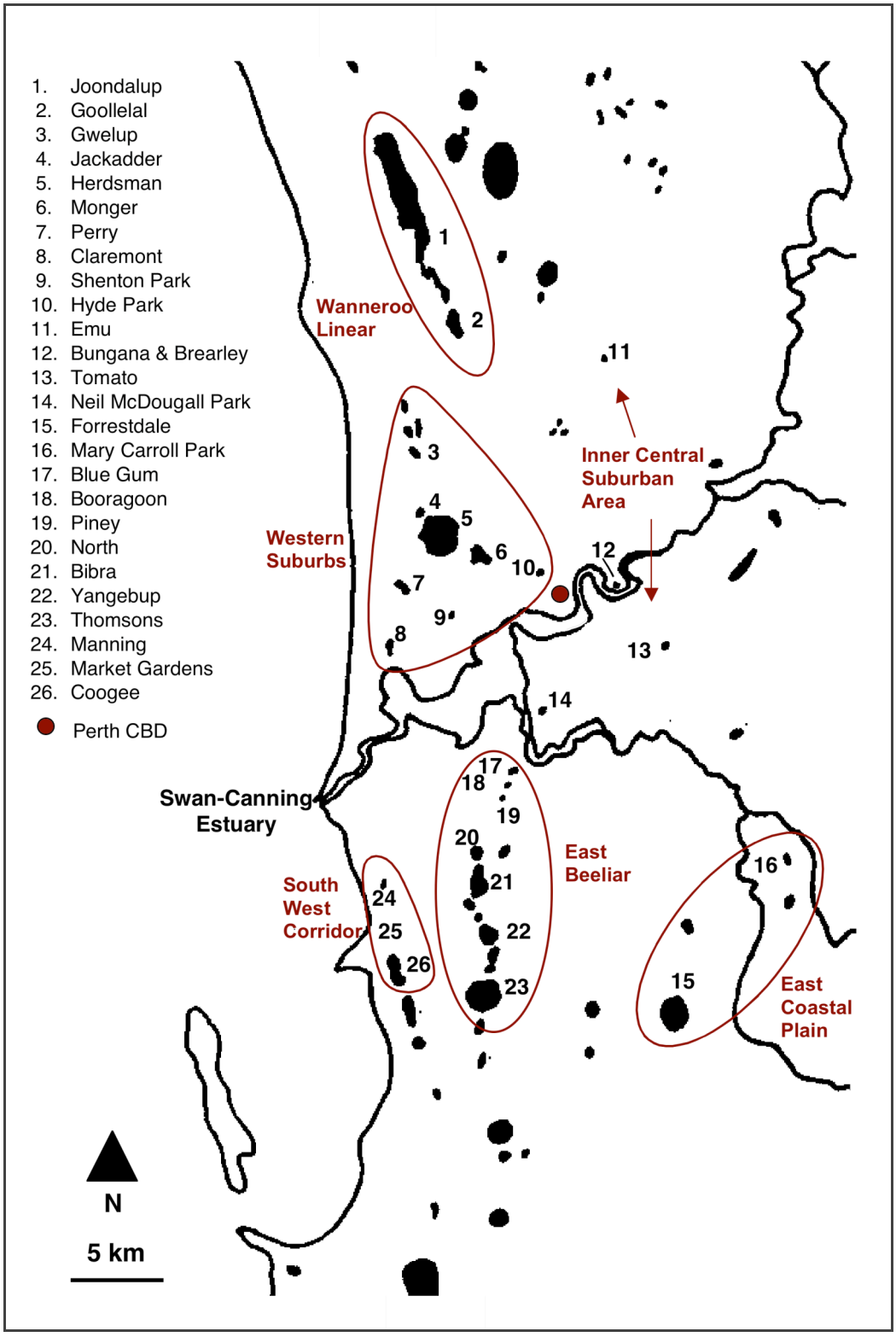



**Figure 2.2.** Map showing the location of the 27 study sites within the wetland groups described in this chapter. Map modified from Davies *et al.* (1993) and Arnold (1990).

## 2.2 Wanneroo Linear Lakes

The Wanneroo Linear Lakes are a chain of wetlands within the Yellagonga Regional Park (YRP), approximately 20 km north of the Perth CBD, and 5 km inland from the coast. Established in 1989, YRP covers an area of approximately 1400 ha, of which 550 ha is wetlands (Kinnear and Garnett 1999). There are five wetlands in total, extending from Loch McNess in the north, to Lake Goolellal in the south. The largest is Lake Joondalup. The wetlands in this group are part of the Yanchep suite of consanguineous wetlands, a linear belt of fresh poikilohaline wetlands occurring in the depression within the Spearwood Dune System on the western edge of the Gnangara Groundwater Mound (Semeniuk 1996). Water movement through the chain occurs in a south to north direction (Plan E 2006).

### *2.2.1 Lake Joondalup*

Lake Joondalup (31°45′41″S, 115°47′21″E) is located in the northern part of the YRP. The lake occupies a total area of 592 ha, with 449 ha of open water (Arnold 1990). Classified as a large elongated freshwater lake (Semeniuk 1987), Lake Joondalup is relatively deep (1.55 m) compared to the other lakes on the SCP (Plan E 2006). Water levels are monitored with a preferred minimum water level of 16.7 m Australian Height Datum (AHD) (Plan E 2006). However, extensive areas can be waterless after a drier than average winter (Plan E 2006). Early reports by Gordon *et al.* (1981) and Congdon (1986) revealed frequent cyanoprokaryota blooms. Lake Joondalup has a management category H classification (high conservation) (Hill and Del Marco 1996). Wetlands in this management category possess a high degree of naturalness and interest for human use. These wetlands need to be actively managed to preserve their natural attributes (Hill and Del Marco 1996).

### *2.2.2 Lake Goollelal*

Lake Goollelal (31°48′36″S, 115°48′41″E) is a permanent wetland 5 km south of Lake Joondalup. The lake occupies a total area 60.7 ha, with 44.9 ha of open water (Arnold 1990). Lake Goollelal is also considered deep compared to the other lakes on the SCP with a preferred minimum water level of 26.4 m AHD (Plan E 2006).

Similar to Lake Joondalup, Lake Goollelal has been assigned a management category H classification, with equal or greater than 95% of the wetland undisturbed (Hill and Del Marco 1996).

## 2.3 Wetlands of the Western Suburbs

The wetlands of the Western Suburbs are located west of the Perth CBD, in areas of intense urbanisation (Plate 2.1). They form part of the Balcatta suite of consanguineous wetlands, a linear belt of fresh stasohaline (constant salinity) wetlands, with irregular elongated or circular shapes, north of the Swan River estuary in the interdunal ridge depression of the SDS (Semeniuk 1996). The wetlands of the western suburbs are situated on the western and southern edge of the Gnangara Groundwater Mound, with groundwater flowing in a southerly to south-west direction discharging into the Swan River (WRC 1997).

### *2.3.1 Lake Gwelup*

Lake Gwelup Reserve (31°52′36″S, 115°47′18″E) contains the northernmost lake sampled in this group. The reserve covers 73 ha, with 20 ha of parkland (Ecoscape 2006). The lake occupies 18.5 ha with 8.5 ha of open water (Arnold 1990). Once classified as a permanent lake (Hill *et al.* 1996b), Lake Gwelup is now seasonal sumpland and has dried out every summer since 2001 (Ecoscape 2006). The maximum water level recorded in Lake Gwelup is 6.5 m AHD (1.5 m) (Ecoscape 2006). There is the potential for increased acidity if groundwater levels decrease. The lowest portions of Lake Gwelup have been identified as having a high risk of acid sulphate soils within 3 metres from the surface (Ecoscape 2006). Five drains enter the lake receiving stormwater from a catchment of approximately 230 ha (Ecoscape 2006). Lake Gwelup has been given a management category H classification (> 95% wetland undisturbed) (Hill and Del Marco 1996).

### *2.3.2 Jackadder Lake*

Jackadder Lake (31°54′30″S, 115°47′36″E) is located approximately 2.8 km south of Lake Gwelup. The lake covers 7.18 ha and is surrounded by 6.6 ha of parkland

(Arnold 1990). Classified as a sumpland (Hill *et al.* 1996b), the water levels are maintained by the input of surface runoff via 10 drain inlets (Rajah 1991). The deepest point is measured at 2.1 m (Rajah 1991). The lake has been subjected to cyanoprokaryota blooms in spring and autumn that led to bird deaths from either algal poisoning or botulism (Arnold 1990). Jackadder Lake has been allocated a management category C1 classification (conservation) (Hill and Del Marco 1996). Wetlands included in this category possess a high degree of naturalness, but with less interest for human use compared to H classified wetlands (Hill and Del Marco 1996).

### *2.3.3 Herdsman Lake*

Herdsman Lake (31°55′18″S, 115°47′50″E) is the largest wetland within this group. It is located within the Herdsman Lake Regional Park, located approximately 7 km northwest of the Perth CBD (ERM Mitchell McCotter 2004). The park covers 340 to 400 ha and is comprised of a large inner wetland, Herdsman Lake (160 ha), surrounded by deep permanent moats connected by small channels (Arnold 1990; ERM Mitchell McCotter 2004). The wetland is a catchment basin for the surrounding urban and industrial areas, which is approximately 3, 000 ha in area (ERM Mitchell McCotter 2004).

For the current project, samples were collected from Floreat Waters in the southwest part of the park (Plate 2.2). Floreat Waters cover approximately 16 to 18 ha and have been artificially deepened to a depth of approximately 12.5 m (Arnold 1990; Schmidt and Rosich 1993; ERM Mitchell McCotter 2004). Long-term nutrient-associated algal blooms are common in these outer moats (Arnold 1990; ERM Mitchell McCotter 2004). Herdsman Lake has a management category C1 classification and is listed on the Directory of Important Wetlands in Australia (Hill and Del Marco 1996).

### *2.3.4 Lake Monger*

Lake Monger (31°55′59″S, 115°49′45″E) is a permanent wetland situated less than one km southeast of Herdsman Lake. The lake is set within a public park covering 110 ha, completely surrounded by urban development and bounded by a freeway on

the eastern side. Directly adjacent to the lake are grassed areas that are used for passive forms of recreation. Lake Monger covers 17 ha and is used as a stormwater drainage basin receiving input from 23 drains that service a catchment of 600 ha (Arnold 1990; Powe and Cameron 1992). Water levels are artificially maintained to keep Lake Monger as a permanent waterbody (Powe and Cameron 1992). Depth ranges from 1 to 1.5 m (Lund and Davis 2000). In summer, the flow from the lake is restricted and in winter excess water is allowed to the Swan River via the Mounts Bay Drain (Arnold 1990; Powe and Cameron 1992). Lake Monger has been assigned a management category C1 classification (Hill and Del Marco 1996).

### *2.3.5 Perry Lakes Reserve*

Perry Lakes Reserve (31°56′47″S, 115°47′9″E) is located approximately 2.4 km southwest from Herdsman Lake and 3 km from the coast. The reserve is a moderately developed recreational parkland occupying 57 ha (PPK Environment & Infrastructure 2000). The area encloses two lakes; the west lake is 5.9 ha and the east lake 6.7 ha (PPK Environment & Infrastructure 2000). Both are shallow (<1.5m) and semi-permanent, experiencing seasonal drying. For the current project, samples were taken from the east lake, near the stadium. The water level of the eastern lake is recharged from irrigation bores during the summer months to prevent the lake from drying out during low-rainfall years. This aims to prevent weed invasion and degradation, which has occurred in the western lake. Four stormwater drains input runoff from the surrounding car park and suburban streets (PPK Environment & Infrastructure 2000). Perry Lakes has been assigned a management category H classification (> 95 % wetland undisturbed) (Hill and Del Marco 1996).

### *2.3.6 Lake Claremont*

Lake Claremont (31°58′39″S, 115°46′39″E) is located approximately 3 km southwest of Perry Lakes, only 2.2 km from the coast. The lake, also known as Butlers Swamp, is bounded by a golf course, recreational parkland and a highschool. Classified as a sumpland (Hill *et al.* 1996b), Lake Claremont covers 18.3 ha with water levels ranging from dry to 2 m AHD (Arnold 1990). Six drains transport surface urban runoff into the lake and a recharge pipe from the adjacent school

removes excess water in winter (Haynes *et al.* 1998). The Claremont Golf Course occupies approximately 57% of the reserve in which Lake Claremont resides (Haynes *et al.* 1998), contributing to the nutrient load in the lake. High levels of phosphorus and nitrogen have been recorded classifying the lake as hypereutrophic (Helleren 1993). The Town of Claremont acts to minimise the quantity of fertiliser and irrigation used on the course (Haynes *et al.* 1998). Saltwater intrusion and evaporative concentration during summer has resulted in salinity greater than 8.9 ppt (Haynes *et al.* 1998). Lake Claremont has been given a management category C1 classification (conservation) (Hill and Del Marco 1996).

### *2.3.7 Shenton Park*

The Shenton Park lake (31°57′34″S, 115°48′93″E), recently renamed Lake Jualbup, is located east of Lake Claremont and Perry Lakes. Shenton Park is recreational parkland of covering 9.2 ha with the lake occupying 2.34 ha (Rockwater 2005). The lake is classified as a small sumpland (Hill *et al* 1996b), with water levels range from dry to 5.5 m AHD (Rockwater 2005). The perimeter of the lake is outlined by a man-made wall of concrete blocks, 1.2 m high at 5.1 to 5.2 m AHD (Rockwater 2005). The lake is a Water Corporation drainage basin with five pipes entering the lake and one outlet pipe to Swanbourne Beach when water levels reach 5.05 m AHD (Rockwater 2005). In 1992 aerators were installed in the lake to prevent stagnant water and botulism (Rockwater 2005). The Shenton Park Lake has a management category R classification for resource enhancement (Hill *et al* 1996b). Wetlands in this classification have been modified but still support substantial functions and attributes (Hill and Del Marco 1996).

### *2.3.8 Hyde Park*

Hyde Park (31°56′18″S, 115°51′43″E) is a large urban parkland located only 1.5 km north of the Perth CBD. The park is of regional importance and has been used for passive recreation since 1896 (Bouma and Powe 1993). The park covers 15.5 ha and encompasses a permanent lake occupying 2.8 ha (Arnold 1990; Kelsall Binet Architects 2003). Once a natural wetland, the lake has been highly modified and is

now classified as an artificial lake (Hill *et al.* 1996b). A causeway separates the lake into two parts (west and east) and a stone retaining wall lines the perimeter (Kelsall Binet Architects 2003). Hyde Park has been allocated a management category C1 classification (Hill and Del Marco 1996), and is listed on the Permanent Register of Heritage Places.

## 2.4 Wetlands of the Inner Central Suburban Area

The wetlands of Inner Central Suburban Area lie within the depressions of the BDS. Neil McDougall Park and Tomato Lake are part of the Jandakot suite of consanguineous wetlands, between the Swan and Canning Rivers, Emu Lake, further north, is part of the Bennet Brook suite of consanguineous wetlands and Lake Bungana and Lake Brearley are part of the Swan River Estuary suite (Semeniuk 1996). The wetlands in this group have not been as extensively studied as the other wetland groups, for example, the Beeliar chain of wetlands. No management plans have been published for lakes Emu, Bungana, Brearley and Tomato.

### *2.4.1 Emu Lake*

Emu Lake (31°50′12″S, 115°52′57″E) is the northern most lake of this area, located 12 km north of the Perth CBD, about 6 to 10 km south east of lakes Joondalup and Goollelal. Emu Lake is comprised of four artificial lakes that cover a total area of 16.9 ha (Plate 2.3) (Hill *et al.* 1996b). Once a natural wetland, Emu Lake has been modified and incorporated into a surrounding housing development (Lakes Estate, Ballajura). The wetland is now comprised of several small lakes, most natural vegetation removed and replaced with manicured gardens. Emu Lake has been assigned a management category C1 classification (Hill and Del Marco 1996).

### *2.4.2 Lake Bungana and Lake Brearley*

Lake Bungana (31°56′34″S, 115°54′16″E) and Lake Brearley (31°56′50″S, 115°54′35″E) are part of the Maylands Peninsula Reserve, situated approximately 200 m from the Swan River. Swan Bank Road, Peninsula Road and Clarkson Road form the boundaries of the reserve (Plate 2.4). Both lakes were previously the sites of

clay-pits for the Maylands Brickwork, which closed in 1983. They have been preserved as artificial lakes separated by a road and walkway approximately 30 m wide. The lakes are surrounded by a residential area, the Maylands Peninsula Golf Course and the Swan River. Due to the close vicinity to the river, the groundwater salinity of in this area is brackish (1500-3000 mg $L^{-1}$) (Department of Environment 2004a).

### *2.4.3 Tomato Lake*

Tomato Lake (31°58′30″S, 115°55′59″E) is located within 13 ha of parkland, approximately 3 km from the Swan River. It has been modified to suit its urban setting, surrounded by lawns and picnic facilities. The lake covers 6.6 ha, with 4.6 ha of open water (Arnold 1990). It is part of the South Belmont Main Drain catchment that discharges into the Swan River. Tomato Lake has been assigned a management category C1 classification (Hill and Del Marco 1996).

### *2.4.4 Neil McDougall Park*

Neil McDougall Park (31°00′22″S, 115°51′47″E) is 9.7 ha of parkland comprised of grassed areas, isolated trees and a shallow lake (1-2 m) located in the northern part of the park (Bowman Bisham Gorham 1995). The lake covers 2.8 ha, with 2.3 ha of open water (Arnold 1990). It was a former marsh, modified to create a compensating basin and permanent lake (Bowman Bisham Gorham 1995). Four stormwater drains enter Neil McDougall Park, receiving drainage from a catchment 100 ha in size (Bowman Bisham Gorham 1995). During summer, groundwater is pumped into the lake to prevent exposure of the lake bed, while in winter; maximum water levels and flooding are controlled by an automatic pumping system discharging into the Swan River (Bowman Bisham Gorham 1995). Poor water quality has resulted in algal blooms and occasional bird deaths due to avian botulism (Bowman Bisham Gorham 1995).

## 2.5 Wetlands of the Eastern Coastal Plain

The wetlands of the Eastern Coastal Plain are positioned on the BDS and Pinjarra Plain along the eastern boundary of the SCP. The two lakes sampled from this group, Forrestdale Lake and Mary Carroll Park, are located south of the Swan-Canning Estuary on the eastern edge of the Jandakot Groundwater Mound. Forrestdale Lake is part of the Bennett Brook suite of consanguineous wetlands located on the eastern side of the Bassendean Dune System (Semeniuk 1996). Mary Carroll Park lies between the Ridge Hill Shelf and Pinjarra Plain and is part of the Mungala suite of consanguineous wetlands (Semeniuk 1996).

### *2.5.1 Forrestdale Lake*

The Forrestdale Lake Nature Reserve (32°9′36″S, 115°55′41″E) is the largest wetland in this group, located 25 km southeast of Perth CBD. The reserve covers a total of 247.5 ha (Arnold 1990). The lake is seasonal with 221.1 ha of open water with a maximum depth of 0.9 m (Arnold 1990; CALM 2005a). During the wetter years water is received from two local drains on the northern and western margins, with an outflow drain on the eastern side, which has not flowed for a number of years (CALM 2005a). The lake has a history of poor water quality with nuisance swarms of midges being an indicator of this (CALM 2005a). Forrestdale Lake has a management category H classification and is listed on the Ramsar Convention on Wetlands and the Directory of Important Wetlands in Australia (Hill and Del Marco 1996).

### *2.5.2 Mary Carroll Park*

Mary Carroll Park (32°4′55″S, 116°00′5″E) lies approximately 20 km southeast of the Perth CB in the low-lying area between the Canning and Southern rivers. The park is 21 ha in area, with two interconnected seasonal wetlands surrounded by 14 ha of remnant native vegetation and parkland (Rodda and Deeley 1990). A main road, Eudoria Street, separates the northern lake (NL) from the southern swamp (SS). The total wetland area is 19.4 ha with 5.7 ha of open water (Arnold 1990). In 1989, a walkway was built through the southern shore of the northern lake, resulting in the

formation of a permanent water body. It was from this area that samples were collected (Plate 2.5).

Mary Carroll Park receives urban runoff via three stormwater drains (Rodda and Deeley 1990). Water flows through the Eudoria Street drain from the SS to the NL when water levels in the SS increase (Rodda and Deeley 1990). When water levels rise in the NL, water is drained to the Canning River (Rodda and Deeley 1990). The salinity of these wetlands ranges from marginal to brackish (1000-7000 µS cm $^{-1}$) (Rodda and Deeley 1990).

In 1989, the water quality was amongst the worst for SCP wetlands (Rodda and Deeley 1990). From 1985 to 1990 a large number of birds (over 2000) suffered botulism (Pederson and Conacher 1991). Blooms of *Microcystis* have been observed in the northern lake (Rodda and Deeley 1990). The high phosphorus and nitrogen loads in the lakes and drains have resulted from current and past land use, which included market gardens, a piggery and a poultry farm (Rodda and Deeley 1990). Mary Carroll Park has been assigned a management category C1 classification (Hill and Del Marco 1996).

## 2.6 East Beeliar Regional Park

The Beeliar Regional Park is located south of the Swan-Canning estuary. It encompasses 19 wetlands that are arranged in two main chains (eastern and western) running parallel to the coastline. The eastern chain is 5 to 6 km from the coast extending 12 km from north to south (Newman 1976; Semeniuk 1996). The lakes North, Bibra, Yangebup and Thomson form part of the Bibra suite of consanguineous wetlands, a linear belt of freshwater poikilohaline lakes formed in the low-lying areas between the SDS and BDS, whereas lakes Blue Gum, Booragoon and Piney are part of the Jandakot suite of consanguineous wetlands (Semeniuk 1996). Located on the western edge of the Jandakot Groundwater Mound, groundwater flows in a west-northwest direction from the mound into the wetlands then towards the Canning River via lakes Booragoon and Blue Gum (Arnold 1990;

WRC 1997). During years of significantly high rainfall, groundwater can flow in a north to south direction within the chain of lakes (Thompson Palmer Pty. Ltd. 2005).

### *2.6.1 Blue Gum Lake*

Blue Gum Lake Reserve (32°2′12″S, 115°50′54″E) is the northernmost body of water in the eastern chain of wetlands, located approximately 560 m from the lower Canning River. The reserve covers approximately 79 ha of bushland, recreational parkland and a central lake (Fox and MacShane 2004a). The lake covers 7 ha with 4.3 ha of open water (Arnold 1990). Blue Gum Lake is classified as a sumpland (Hill *et al* 1996b), with water levels fluctuating seasonally. The western end of the lake is usually dry during summer. However, during the 1970s and 1980s the water level maintained at an average of 6.6 m AHD to produce a permanent lake (Fox and MacShane 2004a). In 1996, the minimum water level was recorded as 2 m AHD (Fox and MacShane 2004a). Water enters the lake via seven drains, receiving water from a catchment of approximately 12 ha (Fox and MacShane 2004a). Blue Gum Lake has a management category C classification (Hill and Del Marco 1996).

### *2.6.2 Booragoon Lake*

Booragoon Lake Reserve (32°2′37″S, 115°50′36″E) is located only 500 m southwest of Blue Gum Lake. As a result, both lakes are closely related due to groundwater movement and drainage from Booragoon Lake to Blue Gum Lake, and the interchange of birdlife between the two lakes (Smith 1986). Groundwater in the area flows northward towards the Canning River. However, Booragoon Lake has rarely experienced problematic algal blooms and nuisance midge swarms, which have been evident in neighbouring Blue Gum Lake (Smith 1986; Fox and MacShane 2004b). This is due to Booragoon Lake having highly coloured water (Fox and MacShane 2004b), which reduces light penetration limiting phytoplankton growth.

The reserve covers 13.24 ha, with 3.8 ha of open water (Smith 1986; Arnold 1990). Similar to Blue Gum Lake, the water level in Booragoon Lake was artificially maintained at 11.4 m AHD during the 1970s and 1980s (Smith 1986; Fox and MacShane 2004b). Since then the minimum water level has been recorded at 10 m

AHD in 1992 (Fox and MacShane 2004b). Five stormwater drains input water into Booragoon Lake from the surrounding suburban area and Leach Highway (Fox and MacShane 2004b). The catchment covers 19 ha, of which, 6.9 ha drains from Leach Highway (Fox and MacShane 2004b). Booragoon Lake has been allocated a management category C1 classification and is included in the Directory of Important Wetlands in Australia (Hill and Del Marco 1996).

### *2.6.3 Piney Lakes Reserve*

Piney Lakes Reserve (32°2′57″S, 115°50′10″E) is situated 300 m south of Booragoon Lake. The reserve covers 67 ha, with 50 ha of bushland, 17 ha of developed parkland and two interconnected wetlands (ATA Environmental 2004). The western wetland is permanent, 3.5 ha in area (ATA Environmental 2004). It has been artificially deepened to a maximum depth of 2.1 m (Schmidt and Rosich 1993). It was from this wetland that samples were collected. The eastern wetland, 12 ha in area, occasionally contains water (ATA Environmental 2004). Stormwater runoff is drained into two drainage basins on the eastern edge of the reserve and not directly discharged into the wetlands (ATA Environmental 2004). Piney Lakes Reserve has been assigned a management category H classification (Hill and Del Marco 1996).

### *2.6.4 North Lake*

North Lake (32°4′ 38″S, 115°49′21″E) is located 14 km south of Perth CBD. The lake occupies a total area 51.9 ha with 24.7 ha of open water (Arnold 1990). North Lake is shallow with a maximum depth of 1.6 m in summer to 3 m in winter (Bayley *et al.* 1989). Stormwater enters North Lake via two drains, which were constructed in the 1970s and led to an increase in water levels (Bayley *et al.* 1989).

In the 1970s, North Lake received groundwater and surface run-off drainage directly from the Murdoch University Veterinary farm (Newman 1976). This resulted in the lake becoming heavily nutrient enriched and experiencing periodic spring and summer algal blooms (Newman 1976; Bayley *et al.* 1989). Toxic blooms of *Microcystis* and *Anabaena* have been recorded and are a potential health risk to the

public and bird-life that used this lake (Bayley *et al.* 1989). North Lake has been given a management category C1 classification (Hill and Del Marco 1996).

### *2.6.5 Bibra Lake*

Bibra Lake (32°5′25″S, 115°49′16″E) is located 500 m south of North Lake. It is classified as a large permanent freshwater lake with salinity that varies seasonally (Semeniuk 1987). The area of open water ranges from 100 to 135 ha (Arnold 1990). The average depth recorded was at 12.56 to 15.61 m AHD (Arnold 1990). Bibra Lake was previously a landfill site (Newman 1976), but the western shore has now been developed as a recreational area. It is also the home to many water birds, which congregate in large numbers on the western shore. The feeding of these birds has localised effects on water quality as uneaten food and faeces contribute to the nutrient loading (Thompson Palmer Pty. Ltd. 2005). Bibra Lake has been assigned a management category C classification (Hill and Del Marco 1996).

### *2.6.6 Yangebup Lake*

Yangebup Lake (32°6′56″S, 115°49′33″E) is a permanently inundated lake located 1 to 2 km south of Bibra Lake. The lake covers a total area 90.5 ha, with 64.4 ha of open water (Arnold 1990). The water levels in Yangebup Lake are monitored for the development of the South Jandakot Drainage Scheme (Thompson Palmer Pty. Ltd. 2005). Yangebup Lake will act as a compensating basin receiving water from Kogolup Lake and Thomsons Lake (Ecoscape 1995). The lake also receives urban runoff from three stormwater drains (Ecoscape 1995). The maximum depth has been recorded at 4 m (Schmidt and Rosich 1993).

The water quality of Yangebup Lake is among the worst in the state due to seepage from a wool scouring plant, which was located near the southeast shore. Prior to 1924, effluent from a woolscourers was discharged directly into Yangebup Lake (Newman 1976). This resulted in elevated levels of arsenic, chromium and iron. In the 1950s, the wool scourers were responsible for the increased salinity levels as water from a deep saline aquifer was used for wool washing (Ecoscape 1995). In addition, Yangebup Lake has a history of algal problems with nuisance blooms of

*Microcystis* and *Anabaena* occurring throughout the year (Newman 1976; Davis *et al.* 1993). *Oscillatoria* is believed responsible for the green stained sands around the shoreline (Newman 1976). Yangebup Lake has been assigned a management category H classification (> 95% wetland undisturbed) (Hill and Del Marco 1996).

### *2.6.7 Thomsons Lake*

Thomsons Lake Nature Reserve (32°9′26″S, 115°49′41″E) is located 2.2 km south of Yangebup Lake and 34 km southwest of the Perth CBD. It is the largest wetland within the eastern chain of Beeliar wetlands. The reserve covers 509 ha in total, with 151 ha of open water (Crook and Evans 1981; Arnold 1990). Thomsons Lake is a seasonal wetland with water levels varying from dry to 3.3 m deep (Crook and Evans 1981). Water levels have been decreasing since 1999 (CALM 2005b). As mentioned, Thomsons Lake is part of the South Jandakot Drainage Scheme to Yangebup Lake. The scheme diverts stormwater from some of the Beeliar wetlands to Thomsons Lake to protect them from nutrient loading as well as maintaining maximum water levels (CALM 2005b). Thomsons Lake has a management category H classification and is included on the Ramsar List of Wetlands of International Importance and the Directory of Important Wetlands in Australia (Hill and Del Marco 1996).

## 2.7 Wetlands of the South West Corridor

The wetlands of the Southern West Corridor are the western chain of wetlands within the Beeliar Regional Park. This chain occupies approximately 1,088 ha, extending from Manning Lake in the north, to Mount Brown Lake, in the south (Newman 1976). There are five lakes in total. They form part of the Coogee suite of consanguineous wetlands, a linear belt of fresh to hypersaline lakes and sumplands situated 1 to 2 km from the coast, in the depressions between the two limestone ridges of the Spearwood Dune System (Semeniuk 1996). Unlike the eastern chain, these wetlands are only partial surface expressions of the groundwater and are more saline (Thompson Palmer Pty. Ltd. 2005). All appear connected to the salt wedge that extends inland from the coast, resulting in higher salinity (O'Brien Planning Consultants 1993). They are located on the western edge of the Jandakot Groundwater Mound with winter groundwater flow is in an east to west direction

(Ecoscape 1996). In summer, a small groundwater mound develops west of Lake Coogee and the groundwater flow is reversed (Ecoscape 1996).

### *2.7.1 Manning Lake*

Manning Lake Reserve (31°5′39″S, 115°46′15″E) is a recreational parkland occupying 11.6 hectares, with 4 hectares of open water (Arnold 1990). Also known as Davilak Lake (Devils Lake), Manning Lake is classified as a small freshwater sumpland (Semeniuk and Semeniuk 1997). Early reports considered water quality to be poor due to high nutrient levels from market garden fertilisers and septic tanks (Newman 1976). As a result blooms of cyanoprokaryota, believed to be toxic and producing shoreline scums, as well as the non-toxic dinoflagellate *Eurococcus* have been reported (Newman 1976). Manning Lake was assigned a management category C classification (Hill and Del Marco 1996).

### *2.7.2 Market Garden Swamps*

The Market Gardens swamps are a string of ephemeral wetlands running north-south direction situated approximately 2.2 km southeast of Manning Lake. The three swamps cover a total area of 75.9 ha with 21.1 ha of open water (Arnold 1990; Ecoscape 1996). Mayor Road separates swamp 1 and 2 from Swamp 3. Classified as sumpland (Hill *et al.* 1996b), these wetlands dry out each summer and do not refill until winter (Ecoscape 1996). Water levels in Swamp 1 are maintained at less than 1.2 m AHD via a drain parallel to Mell Road (Ecoscape 1996). Natural drainage within the system is southwards towards Lake Coogee (Ecoscape 1996). As these swamps are located only 1 km from the coast, the salinity ranges from brackish to saline (1.4 -92.2 mS $cm^{-1}$) (Water Authority 1993, cited in Ecoscape 1996).

These swamps were once completely surrounded by market gardens (Newman 1976, Arnold 1990). As a result, nutrient levels were high due to the surrounding heavily fertilised land (Newman 1976). Similar to Manning Lake, *Eurococcus*, *Microcystis* and *Oscillatoria* has been reported in this lake, as well as, bird deaths reported to be associated with botulism (Newman 1976). For the current project, samples were collected from Market Garden Swamp 1 (37°6′47″S, 115°46′49″E).

### *2.7.3 Lake Coogee*

Lake Coogee (32°8′2″S, 115°46′44″E) is a shallow seasonal lake containing 60.4 ha of open water (Arnold 1990). Maximum water level reaches 1 m AHD, with depth ranging from 0.1 m AHD to 0.9 m AHD (recorded 1981-1987) (O'Brien Planning Consultants 1993). The main input of pollutants and nutrients come from groundwater drainage from the Market Garden swamps (O'Brien Planning Consultants 1993). Evaporation is the major mechanism for water removal (O'Brien Planning Consultants 1993).

Lake Coogee differs from other lakes of the SCP due to salinity and the underlying soils. Located only 750 m from the coast (O'Brien Planning Consultants 1993), Lake Coogee has been classified as a hyposaline (3-20 ppt) (Semeniuk 1987). The salinity can range from brackish in winter to saline in summer (Arnold 1990). As a result, the vegetation and fauna is different from the other freshwater lakes (O'Brien Planning Consultants 1993). Lake Coogee also has a different soil base to the other lakes. The lakebed is comprised of clay, silt and shell debris, which dries every summer (Newman 1976). This clay base suggests the Lake Coogee is originated from a former estuary (Newman 1976). Lake Coogee has a management category C1 classification (Hill and Del Marco 1996).

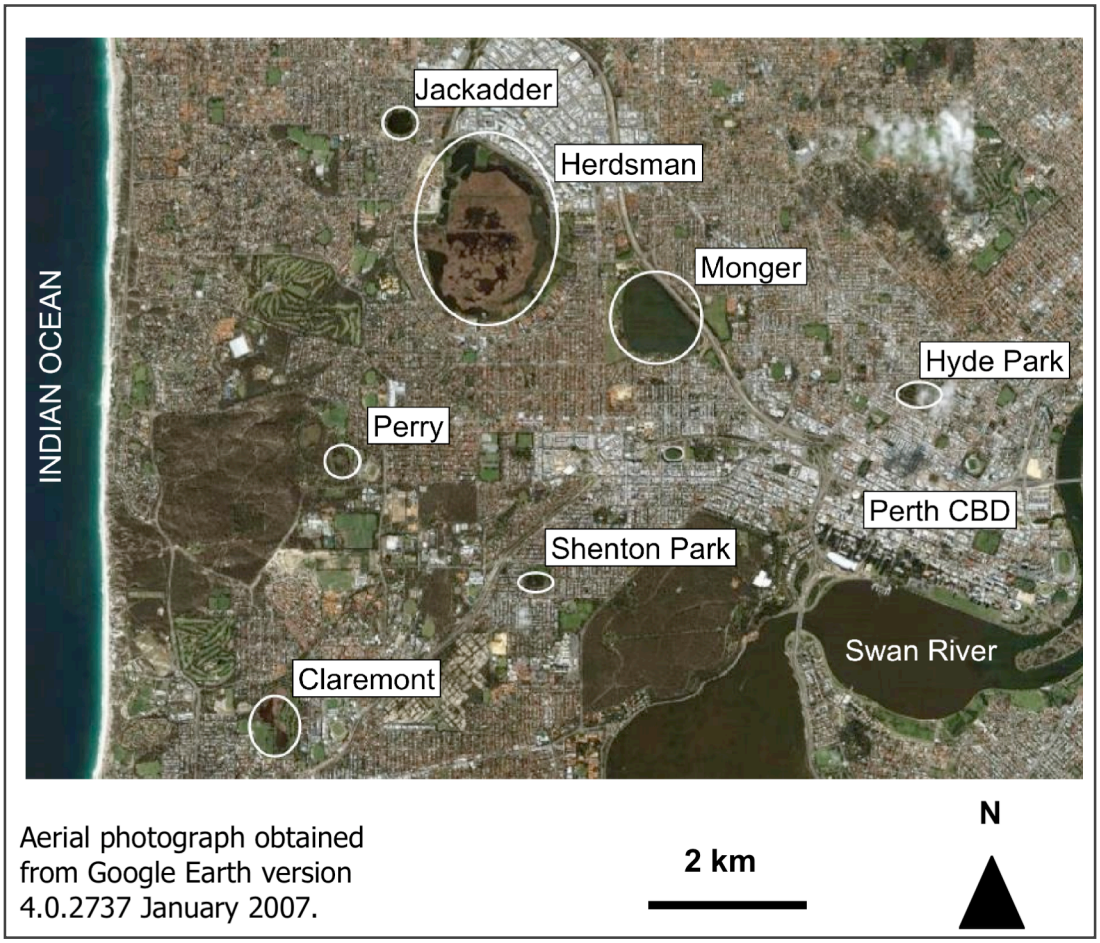



**Plate 2.1.** Aerial view of the wetlands of the Western Suburbs.

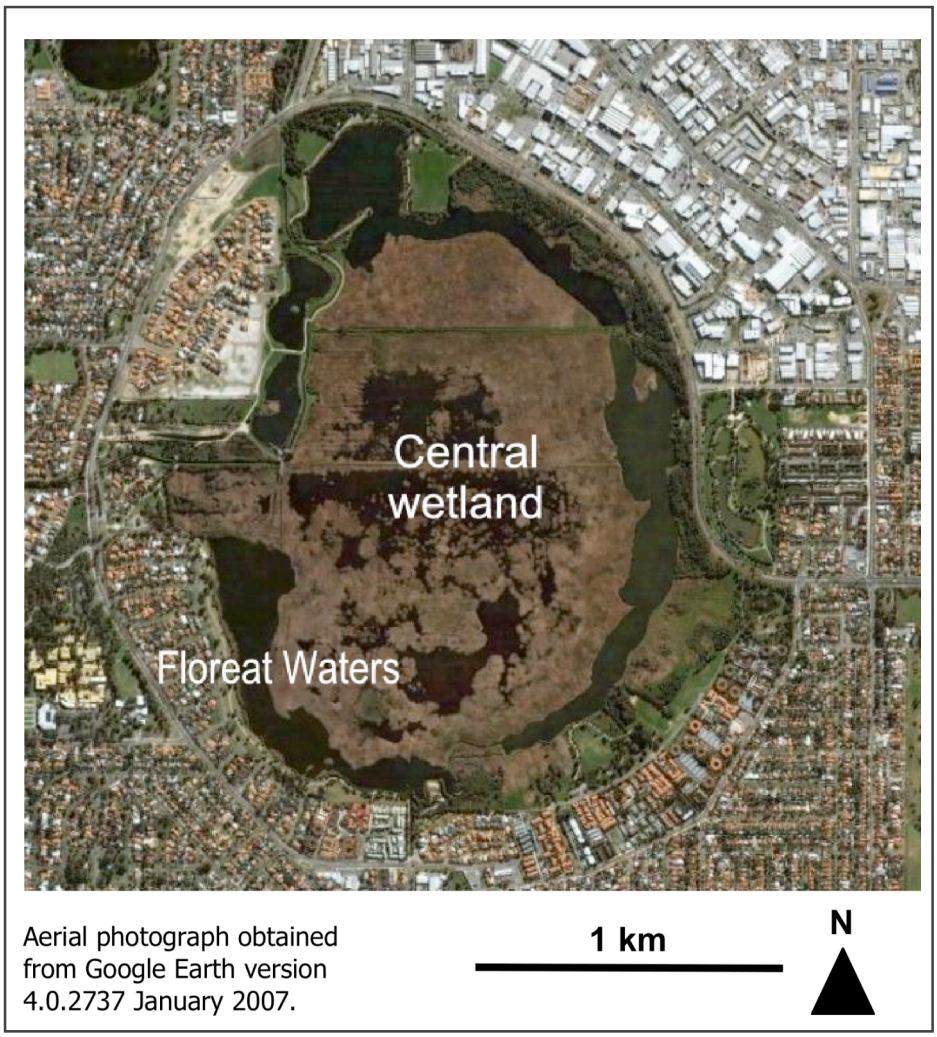



**Plate 2.2.** Aerial view of Herdmans Lake showing the location of Floreat Waters.

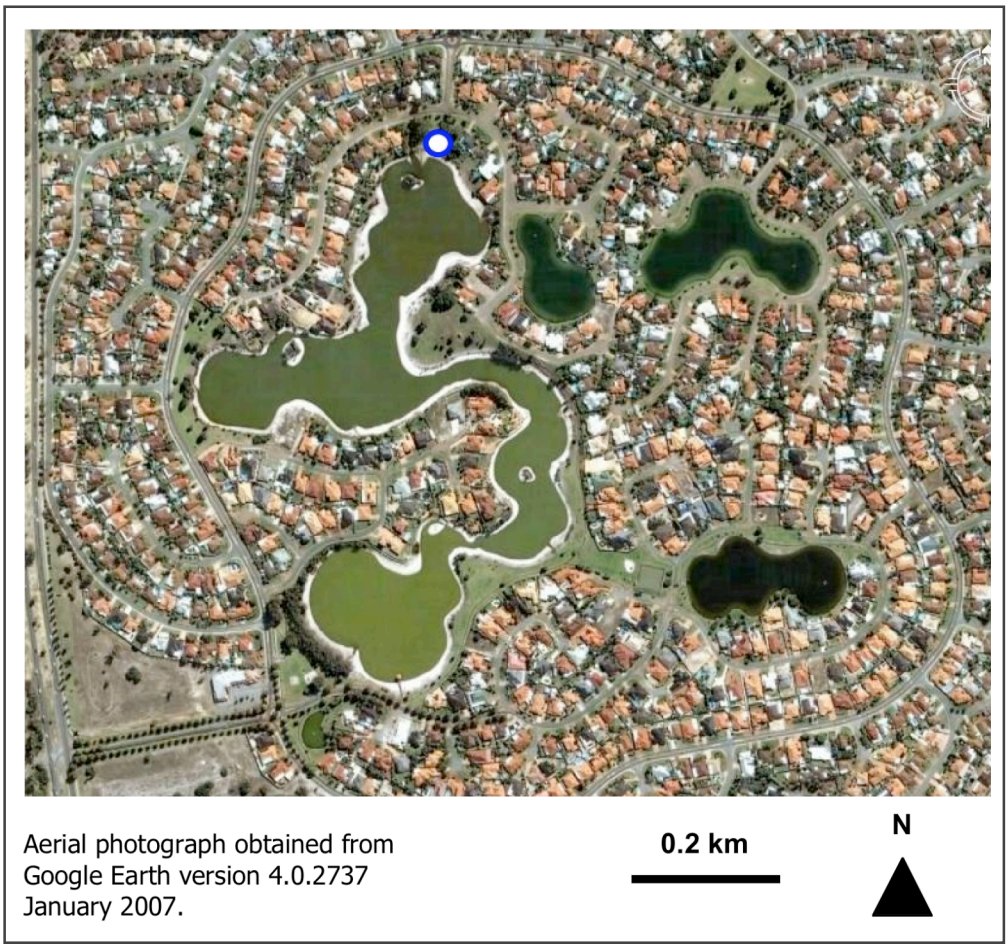



**Plate 2.3.** Aerial view of the small lakes that constitute Emu Lake. Samples were collected from the northern shore of the main water-body (**O**).

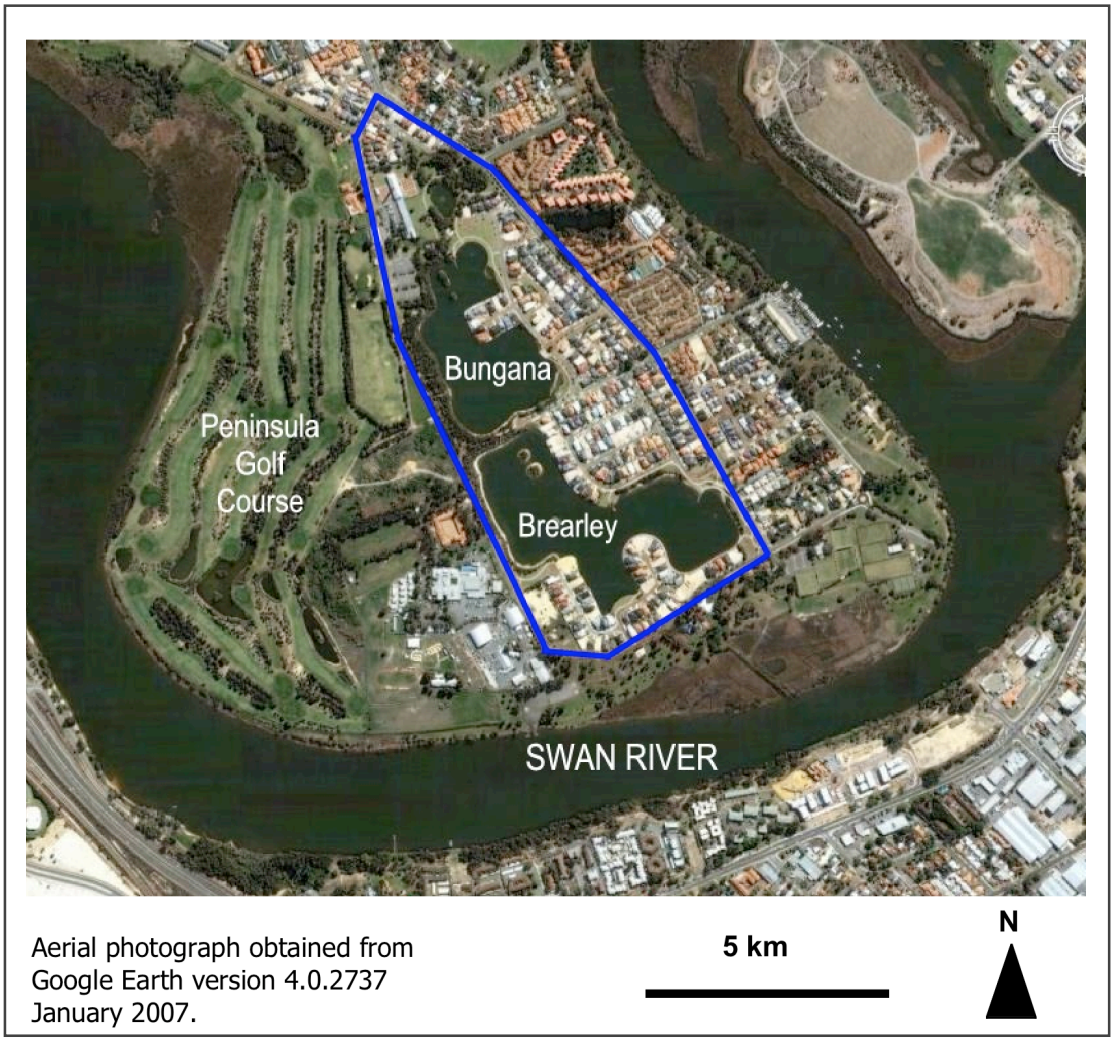



**Plate 2.4.** Aerial view of Lake Bungana and Lake Brearley showing the boundary of the Maylands Peninsula Reserve.

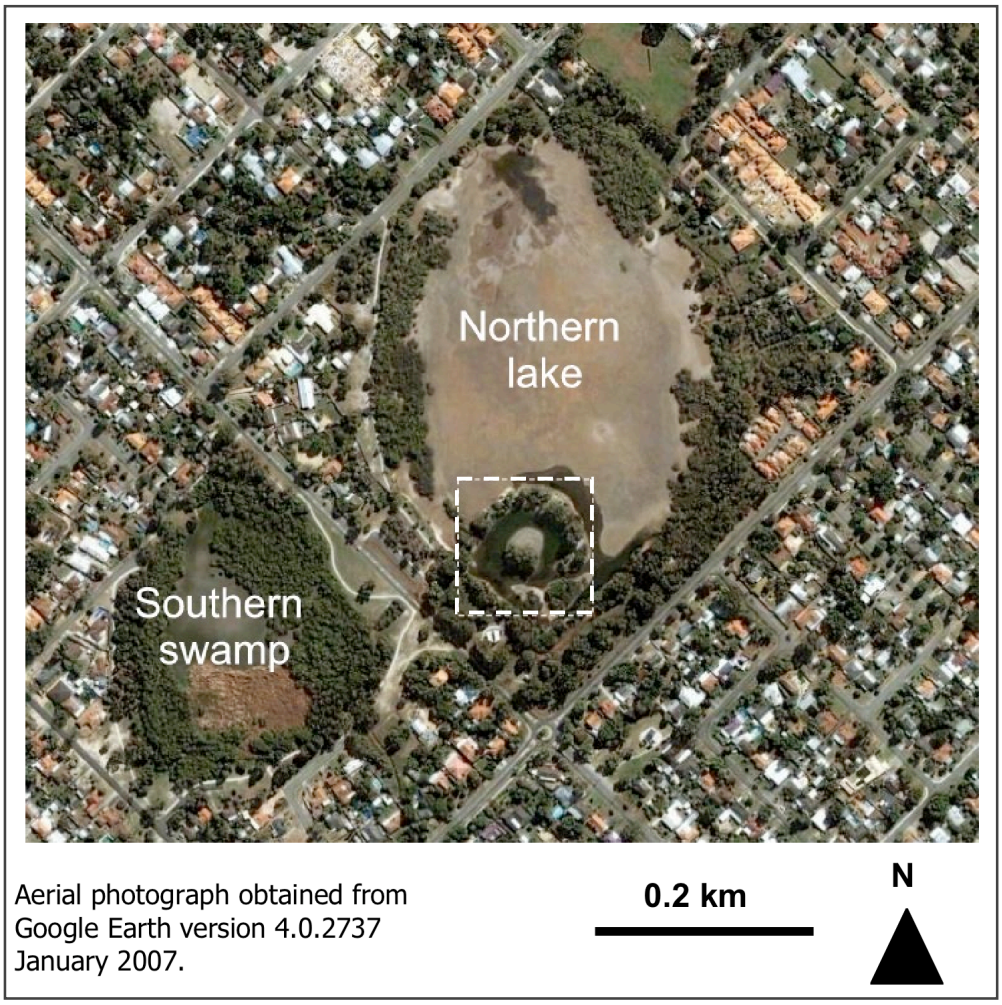



**Plate 2.5.** Aerial view of Mary Carroll Park showing the location of the larger northern lake and smaller southern swamp. Samples were collected from the small permanent body of water indicated on picture.

# Chapter 3: Physico-chemical environment of the Swan Coastal Plain urban lakes

## 3.1 Introduction

The Swan Coastal Plain (SCP) is an area rich in wetlands, accounting for over 25% of its landmass (Hill *et al*. 1996). However, it has been estimated that since European settlement in 1829, 70 to 80% of the wetlands of the SCP have been lost forever (Godfrey 1989). Currently, two million people live in the city of Perth, representing 73% of the state's population (Australian Bureau of Statistics 2005). In common with many cities, urban development has centred around water resources. As a result, the wetlands of the Perth metropolitan area have been filled, drained or modified to allow urban development to continue. Those lakes located within areas of intense urbanisation and functioning as stormwater drainage basins are experiencing repeated nuisance algal blooms (Balla 1994), which is indicative of severe water quality problems (Davis *et al.* 1993).

Although there have been reports of bloom events, studies focusing primarily on cyanoprokaryota blooms in the Perth urban wetlands are sparse. Davis *et al.* (1993) provided a general review of water quality, phytoplankton and macroinvertebrate diversity within 41 SCP lakes and sumplands, while water quality studies by Gordon *et al.* (1981), Congdon (1986), Bayley *et al*. (1989), Lund and Davis (2000) focused on individual lakes. All revealed the occurrence of periodic cyanoprokaryota blooms of potentially toxic species. However, no comprehensive study has focused on the occurrence and distribution of cyanoprokaryota blooms in the SCP wetlands by sampling a large number of sites over consecutive years.

In this chapter, the environmental conditions during spring and summer, when blooms are most likely to occur, and the ordination of the study sites based on this information are described. As explained in Chapter 2, the SCP lakes lie in the depressions developed between the major dune systems forming consanguineous groups based on underlying soils, surrounding landforms and hydrology (Semeniuk

1996). They are characteristically shallow (mean depth < 2 m), alkaline, have average spring to summer water temperatures ranging from 17 to 30 °C and range in salinity from freshwater to marginally fresh (Gordon *et al.* 1981; Schmidt and Rosich 1993; Kinnear and Garnett 1999; Lund and Davis 2000). These physico-chemical variables, as well as nutrient concentration, can potentially control the formation of cyanoprokaryota blooms and determine which genera and species become established and dominant within a wetland (Paerl 1996).

The specific objective of this chapter was to investigate the environmental conditions experienced during spring and summer in the SCP lakes sampled for this study, relating these findings to the formation of cyanoprokaryota blooms. Multivariate statistics (principal component analysis) classified the sites based on pH, temperature, salinity and location on the SCP (consanguineous suite), which will later assist with explaining the distribution pattern of the major bloom-forming species (Chapter 4). For some sites the environmental data collected will provide spring-summer baseline information for future studies.

## 3.2 Materials and Methods

### *3.2.1 Sampling program*

A total of 27 wetlands were sampled from 2000 to 2003 (Table 3.1 and 3.2). Their location with reference map is provided in Chapter 2. These sites represented a range of lakes distributed both south and north of the Swan-Canning Estuary, throughout the metropolitan area. The lakes were sampled at weekly to fortnightly intervals, requiring two days to visit all sites.

In the field, water temperature (°C), salinity (ppt), electrical conductivity (μS $cm^{-1}$; equivalent to EC unit) and pH were measured using a hand-held portable TPS WP-81 meter. Measurements were taken from the surface waters accessible from the shore. The salinity categories were adapted from Williams (1983) and Semeniuk (1987) defined as: freshwater < 1 ppt (g $L^{-1}$), marginally fresh 1 - 3 ppt, hyposaline (brackish) 3 - 10 ppt and saline > 10 ppt.

**Table 3.1.** The months sampled during each seasonal period.

| Period | Months |
|---|---|
| Spring 2000 | August 2000 – November 2000 |
| Summer 2000-01 | December 2000 – February 2001 |
| Spring 2001 | September 2001 – November 2001 |
| Summer 2001-02 | December 2001 – March 2002 |
| Summer 2002-03 | late November 2002 – February 2003 |

Not all lakes were sampled during each period due to time, water availability and financial constraints (Appendix 3.1). Forrestdale Lake and Thomsons Lake were only sampled during one summer period (2000-01) due to low water levels and difficulties in obtaining planktonic samples. For Mary Carroll Park, environmental data were collected from a small section of the northern lake (site number 17a) as explained in section 2.5.2. However, when the entire northern lake was inundated with water, measurements were also taken from the larger seasonal waterbody (site number 17b).

### *3.2.2 Statistical analysis*

For both seasons, the environmental data collected for each lake was averaged and the standard error calculated. Individual data sets for each lake are presented in Appendix B. Differences between the lakes based on the sampling period, season and location were tested using one-way Analysis of Variance (ANOVA) or Kruskall-Wallis H test. Ordination of the lakes based on the physico-chemical variables studied was by principle component analysis (PCA) using the statistical package PRIMER version 6. The Canning River, although described in Chapter 5, was included in the PCA as a comparison to the lakes.

Prior to the PCA analysis, the data were checked for normality and log (n + 1) transformed where applicable. Draftsman plots were generated to assess the linearity of the data. For the summer sampling period, lakes that were only sampled in December due to low water levels were excluded from the PCA. A cumulative

variance for the first and second principle component axis above 70 to 75% was considered good for describing the overall PCA structure (Clarke and Warwick 2001).

The draftsman plots for the three summer periods showed salinity (ppt) and EC (μS $cm^{-1}$) to be highly correlated with each other with pair wise correlations of 0.999, 1.0 and 0.997. Therefore, salinity (ppt) was not included in the PCA dataset and EC was used in the multivariate analyses to define the total dissolved salt concentrations. The Australian and New Zealand Environment and Conservation Council (ANZECC and ARMCANZ) (2000) state freshwaters have an EC less than 1000 μS $cm^{-1}$. Because salinity and EC are terms that should not be strictly used interchangeably (Boulton and Brock 1999), both terms will be used throughout the thesis.

The lakes were assigned a site number and classified based on location on the SCP (Table 3.2). Two-dimensional ordination plots were generated with symbols superimposed to show lakes from similar locations. Hierarchical cluster analysis using Group Average linkage was used to determine the level of similarity between lakes based on pH, water temperature and EC, which were defined on the PCA plots. Similarity matrices were generated using Euclidean distances calculated from the principal component scores. Differences in environmental conditions among the recognised groups were analysed using the non-parametric Kruskall-Wallis H and Mann-Whitney U tests. Separate ordination and clustering analyses were performed for each spring and summer sampling period for seasonal comparison, while a combined PCA analysis of all physico-chemical data collected from each study lake allowed the consanguineous wetland groups to be compared.

**Table 3.2**. Classification of the study lakes based on location (Arnold 1990) and consanguineous wetland suites (Semeniuk 1996). Reference map provided in Chapter 2.

| Location | PCA legend | Lake | Dune system | Wetland suite | Site No. |
|---|---|---|---|---|---|
| Wanneroo Linear Lake | Wanneroo | Joondalup | Spearwood | Yanchep | 1 |
| | | Goollelal | Spearwood | Yanchep | 2 |
| Wetlands of the Western Suburbs | West Sub | Gwelup | Spearwood | Balcatta | 3 |
| | | Jackadder | Spearwood | Balcatta | 4 |
| | | Herdsman | Spearwood | Balcatta | 5 |
| | | Monger | Spearwood | Balcatta | 6 |
| | | Perry | Spearwood | Balcatta | 7 |
| | | Claremont | Spearwood | Balcatta | 8 |
| | | Shenton Park | Spearwood | Balcatta | 9 |
| | | Hyde Park | Spearwood | Jandakot | 10 |
| Wetlands of the Inner Central Suburban Area | Inner | Emu | Bassendean | Bennett Brook | 11 |
| | | Bungana | Bassendean | Swan Estuary | 12 |
| | | Brearley | Bassendean | Swan Estuary | 13 |
| | | Tomato | Bassendean | Jandakot | 14 |
| | | Neil McDougall Park | Bassendean | Jandakot | 15 |
| Wetlands of the Eastern Coastal Plain | East Plain | Forrestdale | Bassendean | Bennett Brook | 16 |
| | | Mary Carroll Park | Bassendean | Mungala | 17a,b |
| East Beeliar Regional Park | East Beeliar | Blue Gum | Interface | Jandakot | 18 |
| | | Booragoon | Interface | Jandakot | 19 |
| | | Piney | Interface | Jandakot | 20 |
| | | North | Interface | Bibra | 21 |
| | | Thomsons | Interface | Bibra | 22 |
| | | Bibra | Interface | Bibra | 23 |
| | | Yangebup | Interface | Bibra | 24 |
| Wetlands of the South West Corridor | West Corridor | Manning | Spearwood | Coogee | 25 |
| | | Market Gardens | Spearwood | Coogee | 26 |
| | | Coogee | Spearwood | Coogee | 27 |

## 3.3 Results

### *3.3.1 Environmental conditions: spring and summer*

The mean physico-chemical parameters measured for spring and summer are presented in Table 3.3. The pH ranged from near neutral to slightly alkaline (7.58-10.44), with a seasonal average of approximately 8. There was no significant difference in pH between the five sampling periods ($p$ = 0.059) even though pH values greater than 10 were recorded for Lake Joondalup, North Lake and Shenton Park in spring 2000. There was also no significant difference in the pH based on the lakes location on the SCP ($p$ = 0.115) or underlying dune system ($p$ = 0.142).

Average water temperatures for the five sampling periods were significantly different ($p$ < 0.001). Average water temperatures for spring were 23.2 ˚C (2000) and 22.1 ˚C (2001), with both years within the range of 20 ˚C to 26 ˚C. Water temperatures greater than 23˚C were recorded in summer with the highest water temperatures exceeding 30 ˚C. Average summer water temperatures were significantly higher than the average temperatures recorded in spring ($p$ < 0.05). An increase in mean water temperature over the three summers from 25.9 ˚C in 2000-01 to 28.3 ˚C in 2002-03 was significant ($p$ < 0.05). Average salinity for both seasons revealed the lakes to be fresh to marginally freshwater with the highest salinity (21.65 ppt) recorded at Lake Coogee. There were no significant differences in salinity between the seasons ($p$ = 0.386) or the sampling periods ($p$ = 0.937).

**Table 3.3.** The mean (± s.e.) and range of physico-chemical parameters measured from the urban lakes during spring and summer.

| | | pH | Temp (°C) | Salinity (ppt) | EC ($\mu S\ cm^{-1}$) |
|---|---|---|---|---|---|
| Spring 2000 (n = 18) | mean | 8.96 ± 0.17 | 23.2 ± 0.45 | 1.78 ± 0.69 | 3374 ± 1323 |
| | range | 7.77-10.44 | 20.2-26.9 | 0.08-12.27 | 190-23620 |
| Spring 2001 (n = 25) | mean | 8.48 ± 0.06 | 22.2 ± 0.28 | 1.63 ± 0.53 | 2661± 826 |
| | range | 7.95-9.18 | 20.07-25.08 | 0.08-11.96 | 150-18590 |
| Summer 2000-01 (n = 17) | mean | 8.58 ± 0.08 | 25.9 ± 0.31 | 2.19 ± 1.04 | 3673 ± 1631 |
| | range | 7.65-9.01 | 23.6-28.4 | 0.24-18.12 | 440-28380 |
| Summer 2001-02 (n = 24) | mean | 8.45 ± 0.09 | 28.05 ± 0.33 | 2.44 ± 0.94 | 4507 ± 1639 |
| | range | 7.58-9.29 | 24.2-32.04 | 0.12-21.65 | 270-37650 |
| Summer 2002-03 (n = 15) | mean | 8.65 ± 0.12 | 28.7 ± 0.3 | 1.71 ± 0.53 | 3280 ± 946 |
| | range | 7.7-9.41 | 26-30.1 | 0.19-8.04 | 400-14170 |

### *3.3.2 Ordination and clustering analysis*

The relationships between the study lakes based on the variables pH, temperature and EC were obtained using PCA ordination and clustering analysis, with Kruskall-Wallis tests confirming statistical differences ($p < 0.05$) between the main groups. The mean physico-chemical variables for the group identified by cluster analysis and the *p*-values obtained using Kruskall-Wallis and Mann-Whitney tests are presented in Appendix 3.2.

#### *3.3.2.1 Spring sampling period*

For both spring seasons (2000 and 2001), the first two principal components accounted for 78.5% and 85.5% of the total variance between the lakes (Table 3.4). For spring 2000, the first axis (PC1) explained 45.7% of the variance and showed a moderate negative relationship with pH (r = -0.689) and water temperature (r = -0.697). The second axis (PC2) showed a very strong relationship with EC (r = 0.978) and explained 32.9% of the variance. Ordination of the environmental data identified four groups, which showed the poor clustering of lakes based on location (Fig. 3.1a). Group 1, comprised mainly of the Western Suburb lakes, recorded significantly higher water temperatures (25.3-26.9 °C) and pH (9.48-10.28), revealing a close association between Hyde Park, Shenton Park and Jackadder Lake. Significantly lower water temperatures (20.6-22.2 °C) and pH (7.77-8.38) were shown in the lakes of Group 4. EC strongly influenced the ordination of the lakes along PC2, with significantly higher EC observed in the lakes of Group 3. This group consisted mainly of the coastal lakes of the South West Corridor (Coogee, Market Gardens and Manning) where EC ranged from 9430 EC to 23620 EC. Included in Group 3 were Yangebup Lake (1.13 ppt; 2360 EC), Lake Thomsons (1.97 ppt; 3710 EC) and Lake Claremont (1.77 ppt; 3490 EC).

For spring 2001, PC1 explained 54% of the total variance. Similar to spring 2000, PC1 showed a negative relationship with pH (r = -0.680) and water temperature (r = -0.632). The 31.5% variation explained in PC2 was strongly related to EC (r = 0.903). Clustering analysis identified seven small groups with Lake Coogee an outlier due to higher salinity (11.96 ppt; 18.59 mS $cm^{-1}$). Significantly higher salinity/EC was also observed in the lakes forming Groups 1 and 2, which included

Mary Carroll Park, Lake Brearley, Lake Bungana, Manning Lake and Yangebup Lake. Although Lake Claremont also recorded higher salinity/EC (2.09 ppt, 3530 EC) it was included with Booragoon Lake in Group 3 due to lower water temperature and pH. In contrast, the lakes of Group 6 recorded higher water temperatures and pH. Lower EC was recorded in Neil McDougall Park (170 EC) and Emu Lake (150 EC) (Group 7). It can be observed on Fig. 3.1b the close association in environmental conditions between Lake Joondalup and Lake Goollelal, part of the Wanneroo Linear lakes. Similarites were also revealed between the Western suburb lakes located in Group 5 (Lake Monger and Shenton Park) and Group 6 (Perry Lakes, Hyde Park and Jackadder Lake).

**Table 3.4.** Coefficients calculated for the environmental variables measured during spring 2000 and 2001. The contribution of each variable to the PC axes is in bold.

| | **PC1** | **PC2** |
|---|---|---|
| 2000 | | |
| pH (log n+1) | **-0.689** | -0.185 |
| Temperature | **-0.697** | -0.100 |
| EC (log n+1) | 0.201 | **0.978** |
| Eigenvalue | 1.36 | 0.99 |
| % variance explained | 45.7 | 32.9 |
| | | |
| 2001 | | |
| pH | **-0.688** | 0.078 |
| Temperature | **-0.632** | 0.422 |
| EC (log n+1) | 0.355 | **0.903** |
| Eigenvalue | 1.62 | 0.94 |
| % variance explained | 54 | 31.4 |

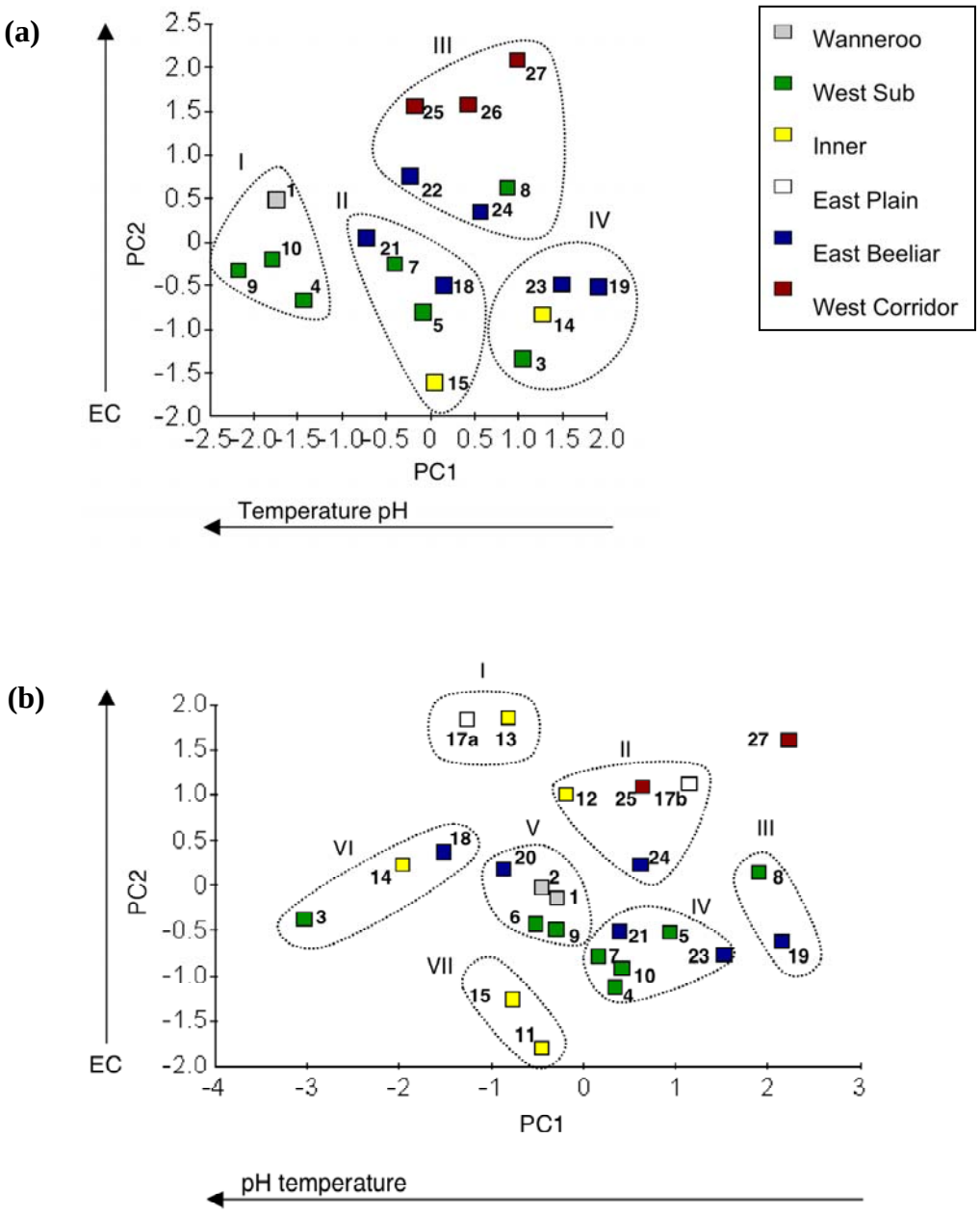



**Figure 3.1.** Principal Component Analysis plots for the lakes sampled in **(a)** spring 2000 and **(b)** spring 2001 showing the environmental parameter associated with PC1 and PC2. Symbols indicate the wetland groups defined by Arnold (1990).

#### *3.3.2.2 Summer sampling period*

The PCA coefficients determined for the summer environmental variables are presented in Table 3.5. The upper Canning River was sampled during all three summers and included in the PCA as a comparison to the lakes. Both Market Garden and Manning Lake was excluded from the summer 2000-01 and 2001-02 PCA analyses due to lack of sufficient water level and consequent lack of data throughout summer. Environmental data were only collected in December and are presented in Table 3.6. In early summer, both lakes are slightly alkaline, hyposaline with moderate water temperatures. For all summer collections, the variation between the lakes could be explained by the first and second principle component axis (PC1 and PC2).

For the summer 2000-01, PC1 and PC2 explained 83% of the total variance. PC1 showed a strong positive relationship with water temperature (r = 0.739), explaining 46.8% of the variance. PC2 explained 33.6 % of the variance and showed a positive relationship with pH (r = 0.613) and EC (r = 0.788). Clustering analysis of the ordination scores identified four groups with Blue Gum Lake an outlier due to the higher mean water temperature of 28.4 ºC. Significantly higher EC and lower water temperatures were observed in Lake Coogee and Lake Claremont (Group 1). Lower EC and higher water temperatures were recorded in Lake Goollelal and Hester Park (Group 4).

The majority of lakes sampled in summer 2000-01 clustered into Group 3, comprised mainly of the lakes of the Western Suburbs and East Beeliar lakes. From this grouping the similarities in environmental conditions between the Canning river sites and the lakes of the Western Suburbs was evident. Hierarchical clustering analysis identified the nearest neighbour to the Kent Street Weir and Liege Street as Hyde Park, Jackadder Lake and Lake Gwelup. For pH, Hester Park and Lake Goollelal (Group 4) recorded significantly lower values (7.26 and 7.65, respectively), while Forrestdale Lake and Lake Joondalup (Group 2) had significantly higher pH (8.9 and 9.01, respectively). Interestingly these two groups separated the two Wanneroo Linear lakes, Goolellal and Joondalup, and also showed a relationship between Lake Joondalup and Forrestdale Lake.

**Table 3.5.** Coefficients calculated for the measured environmental variables measured during summer. Variables with the main contribution to each component (PC) are in bold.

| | **PC1** | **PC2** |
|---|---|---|
| 2000-01 | | |
| pH (log n+1) | 0.553 | **0.613** |
| Temperature | **0.739** | -0.049 |
| EC (log n+1) | -0.384 | **0.788** |
| Eigenvalue | 1.4 | 1.09 |
| % variance explained | 46.8 | 36.2 |
| 2001-02 | | |
| pH (log n+1) | 0.365 | **0.909** |
| Temperature | **-0.681** | 0.112 |
| EC (log n+1) | **-0.635** | 0.402 |
| Eigenvalue | 1.33 | 0.96 |
| % variance explained | 44.4 | 32 |
| 2002-03 | | |
| pH | **0.709** | -0.063 |
| Temperature | -0.052 | **0.989** |
| EC (log n+1) | **0.703** | 0.136 |
| Eigenvalue | 1.44 | 1.01 |
| % variance explained | 48 | 33.6 |

**Table 3.6.** The physico-chemical parameters measured from Market Garden and Manning Lake during December 2000 and 2001.

| | **pH** | **Temp (°C)** | **Salinity (ppt)** | **EC (mS cm$^{-1}$)** |
|---|---|---|---|---|
| Manning Lake | | | | |
| 8/12/00 | 8.81 | 25.7 | 5.24 | 8.68 |
| Market Gardens | | | | |
| 8/12/00 | 8.57 | 24.6 | 8.19 | 13.33 |
| 18/12/01 | 8.2 | 25.5 | 8.33 | 15.32 |

For the summer 2001-02, Lake Coogee was omitted from the PCA as only one set of summer measurements were obtained. In early January 2002, a pH of 8.26, water temperature of 29 ºC, salinity of 21.65 ppt (EC 37.65 mS cm$^{-1}$) was recorded. For the PCA, the first two axes explained 76.4% of the cumulative variance. PC1 explained 44.4% of the total variance with a negative relationship with water

temperature (r = -0.681) and EC (r = -0.635). PC2 explained 32% of the variance with a very strong positive relationship with pH (r = 0.909).

The plotted PC scores revealed four groups with significant differences between the groups only explained by pH. Group 1 had significantly higher pH (8.64-9.29) while Group 4, which included the Kent Street Weir, recorded lower pH values (7.58-8.07). Mary Carroll Park (site 17) was identified as an outlier due to higher EC and water temperature (> 31 ºC, > 5 ppt). With the exclusion of Mary Carroll Park, the lakes of Group 2 recorded significantly higher mean salinity (mean 2.14 ppt, range 0.64-4.27 ppt). This group included lakes Joondalup, Claremont, Brearley and Bungana. As shown in the summer 2000-01 PCA, a close association was observed between the Western Suburban lakes Herdsman, Jackadder, Gwelup, Shenton Park and Hyde Park (Group 3).

For summer 2002-03, only a small selection of lakes was sampled, comprised mostly of lakes from the Inner Central Suburban Area and East Beeliar Regional Park. PC1 and PC2 explained 81.6% of the cumulative variance. PC1 showed strong positive relationships with pH (r = 0.709) and EC (r = 0.703), explaining 48% of the total variance. PC2 explained 33.6% of the variance with a very strong positive correlation with water temperature (r = 0.989).

Clustering analysis identified four groups with Lake Joondalup an outlier due to higher pH (9.35) and lower water temperature (26 ºC). Shenton Park and Emu Lake had significantly lower EC and pH (Group 1), while higher EC and pH were recorded in the lakes forming Group 3, which included Mary Carroll Park, Yangebup Lake, Lake Brearley and Lake Claremont. The Canning River (Kent Street), though included in Group 3, separated out from the lakes due to a higher mean water temperature of 31 ºC. Group 2, consisting of lakes in the inner suburban area and in close proximity to the rivers (Tomato Lake, Neil McDougall Park, and Blue Gum Lake), also recorded significantly higher water temperatures (29.6-30.1 ºC). Significantly lower mean water temperatures were associated with Group 4 (27.3-28.4 ºC).

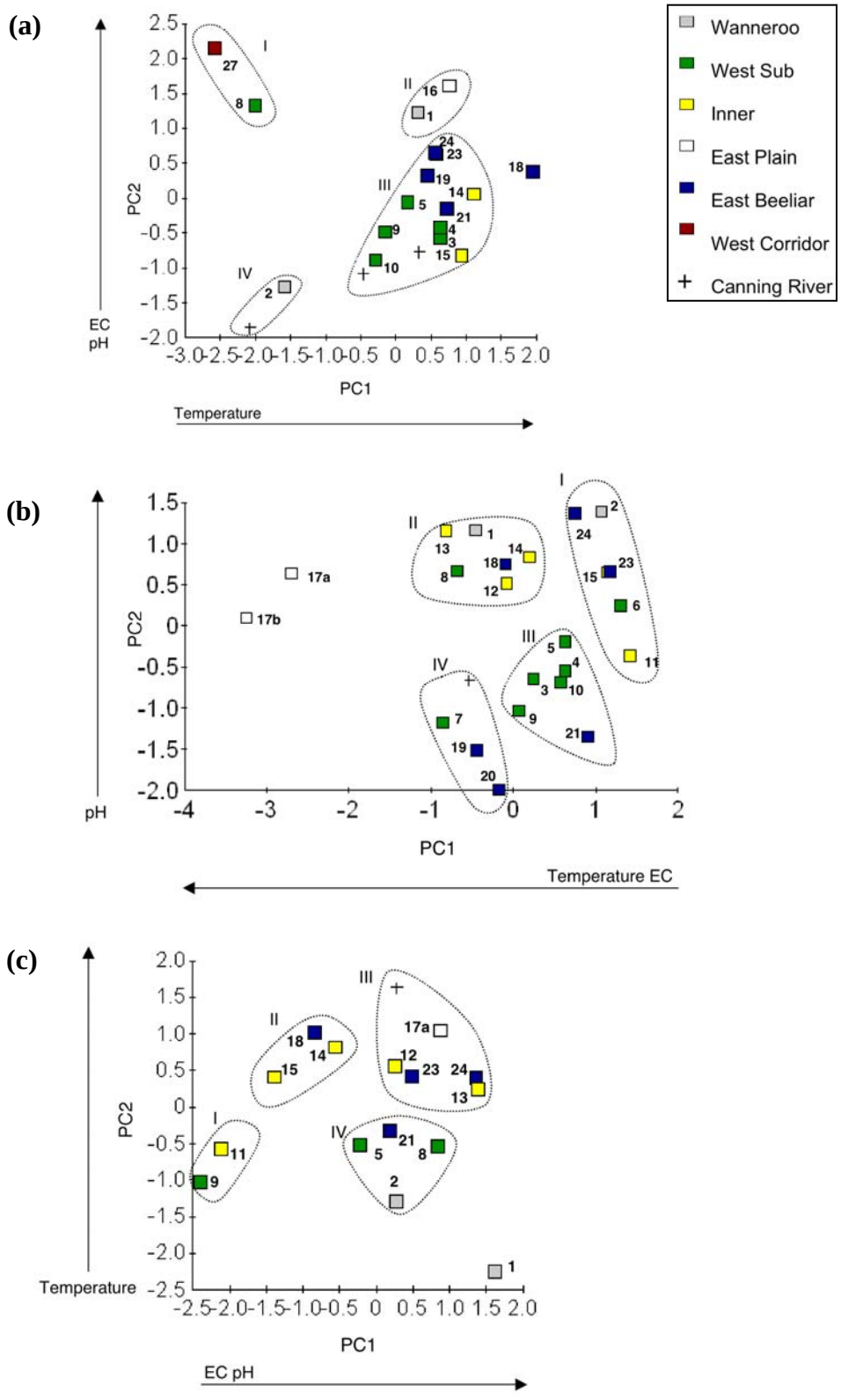



**Figure 3.2.** Principal Component Analysis plots for the lakes sampled in **(a)** summer 2000-01, **(b)** summer 2001-02 and **(c)** summer 2002-03 showing the environmental parameter associated with PC1 and PC2. Symbols indicate the wetland groups defined by Arnold (1990).

#### *3.3.2.3 Overall comparison of sites*

Combining the spring and summer data sets allowed the temporal changes in the physico-chemical data to be investigated (Fig. 3.3). The two principal components accounted for 70.4% of the total variability in the spring and summer data combined, with PC1 explaining 36.8.1% of the variance. Water temperature and EC were the variables showing a strong positive correlation with PC1 with a coefficient of 0.708 and 0.706, respectively. PC2 showed a very strong relationship with pH ($r = 0.968$).

The two dimensional PCA plot showed no visual grouping of sites based on the consanguineous groupings (location) (Fig. 3.3a). The exception was the wetlands of the East Coastal Plain and South West Corridor, which separated out due to higher EC. The spring and summer conditions experienced by the East Coastal Plain and South West Corridor wetlands distinguishes them from the other lakes used in this study (Fig. 3.3b). An overlap of the wetlands of the Western Suburbs, East Beeliar Regional Park and Inner Central Suburban Area was evident.

The similarities in spring and summer conditions are shown in Fig. 3.3b. The close arrangement of sites shows the summer data sets to be more strongly related. However, there are small number of sites from the Inner Suburban Area and Western Suburbs that deviated from the main site group based on their summer conditions.

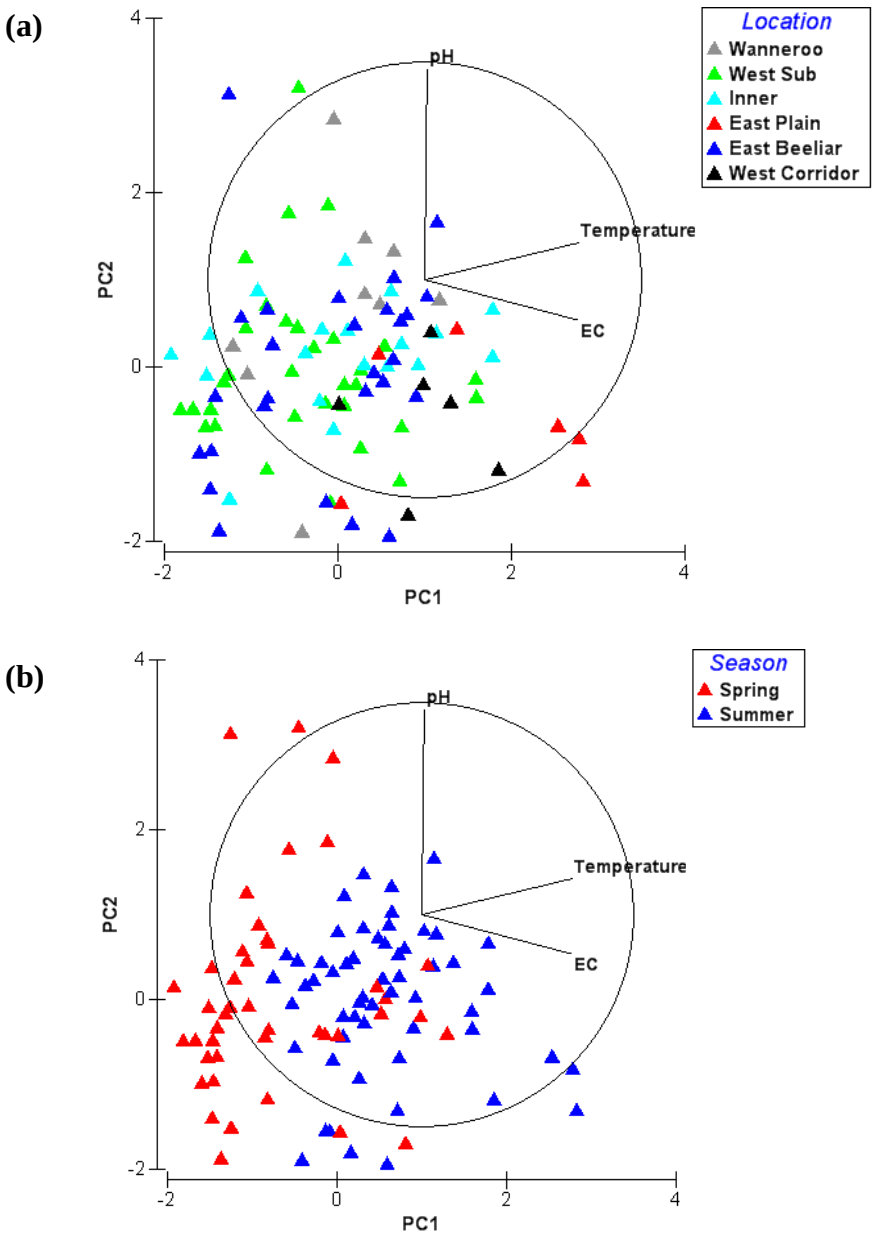



**Figure 3.3.** Principal Component Analysis plots for all sampling periods combined (n = 99) showing the physico-chemical parameters associated with PC1 and PC2. The vector lines indicate water temperature, EC and pH influenced the ordination of sites. Sites classified based on **(a)** location on the SCP and **(b)** season.

## 3.4 Discussion

### *3.4.1 Physico-chemical environment of the SCP lakes*

With the mean summer salinity being less than 3 ppt, the majority of lakes used for this study were fresh to marginally fresh and therefore capable of supporting nuisance cyanoprokaryota blooms. However, salinity is not a variable that directly influences the formation of blooms, but can be a barrier to the development and persistence of many freshwater nuisance bloom-forming species, such as *Microcystis* and *Anabaena*. Robson and Hamilton (2003) reported optimal growth in isolates of *Microcystis aeruginosa* in salinity up to 4 ppt, while less than 2.5 ppt will stimulate the germination of *Anabaena circinalis* akinetes (Baker and Bellifemine 2000). Although at this salinity the potential of *A. circinalis* germlings to develop into trichomes is reduced. Salinity of 1 ppt proved more suitable for cell growth (Baker and Bellifemine 2000).

*Microcystis* and *Anabaena* have already been confirmed as problematic in some Western Australian wetlands, yet the extent of their distribution is unknown, especially regarding the urban lakes used in this study. Because the growth of these freshwater cyanoprokaryotes depends on their salt tolerance, the formation of *Microcystis* and *Anabaena* blooms in the study lakes classified as hyposaline, is unlikely. A community comprised of halotolerant freshwater forms, such as *Nodularia*, *Oscillatoria* and *Anabaenopsis*, can be expected (Williams 1998). *Anabaenopsis* in particular demonstrates moderate salinity tolerance through maintaining a constant growth rate in salinity ranging from 2 to 20 ppt (Moisander *et al.* 2002). This permits a distribution in freshwater, hyposaline and estuarine environments (Huber 1980; Baker 1991; Cronberg and Annadotter 2006).

The pH values recorded during the present study were within the range reported by Schmidt and Rosich (1993) (pH 6-10), and within the ANZECC and ARMCANZ (2000) guideline for natural freshwaters (6.5-8). These alkaline conditions reflect the location of the study lakes with 74% situated on the Tamala limestone sands of the Spearwood Dune System. None of the lakes recorded a mean pH value less than 7, which is expected for the lakes situated on the Bassendean Dune System. Schmidt and Rosich (1993) establish that the high pH (> 7), coupled with nutrient enrichment,

render the wetlands located near the coast on the Spearwood Dune System more prone to cyanoprokaryota blooms, compared to the more acidic wetlands of the Bassendean Dune System. In an environment of high pH and high alkalinity, nuisance cyanoprokaryota are able to dominate and out-compete eukaryotic phytoplankton because they are more efficient in uptaking carbon dioxide when it is present in low concentrations (Fay 1983; Paerl 1988; Shapiro 1997). Cyanoprokaryotes also use bicarbonate directly to support photosynthesis, which is the main form of inorganic carbon at pH 6.5 to 10.5 (Fay 1983; Schmidt and Rosich 1993). Because the study sites were positioned on both dune systems, it would be of interest to see the magnitude of blooms in the wetlands of the Inner Central Suburban Area, which reside entirely on the Bassendean Dune System.

Average water temperatures in the lakes studied ranged from 20 ˚C to 32 ˚C, which is within the range supporting optimal growth (Fay 1983; Robarts and Zohary 1987; Paul 2008), bloom formation (Nalewajiko and Murphy 2001**;** Rahman *et al.* 2005**;** de Figueiredo *et al.* 2006**)** and toxin content (Sivonen and Jones 1999). Any variation in water temperature between sites, as seen in the summer 2002-03 and summer 2000-01 ordinations, are due to differences in size, depth and mixing regime. The SCP lakes are small and shallow (mean depth < 2 m), and can be defined as discontinuous warm polymictic, experiencing intermittently or continuously turbulence (Schmidt and Rosich 1993). Having the water column alternate between states of mixing then followed by stable conditions, allows for the formation of surface blooms (Schmidt and Rosich 1993). This is due to the turbulence suspending the non-buoyant and gas vacuolated cells throughout the water column, when the turbulence has stopped, the gas vacuolated species can migrate upwards to form a surface bloom (Walsby 1987). Surface blooms near shorelines are often associated with cyanotoxin poisoning in humans and pets, thereby presenting a threat in the study lakes.

As a comparison to the freshwater study lakes, the upper Canning River was included in this investigation because the physico-chemical environment and phytoplankton communities of the upstream sections have been compared to a shallow freshwater lake, especially the hypertrophic wetlands of the Beeliar Regional Park (Vincent 1995; John and Kemp 2006). Nearest-neighbour clustering showed a close association between the Canning River sites and the southern lakes North,

Piney and Booragoon, supporting this observation. However, the physico-chemical data recorded in the northern lakes Goollelal, Gwelup, Perry and Hyde Park better resembled the upper Canning River. Of these Lake Goollelal, a Wanneroo Linear Lake located approximately 20 km north of the Perth CBD, was strongly related to the Canning River (Hester Park) in pH (7), water temperature (24 ˚C) and salinity (0.6 ppt). Cyanoprokaryotes form a major portion of the spring-summer phytoplankton in the Canning River (Vincent 1995; Thompson *et al.* 2003), with toxic blooms of *Anabaena* and *Microcystis* during summer rendering the river unsuitable for recreational use.

### *3.4.2 Classification of wetlands*

The PCA groups generated based on pH, temperature and EC did not show a relationship with the conventional consanguineous classification defined by Arnold (1990) and Semeniuk (1996), with the poor grouping of lakes based on location and proximity to each other. A clear example of the dissimilarity between lakes within the same wetland group were Lake Bungana and Lake Brearley, which recorded similar pH and water temperatures but differed in salinity with Lake Bungana being marginally fresh and Lake Brearley classified as hyposaline. Davis *et al.* (1993) has described the wetlands of the SCP as a dynamic and chaotic system in that each may vary in many ways from its nearest neighbour, so such differences are expected. For this reason water quality objectives should be established on an individual basis to reflect the heterogeneous nature of the wetlands.

Only the wetlands from the Western Suburbs and the East Beeliar Regional Park clustered into the same PCA groups, this being more evident in the spring ordination plots (Fig. 3.1) and the combined ordination plot (Fig. 3.3). The summer ordination plots showed great variation between study sites, mostly explained by salinity and EC, demonstrating the changes that can arise over summer due to depth and evaporative salt concentration. PCA identifying similarities between the wetlands of the Western Suburbs and East Beeliar Regional Park is most likely due to a large number of study sites being located in these two regions and adequate water being present throughout the sampling period allowing for routine sampling. However, the current study only focused on three environmental variables that might be too limited

in defining the overall character of a wetland.

The variation in environmental conditions between the study lakes was strongly influenced by salinity and EC, which produced the highest coefficients ($r > 0.9$) in both spring periods and summer 2000-01. The combined PCA analysis, compiled from all data sets, also demonstrated the influence of salinity and EC on the ordination of sites. This result was not observed in summer 2001-02 because lakes with low water levels were omitted from the PCA. This included the wetlands of the South West Corridor, which experience higher salinities than other wetlands on the SCP due to their closeness to the coast. Only a limited number of samples could be collected from this region due to low water levels, which did not sufficiently represent summer conditions. In contrast, only a selected number of lakes were visited in summer 2002-03 to collect concentrated algal samples for toxicity determination. As a result, pH and water temperature produced the strongest coefficients for 2001-02 and 2002-03, respectively. Nevertheless, PCA did identify dissimilarities between lakes sampled and revealed which sites could be considered outliers based on higher salinity, EC and water temperature.

### *3.4.3 Comparison of study sites within consanguineous groups*

The three coastal wetlands of the South West Corridor used in this study recorded the highest salinity and EC. Lake Coogee was the only saline lake used in this study (mean 12-21 ppt; maximum 37.65 mS $cm^{-1}$), which was expected, as it is a perennial saline swamp (Seddon 1972) that has shown an increase in salinity due to uprising of the salt interface, evaporation and recirculation of salts (Commander 1994). The other two lakes of the South West Corridor, Manning Lake and Market Garden, located 2 to 4 km north of Lake Coogee were hyposaline, the result of the salt wedge that extends one kilometre inland from the coast. All three coastal wetlands show seasonal drying, which coupled with the high salinity and EC could limit the formation of cyanoprokaryota blooms during the summer months.

The spring 2000 and summer 2000-01 PCA analysis showed Lake Claremont to be similar in salinity to the South West Corridor lakes, making Lake Claremont unlike the other wetlands of the Western Suburbs. Water levels in Lake Claremont have

been decreasing since 1975 and evaporative concentration during summer has produced salinity levels of up to 8.9 ppt in 1981 and 16 ppt in 1988 (Haynes *et al.* 1998), higher than the maximum 12.03 ppt (2.24 mS $cm^{-1}$) recorded during the current study (Appendix B). The summer 2000-01 ordination plot grouped Lake Claremont with Lake Coogee based on this higher salinity. Similar to the wetlands of the South West Corridor, Lake Claremont is a seasonal swamp that also suffers from saltwater intrusion (Haynes *et al.* 1998).

The Eastern Coastal Plain wetlands located at the base of the Darling Scarp also measured higher EC with 22.67 mS $cm^{-1}$ recorded in Mary Carroll Park in February 2003. This is higher than the EC recorded by Rodda and Deeley (1990) (1-7 mS $cm^{-1}$), Lund *et al.* (1998) (7 mS $cm^{-1}$) and Lund *et al.* (2000) (1.35-1.40 mS $cm^{-1}$), showing how this lake is becoming more saline, most likely the result of declining water levels. The salinity of Forrestdale Lake was also dependent on the declining water level and therefore was hyposaline. With an annual maximum water depth of 0.9 m, Forrestdale Lake will dry by April during wet years and by January in the dry years (CALM 2005a). Similar to the wetlands of the South West Corridor, the season drying and subsequent rise in salinity may save Mary Carroll Park and Forrestdale Lake from severe cyanoprokaryota blooms.

Similarity between Forrestdale Lake and Lake Joondalup was evident in the summer 2000-01 ordination. These two lakes represent the northern-most and southern-most sites used in this study, not located in the same consanguineous group. Both lakes are large, seasonal and differed from the other study sites due to higher pH. Lake Goollelal is the nearest wetland to Lake Joondalup, although the similarity between these two sites was only observed in spring 2001. During summer, lower values for salinity, EC, water temperature and pH were recorded from Lake Goollelal, which related this site to wetlands further south including the upper Canning River (2000-01), Yangebup Lake (2001-02), Herdsman Lake (2002-03) and North Lake (2002-03), which have all experienced nuisance cyanoprokaryota blooms. To the author's knowledge, no blooms have been documented in Lake Goollelal but environmental conditions prove favourable for their formation.

Neighbouring Lake Brearley and Lake Bungana differed in salinity and EC, but

recorded similar pH values and water temperatures. The salinity of Lake Brearley was comparable to Mary Carroll Park (spring 2001), Lake Joondalup (summer 2001-02) and Lake Claremont (summer 2001-02). This can be explained by the close proximity of Lake Brearley to the Swan River and the underlying hyposaline groundwater. The mean summer salinity of Lake Bungana was marginally fresh (1.38ppt in 2002 and 1.69 ppt in 2003) and comparable to lakes Booragoon, Joondalup, Yangebup and Thomsons, even though this lake is also located next to the Swan River. Within the scope of this study, there is no conclusive reason why there was such a difference in salinity between these two adjacent lakes. The variation may be the result of freshwater drainage from the surrounding housing estate into Lake Bungana and not Lake Brearley, but this is not confirmed. Based on this result, the timing, duration and composition of cyanoprokaryota blooms within these two lakes can be expected to be different.

The majority of lakes sampled were located in the East Beeliar Regional Park or Western Suburbs, which allowed for a larger sample size from these regions to be compared. For this reason similarities within these wetland groups were more apparent (Fig. 3.3). The wetlands of the Western Suburbs showed similar pH, water temperature, salinity and EC with only Claremont Lake repeatedly separating from the main group due to higher salinity. Regarding the wetlands of the East Beeliar Regional Park, Yangebup Lake stood out for having higher salinity and EC. The spring ordination grouped Yangebup Lake with Mary Carroll Park, Lake Brearley, Lake Claremont and the lakes of the South West Corridor, which can all be classified as hyposaline during summer. However for the summer periods, Yangebup was closely associated with neighbouring Bibra Lake. The next stage in this research is to use these results obtained to investigate the occurrence of cyanoprokaryota blooms in the study lakes, as well as search for a distribution pattern that can be explained by the physico-chemical data collected and the PCA wetlands groups that were defined.

## 3.5 Conclusions

The urban lakes of the SCP experience spring and summer conditions that are conducive to the formation of cyanoprokaryota blooms. Arranged in consanguineous groups based in hydrology, the lakes within the groups rarely exhibited comparable water quality conditions. Overall there were no significant differences between the spring and summer environmental data. Between site differences were explained by the three variables measured (water temperature, pH and EC), whereas between consanguineous group differences were based on salinity and EC with lakes located on the coast and further inland at the base of the Darling Scarp being more saline. In addition to the wetlands of the South West Corridor and East Coastal Plain, Lake Claremont and Lake Brearley were also recognised as being hyposaline. This distinguished these sites from the other lakes in their consanguineous group, which were fresh. These conditions are likely to favour salt tolerant species, although nuisance blooms are unlikely if water levels in the seasonal wetlands remain low throughout the warmer months. The remaining lakes used in this study were either fresh or marginally fresh. When coupled with nutrient enrichment and stable conditions, nuisance and potentially toxic cyanoprokaryota blooms can develop. The occurrence and distribution of these blooms are explored further in Chapter 4. Similarities between the Canning River and the urban lakes were confirmed, with the upstream sites closely resembling both the nearby southern lakes, lakes located in the Western Suburbs and also Lake Goollelal. This outcome will allow for comparisons to be made between the upper Canning River and the freshwater urban lakes in relation to the cyanoprokaryote bloom.

**Appendix 3.1.** List of lakes visited during each spring and summer sampling period.

| Spring 2000 | Summer 2000-01 | Spring 2001 | Summer 2001-02 | Summer 2002-03 |
|---|---|---|---|---|
| Lake Joondalup | Lake Joondalup | Lake Joondalup | Lake Joondalup | Lake Joondalup |
| Lake Gwelup | Lake Goollelal | Lake Goollelal | Lake Goollelal | Lake Goollelal |
| Jackadder Lake | Lake Gwelup | Lake Gwelup | Lake Gwelup | Herdsman Lake |
| Herdsman Lake | Jackadder Lake | Jackadder Lake | Jackadder Lake | Lake Claremont |
| Perry Lakes | Herdsman Lake | Herdsman Lake | Herdsman Lake | Shenton Park |
| Lake Claremont | Lake Claremont | Lake Monger | Lake Monger | Emu Lake |
| Shenton Park | Shenton Park | Perry Lakes | Perry Lakes | Lake Bungana |
| Hyde Park | Hyde Park | Lake Claremont | Lake Claremont | Lake Brearley |
| Tomato Lake | Tomato Lake | Shenton Park | Shenton Park | Tomato Lake |
| Neil McDougall Park | Neil McDougall Park | Hyde Park | Hyde Park | Neil McDougall Park |
| Blue Gum Lake | Forrestdale Lake | Emu Lake | Emu Lake | Mary Carroll |
| Booragoon Lake | Blue Gum Lake | Lake Bungana | Lake Bungana | Blue Gum Lake |
| North Lake | Booragoon Lake | Lake Brearley | Lake Brearley | North Lake |
| Bibra Lake | North Lake | Tomato Lake | Tomato Lake | Bibra Lake |
| Yangebup Lake | Bibra Lake | Neil McDougall Park | Neil McDougall Park | Yangebup Lake |
| Thomson Lake[a] | Yangebup Lake | Mary Carroll | Mary Carroll | |
| Manning Lake | Thomson Lake | Blue Gum Lake | Blue Gum Lake | |
| Market Gardens | Lake Coogee | Booragoon Lake | Booragoon Lake | |
| Lake Coogee | | Piney Lakes | Piney Lakes | |
| | | North Lake | North Lake | |
| | | Bibra Lake | Bibra Lake | |
| | | Yangebup Lake | Yangebup Lake | |
| | | Manning Lake | Market Gardens | |
| | | Market Gardens | Lake Coogee | |
| | | Lake Coogee | | |

[a]Thomson summer 2000-01 value included in spring as only one sample was collected due to low water levels.

**Appendix 3.2.** Mean physico-chemical parameters for the groups and outliers identified by PCA and clustering analysis and the *p* values obtained from the Kruskal-Wallis and Mann-Whitney tests. *P* values < 0.05 considered significant (ns = not significant).

**(a) Spring 2000**

| | Mean | | | | Kruskal-Wallis Test | Mann-Whitney Test | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Group** | **I** | **II** | **III** | **IV** | | **I** | **II** | **III** | **IV** |
| pH | 9.84 | 9.04 | 8.89 | 8.06 | 0.007 | 0.009 | ns | ns | 0.004 |
| Temperature (ºC) | 25.9 | 23.15 | 22.80 | 21.11 | 0.02 | 0.009 | ns | ns | 0.021 |
| Salinity (ppt) | 0.41 | 0.49 | 4.71 | 0.38 | 0.008 | ns | ns | 0.001 | ns |
| EC (µS $cm^{-1}$) | 820 | 848 | 8976 | 847 | 0.008 | ns | ns | 0.001 | ns |

**(b) Spring 2001**

| | Mean | | | | | | | | Kruskal-Wallis Test | Mann-Whitney Test | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Group** | **I** | **II** | **III** | **IV** | **V** | **VI** | **VII** | **Site 27** | | **I** | **II** | **III** | **IV** | **VI** | **VII** |
| pH | 8.79 | 8.40 | 8.12 | 8.34 | 8.57 | 8.98 | 8.6 | 8.11 | 0.009 | ns | ns | 0.043 | 0.015 | 0.023 | ns |
| Temp (ºC) | 23.84 | 22.17 | 20.16 | 21.24 | 22.53 | 23.82 | 21.76 | 21.06 | 0.013 | 0.047 | ns | 0.007 | ns | 0.037 | ns |
| Salinity (ppt) | 3.63 | 2.46 | 1.46 | 0.4 | 0.45 | 0.32 | 0.09 | 11.96 | 0.007 | 0.037 | 0.013 | ns | ns | ns | 0.014 |
| EC (µS $cm^{-1}$) | 5940 | 4080 | 2480 | 682 | 766 | 573 | 160 | 18590 | 0.006 | 0.037 | 0.013 | ns | ns | ns | 0.007 |

Lake Coogee treated as an outlier and omitted from tests. Group V showed no significant differences.

**Appendix 3.2.** (*continued*)

**(c) Summer 2000-01**

| | Mean | | | | | Kruskal-Wallis Test | Mann-Whitney Test | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Group** | **I** | **II** | **III** | **IV** | **Site 18** | | **I** | **II** | **III** | **IV** |
| pH | 8.39 | 8.96 | 8.51 | 7.46 | 8.84 | 0.026 | ns | 0.034 | ns | 0.024 |
| Temperature (ºC) | 23.6 | 26.48 | 28.18 | 24.7 | 28.37 | 0.024 | 0.024 | ns | 0.054 | ns |
| Salinity (ppt) | 11.51 | 2.68 | 0.66 | 0.65 | 0.88 | 0.027 | 0.024 | ns | 0.018 | ns |
| EC (µS $cm^{-1}$) | 18295 | 4905 | 1213 | 1170 | 1570 | 0.028 | 0.024 | ns | 0.020 | ns |

Blue Gum Lake treated as an outlier and omitted from tests.

**(d) Summer 2001-02**

| | Mean | | | | | | | Kruskal-Wallis Test | Mann-Whitney Test | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Group** | **I** | **II** | **III** | **IV** | **Site 17a** | **Site 17b** | **Site 9** | | **I** | **II** | **III** | **IV** |
| pH | 8.95 | 8.74 | 8.21 | 7.77 | 7.95 | 8.23 | 8.35 | 0.001 | 0.005 | ns | ns | 0.004 |
| Temp (ºC) | 27.17 | 28.67 | 27.64 | 28.55 | 32.59 | 31.5 | 24.21 | ns | ns | ns | ns | ns |
| Salinity (ppt) | 0.69 | 2.14 | 0.45 | 0.78 | 5.97 | 6.97 | 0.13 | ns | ns | 0.01 | ns | ns |
| EC (µS $cm^{-1}$) | 1357 | 4073 | 1000 | 1560 | 11040 | 12960 | 270 | ns | ns | 0.01 | ns | ns |

Mary Carroll Park and Shenton Park treated as outliers and omitted from tests.

**Appendix 3.2.** (*continued*)

**(e) Summer 2002-03**

| | Mean | | | | | Kruskal-Wallis Test | Mann-Whitney Test | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Groups** | **I** | **II** | **III** | **IV** | **Site 1** | | **I** | **II** | **III** | **IV** |
| pH | 7.89 | 8.43 | 8.9 | 8.74 | 9.35 | 0.03 | 0.027 | ns | 0.034 | ns |
| Temperature (ºC) | 27.94 | 29.87 | 29.68 | 27.98 | 26 | 0.013 | ns | 0.043 | ns | 0.019 |
| Salinity (ppt) | 0.24 | 0.56 | 2.73 | 1.55 | 1.91 | 0.18 | 0.042 | ns | 0.01 | ns |
| EC (µS $cm^{-1}$) | 495 | 112 | 5113 | 3065 | 3620 | 0.023 | 0.041 | ns | 0.013 | ns |

Lake Joondalup treated as an outlier and omitted from tests.

# Chapter 4: Distribution of bloom-forming cyanoprokaryotes in the urban lakes of the Swan Coastal Plain

## 4.1 Introduction

Similarities between the study sites based on pH, water temperature, salinity, electrical conductivity (EC) and their grouping within consanguineous suites of wetlands were explored in the previous chapter. The focus of this chapter is the relationship between these abiotic factors and the distribution of the major-bloom forming species.

A fundamental characteristic of the cyanoprokaryotes is their ability to form water blooms - dense populations of planktonic of cells exceeding a threshold of 20 000 cells $mL^{-1}$ (Fay 1983; WHO 2003). Water blooms can cause a noticeable discolouration to the wetland and produce taste and odour compounds such as geosmin and methylisoborneol, producing an earthy, musty smell (Johnstone 1994; Jones and Korth 1995). Of greater concern is the ability of many bloom-forming species to synthesize neurotoxins and hepatotoxins that are harmful to wide range of biota (Saker *et al.* 1999b; Carmichael *et al.* 2001; Lugomela *et al.* 2006). In Australia, cyanoprokaryota blooms are a serious water quality problem and have a significant impact on both drinking water and recreational water use (Burch 2001). To manage the problem, the species, associated toxins and the factors that promote cyanoprokaryota growth need to be understood (Steffensen *et al.* 1999).

Water blooms are produced by the gas vacuolated species belonging to the orders Chroococcales, Nostocales and Oscillatoriales. Gas vacuoles regulate buoyancy allowing for the diurnal vertical migration of cells, which helps maintain a position in the water column that is optimal for both growth and survival. Excessive cell buoyancy is responsible for cell accumulation at the water surface producing a noticeable floating scum (Paerl and Ustach 1982). Surface scums represent a 100 to 1000-fold increase in cell density and pose a high risk of poisoning if the dominant species are toxin producers (WHO 2003). In addition to the buoyancy control,

*Microcystis*, *Anabaena* and *Aphanizomenon* are considered surface-bloom ecostrategists because they form large biomass units, such as colonies or aggregates of filaments, with enhanced sinking or floating abilities (Oliver and Ganf 2000). As a result, these genera are the main cyanoprokaryotes that form noxious blooms in lakes, rivers and estuaries throughout the world.

Geographical differences exist between the species forming nuisance blooms in temperate and tropical regions in Australia. Tropical regions are characterised by blooms of *Cylindrospermopsis*, *Limnothrix* and *Planktolyngbya*, while temperate regions, which includes the Swan Coastal Plain (SCP), Western Australia, experience blooms of *Anabaena* and *Microcystis* (Baker and Humpage 1994; Hosja and Deeley 1994; Bowling and Baker 1996; Bormans *et al.* 2004; Rahman *et al.* 2005). Although *Anabaena* and *Microcystis* have been reported in the freshwater lakes of the SCP (Davis *et al.* 1993), the dominant species and associated toxicity have not been thoroughly studied.

In temperate climates, cyanoprokaryota growth and subsequent bloom formation follows a seasonal pattern of dominance in late spring and summer (Scheffer *et al.* 1997). The warmer months essentially provide calm, clear, nutrient enriched conditions that sustain blooms and allow buoyant migration to the water surface (Oliver and Ganf 2000). However, this time of the year is also the period of highest demand for water. Therefore correctly identifying toxic species and monitoring cell populations is necessary to ensure safe recreational water use.

Unfortunately in Australia most problem-causing cyanoprokaryota blooms remain undocumented (Bowling 1994), including those of the SCP wetlands. Cyanoprokaryota blooms in the Swan-Canning Estuary and Peel–Harvey Estuary have been studied (Huber 1980; John 1987; Vincent 1995; Hale and Paling 1999; Atkins *et al.* 2001; Vincent 2001; Robson and Hamilton 2003; Thompson *et al.* 2003; Orr *et al.* 2004), but there are only a few documented reports of blooms in freshwater urban lakes.

Since the early 1970s, both North Lake and Joondalup Lake have experienced odorous blooms of *Microcystis* and *Anabaena* (Congdon and McComb 1976; Gordon *et al.* 1981; Bayley *et al.* 1989) and in the early 1980s blooms of *Microcystis aeruginosa* were first reported in Lake Monger (Gordon *et al.* 1981). Gordon *et al.* (1981) predicted future blooms in Lake Joondalup if nutrient enrichment continued. Consequently, for all three lakes, blooms still occur more than 20 years later (Lund and Davis 2000). Elsewhere, Hosja and Deeley (1994) documented spring-summer blooms of *Microcystis* and *Anabaena* in Forrestdale Lake, Mary Carroll Park, Blue Gum Lake and Booragoon Lake. However these reports are sporadic and provide limited information on the distribution, ecology and morphology of *Microcystis* and *Anabaena* in SCP wetlands.

This section of the study deals specifically with the occurrence and distribution of cyanoprokaryota blooms in the urban lakes of the SCP. The objective was to collect data on cyanoprokaryota blooms and associated physico-chemical data from a wide range of sites across the metropolitan area, to identify the common bloom-forming species and their spatial distribution throughout this region. The sites were sampled over three years to explore the temporal distribution of blooms and identify the waterbodies that are experiencing repeated blooms. With this information, the species community composition and the environmental parameters associated with the distribution of the common species were investigated.

## 4.2 Materials and Methods

### *4.2.1 Sample collection*

A total of 27 lakes were sampled from 2000 to 2003 as described in section 3.2.1. A reference map showing the location of each site was presented in Chapter 2. In addition, 21 lakes were selected and sampled in autumn-winter 2001 to observe any blooms during the colder months (list of sites is provided in Table 4.4). Environmental parameters were measured *in situ* (see section 3.2.1 for a detailed description of this procedure). Concentrated phytoplankton samples used for taxonomic identification were collected using a 25-µm phytoplankton net (net

samples) and preserved in Transeau's solution (6:3:1 Water: Ethanol: Formalin). Water samples were hand collected and preserved using Transeau's solution to be used for cell counts. All samples were collected near the shore.

### *4.2.2 Phytoplankton identification and abundance measurements*

The phytoplankton were identified to the species level as described in section 7.2.1 using taxonomic guidelines and publications (refer to section 7.2.1 for complete reference list). Cell counts were performed using a calibrated Lund Cell at 400x magnification. A small volume of water was pipetted into the chamber and allowed to stand for a few minutes. Counts were made from 30 fields of view, completed only once when cell numbers in the sample was visually high, and in triplicate when there was a lesser concentration. A count of 30 fields can reveal 90-95% of the species present (Wetzel and Likens 1979). The average count per field was multiplied by the conversion factor to express the count as cells $mL^{-1}$. For colonial species (*Microcystis*), the number of cells per colony was estimated from the area of the colony divided the average diameter of a cell. For filamentous species, where the cell cross-walls were easily recognisable, the number of cells per trichome was counted. Otherwise, the length of the trichome was measured then divided by the average length of the cells. Cell counts are presented in Appendix C (CD-ROM).

### *4.2.3 Statistical analysis*

The physico-chemical and species data were grouped into sampling periods 2000-01, 2001-02 and 2002-03. Because there were no significant differences in pH, water temperature or salinity between spring with summer (refer to section 3.4.1), both seasons were combined for each site. Individual data sets from a lake have only been referred to where necessary. The physico-chemical data were tested for normality and homogeneity of variance, with salinity and EC requiring log (n+1) transformation.

Two-way indicator species analysis (TWINSPAN) using the multivariate statistical program PC-ORD (version 3.17) was used to identify lakes with similar species assemblages. The analysis was based on the presence (1) and absence (0) of species

and was performed separately on the three sampling periods. Species detected in only one site were excluded. The Canning River (all sampling locations combined) was also included. Due to salinity and EC still showing a skewed distribution, even after transformation, non-parametric tests were used to search for differences in environmental conditions between the TWINSPAN groups. The relationship between physico-chemical data and the first divisional TWINSPAN group were analysed using the Mann-Whitney Test, with the succeeding divisions tested using the Kruskal-Wallis Test. Both analyses were performed using SPSS 11 for Mac OS X. Clustering analysis was used to compare the species community over all three sampling periods. A dendrogram based on presence/absence species data was generated using the Bray-Curtis similarity measure.

## 4.3 Results

### *4.3.1 Major bloom-forming species*

A total of 24 species were identified, representing six genera (Table 4.1). The morphological data used to identify each species, including micrographs, are presented in Chapter 7. No cyanoprokaryotes were detected in the samples from the South West Corridor wetlands, Manning Lake and Market Garden. All other sites contained nuisance bloom-forming cyanoprokaryota in at least one stage of the study. A high number of species (6-8 in total) were collected from Lake Joondalup, Tomato Lake, McDougall Park, Blue Gum Lake, North Lake, Bibra Lake and Mary Carroll Park. In contrast, only one species was found in Forrestdale Lake (*Planktothrix planctonica*) and Thomson Lake (*Oscillatoria sancta*). However, this only represented one summer period (2000-01).

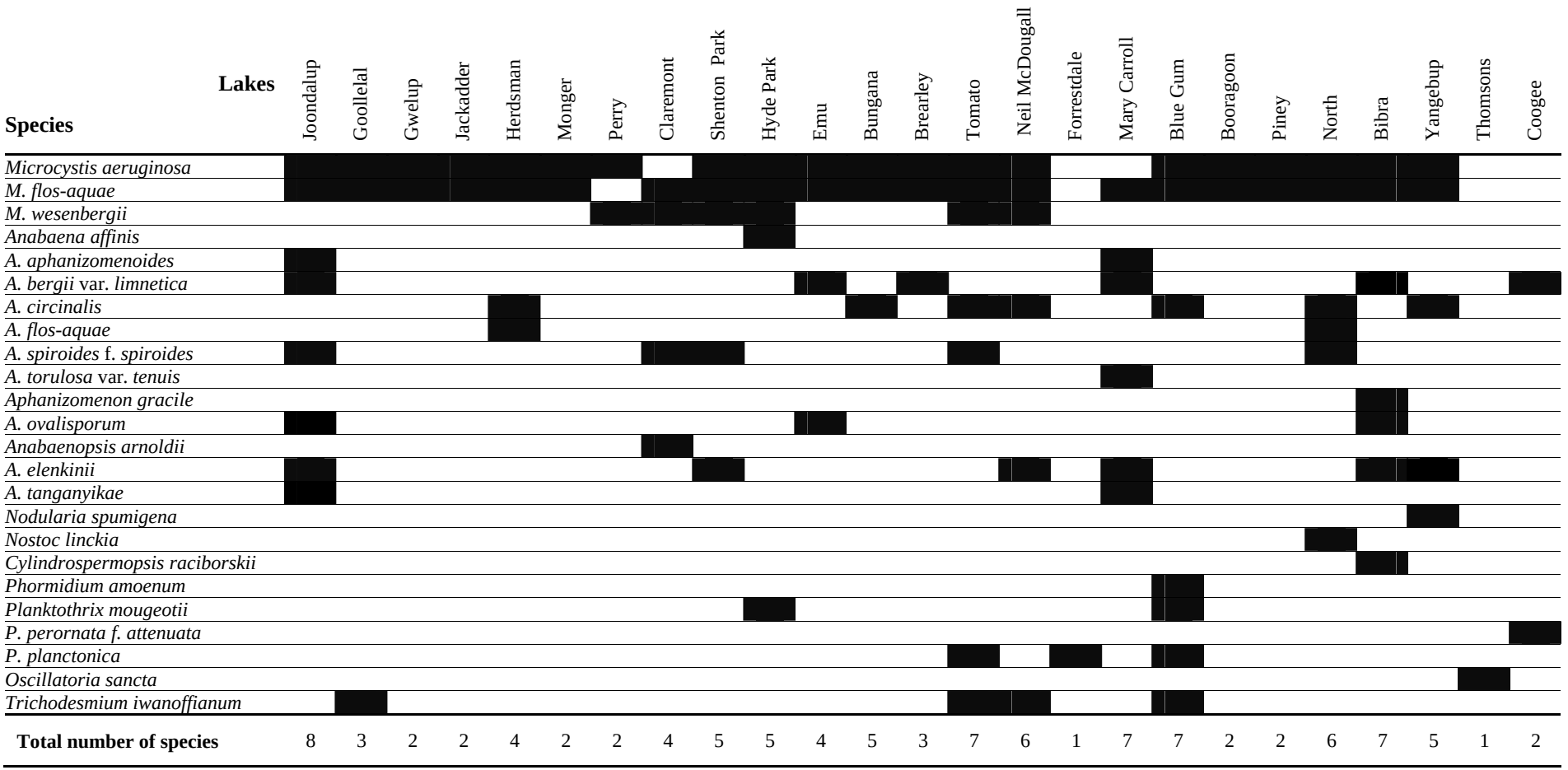
Colonial *Microcystis* was the most common genus, found in all but three lakes (Coogee, Forrestdale and Thomson) (Table 4.1). The most widely distributed species were *Microcystis flos-aquae* (84% sites) and *M. aeruginosa* (80% sites). Both species coexisted in all lakes except for Perry Lake, Lake Claremont and Mary Carroll Park, with *M. flos-aquae* dominating at higher salinities (Fig. 4.1). *Microcystis wesenbergii* was frequently collected from Hyde Park with additional

occurrences in Tomato Lake, McDougall Park, Shenton Park, Perry Lakes and Lake Claremont.

Seven species of *Anabaena* were identified, with *Anabaena circinalis* the most frequently observed species, recorded in seven lakes. *Anabaena bergii* var. *limnetica* and *A. spiroides* f. *spiroides* were also common, but only observed in five sites. The other Nostocales identified were *Anabaenopsis* and *Aphanizomenon. Anabaenopsis elenkinii* was found in six lakes, in a salinity ranging from 0.15 to 9.56 ppt (Fig. 4.2). Two species of *Aphanizomenon* were collected, both being present in Bibra Lake. *Aphanizomenon gracile* was only observed in Bibra Lake in spring 2000 (Table 4.2), while *Aphanizomenon ovalisporum* was present during summer 2001 to 2003, as well as winter 2001. *Aphanizomenon ovalisporum* was also present in Emu Lake and Lake Joondalup (Table 4.3). *Trichodesmium iwanoffiana* Nygaard was the most frequently observed Oscillatoriales.

Twelve of the 21 lakes sampled in late autumn-early winter experienced blooms (Table 4.4) (Bibra Lake omitted, results presented in section 4.3.3). Water temperatures ranged from 13.5 to 16.5 °C. The dominant species were *M. flos-aquae* and *M. aeruginosa*. *Microcystis wesenbergii*, present again in Hyde Park, was also detected in two additional sites, Perry Lakes and Lake Claremont. *Microcystis* cell counts were high in lakes Herdsman, Jackadder, Yangebup, Booragoon, and Blue Gum, exceeding $1 \times 10^7$ cells $mL^{-1}$. Filamentous species were only observed in Bibra Lake.

**Table 4.1.** The dominant species of cyanoprokaryota collected from the urban lakes of the Swan Coastal Plain, Western Australia.



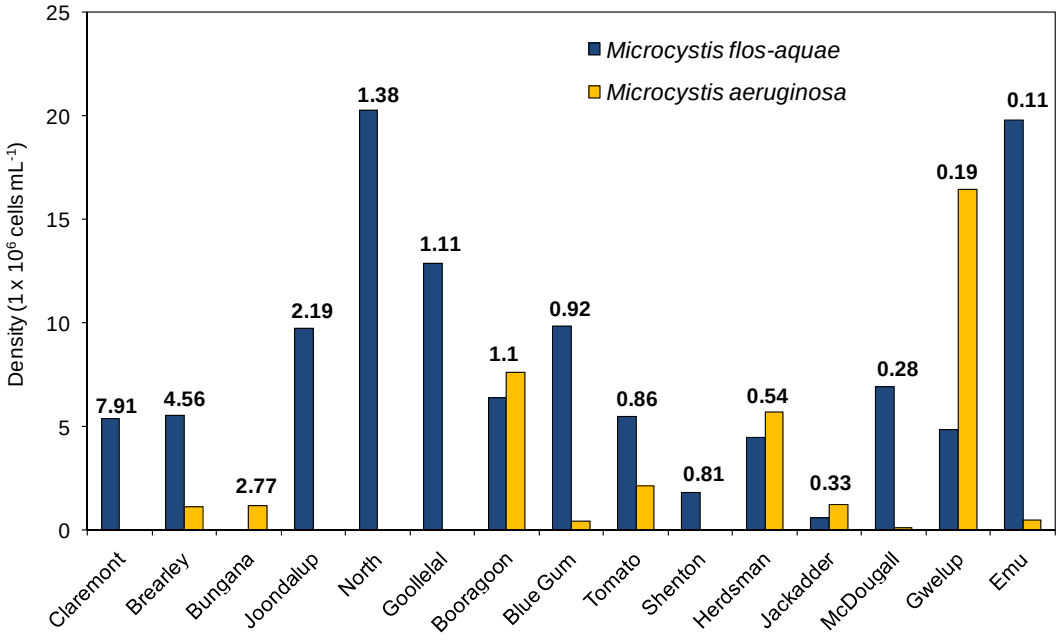



**Figure 4.1.** Distribution of *M. flos-aquae* and *M. aeruginosa* according to salinity (ppt) in 15 lakes from 2000 to 2003, arranged in decreasing salinity. Value presented above the bar indicates salinity.

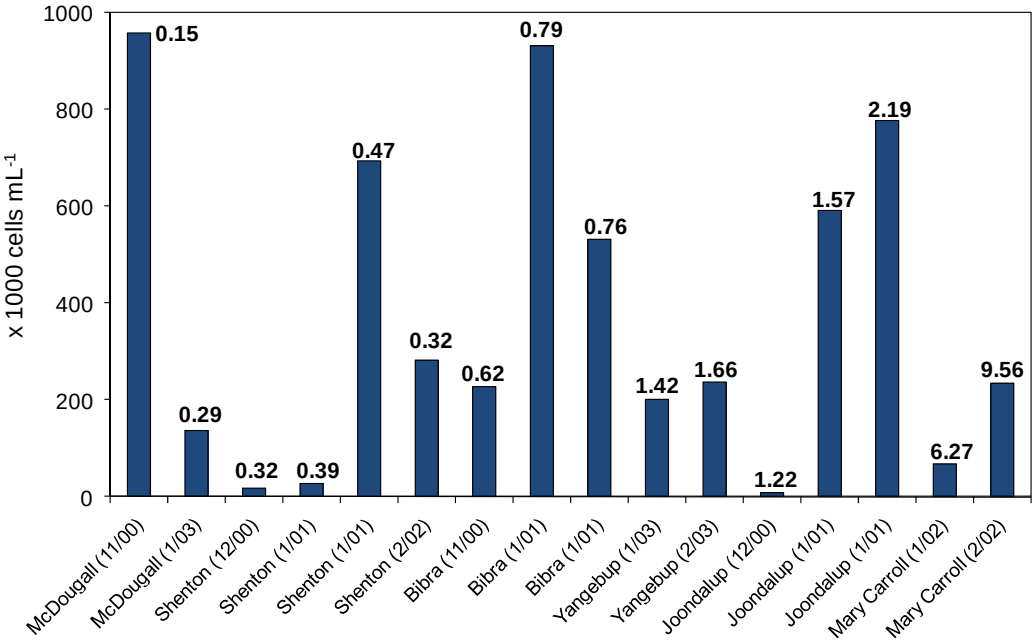



**Figure 4.2.** Distribution of *A. elenkinii* according to salinity (ppt), arranged by site (month/year). Value presented above the bar indicates salinity.

**Table 4.2.** Cell numbers of *Aphanizomenon gracile* and physico-chemical parameters measured from Bibra Lake during spring 2000.

| | **Temp. (°C)** | **pH** | **Salinity (ppt)** | **EC (µS cm$^{-1}$)** | **Cells mL$^{-1}$** | **Additional species** |
|---|---|---|---|---|---|---|
| 8/9/00 | 18.4 | 7.97 | 0.62, | 922 | $3.52 \times 10^5$ | *M. flos-aquae*<br>*A. elenkinii* |
| 21/9/00 | 18.6 | 8.43 | 0.38, | 1081 | $2.05 \times 10^5$ | *M. flos-aquae*<br>*M. aeruginosa*<br>*A. elenkinii* |
| 4/10/00 | 22.8 | 8.33 | 0.54, | 1093 | $7.53 \times 10^4$ | *M. flos-aquae*<br>*A. elenkinii* |

**Table 4.3.** Cell numbers of *Aphanizomenon ovalisporum*, and associated environmental conditions, collected from lakes Bibra, Emu and Joondalup during spring-summer.

| | **Temp. (°C)** | **pH** | **Salinity (ppt)** | **EC (µS cm$^{-1}$)** | **Cells mL$^{-1}$** |
|---|---|---|---|---|---|
| Bibra Lake | | | | | |
| 10/1/02[a] | 25.2 | 8.56 | 0.8 | 1627 | $6.45 \times 10^4$ |
| 23/3/02 | 26.7 | 9.15 | 1.34 | 2630 | $4.45 \times 10^5$ |
| 31/1/03[a] | 23.5 | 8.85 | 1.35 | 2544 | - |
| 20/2/03 | 33.1 | 9.08 | 1.66 | 3120 | $7.20 \times 10^5$ |
| Additional species: *M. flos-aquae, M. aeruginosa, A. bergii* var. *limnetica, A. elenkinii* | | | | | |
| Emu Lake | | | | | |
| 9/1/02[a] | 28.1 | 9.29 | 0.11 | 234 | - |
| 22/1/02 | 31.6 | 9.08 | 0.11 | 238 | $8.79 \times 10^4$ |
| 7/11/02[a] | 28.2 | 7.81 | 0.11 | 246 | $1.15 \times 10^5$ |
| 20/12/02 | 29.8 | 7.65 | 0.14 | 328 | $4.64 \times 10^5$ |
| Additional species: *M. flos-aquae, M. aeruginosa, A. bergii* var. *limnetica, A. affinis* | | | | | |
| Lake Joondalup | | | | | |
| 7/1/03 | 26.8 | 9.11 | 1.68 | 3150 | $6.77 \times 10^5$ |
| Additional species: *M. flos-aquae, A. spiroides* f. *spiroides* | | | | | |

[a] first observed in net samples during that sampling period.

**Table 4.4.** The lakes containing cyanoprokaryota blooms in June 2001 with corresponding environmental parameters.

| Lake | Temp (°C) | pH | Salinity (ppt) | EC ($\mu S\ cm^{-1}$) | Additional species |
|---|---|---|---|---|---|
| Herdsman | 15 | 8.77 | 0.52 | 951 | *M. flos-aquae, M. aeruginosa* |
| Yangebup | 15.3 | 9.01 | 1.89 | 3314 | *M. flos-aquae, M. aeruginosa* |
| Hyde | 14.8 | 8.16 | 0.3 | 475 | *M. aeruginosa, M. wesenbergii* |
| Claremont | 13.4 | 8.83 | 4.37 | 6070 | *M. aeruginosa, M. wesenbergii* |
| Tomato | 15 | 7.70 | 0.56 | 911 | *M. aeruginosa* |
| Jackadder | 13.8 | 8.69 | 0.29 | 617 | *M. aeruginosa, M. flos-aquae* |
| Emu | 13.8 | 7.74 | 0.12 | 187 | *M. aeruginosa, M. flos-aquae* |
| Booragoon | 13.9 | 9.78 | 1.54 | 2905 | *M. aeruginosa, M. flos-aquae* |
| Blue Gum | 15 | 9.38 | 0.65 | 1104 | *M. aeruginosa* |
| Bungana | 16.2 | 8.79 | 2.14 | 3323 | *M. aeruginosa, M. flos-aquae* |
| Perry | 16.5 | 7.74 | 0.22 | 484 | *M. aeruginosa, M. wesenbergii* |

Bibra Lake omitted. Refer to Table 4.7.

#### *4.3.1.1 Nodularia spumigena in Yangebup Lake*

*Nodularia spumigena* was first identified in net samples collected from Yangebup Lake in October 2001 and was present again in November 2002. In 2001, Yangebup Lake was experiencing a spring bloom of *Microcystis* with maximum cell numbers recorded in early October (*M. flos-aquae*, $1 \times 10^8$ cells $mL^{-1}$; *M. aeruginosa* $2 \times 10^7$ cells $mL^{-1}$). On the 22nd October 2001, *N. spumigena* appeared in the water samples at 27000 cells $mL^{-1}$. During this time, *Microcystis* concentration was still high (*Microcystis flos-aquae*, $6.13 \times 10^7$ cells $mL^{-1}$; *M. aeruginosa*, $1.53 \times 10^7$ cells $mL^{-1}$). Later in November and December 2001, *N. spumigena* was observed in the concentrated net samples, but there were no traces of *N. spumigena* in the water samples. In relation to the physico-chemical data, only pH and water temperature changed from October to December (Table 4.5).

*Nodularia spumigena* was found again in the net samples collected from November 2002 to February 2003 (Fig. 4.4). However, unlike the previous year when the highest biomass of *N. spumigena* was observed in October, it was not until early

January 2003 that *N. spumigena* became prominent in the water samples. As with the previous year, Yangebup Lake was experiencing a bloom of *M. flos-aquae* and *M. aeruginosa*. *Anabaena circinalis* was also present as the dominant filamentous species. On the 12th December 2002 all three species recorded their highest cell numbers. By the 18th December cell numbers had began to decline, with cell concentrations considerably lower by the 24th January 2003.

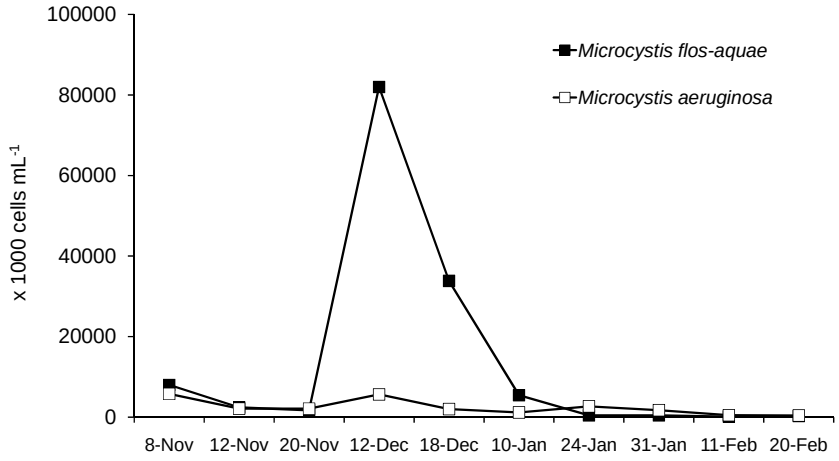
On the 10th January 2003, *N. spumigena* and *Anabaenopsis elenkinii* were recorded in the water samples at $2.60 \times 10^4$ cells $mL^{-1}$ and $2 \times 10^5$ cells $mL^{-1}$, respectively. However, this only constituted a small percentage (1% and 6%) of the total cyanoprokaryota volume. By February 2003, the concentration of both species had increased, now comprising 37% (*A. elenkinii*) and 8% (*N. spumigena)* of the total cyanoprokaryota biovolume. The water sample collected on the 20th February contained 70 000 cells $mL^{-1}$ of *N. spumigena*.

**Table 4.5.** The physico-chemical parameters associated with *Nodularia spumigena* blooms in Yangebup Lake.

| Date | | Temp (°C) | pH | Salinity (ppt) | EC (µS $cm^{-1}$) |
|---|---|---|---|---|---|
| 2001 | 3/10 | 20.2 | 8.55 | 1.32 | 2264 |
| | 22/10[a] | 19.1 | 8.82 | 1.31 | 2231 |
| | 1/11[a] | 22.9 | 8.53 | 1.34 | 2289 |
| | 14/11 | 25 | 8.32 | 1.38 | 2344 |
| | 18/12[a] | 27.8 | 9.06 | 1.25 | 2487 |
| 2002 | 8/11[a] | 28.6 | 8.87 | 1.07 | 2251 |
| | 12/11 | 27.8 | 8.7 | 1.09 | 2272 |
| | 20/11 | 25.9 | 8.73 | 1.07 | 2256 |
| | 27/11 | 28.9 | 8.75 | 1.06 | 2293 |
| | 12/12[a] | 31.2 | 9.25 | 1.15 | 2422 |
| | 18/12[a] | 29.9 | 8.98 | 1.21 | 2533 |
| 2003 | 10/1[a] | 31.4 | 9.7 | 1.42 | 2686 |
| | 24/1 | 26.2 | 9.45 | 1.49 | 2800 |
| | 31/1[a] | 25.1 | 9.53 | 1.51 | 2830 |
| | 5/2 | 29 | 9.75 | 1.32 | 2496 |
| | 11/2 | 30.1 | 9.49 | 1.74 | 3230 |
| | 20/2[a] | 31.3 | 9.37 | 1.66 | 3090 |

[a] Dates when *Nodularia spumigena* was present in net samples.

**(a)**




**(b)**

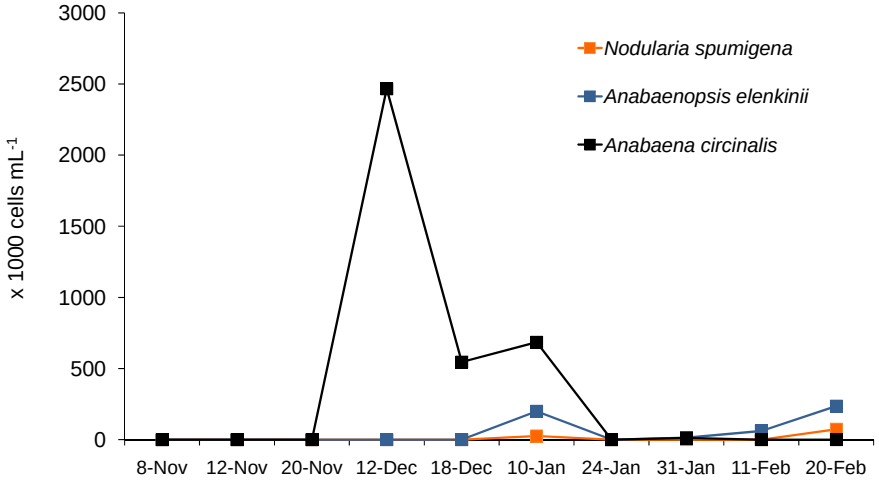



**Figure 4.3.** Cell numbers (cells $mL^{-1}$) of the **(a)** colonial and **(b)** filamentous species recorded in Yangebup Lake from November 2002 to February 2003.

#### *4.3.1.2 Cylindrospermopsis raciborskii in Bibra Lake*

In late autumn-early winter 2001 the tropical species *Cylindrospermopsis raciborskii* was sampled in Bibra Lake on two occasions with *A. ovalisporum*, *M. aeruginosa* and *M. flos-aquae* (Table 4.6). *Cylindrospermopsis raciborskii* had not been present in previous spring-summer samples collected from Bibra Lake. The concentration of *C. raciborskii* was high ($6.43 \times 10^6$ cells $mL^{-1}$, 25% total biovolume), although *M. flos-aquae* was the dominant species (40% and 76% of total biovolume).

**Table 4.6.** The concentration of each species collected from Bibra Lake in autumn-winter 2001 with concurrent environmental conditions.

| Date | Species | Cells $mL^{-1}$ | Temp. (°C) | pH | Salinity (ppt) | EC ($\mu S\ cm^{-1}$) |
|---|---|---|---|---|---|---|
| 8/5 | *A. ovalisporum* | $7.93 \times 10^5$ | 17.7 | 9.14 | 1.56 | 2690 |
| | *C. raciborskii* | $6.44 \times 10^6$ | | | | |
| | *M. aeruginosa* | $9.20 \times 10^6$ | | | | |
| | *M. flos-aquae* | $4.03 \times 10^7$ | | | | |
| 21/6 | *A. ovalisporum* | $4.99 \times 10^5$ | 14.9 | 9.2 | 1.03 | 1714 |
| | *C. raciborskii* | $3.87 \times 10^5$ | | | | |
| | *M. aeruginosa* | $4.42 \times 10^5$ | | | | |
| | *M. flos-aquae* | $1.53 \times 10^7$ | | | | |

### *4.3.2 Species community composition*

Multivariate analysis (TWINSPAN) was used to distinguish the species composition found in each lake. Prior to the TWINSPAN analyses, species detected in only one site were removed (Appendix 4.1). Following is a description of the wetland (site) groups and species groups generated for each sampling period. The indicator species for each TWINSPAN division are stated.

#### *4.3.2.1 Sampling period 2000-2001*

A total of 13 species recorded, nine species were repeatedly observed (Table 4.7). *Microcystis flos-aquae* and *M. aeruginosa* were the most common species recorded in 88% and 72% of the sites, respectively. The third TWINSPAN division resulted in

four site groups that did not differ significantly in environmental conditions (Kruskall-Wallis $p > 0.05$) (Table 4.8). The non-parametric tests revealed no significant differences between the site groups for any of the TWINSPAN divisions. The indicator species for the three levels of divisions were *A. bergii* var. *limnetica, M. flos-aquae* and *M. aeruginosa.*

The first two TWINSPAN divisions separated out Lake Coogee (Group 1) and Forresdale Lake (Group 00) due to the absence of *M. flos-aquae.* Lake Coogee contained only *A. bergii* var. *limnetica* and Forrestdale Lake contained *P. planctonica*. Group 00 and Group 1 showed significantly higher salinity and EC when compared to the other sites ($p = 0.035$, $p < 0.05$). The third division separated lakes Joondalup, Claremont and Shenton Park (Group 011) based on the absence of *M. aeruginosa*. *Microcystis flos-aquae* and *A. elenkinii* were the dominant species of this group.

For the species data, the second TWINSPAN division resulted in two species groups that were defined by *M. aeruginosa* and *M. flos-aquae*. *Microcystis aeruginosa* was associated with *A. circinalis, T. iwanoffianum* and *M. wesenbergii*, while *M. flos-aquae* grouped with *A. elenkinii, A. spiroides* f. *spiroides* and *P. planctonica*. For this period, *A. bergii var. limnetica* did not form an assemblage with any other species.

#### *4.3.2.2 Sampling period 2001-2002*

A total of 19 species were recorded with 12 species observed more than once (Table 4.9). Again, the dominant species were *M. flos-aquae* (91% sites) and *M. aeruginosa* (74% sites). The third TWINSPAN division produced a total of five site groups with no significant difference in environmental conditions between any of the groups (Kruskall-Wallis $p > 0.05$) (Table 4.10). The indicator species divisions were *A. elenkinii, A. spiroides* f. *spiroides, A. bergii* var. *limnetica, A. circinalis* and *P. planctonica.*

The first TWINSPAN division separated out Lake Joondalup, Lake Claremont, Shenton Park and Mary Carroll Park based on the presence of both *A. elenkinii* and

*A. spiroides* f. *spiroides* (Group 1). The second division separated the remaining sites into those containing *A. circinalis* (Group 01) from the sites with *A. bergii* var. *limnetica* (Group 00). Group 00 included Bibra Lake, Emu Lake and Lake Brearley. Group 01 was further divided based on the presence of *P. planctonica* (Group 011) and *T. iwanoffianum* (Group 010). The fourth level of division resulted in two species groups with *M. flos-aquae, M. aeruginosa*, *A. ovalisporum* and *A. bergii* var. *limnetica* not part of any assemblage. *Anabaena circinalis* grouped with *Planktothrix* and *Trichodesmium*, while *A. spiroides* f. *spiroides* was associated with *Anabaenopsis*.

#### *4.3.2.3 Sampling period 2002-2003*

A total of 16 species were recorded with 11 species observed in more than one site (Table 4.11). No cyanoprokaryotes were recorded in Shenton Park and Lake Claremont. Similar to the other two periods, *M. flos-aquae* (93% sites) and *M. aeruginosa* (71% sites) were the most commonly observed species. However, this year *A. circinalis* was observed in over 50% of the sites. At the third TWINSPAN division there were a total of five site groups with *A. bergii* var. *limnetica, M. aeruginosa*, *A. flos-aquae* and *A. spiroides* f. *spiroides* indicators for each division (Table 4.12). There were no significant differences in environmental conditions between any of the recognised groups (Kruskall-Wallis $p > 0.05$)

The first division identified the five sites recording *A. bergii* var. *limnetica* (Bibra, Brearley, Emu, Mary Carroll and Joondalup). This group was further subdivided by the presence of *M. aeruginosa* in Bibra Lake, Lake Brearley and Emu Lake (Group 10). The remaining nine sites were grouped at the second level of division by the presence of *A. flos-aquae* (Group 01) and at the third level of division by the absence of *A. spiroides* f. *spiroides* (Group 000). The second level of division for the species data resulted in three species groups. Unlike the other periods, *M. flos-aquae* and *M. aeruginosa* were in the same assemblage and *Anabaena bergii* var. *limnetica* grouped with *A. ovalisporum*, *A. tanganyikae* and *A. aphanizomenoides*.

**Table 4.7.** Summary of the TWINSPAN site and species groups based on the species collected in 2000-2001. The number of sites in each group is indicated in parentheses.

| | **TWINSPAN site group** | | | |
|---|---|---|---|---|
| | **00 (1)** | **010 (13)** | **011 (3)** | **1 (1)** |
| *Microcystis aeruginosa* | | 13 | 1 | |
| *Anabaena circinalis* | | 5 | | |
| *Trichodesmium iwanoffianum* | | 3 | | |
| *Microcystis wesenbergii* | | 2 | | |
| *Microcystis flos-aquae* | | 13 | 3 | |
| *Anabaenopsis elenkinii* | | 2 | 3 | |
| *Anabaena spiroides* f. *spiroides* | | 2 | 1 | |
| *Planktothrix planctonica* | 1 | 2 | | |
| *Anabaena bergii* var. *limnetica* | | | 1 | 1 |

**Table 4.8**. Mean environmental variables for the 2000-2001 TWINSPAN site groups.

| | **TWINSPAN site group** | | | |
|---|---|---|---|---|
| | **00** | **010** | **011** | **1** |
| pH | 8.9 | 8.60 ± 0.37 | 8.96 ± 0.40 | 8.55 |
| Temp. (ºC) | 27.18 | 25.84 ± 1.07 | 24.87 ± 1.02 | 22.82 |
| Salinity (ppt) | 3.66 | 0.67 ± 0.39 | 2.09 ± 2.15 | 15.19 |
| EC (mS $cm^{-1}$) | 6.65 | 1.31 ± 0.62 | 3.58 ± 3.65 | 26 |

**Table 4.9.** Summary of the TWINSPAN site and species groups based on the species collected in 2001-2002. The number of sites in each group is indicated in parentheses.

| | **TWINSPAN site group** | | | |
|---|---|---|---|---|
| | **00 (4)** | **010 (9)** | **011 (6)** | **1 (4)** |
| *Microcystis flos-aquae* | 4 | 8 | 6 | 3 |
| *Microcystis aeruginosa* | 2 | 9 | 5 | 1 |
| *Anabaena circinalis* | | 2 | 6 | |
| *Trichodesmium iwanoffianum* | | 3 | | |
| *Planktothrix planctonica* | | | 3 | |
| *Planktothrix mougeotii* | | 1 | 1 | |
| *Aphanizomenon ovalisporum* | 2 | | | |
| *Anabaenopsis elenkinii* | | | | 3 |
| *Anabaena spiroides* f. *spiroides* | | | 1 | 3 |
| *Microcystis wesenbergii* | | 2 | | 1 |
| *Anabaenopsis tanganyikae* | | | | 2 |
| *Anabaena bergii* var. *limnetica* | | | | 1 |

**Table 4.10.** Mean environmental variables for the 2001-2002 TWINSPAN site groups.

| | **TWINSPAN site group** | | | |
|---|---|---|---|---|
| | **00** | **010** | **011** | **1** |
| pH | 8.66 ± 0.1 | 8.36 ± 0.39 | 8.53 ± 0.37 | 8.50 ± 0.24 |
| Temp. (ºC) | 25.19 ± 1.7 | 24.97 ± 1.07 | 25.58 ± 2.29 | 25.24 ± 1.41 |
| Salinity (ppt) | 1.34 ± 1.97 | 0.61 ± 0.51 | 0.8 ± 0.31 | 2.25 ± 2.08 |
| EC (mS $cm^{-1}$) | 2.31 ± 3.50 | 1.05 ± 0.84 | 1.36 ± 0.23 | 3.96 ± 3.88 |

**Table 4.11.** Summary of the TWINSPAN site and species groups based on the species collected in 2002-2003. The number of sites in each group is indicated in parentheses.

| | **TWINSPAN site group** | | | | |
|---|---|---|---|---|---|
| | **000 (1)** | **001 (4)** | **01 (4)** | **10 (3)** | **11 (2)** |
| *Anabaena circinalis* | 1 | 3 | 3 | | 1 |
| *Anabaenopsis elenkinii* | | 2 | | | |
| *Planktothrix mougeotii* | 1 | 1 | | | |
| *Anabaena flos-aquae* | | | 3 | | |
| *Microcystis aeruginosa* | 1 | 4 | 2 | 3 | |
| *Microcystis flos-aquae* | 1 | 3 | 4 | 3 | 2 |
| *Anabaena spiroides* f. *spiroides* | 1 | | 1 | | 1 |
| *Anabaena bergii* var. *limnetica* | | | | 3 | 2 |
| *Aphanizomenon ovalisporum* | | | | 2 | 1 |
| *Anabaenopsis tanganyikae* | | | | | 2 |
| *Anabaena aphanizomenioides* | | | | | 2 |

**Table 4.12.** Mean environmental variables for the 2002-2003 TWINSPAN sites groups.

| | **TWINSPAN site group** | | | | |
|---|---|---|---|---|---|
| | **000** | **001** | **01** | **10** | **11** |
| pH | 8.81 | 8.66 ± 0.4 | 8.78 ± 0.19 | 8.60 ± 0.48 | 8.78 ± 0.7 |
| Temp. (ºC) | 30.01 | 29.32 ± 0.63 | 28.43 ± 1.73 | 28.45 ± 0.24 | 28.15 ± 2.43 |
| Salinity (ppt) | 0.55 | 0.89 ± 0.54 | 0.81 ± 0.08 | 1.61 ± 1.79 | 3.80 ± 3.22 |
| EC (mS $cm^{-1}$) | 1.12 | 1.77 ± 1.05 | 1.58 ± 0.16 | 3.10 ± 3.36 | 6.96 ± 5.68 |

### *4.3.3 Site comparison: species assemblages*

Species data were collated from each lake over the three sampling periods and examined using clustering analysis to allow the main species assemblages to be identified. Clustering analysis also enabled the species assemblages from each site to be compared. The upper Canning River was included in the analysis as a comparison to the freshwater lakes.

Excluded from the analysis were species only recorded from one site - *A. affinis* (Emu), *A. arnoldii* (Claremont), *A. torlusa* var. *tenuis* (Mary Carroll), *N. spumigena* (Yangebup), *N. linkia* (North) and *O. sancta* (Thomson). For each group, the dominant species, defined as those represented in a high percentage of samples, were identified. The dendrogram showing the summarised results is presented in Figure 4.4. A map showing the location of the study lakes and an overview of the distribution of the main genera; *Microcystis*, *Anabaena*, *Aphanizomenon* and *Anabaenopsis* is presented in Figure 4.5.

A description of five major site groups follows;

**Outliers:** Forrestdale Lake (2000-01) and Lake Claremont (2001-02) were outliers due to distinct assemblages. Forrestdale Lake contained only *P. planctonica* and Lake Claremont contained only *A. spiroides* f. *spiroides*. The 2001-02 samples from Lake Claremont differed from 2000-01 where a bloom of *M. flos-aquae* was recorded.

**Cluster A:** Coogee Lake (2000-01) was linked with Lake Brearley (2001-02) as both contained *A. bergii* var. *limnetica*. Lake Brearley also recorded *M. flos-aquae*.

**Cluster B:** Lake Joondalup, Mary Carroll Park and Shenton Park all contained *M. flos-aquae* with *Anabaenopsis* (*elenkinii* and/or *tanganyikae*). *Anabaena bergii* var. *limnetica* and *A. aphanizomenoides* were also recorded in Lake Joondalup and Mary Carroll Park.

**Cluster C:** This cluster recognised sites containing only *Microcystis*. All sites except Perry Lakes contained *M. flos-aquae* (80%). The 2002-03 samples from Lake Bungana also contained *M. aeruginosa*, which differed from the 2001-02 samples where *A. circinalis* and *T. iwanoffiannum* were recorded.

**Cluster D:** All the sites in this cluster contained *M. flos-aquae* and *M. aeruginosa*. Similarity between Emu Lake and Bibra Lake was evident, this due to the presence of *A. bergii* var. *limnetica* and *A. ovalisporum*. *Anabaena bergii* var. *limnetica* was again observed in Lake Brearley (2002-03), as shown in Cluster A, but in 2002-03 *M. aeruginosa* was also present. A high level of similarity (100%) between the northern lakes Goollelal, Gwelup, Monger, Herdsman and Jackadder, and the southern lakes Booragoon and Piney was revealed.

**Cluster E:** This cluster was comprised of 21 samples representing eight sites. All the sites contained *M. flos-aquae*, and 90% with *M. aeruginosa* and *A. circinalis*. North Lake separated out from the majority of lakes in Cluster E due to the absence of *M. aeruginosa*. Within this cluster, a relationship between the inner suburban wetlands, Tomato Lake and Neil McDougall Park, was shown due to the presence of *T. iwanoffiannum*, *M. wesenbergii* and *P. mougeotii*. A high level of similarity (> 80%) was revealed between the successive years of sampling for Herdsman Lake, Tomato Lake, Neil McDougall Park, Yangebup Lake, Blue Gum Lake, North Lake and the Canning River. The Canning River was strongly related to Herdsman Lake and Blue Gum Lake.

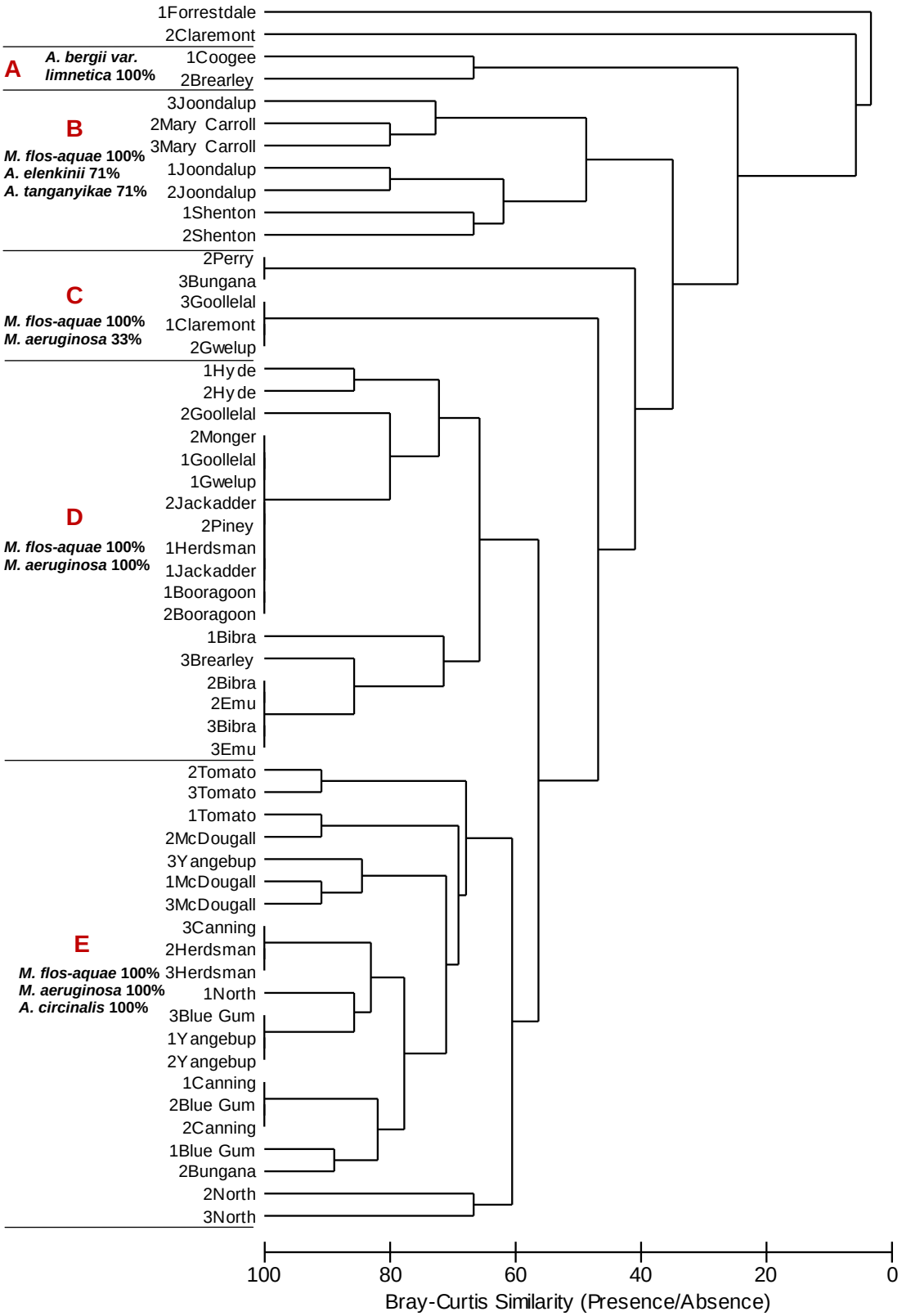



**Figure 4.4.** Dendrogram showing the relationship among sites based on species. For each cluster the dominant species (% samples) is indicated. Number preceding site name indicates sampling period, (1) 2000-01, (2) 2001-02 and (3) 2002-03.

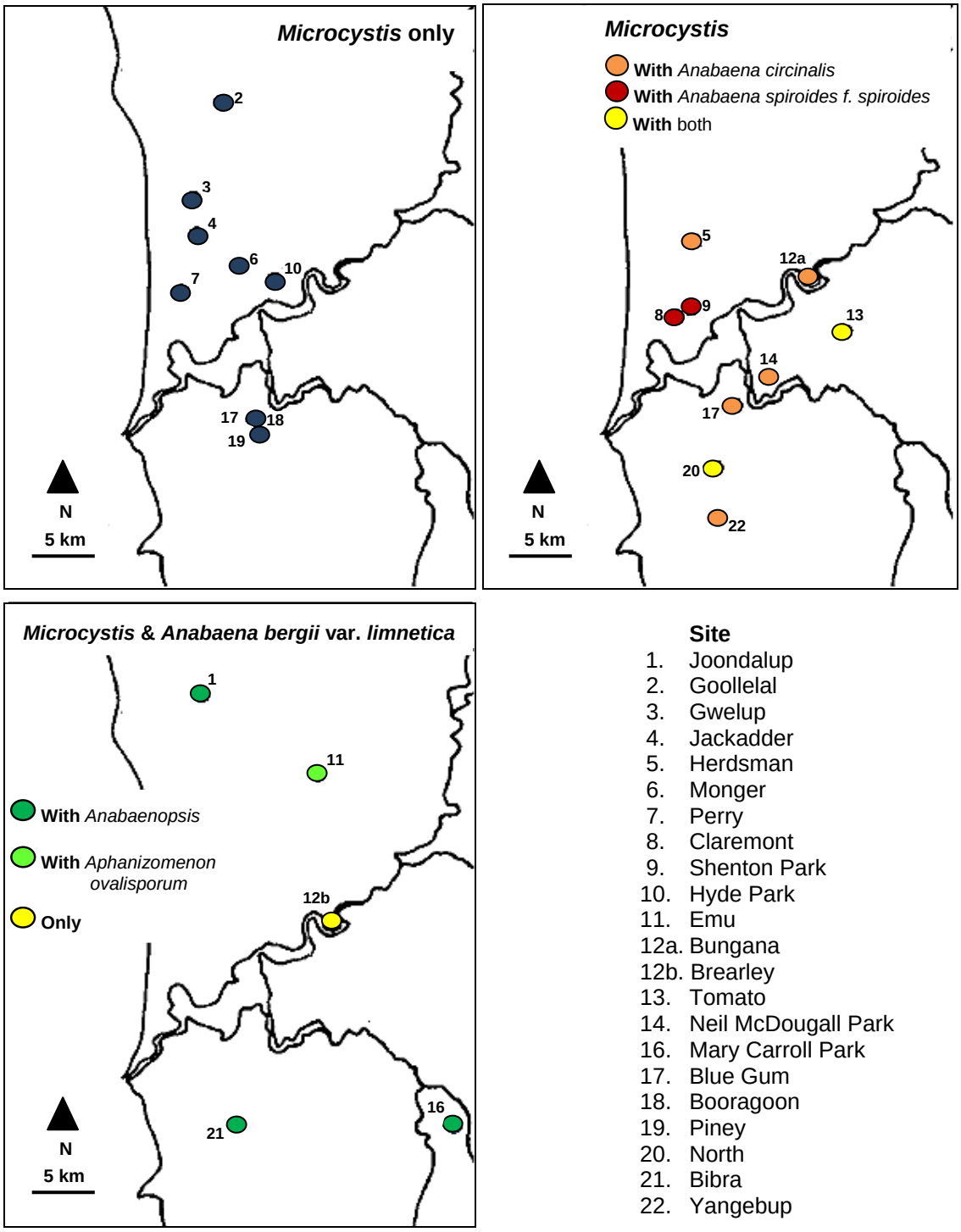



**Figure 4.5.** Maps of the Swan Coastal Plain, Western Australia, summarising the distribution of the major genera; *Microcystis*, *Anabaena*, *Aphanizomenon* and *Anabaenopsis* within the SCP lakes. Lakes excluded due to absence of cyanoprokaryotes or unique assemblages; Coogee, Manning, Market Gardens, Forrestdale Lake and Thomson Lake.

## 4.4 Discussion

### *4.4.1 Cyanoprokaryota blooms in the SCP lakes: an overview*

This study provides the first extensive survey of cyanoprokaryota blooms in the lakes of the SCP. Clustering analysis was successful in identifying lakes with similar species assemblages, allowing the distribution of the dominant species to be examined. In addition, the lakes experiencing recurring blooms of the same species were revealed. These insights into the spatial and temporal distribution of the major bloom-forming species, especially those that are potentially harmful, are important in managing cyanoprokaryota blooms in the SCP wetlands.

The three common species *Microcystis flos-aquae*, *M. aeruginosa* and *Anabaena circinalis* identified in the lake samples are typical of the temperate regions of Australia, being previously reported in the southwest wetlands. However the minor species - *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum* and *Anabaena bergii* var. *limnetica*, have not been previously described in the area. All three species are toxic in Australia and represent a future threat in Western Australian wetlands, as already demonstrated in the eastern states (Shaw *et al.* 1999; Heresztyn and Nicholson 1997; Saker and Griffiths 2001) and throughout the world (Mazur and Pliński 2003; Bouaïcha and Nasri 2004; Manti *et al.* 2005; Gkelis *et al.* 2005; Stüken *et al.* 2006). In addition, the occurrence of *N. spumigena* in Yangebup Lake demands further attention as this toxic halotolerant species, which is more common in an estuarine environment, was capable of forming a toxic bloom in an inland freshwater Yangebup Lake. This is the second account of *N. spumigena* in an East Beeliar Regional Park wetland (John and Kemp 2006).

All the lakes studied contained a bloom-forming species at some stage of the research, irrespective of location on the SCP. The exception was Manning Lake and Market Garden Swamp. There is no conclusive reason as to why no cyanoprokaryotes were identified in these two wetlands of the South West Corridor. Both were hyposaline (3-9 ppt), which would explain the absence of freshwater species; however neighbouring Lake Coogee experiences higher salinity and recorded *A. bergii* var. *limnetica* and *Planktothrix perornata* f. *attenuata*. A possible

explanation is that Manning Lake and Market Garden Swamp are examples of highly coloured wetlands that do not support phytoplankton activity (Davis *et al.* 1993). Cyanoprokaryota blooms were previous observed in both lakes in the 1970s (Newman 1976), but since this time, both lakes have dried out each summer, which would naturally limit the formation of cyanoprokaryota blooms.

### *4.4.2 Distribution of Microcystis*

*Microcystis* spp. dominated the spring-summer phytoplankton and coexisted in over 80% of the lakes sampled. Worldwide, *Microcystis* is also the most widespread bloom-forming cyanoprokaryote and the genus most commonly associated with hepatotoxicity. The only sites not to record *Microcystis* were Lake Coogee, Forrestdale Lake and Thomsons Lake. All three are seasonal wetlands that recorded higher salinity than the other sites, which would inhibit the formation of freshwater blooms but favour the growth of more salt tolerant species. This was evident in the results with Oscillatoriales observed in the abovementioned lakes (*P. perornata* f. *attenuata, P. planctonica* and *O. sancta*, respectively). Both *Planktothrix* species have a wide distribution across varying salinities in south-eastern Australia (Baker 1992), while *O. sancta* has a wide salinity tolerance from fresh to salt water (Komárek and Anagnostidis 2005; McGregor 2007). Lake Coogee, also recorded *A. bergii* var. *limnetica*, a species common in brackish waters (Cronberg and Annadotter 2006).

The absence of *Microcystis* in Forrestdale Lake and Thomsons Lake differs from past reports, with *M. flos-aquae* and *M. aeruginosa* producing blooms in 1993 (John and Kemp 2006). For both sites, *Microcystis* persisted throughout the year, with the highest biomass achieved in winter in lower salinity. However, the inability of *Microcystis* to produce blooms in Forrestdale Lake and Thomsons Lake during the current study cannot be assured as these sites were only sampled during a single period (2000-01) and no further sampling was possible due to low water levels. Winter sampling of these site were also not conducted. Nevertheless, in the present day, declining water levels with increasing salinity commonly observed in

Forrestdale Lake and Thomsons Lake would limit the growth of freshwater species such as *M. aeruginosa* during the warmer months.

Although *Microcystis flos-aquae* and *M. aeruginosa* coexisted in the majority of sites sampled, *M. flos-aquae* did tend to dominate only at higher salinity (2-8 ppt) and was associated with species with wide salinity tolerance (*A. elenkinii* and *P. planctonica*). *Microcystis flos-aquae* dominated Lake Joondalup, Lake Claremont and Lake Brearley, which were hyposaline and experienced seasonal drying with increased salinity. *Microcystis flos-aquae* was also present in net samples collected from Mary Carroll Park associated with *A. elenkinii*, *A. tanganyikae* and *A. bergii* var. *limnetica*, the latter two species also showing a wide salinity tolerance (Hindák 2000; Cronberg and Annadotter 2006). This observed distribution of *M. flos-aquae* suggests this species is more salt tolerant than *M. aeruginosa* in Western Australia**.** However, this does not support what is currently known about the distribution and growth requirements of these two species.

Both Komárek and Anagnostidis (1998) and Cronberg and Annadotter (2006) describe *M. aeruginosa* as a brackish and fresh species, while *M. flos-aquae* is confined to freshwater. This has been demonstrated in the upper salt tolerance level for *M. aeruginosa,* which ranges from 4 to 10 ppt depending on the strain (Atkins *et al.* 2001; Robson and Hamilton 2003; Orr *et al.* 2004; Albay *et al.* 2005; Verspagen *et al.* 2006; Tonk *et al.* 2007). The upper salt tolerance level for strains of *M. flos-aquae* has not been investigated. However natural bloom samples containing both *M. aeruginosa* and *M. flos-aquae* can tolerate higher salt concentrations when compared to pure cultures of *M. aeruginosa*. For example, Verspagen *et al.* (2006) found *M. aeruginosa* and *M. flos-aquae* from Lake Volkerak, The Netherlands, were salt tolerant at 8 to 10 ppt, while samples from the 2000 Swan River bloom were salt tolerant to 9.8 ppt (Orr *et al.* 2004). Robson and Hamilton (2003) previously reported a 4 ppt limit for *M. aeruginosa* collected from the Swan River bloom. Although the salt tolerance of *M. flos-aquae* is yet to be defined, in natural populations it appears to be higher than that of *M. aeruginosa*. This is supported by the field data collected during the present study with *M. flos-aquae* being more wide

spread and attaining higher cell numbers in wetlands that were hyposaline. Further research is needed to genetically separate Western Australian populations of *M. aeruginosa* and *M. flos-aquae* and establish their potential distribution based on salt tolerance.

The unseasonable blooms of *Microcystis flos-aquae* and *M. aeruginosa* observed in autumn-winter 2001 illustrates the need to better understand the ecology of this genus in the SCP wetlands. In Chapter 8, it will be shown that microcystin concentrations were much higher in the winter samples than the spring and summer samples collected the same year. Roberts and Zohary (1987) reported *Microcystis* blooms in water temperatures as low as 13 °C, below the 15 °C limit for optimal growth. Although growth should be minimal in such low temperatures, pre-existing standing stocks were maintained throughout winter by remaining in suspension and experiencing low loss rates. Furthermore *Microcystis* is an example of a K-selected genus, adapted to withstand the range of environmental conditions experienced through summer to winter through competition with other phytoplankton (Reynolds 2006; Zohary *et al.* 1995). Such adaptive advantages will allow *Microcystis* to form harmful blooms in the SCP lakes throughout the year and this has been demonstrated by the autumn-winter 2001 blooms. These winter blooms of *M. aeruginosa* are not a unique occurrence as there have been previous reports in Lake Joondalup **(**14 °C), Thomsons Lake (14 °C) and Forrestdale Lake (12 °C) (Gordon *et al.* 1981; John and Kemp 2006). These reports, coupled with the findings of the current study, further show the potential for *Microcystis* blooms to persist throughout the year in the SCP wetlands.

### *4.4.3 Distribution of the Nostocales*

The Nostocales identified were very diverse in morphology and distribution. The most common species, *A. circinalis*, has been previously described in the area from both river and lake populations. Similarly, *A. spiroides* f. *spiroides* and *A. elenkinii* have appeared in the Canning River (Vincent 1995) and Peel-Harvey Estuary (Huber 1980). This study shows both species to have a distribution that extends outside of the major river systems with repeated occurrences of *A. spiroides* f. *spiroides* in

North Lake, Tomato Lake and Lake Joondalup, and *A. elenkinii* in Neil McDougall Park, Joondalup Lake and Yangebup Lake. Within the Canning River and Peel-Harvey Estuary both species have coexisted, a similar observation was made in Lake Joondalup, Lake Claremont, Shenton Park and Mary Carroll Park.

*Anabaena bergii* var. *limnetica* and *A. ovalisporum*, observed in more than three sites, are lesser known species in Australia and are reported here for the first time in Western Australia. In addition, the single occurrence of *Cylindrospermopsis raciborskii* and *Nodularia spumigena* expands on what is currently known about these species in Australian wetlands.

#### *4.4.3.1 Anabaena bergii var. limnetica*

There is little information regarding *A. bergii* var. *limnetica* in Australia with the only described occurrence in the Murray-Darling Basin (Baker and Humpage 1994; Baker and Fabbro 2002). In this study *A. bergii var. limnetica* was sampled from six lakes, ranging from fresh (< 1 ppt) to hyposaline (3-12 ppt). It was the dominant Nostocales in Lake Coogee, which was shown to have significantly higher salinity than the other lakes, and Lake Brearley. In Mary Carroll Park it was present with *Anabaenopsis*. In Emu Lake and Bibra Lake, where salinity was lower, *A. bergii* var. *limnetica* was commonly observed with *A. ovalisporum*.

This distribution pattern of *A. bergii* var. *limnetica* was wide spread across the study sites, although never observed in two neighbouring wetlands within the same consanguineous suite. The distribution in Emu Lake and Lake Coogee supports the current reports of this morphospecies inhabiting both freshwater and hyposaline environments. *Anabaena bergii* var. *limnetica* is a morphotype of *Anabaena bergii* Ostenfeld and has been observed in freshwater lakes and rivers in France, Switzerland and Slovakia (Hindák 2000). *Anabaena bergii* Ostenfeld also grows in higher conductivity, hyposaline waters (Cronberg and Annadotter 2006), and is endemic to the Caspian and Aral Sea (Orlova and Rusakova 1999). *Anabaena bergii* var. *limnetica* is also synonymous with *A. minderi* and *A. bergii* var. *minor*, the later also occurring in the hyposaline waters of the Caspian and Aral Sea (Hindák 2000).

Morphologically, *A. bergii* var. *limnetica* and *A. ovalisporum* are very similar; both producing solitary, attenuated trichomes with elongated apical cells. Theses similarities suggest *A. ovalisporum* and *A. bergii* var. *limnetica* to be one species (Shaw *et al.* 1999; Hindák 2000). Both species also occur in fresh to slightly brackish waters and both contain genes for the production of cylindrospermopsin (Shaw *et al.* 1999; Schembri *et al.* 2001). Despite these similarities, the *A. bergii* var. *limnetica* and *A. ovalisporum* populations observed in Bibra Lake, Emu Lake and Lake Joondalup could be distinguished by the cell dimension, with *A. ovalisporum* producing more cylindrical vegetative cells (refer to section 7.3.2).

#### *4.4.3.2 Aphanizomenon ovalisporum*

*Aphanizomenon ovalisporum* is a newly identified species in Australia, first reported in a series of newly constructed lakes within a coastal housing estate in Queensland (Shaw *et al.* 1999). Worldwide it is an uncommon bloom-forming cyanoprokaryote with reported blooms only in Israel (Pollingher *et al.* 1998), Greece (Gkelis *et al.* 2005), Germany (Stüken *et al.* 2006) and Spain (Quesada *et al.* 2006). *Aphanizomenon ovalisporum* can potentially produce toxic blooms with strains from Australia, Israel and Spain shown to produce cylindrospermopsin – a heptotoxin commonly associated with *Cylindrospermopsis raciborskii* (Banker *et al.* 1997; Shaw *et al.* 1999; Quesada *et al.* 2006). Cylindrospermopsin is already a major health concern and water quality problem in the eastern states (McGregor and Fabbro 2000; Falconer 2005), becoming an important area of toxicology research. For this reason, future research needs to focus on the production and distribution of cylindrospermopsin in Western Australia populations of *A. ovalisporum* and *C. raciborskii,* and study the factors that promote their growth and ability to form blooms.

*Aphanizomenon ovalisporum* has not been previously identified in Western Australia, with a distribution limited to Queensland and South Australia (Baker and Fabbro 2002). Published research has focused on the blooms in tropical Queensland (Shaw *et al.* 1999), so factors promoting *A. ovalisporum* growth in temperate

Australia in unknown. *Aphanizomenon ovalisporum* blooms have been associated with waters that were slightly hyposaline (1500-3550 µS $cm^{-1}$), have high temperatures (25-30°C) and high pH (7-9) (Hadas *et al.* 1999; Shaw *et al.* 1999; Gkelis *et al.* 2005; Quesada *et al.* 2006). Only the single occurrence in Lake Joondalup complements these findings. In particular, the repeated blooms of *A. ovalisporum* in Bibra Lake and Emu Lake were associated with much lower salinity, as low as 0.1 ppt (EC 200 µS $cm^{-1}$) in Emu Lake. As far as it could be established, *A. ovalisporum* has not been reported in such low salinity. This requires further research, aimed at clearly defining the geographical range of this species outside of tropical Australia and the conditions that favour the growth of temperate strains.

Of importance was the presence of *A. ovalisporum* in Bibra Lake in May and June 2001 in association with *C. raciborskii*. *Cylindrospermopsis raciborskii* is a species similar to *A. ovalisporum* in relation to an occurrence mostly known to tropical regions of Australia and the production of cylindrospermopsin. Furthermore, *C. raciborskii* has become problematic in temperate regions of the world due to its invasive nature. Therefore the presence of both species in Bibra Lake is a concern. During the autumn-winter bloom, cell concentrations, salinity and pH were comparable to summer conditions, but as expected, water temperatures were lower (14 °C and 17 °C). The favourable salinity and pH, together with an adequate supply of nutrients supplied by winter runoff, may allow *A. ovalisporum* to persist throughout the colder months. This is of great concern as cyanoprokaryota blooms are generally not monitored during winter. The autumn-winter 2001 bloom in Bibra Lake recorded cell counts for *M. aeruginosa*, *A. ovalisporum* and *C. raciborskii* in excess of the safe recreational guidelines (WHO 2003).

#### *4.4.3.3 Cylindrospermopsis raciborskii*

The discovery of *C. raciborskii* in Bibra Lake in 2001 is one of the first reports of this species in a SCP wetland. During the same winter period, *C. raciborskii* was detected in a small shallow lake within a newly established housing estate in Canning Vale, approximately 8 kilometres east of Bibra Lake. *Cylindrospermopsis raciborskii* is a freshwater cyanoprokaryote with early reports from sub-tropical to

tropical regions (Chapman and Schelske 1997; Saker *et al.* 1999a), but in recent years this distribution has expanded to temperate areas, such as France (Briand *et al.* 2002), Portugal (Saker *et al*. 2003), New Zealand (Wood and Stirling 2003), Algeria (Bouaïcha and Nasri 2004), Italy (Manti *et al.* 2005) and Germany (Stüken *et al.* 2006). In Australia, *C. raciborskii* blooms are more common in the eastern states, especially in drinking water sources in subtropical and tropical Queensland with cell concentrations exceeding 600 000 cells $mL^{-1}$ (McGregor and Fabbro 2000). In southern temperate Australia, *C. raciborskii* is endemic, but usually at low cell densities (Falconer 2005). Unlike tropical blooms where there is continual dominance of *C. raciborskii*, in temperate waters *C. raciborskii* forms part of the species succession with higher growth in the warmer months (Bowling 1994; Baker and Humpage 1994).

The 2001 record of *C. raciborskii* in Bibra Lake is a threat due to the invasive potential of this species in temperate areas and reports of both animal and human poisonings (Thomas *et al.* 1998; Griffiths and Saker 2003). *Cylindrospermopsis raciborskii* is the main producer of the hepatotoxin cylindrospermopsin (Hawkins *et al.* 1997), however paralytic shellfish toxins, similar to those from *A. circinalis*, have been reported in Brazilian strains (Lagos *et al.* 1999; Molica *et al.* 2002). Because Bibra Lake is a popular picnic and waterbird area, future blooms of *C. raciborskii*, *A. ovalisporum* and *M. aeruginosa* need to be tested for cylindrospermopsin and microcystins. Cylindrospermopsin is yet to be identified in Western Australian populations of *C. raciborskii*, *A. ovalisporum* or *A. bergii* var. *limnetica*. The high cell count ($6 \times 10^6$ cells $mL^{-1}$) recorded for *C. raciborskii* indicates a high probability for adverse health effects (WHO 2003). These effects can be exhibited in the lungs, liver, kidney and gastrointestinal tract showing the severity of this species (Hawkins *et al.* 1985).

Since the first observation in 2001, *C. raciborskii* has reappeared again in summer 2004, in addition to sightings in Emu Lake and Hyde Park (ARNAT 2004). The sighting in Emu Lake shows how similar Bibra Lake and Emu Lake are in relation to species composition (*A. ovalisporum*, *M. aeruginosa* and *M. flos-aquae*), although

they are not close in location (Fig. 4.5). Regarding Hyde Park, the colonial genus *Microcystis* usually dominates spring-summer blooms. The presence of *C. raciborskii* in Emu Lake and Hyde Park adds to the concern that it is an invasive species in newly constructed lakes and ornamental lakes (Hawkins *et al.* 1997). For example, long term monitoring of Lake Julius, Queensland, has shown a shift from *M. aeruginosa* to *C. raciborskii* dominance (Saker and Griffiths 2001). Because cyanoprokaryota blooms in Hyde Park and Emu Lake have not been previously documented it is uncertain whether this is the first occurrence of *C. raciborskii* in a SCP wetland. What is established is that these sites, including Bibra Lake, will require future monitoring for *C. raciborskii*.

The shift in bloom dominance is due to *C. raciborskii* ability to tolerate a wide range of temperatures, with the minimal and optimal temperatures for growth outside the range for *Anabaena* and *Microcystis.* In vitro studies show optimum temperature for growth is between 25 and 30 °C, regardless of tropical or temperate origin (Saker and Griffiths 2000; Briand 2004). However, natural populations collected from temperate regions are capable of withstanding much lower water temperatures (>25 °C). In temperate regions, *C. raciborskii* has been observed in water temperatures ranging from 17 to 25 °C (Briand *et al.* 2002), to less than 20 °C (Ryan *et al* 2003). In German waters, the lower limit for seasonal germination and growth is 17 °C (Mischke 2003), while Chonudomkul *et al.* (2004) showed Japanese and Thai strains could adapt to low temperatures (15 °C) showing moderate to good growth. Strains from Africa, Asia, Europe and Australia have shown the same growth rate in temperatures ranging from 15 to 20 °C (Briand 2004). Therefore, the low water temperatures (14.9 and 17.8 °C) recorded in Bibra Lake shows cold adaptation in this temperate population of *C. raciborskii*. This contrasts to the higher water temperatures (25 to 32 °C) required by tropical populations, with growth reduced below 20 °C (Fabbro and Duivenvoorden 1996; Saker and Griffiths 2001; Berger *et al.* 2006).

*Cylindrospermopsis raciborskii* may show good adaptability to a range of temperatures but it is a poor competitor in temperate zones especially in the presence

of other species (Briand *et al.* 2002). This species is capable of producing monospecific blooms in the absence of competitors (Briand *et al.* 2002; Ryan *et al.* 2003). This may explain why *C. raciborskii* failed to produce any spring-summer blooms in Bibra Lake. However, in contrast to this, higher cell counts were recorded for *Microcystis* and *Aphanizomenon* in Bibra Lake during autumn-winter 2001 when *C. raciborskii* was present, compared to spring-summer 2001. Competition between species for resources would be enhanced during the colder months, which would not benefit *C. raciborskii*. Therefore, lower water temperatures in Bibra Lake probably contributed to the presence of *C. raciborskii*.

#### *4.4.3.4 Anabaenopsis elenkinii*

*Anabaenopsis* is a genus that has been rarely reported in Western Australia, with *A. elenkinii* previously identified in the Peel-Harvey Estuary and Canning River (Huber 1980; Vincent 1995) and *A. arnoldii* reported in the Vasse River (Department of Environment 2005). To the author's knowledge, no other occurrence has been documented, particularly in the urban lakes, and as a result, little is known about the distribution of this genus in SCP wetlands.

Worldwide, *Anabaenopsis* is common in stagnant brackish and saline waters, rare in low conductivity and low nutrient waters (Komárek *et al.* 2003; Cronberg and Annadotter 2006). In Australia, *Anabaenopsis* blooms are common in the backwaters of the Murray River where salinity is moderately high (Baker 1991). This preference for higher salinity was only partly supported by the current study, with *A. elenkinii* showing a wide distribution in varying salinity from fresh (Shenton Park, Bibra Lake and Neil McDougall Park), marginally fresh (Joondalup Lake and Yangebup Lake) and hyposaline (Mary Carroll Park). The highest cell counts were found in salinity equal to, or less than 2 ppt. This opposes the findings of Moisander *et al.* (2002) who observed a similar growth rate in salinity ranging from 2 to 20 ppt for *A. elenkinii* isolates from the Neuse River Estuary, with slower growth rates below 2 ppt. Due to this degree of salt tolerance, *A. elenkinii* is considered both a freshwater and estuarine species (Moisander *et al.* 2002).

In the SCP lakes *A. elenkinii* did not dominate a bloom and was commonly found in association with more prominent filamentous species, such as *A. circinalis* and *A. ovalisporum*. However, *A. elenkinii* did become more noticeable in the net samples from mid to late summer (January-February), corresponding to the time when water levels are lower and salinity is subsequently higher. This late summer succession can result in the replacement of freshwater forms, such as *A. circinalis*, with more salt tolerant species like *A. elenkinii*. This was evident in Yangebup Lake, especially during the time when *N. spumigena* bloomed. This late summer succession has also been observed in the Canning River where *Anabaenopsis* replaced *Anabaena* as the dominant species in February and March (Vincent 1995). Despite this distribution pattern, *A. elenkinii* is non-toxic in Australian waters (Baker and Humpage 1994).

#### *4.4.3.5 Nodularia spumigena*

*Nodularia spumigena* is a halotolerant species with a global distribution in environments ranging from fresh (Lake Alexandrina, South Australia), hyposaline (Baltic Sea; Lake Ellesmere, New Zealand) and saline (Orielton Lagoon, Tasmania; Lake Alchichica, Mexico) (Carmichael *et al.* 1988; Baker and Humpage 1994; Jones *et al.* 1994; Kankaanpää *et al.* 2001; Oliva *et al.* 2001; Mazur and Pliński 2003). Yet, in spite of this distribution in wide-ranging salinity, *N. spumigena* blooms appear to be sporadic and confined to certain waterbodies (Moisander and Paerl 2000). This has been the case for the SCP lakes with sporadic short-lived blooms observed in Yangebup Lake during this study, as well as spring blooms observed in two nearby wetlands, Thomsons Lake and Forrestdale Lake in 1993 (John and Kemp 2006). In Western Australia, recurring extensive blooms of *N. spumigena* are commonly associated with the Peel-Harvey and Vasse-Wonnerup estuarine systems.

*Nodularia spumigena* is not commonly encountered in the freshwater wetlands of Australia. The only well-known freshwater occurrences are the frequent blooms in the Lower Lakes (Lake Alexandrina and Lake Albert) at the base of the Murray River, South Australia (Baker and Humpage 1994; Heresztyn and Nicholson 1997; Baker 2000). It was here in 1878 that the first toxic cyanoprokaryota blooms (*N. spumigena*) were observed by George Francis (Codd *et al.* 1994). At the time of

Francis, the lakes were semi-tidal estuaries, but between 1935 and 1940 a series of barrages were built isolating the estuarine water of the Coorong, a long shallow lagoon, from the Lower Lakes (MDBC 2005). This prevents seawater intrusion into the lakes, except in the immediate vicinity of the barrages (Walker and Jessup 1992). Nevertheless, these lakes are predominantly fresh water with salinity ranging from 0.16 to 1.7 ppt (EC 400-1100 µS $cm^{-1}$) being associated with the *N. spumigena* blooms in Lake Alexandrina (Baker 2000).

Salinity up to 1.25 to 1.65 ppt (EC 3000 µS $cm^{-1}$) was measured when *N. spumigena* was present in Yangebup Lake, higher than that recorded in the Lower Lakes. In spite of this, Yangebup Lake provides another example of Australian populations of *N. spumigena* being halotolerant, occurring in salinity ranging from 1 ppt (Lower Lakes; Yangebup Lake) to 30 ppt (Serpentine River, this study). Outside of Australia *N. spumigena* has not been reported in a freshwater wetland until recently when Akcaalan *et al.* (2008) provided the first report of toxic *N. spumigena* in a European freshwater lake (0.5 ppt). During summer 2005, *N. spumigena* was recorded in Iznik Lake, a deep (80 m) freshwater lake in southeast Turkey. *Nodularia spumigena* comprised 60% of the total cyanoprokaryote abundance. Similar to Yangebup Lake, previous cyanoprokaryota blooms contained well-known fresh water genera, with *Anabaena*, *Aphanizomenon*, *Anabaenopsis* and *Cylindrospermopsis* appearing in Iznik Lake. However, unlike Yangebup Lake, *N. spumigena* had been previously identified in Iznik Lake, but was not thoroughly studied (Akcaalan *et al.* 2008). The presence of *N. spumigena* in Yangebup Lake prior to 2001 has not been documented.

Yangebup Lake and the Lower Lakes, although both are temperate freshwater lakes, are different due to location in relation to the coast, size and drainage. The Lower Lakes are large shallow coastal lakes that form at the mouth of the Murray River. The river discharges into Lake Alexandrina maintaining freshwater condition; however during periods of drought, salinity can increase enough to compromise the use of the lakes for domestic and agricultural purposes. In contrast, Yangebup Lake is a small (64.4 ha open water), permanently inundated lake that forms part of the eastern chain of lakes within the Beeliar Regional Park (refer to Fig. 2.2). Unlike the

hyposaline to hypersaline lakes of the western chain, the eastern chain lakes are predominantly fresh water and located approximately 5 to 6 km from the coast. Discharge into the lake is mostly received from urban runoff & drainage from Thomsons Lake during very wet years. Furthermore, there are no previous reports of *N. spumigena* in Yangebup Lake, whereas in the Lower Lakes blooms were present before and after the construction of the barrages.

The reoccurrence of *N. spumigena* in Yangebup Lake provides the first account of this species in an inland freshwater lake. Besides Iznik Lake, the other lake occurrences include the deep saline lakes of Mexico and North America (Beutal *et al.* 2001; Oliva *et al*. 2001; Falcón *et al.* 2002), Orielton Lagoon in Tasmania, a shallow coastal embayment (Jones *et al*. 1994) and Lake Ellesmere in New Zealand, a shallow coastal lake with seasonal breakthrough to the Pacific Ocean (Carmichael *et al.* 1988). The source of *N. spumigena* into Yangebup Lake is most likely Thomsons Lake, which experienced a spring *N. spumigena* bloom in 1993, the same year as Forrestdale Lake (John and Kemp 2006). Yangebup Lake is situated approximately 2 km north of Thomsons Lake and during periods of high rainfall a series of connections and groundwater flow connect Yangebup Lake with Thomsons Lake (Thomson Palmer Pty 2005). Thomsons Lake has the highest salinity of the lakes in the East Beeliar Regional Park (Davis *et al.* 1993) and rarely supports blooms of freshwater cyanoprokaryotes. In November 1999 a nodularin concentration of 2.11 µg $L^{-1}$ was measured in concentrated algal samples collected from Thomsons Lake even though *N. spumigena* itself was not observed (Kemp, unpublished data).

Yangebup Lake usually experiences nuisance blooms of *Microcystis* and *Anabaena*, so the recurring presence of *N. spumigena* may be indicative of increasing salinity. This may lead to the replacement of *Microcystis* and *Anabaena* by *Nodularia* and other salt tolerant genera such as *Anabaenopsis*. For this reason, Yangebup Lake should be monitored for future blooms of *N. spumigena*, focusing on cell concentration, akinete abundance and trichome morphology as weak yellow trichomes indicate unfavourable growth conditions (Jones *et al*. 1994). More field

surveys should be undertaken to see if *N. spumigena* is still present in Yangebup Lake and the physico-chemical variables that are promoting growth. Salinity is not the only influential variable, the availability of inorganic nitrogen and phosphorus throughout summer are two factors that also need to be addressed. The onset of the 1993 bloom in Forrestdale Lake coincided with a decrease in both salinity and water temperature, and an increase in the availability of phosphorus (John, unpublished data). In Lake Alexandrina low turbidity, reduced flow from the Murray River, high light availability and moderate to low nutrient levels have been shown to favour the growth of *N. spumigena* (Baker 2000).

Low salinity (0-10 ppt) does prove beneficial to the germination of *N. spumigena* akinetes (Huber 1985), however salinity higher than this range is required to maintain the bloom. Blackburn *et al.* (1996) studied and compared the growth and cell yield of isolates from the Peel-Harvey Estuary, Orielton Lagoon and Lake Alexandrina in salinity ranging from fresh (0 ppt) to seawater (35 ppt). There were no significant differences in growth rate between these three populations, even though the Lake Alexandrina isolates were expected to be more tolerant to fresh water. Growth rate was significantly slower in freshwater than at any other salinity showing bloom development to be restricted in low salinity. Cell yield was also lower in the 0 g $kg^{-1}$ salinity. In spite of these findings, *N. spumigena* is capable of developing blooms in freshwater conditions (Lake Alexandrina) so further research into the salt tolerance of Australian strains is needed. Yangebup Lake now provides another freshwater population that can be used to examine this response. The natural population and cultured strain of *N. spumigena* from Yangebup Lake should be compared to the Lower Lake population with regards to the lower limit for salt tolerance. Genetic diversity within Australian populations should also be investigated because *N. spumigena* can persist in both freshwater and estuarine environments.

The two Western Australian populations sampled during this research project clearly show the differences between freshwater and estuarine strains of *N. spumigena.* The cell counts recorded for the Serpentine River exceeded $1 \times 10^6$ cells $mL^{-1}$, while a maximum of 70, 000 cells $mL^{-1}$ were counted in Yangebup Lake. A similar

maximum cell count (80, 000 cells $mL^{-1}$) was recorded during the 1995 bloom in Lake Alexandrina (Heresztyn and Nicholson 1997). When comparing the morphology of the species from the two Western Australian sites, akinetes were abundant in the Serpentine River samples, while in the 2002-03 Yangebup Lake samples no akinetes were observed. Akinete production is considered a response to conditions unfavourable for vegetative growth (Fay 1983). In Orielton Lagoon Jones *et al.* (1994) observed trichomes that were an "unhealthy" yellow-green colour and a greater abundance of akinetes in conditions that did not support optimal growth. This was not evident in the samples collected from Yangebup Lake. *Nodularia spumigena* in Yangebup Lake appears opportunistic making use of the freshwater conditions to support initial growth; however higher salinity appears to be a requirement to sustain this growth. The two reported *N. spumigena* blooms in Orielton Lagoon were also stimulated by low salinity (Jones *et al.* 1994). The first bloom in 1986 correlated with heavy rainfall and subsequent decrease in salinity, while the early stages of the second bloom in 1992 coincided with unusually low salinity in the lagoon. However, unlike Yangebup Lake cell counts in Orielton Lagoon reached $1 \times 10^7$ cells $mL^{-1}$ as salinity in the lagoon increased.

## 4.5 Conclusions

This investigation showed how common and extensive cyanoprokaryota blooms are in the urban lakes of the SCP. A high percentage of lakes sampled experienced recurring blooms of potentially harmful species, evidence that these wetlands are under significant stress. *Microcystis flos-aquae* and *Microcystis aeruginosa* were dominant in the cyanoprokaryote community, widespread in their distribution irrespective of geographical location. Salinity was variable that could separate the distribution of these two species, with *M. flos-aquae* demonstrating a higher salinity tolerance. The presence of *Microcystis* dominated blooms in both autumn and winter implies that growth and bloom formation are not restricted to the warmer months. Due to their prevalence in the SCP wetlands, the next stage in this research is to analyse these *Microcystis* bloom samples for the hepatotoxin microcystin. Likewise, the *Anabaena circinalis* populations that were repeatedly observed in lakes such as

Herdsman, Tomato, Blue Gum and Neil McDougall Park need to be assessed for saxitoxins.

Other species of national interest, *C. raciborskii*, *A. ovalisporum* and *A. bergii* var. *limnetica* were found for the first time in the SCP wetlands. Both species can potentially cause continual blooms throughout the year in Bibra Lake and Emu Lake where they are both common. The occurrence of *A. ovalisporum* and *A. bergii* var. *limnetica* in salinity ranging from fresh to hyposaline shows their potential to populate other wetlands, whereas *A. circinalis* was found predominantly in fresh water.

The presence of *N. spumigena* in Yangebup Lake also warrants further investigation due to the marginally fresh conditions associated with the short-lived blooms. Concern arises because of the placement of Yangebup Lake within the eastern chain of lakes of the Beeliar Regional Park and the previous bloom of *N. spumigena* in neighbouring Thomson Lake. In the SCP wetlands, the increasing salinity and water temperatures associated with summer conditions are likely to support the future growth of salt tolerant species like *N. spumigena*, *A. elenkinii* and *A. bergii* var. *limnetica*.

**Appendix 4.1.** Species observed in only one site during each period and therefore not included in the TWINSPAN classification.

| | **Species** | **Site** |
|---|---|---|
| 2000-01 | *A. gracile* | Bibra |
| | *A. ovalisporum* | Bibra |
| | *A. tanganyikae* | Joondalup |
| | *O. sancta* | Thomson |
| 2001-02 | *A. affinis* | Emu |
| | *A. aphanizomenoides* | Mary Carroll |
| | *A. flos-aquae* | Herdsman |
| | *A. torlusa* var. *tenuis* | Mary Carroll |
| | *A. arnoldii* | Claremont |
| | *N. spumigena* | Yangebup |
| | *P. perornata f. attenuata* | Coogee |
| 2002-03 | *A. affinis* | Emu |
| | *A. torlusa* var. *tenuis* | Mary Carroll |
| | *N. spumigena* | Yangebup |
| | *N. linkia* | North |
| | *T. iwanoffianum* | Neil McDougall |

# Chapter 5: Cyanoprokaryota blooms in the Swan, Canning and Serpentine Rivers

## 5.1 Introduction

Cyanoprokaryota blooms are not only restricted to the freshwater wetlands of the Swan Coastal Plain (SCP); species confirmed to be toxic in Australian waters have produced extensive blooms in some of Western Australia's most important river systems. Therefore, bloom events in the Swan River, Canning River and upper Serpentine River were investigated. These rivers have experienced either short-lived or ongoing blooms of genera commonly found in the surrounding freshwater lakes, for example *Microcystis*, *Anabaena* and *Anabaenopsis* (Huber 1980; John 1987; Hosja and Deeley 1994; Vincent 1995; Atkins *et al.* 2001).

The most prominent feature of the Perth metropolitan area is the Swan-Canning Estuary, a shallow 'seasonal estuary' characterised by the seasonal pattern of rainfall and flow, that make parts of the river fresh in winter and hyposaline to marine in summer (Hodgkin 1987). The estuary plays an important role in the history, economy and recreational enrichment of Western Australia. Climate change, urbanisation and industrialisation have changed the character of the Swan-Canning Estuary, with the river system showing signs of deterioration (SRT 1999a; SRT 2007). In the early 1990s the first signs of environmental stress became evident with fish deaths, algal blooms and wide-scale water quality problems (SRT 1999b). An increase in the frequency of such problems is expected (SRT 2007).

Cyanoprokaryota blooms in the estuary were once considered rare (John 1987), with temporary blooms of *Nodularia*, *Microcystis* and *Anabaena* observed in the hyposaline upper reaches of the Swan River in the 1990s (SRT 1999a; John 2000). However in February 2000, an extensive bloom of *Microcystis* caused the closure of the estuary for 12 days due to the extremely high microcystin concentrations. This historically significant bloom event brought attention to the ongoing problems of eutrophication in the SCP wetlands and the resultant cyanoprokaryota blooms.

However potentially harmful blooms have been occurring in the area for the past 30 years, yet little had been documented about their occurrence. In all accounts, the cyanoprokaryota blooms in the river system most likely originated from overflow in the surrounding riverine wetlands and the urban drains and streams that discharge into the upper estuary following extremely high rainfall events (John 1994; 2000). Therefore establishing a link between these wetland systems in relation to community structure, timing of bloom events and factors promoting bloom formation is important.

In general, cyanoprokaryota blooms in the Swan-Canning Estuary are confined to the upper Canning River. Once the weir boards are installed at Kent Street Weir in late October, the upstream impoundment produces conditions similar to a shallow freshwater lake, with poor flushing and flow rate (SRT 2007). The area becomes a prime site for ongoing blooms of *Anabaena* and *Microcystis* (Vincent 1995; Thompson *et al.* 2003). The first major blooms event occurred in the summer of 1993-94 following the removal of a massive *Hydrocotyl* infestation (Vincent 1995), with another large bloom recorded later in summer 1997-98. Similar to the Swan River, *Nodularia spumigena* has also been detected in the upper regions. Historically *N. spumigena* blooms are more commonly associated with the Peel-Harvey Estuary, which experienced extensive summer blooms annually from 1970 to 1994 until construction of the Dawesville Channel in 1994. Since then, no blooms have been detected in the estuary showing the effectiveness of the intrusion of the saline water and increased salinity (Hale and Paling 1999). However *Nodularia* blooms are now present in the upper Serpentine River and interconnected lakes where the influence of flushing with marine water from the channel is reduced.

The objective of this chapter was to examine cyanoprokaryota blooms in the Swan, Canning and Serpentine rivers focusing on associated physico-chemical conditions and species morphology, compared to the freshwater metropolitan lakes previously examined in Chapter 4. John (1987) and Vincent (1995) have revealed a link between the rivers and lakes of the Perth metropolitan area in relation to phytoplankton succession. The Canning River in particular shows a phytoplankton

pattern that closely resembles the southern metropolitan lakes, which includes the series of cyanoprokaryota blooms throughout summer (Vincent 1995). Evidently, the lakes of the SCP have been experiencing cyanoprokaryota blooms even before the first bloom in the Canning River was reported (John, unpublished data), yet very little information regarding this has been published.

## 5.2 Materials and Methods

Water quality parameters and phytoplankton samples were collected from these rivers during blooms events. Seven sites along the Swan River were sampled in February 2000 during the massive *Microcystis* bloom (Fig. 5.1). The upper Canning was sampled in summer from January to February, 2001 to 2003. Three collection sites were chosen upstream from the Kent Street Weir (Fig. 5.1). Four sites along the upper reaches of the Serpentine River were sampled in November 2000 and two sites were sampled in December 2001 (Fig. 5.2).

In the field, surface water temperature, salinity (ppt), electrical conductivity (μS $cm^{-1}$) and pH were measured *in situ* using a hand-held portable TPS WP-81 meter, with all measurements taken from off the shore, or measured from a collected volume of water. One-litre water samples were collected from the Swan River and sent to SGS Environmental Services (Welshpool) for determination of total phosphorus (TP), ortho-phosphate ($PO_4$-P), total nitrogen (TN) and dissolved inorganic nitrogen ($NO_x$-N, $NH_3$-N).

A depth-integrated phytoplankton sample was collected by vertically dragging a 25μm mesh size plankton net through the water column for a distance of 5 m. Samples were identified to species level using the procedure described in section 7.2.1. Cell counts were made from hand collected water samples using a calibrated Lund Cell at 400x magnification as described in section 4.2.2.

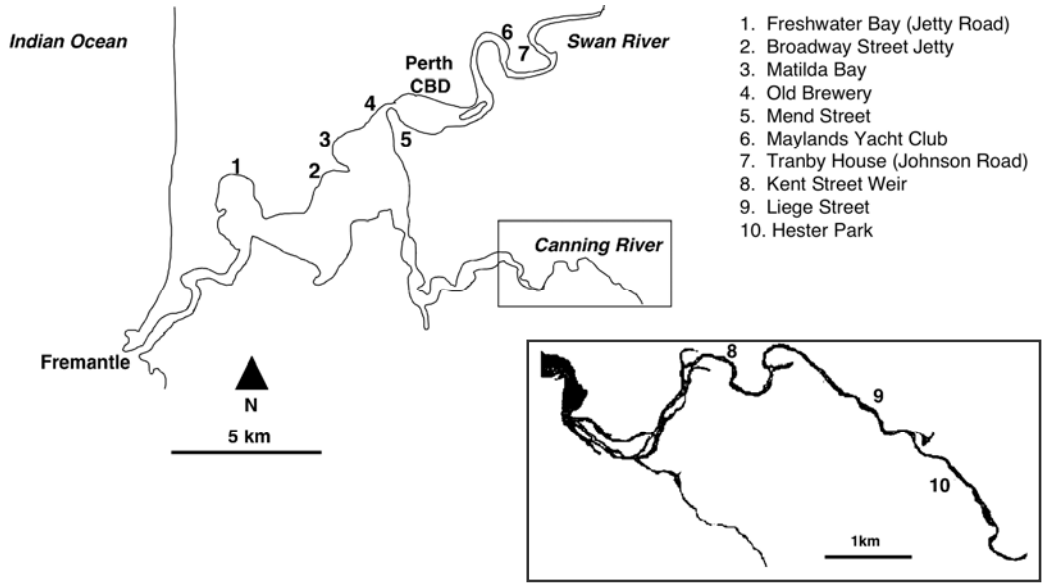



**Figure 5.1.** The location of the Swan River and Canning River collection sites.

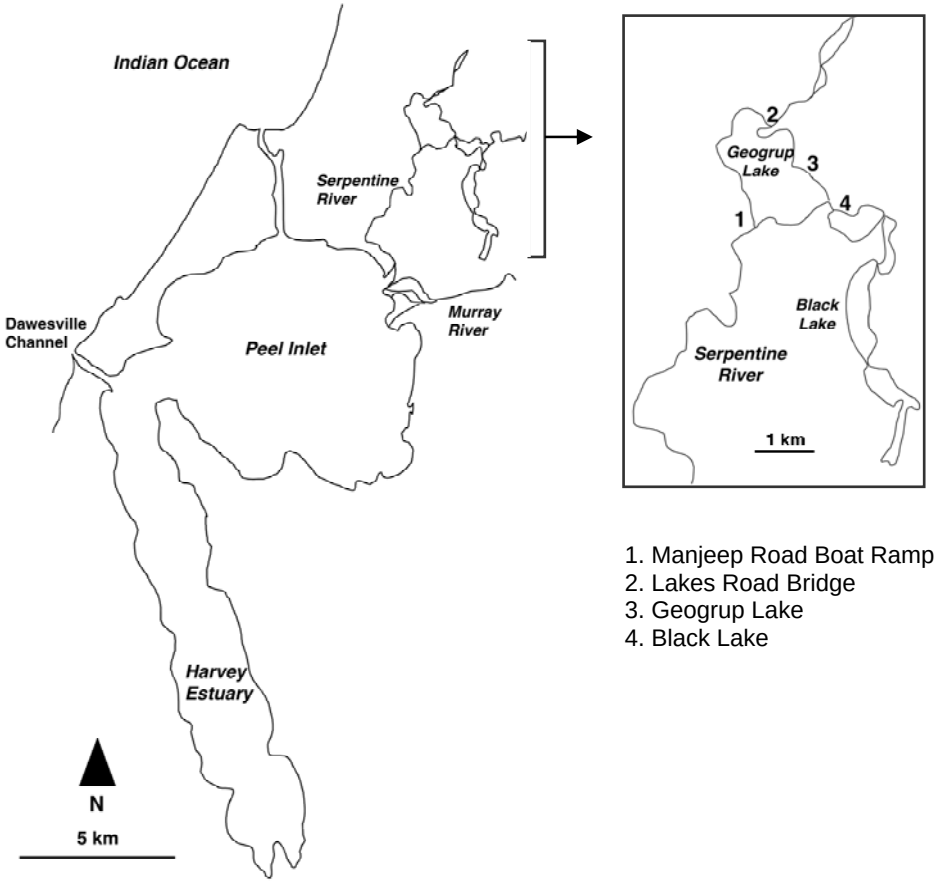



**Figure 5.2.** The sampling sites along the Serpentine River in relation to the Peel-Harvey Estuary, approximately 70 km south of Perth.

## 5.3 Results

### *5.3.1 Swan River Estuary*

The massive bloom of *Microcystis* was reported in the Swan River estuary on 10th February 2000. It extended throughout the estuary from Fremantle upstream to the Helena River, and as far as Saltwater Point in the Canning River. For this study, samples were collected on the 11th, 14th and 16th February 2000. Cell concentrations reportedly peaked on the 16th February, three weeks after the maximum river discharge into the estuary (Robson and Hamilton 2003). The main species identified in the net samples was *Microcystis flos-aquae* (Wittrock) Kirchner represented by spherical colonies of compactly arranged cells, 1.85 μm to 4 μm in diameter (mean 3.35 μm, n = 200). The colonies were without fenestrations and the daughter colonies tended to bud-off with compactly arranged spherical cells. Cell concentrations exceeded $1 \times 10^7$ cells $mL^{-1}$ with the highest cell density recorded upstream at Tranby House (Table 5.1). High cell concentrations were also recorded at Matilda Bay indicating extensive scum accumulation in sheltered locations.

Lower water temperature and salinity was recorded on the 11th February, one day after the bloom was first reported (Table 5.1). The salinity of the surface water ranged from 3.60 ppt (7135 μS $cm^{-1}$) at Maylands Yacht Club (MYC) to 10.43 ppt (19670 μS $cm^{-1}$) at Freshwater Bay. The concentration of total dissolved salts (TDS) indicated hyposaline conditions with concentrations decreasing upstream (Table 5.2). Surface water temperature varied from 24.2 °C to 28.4 °C. Temperatures measured on the 11th February varied only by 0.7 °C, with three sites recording 25.3 °C. The pH was consistent ranging from 7.58 to 8.75. Ambient nutrient concentrations collected on the 11th February are presented in Table 5.2. Chlorophyll-a concentrations indicated water quality conditions ranging from oligotrophic (< 1 μg $L^{-1}$) at MYC and Mend Street, to eutrophic at Matilda Bay. The higher Chl-a concentrations measured in Matilda Bay and Old Brewery corresponded to the higher cell density present in these areas. The $PO_4$-P concentrations were low for all sites. The bioavailable forms of nitrogen ($NH_3$-N and $NO_x$-N) were in greater supply especially for the Old Brewery and Mend Street sites. This varied considerably from

nearby Matilda Bay, which contained the lowest concentrations of $NH_3$-N, $NO_x$-N and TN.

**Table 5.1.** The physico-chemical parameters and *M. flos-aquae* cell counts measured in the Swan-Canning Estuary during February 2000.

| Date | Site | pH | Temp (°C) | Salinity (ppt) | EC ($\mu S\ cm^{-1}$) | Cells $mL^{-1}$ |
|---|---|---|---|---|---|---|
| 11/2/00 | Matilda Bay | 8.15 | 25.3 | 4.56 | 9280 | $1.26 \times 10^7$ |
| | Old Brewery | 7.87 | 24.8 | 4.28 | 8402 | - |
| | Mend Street | 8.23 | 25.3 | 4.39 | 8613 | - |
| | Maylands Yacht Club | 8.48 | 24.6 | 3.60 | 7135 | - |
| | Tranby House | 7.58 | 25.3 | 3.89 | 7663 | $6.88 \times 10^7$ |
| 14/2/00 | Broadway St. | 8.75 | 26.8 | 8.27 | 15490 | - |
| | May Yacht Club | 8.26 | 28.4 | 3.72 | 7560 | - |
| 16/2/00 | Freshwater B. | 8.72 | 25.2 | 10.43 | 19670 | $2.94\times 10^7$ |
| | Broadway St. | 8.46 | 24.8 | 8.35 | 15460 | $1.38 \times 10^7$ |
| | Matilda Bay | 8.29 | 26.1 | 7.42 | 14220 | $4.97 \times 10^7$ |
| | Old Brewery | 8.36 | 26.2 | 5.70 | 11010 | $3.12 \times 10^7$ |

**Table 5.2.** Ambient nutrient concentrations and Chl-a measured from the Swan-Canning Estuary on the 11th February 2000. LOD, Limit of Detection.

| Site | TDS ($mg\ L^{-1}$) | Chl-a ($\mu g\ L^{-1}$) | $NH_3$-N ($\mu g\ L^{-1}$) | $NO_x$-N ($\mu g\ L^{-1}$) | TN ($\mu g\ L^{-1}$) | $PO_4$-P ($\mu g\ L^{-1}$) |
|---|---|---|---|---|---|---|
| Matilda Bay | 6200 | 10 | 9 | < 5 | 1200 | 4 |
| Old Brewery | 5200 | 6.6 | 100 | 296 | 1500 | 5 |
| Mend Street | 5400 | 1.5 | 33 | 343 | 1600 | 13 |
| Maylands Yacht Club | 4900 | < 5 | 29 | 239 | 1400 | 3 |
| Tranby House | 4700 | 3 | 62 | 170 | 1400 | 3 |

### *5.3.2 Upper Canning River*

The algal samples from the Kent Street Weir were dominated by *Anabaena circinalis* Rabenhorst ex Bornet et Flahault. The samples also contained *Microcystis aeruginosa* (Kützing) Kützing, *M. flos-aquae*, *Planktothrix planctonica* (Elenkin) Anagnostidis et Komárek and *A. flos-aquae* (Lyngbye) Brébisson ex Bornet et Flauhault. The morphology of *A. flos-aquae* differed from *A. circinalis* by smaller, more spherical, vegetative cells and smaller heterocytes (Table 5.3).

**Table 5.3.** Mean cell measurements (L, length; B, breadth), standard deviation (SD) and range for *Anabaena circinalis* and *Anabaena flos-aquae* collected from the Canning River sites.

| Species | Cell Type | | Mean diameter (μm) | SD | Range (μm) |
|---|---|---|---|---|---|
| *A. circinalis* | Vegetative | L | 5.43 | 0.68 | 5-7 |
| | | B | 7.53 | 0.63 | 6-8 |
| | Heterocyst | L | 7.83 | 0.79 | 6-10 |
| | | B | 8.10 | 0.80 | 7-10 |
| | Akinete | L | 21 | 2.17 | 16-25 |
| | | B | 13.48 | 1.50 | 11-17 |
| *A. flos-aquae* | Vegetative | L | 4.53 | 0.51 | 4-5 |
| | | B | 5.27 | 0.61 | 4-7 |
| | Heterocyst | L | 5.42 | 0.49 | 5-6 |
| | | B | 5.73 | 0.58 | 5-7 |
| | Akinete | L | 16.95 | 1.68 | 14-20 |
| | | B | 10.14 | 1.42 | 8-12 |

The greatest cell numbers were recorded at the Kent Street Weir with lower numbers upstream at the other two sampling sites. An *Anabaena* bloom recorded in February 2001, produced high cell concentrations of $7.35 \times 10^6$ cells $mL^{-1}$ (Kent Street Weir) and $3.08 \times 10^6$ cells $mL^{-1}$ (Hester Park) (Table 5.4). In March of the following year, a greater number of species were observed with *Microcystis* and *P. planctonica* being prominent in the water samples. In 2003, an oxygenation plant was functional upstream from Kent Street Weir, at Bacon Street, which dispersed the development of scum in the immediate area. The oxygenated area extended 2 km to Liege Street. Cyanoprokaryote samples were collected from an area of the river adjacent to Balbiri Close, approximately 500 m from the oxygenation plant. Although no scums were evident, the samples contain *A. circinalis* at an abundance of $2.58 \times 10^6$ cells $mL^{-1}$.

Summer water temperatures ranged from 24.3 °C at Hester Park to 33.3°C at Kent Street Weir (Balbiri Close). Consistently higher water temperatures were recorded at the Kent Street Weir and Balbiri Close (26-31 °C). The low salinity was indicative of a freshwater environment (Table 5.4). Kent Street showed the greatest variation in salinity ranging from 0.67 ppt to 1 ppt. The pH indicated alkaline conditions (6.6-9.32) with lower pH measured at Hester Park (6.6-7.73).

**Table 5.4.** Mean physico-chemical parameters and cell counts measured along the upper Canning River from 2000 to 2003.

| Site | Date | pH | Temp (°C) | Salinity (ppt) | EC ($\mu S\ cm^{-1}$) | Species | Cells $mL^{-1}$ |
|---|---|---|---|---|---|---|---|
| Kent Street Weir | 23/1/01 | 7.36 | 26 | 0.67 | 1130 | | - |
| | 23/2/01 | 8.42 | 27.2 | 0.98 | 1681 | *A. circinalis* | $7.35 \times 10^6$ |
| | 18/12/01 | 7.71 | 28 | 0.63 | 1287 | | - |
| | 12/2/02 | 7.96 | 30.03 | 0.8 | 1625 | | - |
| | 21/3/02 | 8.54 | 29.1 | 1 | 1982 | *A. circinalis* | $6.91 \times 10^5$ |
| | | | | | | *M. aeruginosa* | $9.84 \times 10^5$ |
| | | | | | | *M. flos-aquae* | $2.72 \times 10^5$ |
| | | | | | | *P. planctonica* | $1.94 \times 10^6$ |
| Balbiri Close | 10/1/03 | 8.63 | 30.7 | 0.82 | 1572 | *A. circinalis* | $2.58 \times 10^6$ |
| | 30/1/03 | 9.32 | 31 | 0.98 | 1877 | *A. circinalis* | $1.06 \times 10^6$ |
| Liege Street | 8/1/01 | 8.56 | 33.3 | 0.62 | 1255 | *M. flos-aquae* | $3.03 \times 10^6$ |
| | 23/1/01 | 7.36 | 24.7 | 0.67 | 1130 | | - |
| | 2/2/01 | 8.59 | 25.1 | 0.63 | 1190 | *A. circinalis* | $2.81 \times 10^6$ |
| | | | | | | *M. flos-aquae* | $1.52 \times 10^7$ |
| | 23/2/01 | 7.73 | 25.1 | 0.78 | 1344 | *A. circinalis* | $5.16 \times 10^6$ |
| Hester Park | 23/1/01 | 6.6 | 26.2 | 0.66 | 1160 | | - |
| | 2/2/01 | 7.22 | 24.3 | 0.63 | 1190 | *A. circinalis* | $1.62 \times 10^6$ |
| | 23/2/01 | 7.66 | 24.6 | 0.72 | 1238 | *A. circinalis* | $3.09 \times 10^6$ |

### *5.3.3 Upper Serpentine River*

The environmental data and cell density counts recorded from the Serpentine River sites are presented in Table 5.5. Bloom samples were dominated by *Nodularia spumigena* Mertens ex Bornet et Flahault and *Anabaenopsis elenkinii* Miller. Cell counts for *N. spumigena* exceeded $2 \times 10^6$ cells $mL^{-1}$ at all sites. The highest cell count for both species was recorded at Lake Roads Bridge on the 12th December 2001. Surface water temperatures ranged from 20.7 °C to 25.9 °C.

Salinity varied between sites ranging from marginally fresh (2.30 ppt) to hypersaline (31.6 ppt). Overall, hyposaline conditions were experienced in November 2000, with considerably higher salinity recorded in December 2001. The pH showed moderately alkaline conditions.

**Table 5.5.** Physico-chemical parameters and *Nodularia spumigena* cell densities (unless stated) measured from sites along the upper Serpentine River.

| Date | pH | Temp (°C) | Salinity (ppt) | EC (µS $cm^{-1}$) | Cells $mL^{-1}$ |
|---|---|---|---|---|---|
| **Lakes Road Bridge** | | | | | |
| 3/11/00 | 9.77 | 22.2 | 3.40 | 6937 | $3.83 \times 10^6$ |
| 12/12/01 | 8.35 | 25.8 | 19.6 | 29600 | $2.41 \times 10^6$ |
| | | | | | $4.50 \times 10^4$ *A. elenkinii* |
| **Manjeep Road** | | | | | |
| 3/11/00 | 10.07 | 20.7 | 10.07 | 19592 | $3.72 \times 10^6$ |
| 12/12/01 | 8.29 | 24.2 | 31.6 | 46425 | no count |
| **Black Lake** | | | | | |
| 3/11/00 | 9.84 | 22.5 | 10.87 | 21085 | $3.07 \times 10^6$ |
| **Geogrup Lake** | | | | | |
| 3/11/00 | 9.06 | 24.7 | 2.30 | 4737 | $2.20 \times 10^6$ |
| | | | | | $4.14 \times 10^5$ *A. elenkinii* |

## 5.4 Discussion

### *5.4.1 Swan River Estuary (Summer 2000)*

The *Microcystis* bloom was considered the largest algal bloom in the history of the Swan-Canning River system (Atkins *et al.* 2001; Robson and Hamilton 2003). It was caused by two unseasonably large rain events in January 2000, which produced unusual freshwater conditions (Robson and Hamilton 2003). Cell numbers, in relation to surface accumulation, exceeded $1 \times 10^7$ cells $mL^{-1}$ for this study, and $1.3 \times 10^8$ cells $mL^{-1}$ reported by Atkins *et al.* (2001). Atkins *et al.* (2001) recorded integrated water column counts of 15 000 cells $mL^{-1}$. In the current research project, *Microcystis flos-aquae* cell counts to this magnitude were recorded in some of the highly eutrophic urbanised lakes, for example Emu Lake, Herdsman Lake, Bibra Lake and Yangebup Lake. This cell density presents a high probability of adverse health effects if ingested through recreational exposure (WHO 2003). As a precaution extensive areas of the Swan River estuary were closed to recreational and commercial activities due to the health concerns related to the toxic nature of this species. Microcystin concentration reached 124.16 $\mu g\ L^{-1}$ in scum samples used for this study (refer to section 8.3.1) and Atkins *et al.* (2001) reported a concentration of 8 $\mu g\ L^{-1}$ in water samples.

Microscopic examination of the bloom material collected during this study identified the dominant species to be *Microcystis flos-aquae*, also referred to as *M. aeruginosa* forma *flos-aquae* (Wittrock) Elenkin. Other reports also identified *M. aeruginosa* forma *aeruginosa* Kützing in the bloom (Atkins *et al.* 2001; Orr *et al.* 2004). Both species of *Microcystis* are primarily considered freshwater in their occurrence and generally not associated with estuarine conditions. However in the current study *M. flos-aquae* was the dominant *Microcystis* in lakes of higher salinity (> 3 ppt) indicating a wider salinity tolerance compared to *M. aeruginosa*. However this distribution is not supported by the current literature. Unlike *M. flos-aquae, M. aeruginosa* has been associated with both fresh and hyposaline (brackish) waters (Komárek and Anagnostidis 1998; Cronberg and Annadotter 2006), producing blooms in a number of estuaries worldwide including the Patos Lagoon Estuary

(Yunes *et al.* 1997), San Francisco Bay Estuary (Lehman *et al.* 2005), Guadiana Estuary (Domingues *et al.* 2005) and the Golden Horn Estuary (Tas *et al.* 2006).

In the Swan River Estuary, there is a salinity gradient established by the salt wedge and river flow, which is present during most of the year, preventing cyanoprokaryotes from producing nuisance blooms. During summer the gradient is mainly in the upper estuary where salinity varies from fresh (~ 3 to 4 ppt) to near marine salinities (> 30) by late summer and autumn (John 1987; Chan and Hamilton 2001). During this time marine diatoms are usually the dominant phytoplankton with periodic blooms of dinoflagellates (John 1987; Thompson 1998). Therefore, the *M. flos-aquae* bloom in February 2000 was a drastic deviation from the typical phytoplankton pattern. Prior to the bloom, salinity in the estuary was between 25 and 35 ppt with dinoflagellates and diatoms the dominant phytoplankton in the lower estuary and chlorophytes in the upper reaches (Robson and Hamilton 2003). Following the heavy rainfall in January 2000 surface water salinity decreased to around 5 ppt and low numbers of diatoms and dinoflagellates were recorded (Robson and Hamilton 2003).

The seasonal variation of rainfall, nutrient input and the movement of the salt wedge influence the distribution and succession of phytoplankton within the Swan-Canning Estuary (SRT 1999b; Twomey and John 2001). It is evident that unusual patterns of rainfall can trigger nuisance blooms of opportunistic species, such as *Microcystis* and *Nodularia* that take advantage of the lowered salinity levels (John 1994; SRT 1999b). January 2000 was the third wettest summer on record with 102 to 206 mm of rainfall. A large proportion of that rainfall was in the Avon River catchment causing 270 GL of fresh water and nutrients to be flushed into the system (Atkins *et al.* 2001). In addition, this population of *Microcystis* most likely originated from the surrounding wetlands in the Avon River Catchment where the highest rainfall was recorded. Due to the high rainfall, most parts of the estuary, which should have been saline, had become fresh. A similar event in April 1955 showed heavy unseasonal rains depositing a layer of fresh water in the Swan River basin causing salinity

stratification and massive fish mortalities due to deoxygenation (Middleton 1955, cited in Hodgkin 1987).

The salinity regime of the Swan River estuary is also dependant on the duration, intensity and timing of winter rainfall (John 1994). Twomey and John (2001) found higher rainfall in early winter 1980 resulted in the upper estuary being under the influence of freshwater for a greater period of time. From the beginning of winter 1980 until autumn 1981 salinity was less than 10 ppt (Twomey and John 2001). As a result, the phytoplankton in the upper estuary was characteristic of hyposaline water with the absence of marine species. Species like *N. spumigena*, that can tolerate large and sustained changes in salinity would benefit from these conditions, although there were no reports of *N. spumigena* at this time. It was later during December 1981 that a bloom of *N. spumigena* was detected in the upper Swan River (SRT 1999b). Likewise, in the Harvey Estuary where *N. spumigena* usually flourishes, prolonged winter rain in 1983 resulted in an *Anabaena*, common freshwater species, blooming during spring (Hosja and Deeley 1994).

The inflow of fresh water into the Swan-Canning Estuary lowered the salinity to within the range tolerated by *M. flos-aquae*. Laboratory salinity trials using cultured Swan River bloom material collected from this showed growth to be optimum up to 4 ppt, with no growth at 25 ppt (Robson and Hamilton 2003). Orr *et al.* (2004) reported cultures to be salt tolerant up to 9.8 ppt, which is within the salinity range recorded in this study (3-10 ppt) and by Atkins *et al.* (2001) (5-10 ppt). This is well within the range that would allow for adequate growth of *Microcystis*. Seawater intrusion naturally increased the salinity to above 10 ppt, which is the upper tolerance limit for this strain, resulting in the collapse of the bloom (Atkins *et al.* 2001).

Worldwide, other case studies have shown *Microcystis* to inhabit water with a similar tolerance range. A model developed to describe the dynamics of annual *Microcystis* blooms in Lake Volkerak, Netherlands, predicted the summer blooms would persist in salinities up to 8 to 10 ppt (Verspagen *et al.* 2006). Salinity above 14

ppt would suppress these blooms (Verspagen *et al.* 2006). In the San Francisco Bay Estuary, California, *M. aeruginosa* was found in both freshwater and hyposaline areas at salinities from 0.1 to 18 ppt (Lehman *et al* 2005). In Kucukcekmece Lagoon, Istanbul, the highest biomass of *M. aeruginosa* occurred at the highest salinity (8.8 ppt), which was where the lagoon is connected to the Marmara Sea (Albay *et al.* 2005).

The latest *Microcystis* bloom and the increase in dinoflagellate blooms in the upper estuary (Twomey and John 2001) are signs of a degraded eutrophic system. Further deterioration of water quality can greatly affect the value of the estuary as a recreation and conservation resource (John 1994). The Golden Horn Estuary in Turkey illustrates this point. Poor water quality has allowed *M. aeruginosa* to be present during most of the year throughout the system, with persistent blooms occurring in the upper estuary where salinity ranges from 1.8 to 3.9 ppt (Tas *et al.* 2006). The highest abundance of *M. aeruginosa* ($2.7 \times 10^6$ cells $mL^{-1}$) was recorded in winter when salinity was low and nutrient concentration had increased in relation to freshwater inflow (Tas *et al.* 2006).

Although ambient nutrient concentrations were lower than the levels cited by Atkins *et al.* (2001) and Robson and Hamilton (2003); TN, $NH_3$-N and $NO_x$-N were greater that the ANZECC and ARMCANZ (2000) recommendation for healthy rivers and estuaries. For all sites except Mend Street the soluble reactive phosphorus ($PO_4$-P) concentration was below the trigger value of 5-15 μg $L^{-1}$. The lower nutrient concentrations reported in the current study show they were being utilised by *Microcystis* to support exponential growth. Routine monitoring found higher concentrations of inorganic nitrogen (>2000 $NH_3$-N μg $L^{-1}$) and phosphorus (> 400 μg $L^{-1}$) followed a major discharge event on the 22nd January 2000 (Robson and Hamilton 2003). *Microcystis*, not capable of nitrogen fixation, would benefit from the readily available source of freshwater and $NH_3$-N to achieve optimal growth. The nutrient data used for the current study were collected on the 11th February 2000, almost three weeks after the major inflow event. Nutrient levels were shown to have decreased before *Microcystis* density increased to peak on the 16th February 2000.

### *5.4.2 Summer blooms in the upper Canning River*

*Anabaena* blooms have persisted upstream of the Kent Street Weir since the early 1990s. Confined to the freshwater sections of the upper Canning River they flourish from January through to April (Vincent 1995; Thompson *et al.* 2003). These blooms have been associated with low stream flow, low salinity, high water temperature and low concentrations of dissolved inorganic nitrogen (Vincent 1995; Thompson *et al.* 2003). Similar ongoing problems have occurred in the Barwon-Darling River, New South Wales, where extensive *Anabaena* blooms are triggered by low flow conditions, high nutrient concentrations, elevated pH and reduced turbidity (Bowling and Baker 1996).

There were five species identified in the summer phytoplankton with *Anabaena circinalis* being the dominant species observed each year. The first detailed account of phytoplankton succession in the Canning River by Vincent (1995) recorded 15 cyanoprokaryote species with *A. circinalis, A. spiroides* and *Anabaenopsis sp.* forming a major component of the summer phytoplankton. In the current study, *A. flos-aquae* was the only other Nostocales species identified. *Anabaena flos-aquae* was previously reported in the Canning River when it caused a bloom in January 1998 (SRT 1999a).

In February 2001, *A. circinalis* cell density reached $7.35 \times 10^6$ cells $mL^{-1}$. For previous blooms, *Anabaena* density reached $2.20 \times 10^5$ cells $mL^{-1}$ in April 1994 (Vincent 1995), and $150 \times 10^9$ cells $L^{-1}$ in January (Thompson *et al.* 2003). All are above the recommended guideline value for a moderate health alert in recreational waters (WHO 2003). Persistently high cell numbers have resulted with warning signs against swimming becoming a permanent feature of the upper Canning River. During both major bloom events in 1993 and 1997, areas of the Canning River were closed to the public for swimming and fishing.

*Microcystis flos-aquae* and *M. aeruginosa* were detected in the summer-autumn 2001 samples at a combined concentration higher than what was recorded in the

Swan River estuary. Both species are also common the freshwater lakes sampled for this study. Interestingly, Vincent (1995) reported *Microcystis sp.* to be a minor taxon in the summer phytoplankton in 1994-95 (< 30 % total phytoplankton) with *A. circinalis* the dominant species at $2.20 \times 10^5$ cells $mL^{-1}$. This change in *Microcystis* density may be associated with greater abundance of dissolved inorganic nitrogen resulting from the breakdown of the *Anabaena* blooms. However, nutrient samples were not collected during the current study, so this can not be confirmed. Another possible explanation is higher water temperatures. *Microcystis* has been shown to be more abundant than *Anabaena* in a higher temperature range of 28 to 32 °C (Nalewajiko and Murphy 2001), and this was evident in the results. *Anabaena circinalis* was dominant at Hester Park where lower water temperatures were recorded (24-26 °C), while Kent Street Weir experienced higher water temperatures (26-31 °C) and higher density of *Microcystis* and greater diversity of species.

*Planktothrix planctonica* had not been previously identified in the upper reaches of the Canning River. *Oscillatoria* was identified as the main Oscillatoriales occurring in the area, present throughout the year in a predominately estuarine environment (Vincent 1995; SRT 1999a). The slightly wavy appearance of the trichomes illustrated in Hosja and Deeley (1994) suggests this species to be *Oscillatoria sancta*, a benthic species that grows in both stagnant or flowing fresh or slightly hyposaline water (Komárek and Anagnostidis 2005). *Planktothrix planctonica* appears to have a similar distribution in the SCP wetlands. In addition to the Canning River, this species was also recorded in the freshwater lakes Blue Gum and Tomato, as well as Forrestdale Lake, which is a shallow seasonal wetland that becomes hyposaline in summer.

In relation to physico-chemical parameters, high water temperature and low salinity may be implicated in controlling the formation of *Anabaena* blooms in the upper Canning River (Vincent 1995). Average water temperatures (24.7 to 31 ºC) were within the range reported by Vincent (1995) (23.1-31.1 ºC), who found optimal growth of *A. circinalis* could be achieved at 24°C. However, the optimal temperature range for akinete germination is lower (20-25 ºC) (Baker and Bellifemine 2000).

Prolonged exposure to high temperatures, similar to those experienced at Balbiri Close, may reduce the severity of these blooms. Essentially the upper Canning River becomes a freshwater impoundment from spring to autumn and this evident in these results. Surface salinity was uniform across the three collection sites with the blooms developing in salinity ranging from 0.6 to 1 ppt. Lower salinity (0.3-0.6 ppt) was associated with the 1994 bloom studied by Vincent (1995).

### *5.4.3 Nodularia in the upper Serpentine River*

The *N. spumigena* blooms and related water conditions in the Peel-Harvey Estuary have been studied previously (Huber 1980; Huber 1984; Hamel and Huber 1985; Hale and Paling 1999). The distribution of this species in Western Australia has extended from Peel-Harvey Estuary and Swan River Estuary to include three metropolitan lakes; Yangebup Lake, Forrestdale Lake and Thomsons Lake.

Salinity recorded from the upper Serpentine River was comparable to the South West Corridor and East Coastal Plain wetlands sampled in this study (Table 5.6). Hyposaline environments favour halotolerant species like *N. spumigena* and there is the potential for this species to expand its distribution to wetlands other than the Serpentine River system. This has been the case with the occurrences in Yangebup Lake, Forrestdale Lake and Thomsons Lake. Of concern are the near freshwater conditions that are beneficial to the germination of *N. spumigena* akinetes. Low salinity and high nutrient concentrations present during spring are sufficient to initiate a *N. spumigena* bloom in the aforementioned lakes, with subsequent increases in salinity and water temperatures experience during summer capable of sustaining the bloom.

**Table 5.6.** Salinity of the Serpentine River sites compared to the study lakes described in Chapter 3. Sites arranged by increasing salinity. Serpentine River sites are in bold.

| Site | Month/Year | EC ($\mu S\ cm^{-1}$) |
|---|---|---|
| Thomsons Lake | Dec 2000 | 3890 |
| **Geogrup Lake** | **Nov 2000** | **4737** |
| **Lakes Road Bridge** | **Nov 2000** | **6937** |
| Lake Brearley | Feb 2002 | 7990 |
| Manning Lake | Nov 2000 | 10710 |
| Forrestdale Lake | Jan 2001 | 11470 |
| Lake Claremont | Jan 2001 | 12030 |
| Market Gardens | Dec 2001 | 15320 |
| **Manjeep Road** | **Nov 2000** | **19592** |
| **Black Lake** | **Nov 2000** | **21085** |
| Mary Carroll Park | Feb 2003 | 22670 |
| **Lake Road Bridge** | **Dec 2001** | **29600** |
| Lake Coogee | Jan 2002 | 37650 |
| **Manjeep Road** | **Dec 2001** | **46425** |

*Anabaenopsis elenkinii* was also found at the two of the upstream sites. This species shows moderate salinity tolerance and often occurs in stagnant hyposaline or saline waters (Cronberg and Annadotter 2006). *Anabaenopsis elenkinii* had been previously isolated in the estuary by Huber (1980) but there is no mention of distribution or abundance. Abundance in the current study reached $4 \times 10^5$ cells $mL^{-1}$ showing *A. elenkinii* to be a major component of the bloom.

However the occurrence of *A. elenkinii* in the Serpentine River may be limited to areas upstream of Geogrup Lake where salinity is generally lower. With no river flow in summer the water in this area can become stagnant. The spring salinity measured at Lakes Road Bridge and Geogrup Lake (~ 3 ppt) was lower than downstream at Manjeep Road (~ 10 ppt). The lower sections of the Serpentine River can reach 55 mg $L^{-1}$ in summer, higher than the salinity of seawater (Serpentine River Working Group 1998). The lower cell numbers of *A. elekinii* recorded in December 2001 at Lakes Road Bridge may be due to the higher salinity (19.6 ppt), which would then favour the growth of *N. spumigena*. Blooms of *Anabaenopsis*,

with *Anabaena*, near Lakes Road Bridge were reported again in November 2001, December 2004 and January 2005 (WRC 2001; Department of Environment 2004b, 2005). There were low numbers of *N. spumigena*, which were predicted to increase once the water became more saline and water temperatures increased (WRC 2001).

## 5.5 Conclusions

The *M. flos-aquae* bloom in the Swan-Canning Estuary was a deviation from the characteristic phytoplankton succession, caused by unseasonable rain that reduced the salinity to less than 10 ppt. *Microcystis flos-aquae* was recorded in salinities greater than 3 ppt, confirming this species to have a moderate salinity tolerance and capability of forming opportunistic blooms in the most unlikely environments. The source of *Microcystis* was most likely the surrounding wetlands, which was the case with the previous minor blooms, demonstrating the need to monitor and control blooms in the metropolitan lakes. Management solutions aimed at reducing cyanoprokaryota blooms in the Swan and Canning rivers need to include the surrounding lakes and riverine wetlands, which are providing the source of these blooms.

*Microcystis flos-aquae* and *M. aeruginosa* have been identified as the most widespread bloom-forming cyanoprokaryotes in the SCP lakes during summer. Both are also common in the urban streams and drains throughout the metropolitan area. This distribution can be extended to the upper Swan and Canning rivers where blooms, whether temporary or extensive, seem common. Nevertheless, *A. circinalis* is still the dominant species associated with the spring-summer cyanoprokaryota blooms in the upper Canning River. The presence of *Microcystis* and *Anabaena* in both the SCP lakes and upper Canning River clearly demonstrates how similar both wetlands are in relation to community structure and physico-chemical environment. Reoccurring *Anabaena* blooms in the Canning River are an ecological problem that is likely to continue, if eutrophication persists.

Likewise, *N. spumigena* blooms are still an ongoing occurrence in the Serpentine River. However the distribution of *N. spumigena*, which was previously only associated with river systems, now includes inland freshwater and hyposaline lakes. The similar range in salinity observed between the Serpentine River sites and the coastal and inland lakes shows the potential for *N. spumigena* to bloom in the SCP lakes during the summer months. This view is supported by the previous occurrences of *N. spumigena* within three of the southern study lakes. Of concern are the high cell concentrations, in excess of 100 000 cells $mL^{-1}$, recorded for *Microcystis*, *Anabaena* and *Nodularia*. All three are capable of producing toxic blooms, which at this cell concentration could produce allergic reactions in recreational users.

# Chapter 6: Influence of phosphorus and nitrogen availability on the succession and abundance of bloom-forming cyanoprokaryotes in five eutrophic urban lakes

## 6.1 Introduction

The role of nitrogen (N) and phosphorus (P) in regulating cyanoprokaryote community structure has been investigated. Nutrient data and cyanoprokaryote samples were collected from five eutrophic lakes in the summer of 2002-03 to investigate changes in the abundance of N-fixing and non-N-fixing species in relation to N and P in ambient water. In temperate lakes, eutrophic conditions can lead to excessive phytoplankton growth, prolonged blooms and a transition in community structure (Watson *et al.* 1997; Huszar and Caraco 1998; Vezie *et al.* 2002; Reynolds 2006). In particular, the ratio between total N and P (TN:TP), as well as dissolved inorganic N and P (DIN:SRP), are important in the development and dominance of cyanoprokaryota blooms, as low TN:TP, or limiting concentrations of dissolved inorganic N, favour the growth and dominance of vacuolated N-fixing species (Smith 1983; Pick and Lean 1987; Levine and Schindler 1999; Havens *et al.* 2003).

Nitrogen is of particular importance to bloom-forming cyanoprokaryotes, as an essential component in the formation of gas-vesicles, consequently affecting the regulation of cell buoyancy (Oliver and Ganf 2000). Nitrogen limitation can result in a decrease in gas vesicle volume and a loss of buoyancy preventing vertical migration through the water column (Brookes and Ganf 2001). Buoyancy regulation allows cells to maintain suspension in the euphotic zone. Nitrate and ammonia are assimilated as the preferred source of N, with heterocytic species having the additional ability to fix atmospheric N, reducing it to ammonia. Nitrogen fixation contributes to the internal N load and influences N availability. Additionally, this process provides an adaptive advantage over non-heterocytic species when epilimnetic concentrations of dissolved inorganic N are low. Therefore, controlling N-fixing taxa is strongly dependent on P supply (Paerl 2008). Past studies have

shown N limitation can favour the dominance of N-fixing species, such as *Anabaena*, in the freshwater wetlands of the SCP (Congdon 1986; Bayley *et al.* 1989; Lund and Davis 2000).

Conversely, P is required for nucleic acid and lipid synthesis (Simon 1987) and is often in limiting supply in fresh water lakes (Reynolds 2006). In P-limited conditions, cyanoprokaryotes can synthesize periplasmic or extracellular phosphatases and exhibit an increased capacity to take up phosphate (Torriana-Gorini *et al.* 1994). Cyanoprokaryotes are more efficient than other phytoplankton species in converting available P into their biomass (Dokulil and Teubner 2000), with low concentrations of inorganic P favouring species with a greater capacity to store P (Oliver and Ganf 2000; Xie *et al.* 2003). For example, under P limitation, *Anabaena* is more efficient than *Microcystis* in P uptake and has a higher growth rate (Nalewajiko and Murphy 2001). Compared to *Aphanizomenon*, *Anabaena* again has a greater affinity for P, and can produce more biomass in P-limited and N-fixing conditions (De Nobel *et al.* 1997). This allows *Anabaena* to be a superior competitor and dominant within a bloom containing multiple genera.

Both N and P are in excessive amounts in most of the wetlands of the SCP (Davis and Rolls 1987; Helleren 1993; Kinnear and Garnett 1999), as the result of excessive nutrient run-off in the Swan-Avon catchment. Changes in land use since European settlement have seen large areas of the coastal plain cleared to support agriculture, horticulture, industry and urban expansion. The naturally infertile sandy soils of the SCP readily lose water and nutrients into an extensive drainage system that is directed into the urban wetlands (Hodgkin and Hamilton 1993). This has a detrimental effect with many SCP lakes becoming heavily nutrient enriched and experiencing frequent spring-summer cyanoprokaryota blooms as demonstrated in Chapter 4.

The shallow polymictic freshwater lakes of the SCP are predisposed to eutrophication due to their small volume and the relatively large surrounding catchment. The pelagic zone of shallow lakes does not show the systematic loss of nutrients during summer due to the close sediment-water contact producing a rapid

cycle of sedimentation and resuspension, ensuring a quick return of most sedimented nutrients back into the water column (Scheffer 1998). Furthermore, being shallow means the water column is frequently mixed by wind and the loose surface-sediment layer allows significant resuspension of stored nutrients into the water column (Arnold and Oldham 1997; Boulton and Brock 1999). Whether this internal loading of nutrients within the SCP lakes is sufficient to prolong summer cyanoprokaryota blooms is examined in this chapter.

The objective of this chapter was to firstly investigate whether nutrient concentrations decrease from late spring (November) to mid-summer (February), once catchment runoff has ceased, or if concentrations remain sufficient to fuel summer cyanoprokaryota blooms. The temporal changes in species composition (N-fixing versus non-N fixing) in relation to the availability of dissolved inorganic N and P were then examined. The five study sites chosen all experience blooms of both heterocytic (*Anabaena*, *Anabaenopsis*, *Aphanizomenon* and *Nodularia*) and non-heterocytic species (*Microcystis* and *Planktothrix*), so would best represent changes in community structure. Finally, the nutrient data collected during this study was compared to baseline data collected in spring 1998 (John, unpublished data) to assess changes in trophic status of these lakes.

## 6.2 Materials and Methods

### *6.2.1 Study sites*

The five sites are located in popular parks and recreation reserves within the metropolitan city of Perth. A full site description for each lake is provided in Chapter 2. Here the location and species associated with the spring-summer blooms, as described in Chapter 4, are summarised.

The three southern lakes, Blue Gum, Bibra and Yangebup, are within the Beeliar Regional Park, southwest of the Perth metropolitan area. They form the eastern chain of lakes that also includes Booragoon Lake, Piney Lake Reserve and North Lake (Fig. 6.1). All are permanent with their water levels artificially maintained. Spring

nutrient levels for these three lakes are indicative of eutrophic to hypertrophic systems (Table 6.1). Blue Gum Lake is the northernmost water body in this chain of lakes, 1 km northeast of Booragoon Lake. Blooms containing *Microcystis aeruginosa*, *M. flos-aquae* and *Anabaena circinalis* are common during spring and summer, with five species of Oscillatoriales also recorded in this site. Bibra Lake is approximately 5 km south of Blue Gum Lake and is home to many water birds. The feeding of birds on the western shore, where samples were collected, has localised effects on water quality as uneaten food and bird faeces contribute to the nutrient loading (Thompson Palmer Pty Ltd 2005). *Microcystis aeruginosa* blooms have persisted since 1994 and in recent years *M. flos-aquae*, *Aphanizomenon ovalisporum* and *Anabaena bergii* var. *limnetica* have been observed throughout spring and summer. Yangebup Lake is located 1 to 2 km south of Bibra Lake. Poor water quality has resulted in frequent blooms of *M. aeruginosa, M. flos-aquae* and *A. circinalis*. The halotolerant species *Nodularia spumigena* and *Anabaenopsis elenkinii* also inhabit Yangebup Lake.

Emu Lake is the northern most lake used in the study, located 12 km north of the Perth metropolitan area, about 6 to 10 km south east of Lake Joondalup and Lake Goollelal (Fig. 6.1). Similar to Bibra Lake, the common bloom-forming species in Emu Lake are *M. flos-aquae*, *M. aeruginosa*, *A. ovalisporum* and *A. bergii* var. *limnetica*. Spring nutrient concentrations are lower than those of the southern lakes but are still indicative of a eutrophic system. Tomato Lake is located east of the Perth metropolitan area, as part of the wetlands of the Inner Central Suburban Area, which also includes Neil McDougall Park. Similar to the southern lakes Yangebup and Blue Gum, Tomato Lake experiences frequent blooms of *M. aeruginosa*, *M. flos-aquae* and *A. circinalis*. *Anabaena spiroides* f. *spiroides* is also common to Tomato Lake.

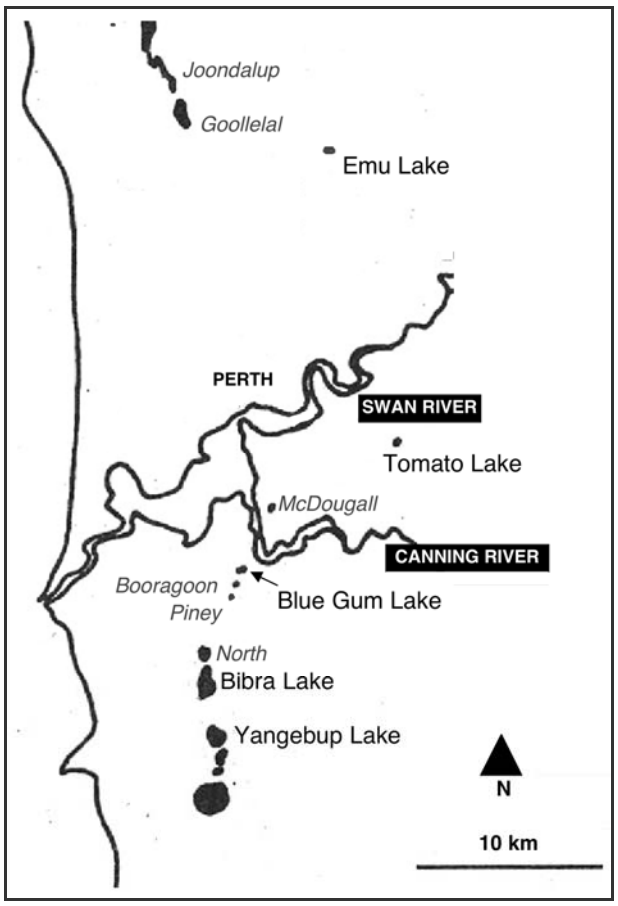



**Figure 6.1.** Reference map showing the location of the five study lakes in the Swan Coastal Plain (Emu, Tomato, Blue Gum, Bibra and Yangebup).

**Table 6.1.** Spring nutrient concentrations for the study sites and the surrounding lakes. Classification based on the OECD boundary values (Rast *et al.* 1989). All samples collected in November 2001 except Bibra Lake sample, collected in October 1999.

| | TP (μg $L^{-1}$) | TN (μg $L^{-1}$) | Classification |
|---|---|---|---|
| Blue Gum Lake | 140 | 1400 | Eutrophic/Hypertrophic |
| Booragoon Lake | 6500 | 8600 | Hypertrophic |
| Piney Lake Reserve | 110 | 2600 | Eutrophic/Hypertrophic |
| North Lake | 230 | 2400 | Hypertrophic |
| Bibra Lake | 130 | 960 | Eutrophic/Hypertrophic |
| Yangebup Lake | 200 | 3100 | Hypertrophic |
| Tomato Lake | 150 | 3600 | Hypertrophic |
| Emu Lake | 50 | 790 | Eutrophic |

### *6.2.2 Data collection and nutrient analysis*

The samples were collected at weekly intervals from November 2002 to February 2003, except December 2002 when the lakes were sampled fortnightly. One-litre water samples were collected monthly (7$^{th}$ or 8$^{th}$ November, 12$^{th}$ or 13$^{th}$ December, 10$^{th}$ January, 5$^{th}$ February) and sent to SGS Environmental Services (Welshpool) for determination of total phosphorus (TP), soluble reactive phosphorus (SRP, $PO_4$-P), total nitrogen (TN) and dissolved inorganic nitrogen ($NO_3$-N, $NO_2$-N, $NH_3$-N). In the field, electrical conductivity (EC; μS cm$^{-1}$) and water temperature (°C) were measured using a TPS WP-81 portable meter during each visit. Both parameters influence the community structure of a bloom and also vary significantly between study sites, therefore they were included in the study. A 25-μm phytoplankton net was dragged through the water column to collect concentrated cyanoprokaryote samples that were used for identification. Water samples used for cell counts were hand collected. All samples were preserved in Transeau's solution (6:3:1 Water: Ethanol: Formalin).

### *6.2.3 Phytoplankton identification and estimation of biomass*

Cyanoprokaryota samples were identified to the species level using recent taxonomic guidelines (refer to section 7.2.1 for complete reference list). Cell numbers (cells mL$^{-1}$) were determined from the water samples using a Lund Cell at 400x magnification. A volume of water was pipetted into the chamber and allowed to stand for ten minutes. Counts were made from 30 fields of view, completed only once when cell density in the water sample was visually high, and in triplicate when there was a lesser concentration. The average count per field was multiplied by the conversion factor to express the count as cells mL$^{-1}$. For colonial species (*Microcystis*), the number of cells per colony was estimated from the area of the colony divided by the diameter of a cell. For filamentous species where cell walls were easily recognised the number of cells per trichome was counted. Otherwise, the number of cells per trichome was calculated by dividing the length of the trichome by the length of one cell.

To allow the abundance of various genera to be compared**,** the cell counts were converted into volumetric biomass by multiplying mean cell biovolume ($\mu m^3$) by the total cell count (cells $mL^{-1}$). The mean cell biovolume was determined by finding the nearest geometric shape and measuring the appropriate cell dimensions (Sun and Lui 2003). The dimensions were measured from 30 cells. The final value is expressed as population biovolumes ($\mu m^3$ $mL^{-1}$). The biovolumes were divided into two groups, heterocytic species and non-heterocytic species to represent N-fixing and non-N fixing groups. The common heterocytic species observed in the SCP belong to *Anabaena*, *Anabaenopsis*, *Aphanizomenon* and *Nodularia*, while the common non-heterocytic genera are *Microcystis* and *Planktothrix*. However, the absence of heterocytes does not imply the inability to fix N (Oliver and Ganf 2000). Non-heterocytic terrestrial and marine taxa of Oscillatoriales have been shown to fix N (Bergman *et al.* 1997; Capone *et al.* 2005), but the extent to which N-fixation takes place in freshwater systems is not known. Therefore these Oscillatoriales were included with non-nitrogen fixing species and not separated into a third group.

### *6.2.4 Statistical analysis*

One-way ANOVA's were used to test for significant differences between the five lakes in relation to monthly water quality measurements. Scheffe's post hoc tests were used to make comparisons when significant differences were detected. The variance of the data was tested for homogeneity and log transformed, when required. The relationship between the species biovolume (heterocytic, non-heterocytic and total) and nutrient concentration were determined using Spearman's rank correlation, as the data differed significantly from a normal distribution even after transformation. The nutrient data were log transformed and species abundances were transformed into log (n + 1).

## 6.3 Results

The mean nutrient concentrations, pH and EC are presented in Table 6.2. The analysis of variance revealed significant differences among the five lakes ($p < 0.05$). Electrical conductivity was significantly higher in the southern lakes, Yangebup and Bibra, and was significantly lower in Emu Lake (Scheffe's, $p < 0.05$). The pH was also significantly higher in Yangebup Lake compared to Emu Lake (Scheffe's, $p < 0.05$). Overall, Yangebup Lake recorded significantly higher concentrations of TN and TP. Yangebup Lake produced significantly higher SRP concentrations compared to Bibra Lake and Emu Lake (Scheffe's, $p < 0.05$).

For further analysis, a separate one-way ANOVA was performed using only Blue Gum Lake, Bibra Lake and Yangebup Lake. Significant differences between these southern lakes in relation to EC and selected nutrient concentrations were revealed (Table 6.3). Post-hoc comparisons indicated significantly lower concentrations of TP and SRP in Bibra Lake, resulting in a higher TN:TP ratio compared to the other two lakes (Scheffe's, $p < 0.05$). The EC of Blue Gum Lake was significantly lower than Yangebup Lake and Bibra Lake (Scheffe's, $p < 0.05$).

Water temperature was the only variable showing significant difference between the months (Table 6.4), with significantly higher water temperatures experienced in December and January (Scheffe's, $p < 0.05$). Each site experienced a decrease in water temperature in late January 2003 (Appendix 6.1-6.3). Tomato Lake and Emu Lake showed the greatest decrease, by 5.8 °C and 4.2 °C, respectively.

Spearman rank correlation revealed a significant relationship between total algal biovolume (non-heterocytic and heterocytic) and TP and SRP (Table 6.5). The combined biovolume of heterocytic species in all five lakes was positively correlated with TP, SRP and TN and negatively correlated to DIN:SRP. There was no correlation between the combined biovolume of non-heterocytic species and any of the nutrient concentrations. No relationship between TN:TP and non-heterocytic biovolume or heterocytic biovolume was shown.

**Table 6.2.** Summary of the water quality parameters measured at the five study lakes. Values represent average (± s.e.). All values were log transformed, except for pH, TN and TP. Water temperature was omitted from the analysis as data differed significantly from a normal distribution, even after transformation. Significant values ($p < 0.05$) in bold type.

| Variable | | Yangebup Lake | Bibra Lake | Blue Gum Lake | Tomato Lake | Emu Lake | *p* |
|---|---|---|---|---|---|---|---|
| pH | | 9.39 (0.21) | 8.83 (0.12) | 8.41 (0.37) | 8.77 (0.31) | 8.07 (0.11) | **0.019** |
| EC | $\mu S\ cm^{-1}$ | 2464.25 (90.04) | 1925 (252.36) | 1374 (216.26) | 1066.5 (118.39) | 338 (37.13) | **< 0.001** |
| Temp | °C | 30.1 (0.74) | 28 (1.88) | 30.8 (0.59) | 29.7 (1.15) | 28.4 (0.71) | |
| TP | $\mu g\ l^{-1}$ | 190 (30.28) | 22.5 (7.5) | 72.50 (22.97) | 115 (25) | 60 (14.72) | **0.001** |
| SRP | $\mu g\ l^{-1}$ | 92.75 (18.74) | 6.5 (0.65) | 29.50 (8.27) | 58.25 (24.74) | 14.50 (4.25) | **< 0.001** |
| TN | $mg\ l^{-1}$ | 4.60 (0.53) | 1.90 (0.25) | 1.74 (0.53) | 2.65 (0.37) | 1.22 (0.28) | **< 0.001** |
| $NH_3$-N | $\mu g\ l^{-1}$ | 71 (34.35) | 25.50 (7.58) | 294.75 (268.44) | 791.50 (512.19) | 15.75 (5.92) | 0.059 |
| TN:TP | | 24.83 (1.5) | 108.96 (24.85) | 26.27 (8.21) | 25.74 (4.84) | 20.45 (0.53) | **< 0.001** |
| DIN:SRP | | 1.06 (0.62) | 4.04 (1.18) | 20.57 (19.67) | 15.71 (7.77) | 1.39 (0.79) | 0.232 |

**Table 6.3.** One-way ANOVA results for mean environmental variables recorded from the southern lakes Blue Gum, Bibra and Yangebup. All values were log transformed, except for pH and TN. Significant values ($p < 0.05$) in bold type.

| | **df** | **MS** | ***F*** | ***p*** |
|---|---|---|---|---|
| pH | 2 | 0.97 | 3.79 | 0.064 |
| EC | 2 | 0.07 | 6.42 | **0.018** |
| TP | 2 | 0.98 | 16.59 | **0.001** |
| SRP | 2 | 1.28 | 38.51 | **0.000** |
| TN | 2 | 10.34 | 12.42 | **0.003** |
| $NH_3$-N | 2 | 0.25 | 0.81 | 0.478 |
| TN:TP | 2 | 0.51 | 13.33 | **0.002** |
| DIN:SRP | 2 | 0.59 | 1.37 | 0.302 |

**Table 6.4.** One-way ANOVA results for mean monthly nutrient concentrations. All values were log transformed. Significant values ($p < 0.05$) in bold type.

| | **df** | **MS** | ***F*** | ***p*** |
|---|---|---|---|---|
| pH | 3 | 0.001 | 1.147 | 0.360 |
| EC | 3 | 0.045 | 0.395 | 0.758 |
| Temp | 3 | 0.005 | 7.614 | **0.002** |
| TP | 3 | 0.074 | 0.423 | 0.739 |
| SRP | 3 | 0.004 | 0.014 | 0.998 |
| TN | 3 | 0.07 | 0.931 | 0.449 |
| $NH_3$-N | 3 | 0.712 | 1.449 | 0.266 |

**Table 6.5**. Significant coefficients from Spearman's rank correlation analysis for individual and total biovolume and nutrient concentrations for all lakes combined. Text denotes significance at * $p < 0.05$ level and ** $p < 0.01$.

| **Biovolume** | **Nutrient concentration** | **r** |
|---|---|---|
| Total | TP | $0.471^{*}$ |
| | SRP | $0.537^{*}$ |
| Heterocytic | TP | $0.577^{**}$ |
| | SRP | $0.569^{*}$ |
| | TN | $0.588^{**}$ |
| | DIN:SRP | $-0.527^{*}$ |

### 6.3.1 Yangebup Lake

Both TP and TN followed a similar fluctuating pattern with higher concentrations recorded in December and February (Fig. 6.2). TN increased over the months with the highest concentration measured in December (5.6 mg $L^{-1}$), although the increase in TN coincided with a decrease in $NH_3$-N and the availability of DIN remained low. DIN was less than 1% of TN from December to February. Comparing all sites, the concentration of TP (250 μg $L^{-1}$) and SRP (130 μg $L^{-1}$) was higher in Yangebup Lake, reaching 250 and 130 μg $L^{-1}$, respectively. Similar to TN, TP and SRP both showed a fluctuating pattern with increases observed in December and February. For both months SRP made up 52% of the TP. The availability of the dissolved inorganic fractions (DIN:SRP) was higher in November, then decreased throughout the sampling period from 2.88 to 0.23. Water temperature ranged from 25.1 to 31.4 °C. Lowered water temperatures were measured in November (25.9 °C) in and January (25.1 °C). The EC ranged from 2251 to 3230 μS $cm^{-1}$, increasing over the four months.

*Microcystis* was present throughout the sampling period and was the only genus recorded in November (Fig. 6.3). Cell numbers peaked at $8.76 \times 10^7$ cells $mL^{-1}$ ($1.81 \times 10^9$ $\mu m^3$) in early December. The heterocytic cyanoprokaryotes first appeared in December with *Anabaena* being the dominant form until the 11th February. *Anabaenopsis* and *Nodularia* were observed on the 10th January, though they only comprised 15% and 1% of the total heterocytic cyanoprokaryote volume. However, on this date, the combined volume of heterocytic species was nearly that of the non-heterocytic species. By the 11th February *Anabaenopsis* and *Nodularia* were the only heterocytic species present.

### 6.3.2 Bibra Lake

The TN:TP ratio was significantly higher in Bibra Lake compared to the other sites (Scheffe's, $p < 0.05$). Similar to Blue Gum Lake, the TN increased over the sampling period (Fig. 6.4). Both $NO_2$-N and $NO_3$-N were below the level of detection (LOD; < 5 μg $L^{-1}$) and $NH_3$-N remained low, decreasing from 38 to 10 μg $L^{-1}$. DIN

comprised less than 1% of the TN in January and February. Bibra Lake produced the lowest concentrations of TP (< 40 μg $L^{-1}$) and SRP was significantly lower compared to the other lakes (Scheffe's, $p < 0.05$). The TP did not change from November to December, with SRP comprising 60 to 70% of TP. By January TP had reached the maximum concentration recorded for this site, which coincided with a decrease in SRP (SRP:TP; 0.12). The DIN:SRP peaked in December at 6.50, decreasing to 1.25 in February. Water temperatures ranged from 23.5 to 33.1 °C, with higher temperatures observed in December (30.4-31.5 °C) and February (29-33.1 °C). The EC ranged from 1397 to 3120 μS $cm^{-1}$, increasing over the sampling period.

Cell counts were low in Bibra Lake. *Microcystis* dominated the water samples, reaching $4.02 \times 10^5$ cells $mL^{-1}$ in November ($3 \times 10^7$ $\mu m^3$) (Fig. 6.5). By December and throughout January the number of *Microcystis* cells was less than 1000 cells $mL^{-1}$. The highest *Microcystis* biovolume was recorded on the 11th February and associated with a *M. aeruginosa* bloom. At this point, *Aphanizomenon* appeared in the water samples, but only comprised 15% of total biovolume. This was after the last nutrient sample had been collected (5th February) so the N and P concentration at this time is unknown. By the following week *Aphanizomenon* had become the dominant species making up 84% of the total cyanoprokaryota biovolume.

### *6.3.3 Blue Gum Lake*

Total nitrogen increased over the sampling period from 0.74 to 2.5 mg $L^{-1}$ with the greatest increase observed from December to January (Fig. 6.6). The concentration of $NH_3$-N was consistently low (20-30 μg $L^{-1}$) until February when both $NH_3$-N and $NO_2$-N increased in concentrations (8-14 μg $NO_2$-N $L^{-1}$). The $NO_2$-N concentrations had previously been below the LOD. In November, DIN comprised only 5% of TN, increasing to 44% in February. The highest concentration of TP and SRP was recorded in January (140 and 52 μg $L^{-1}$ respectively). However only a small portion of TP was SRP (SRP:TP, 0.37). There was a greater availability of SRP in November (SRP:TP, 0.52) and December (SRP:TP; 0.53). A considerable increase in DIN:SRP was recorded in February (79.57). Prior to this, DIN:SRP was consistently low (0.59-

1.19). Water temperature reached 33.1 °C in November, before decreasing to 30.9°C in early January. January recorded the lowest water temperatures (27.2-29.3 °C). Electrical conductivity increased over the sampling period from 887 μS $cm^{-1}$ in November to 1888 μS $cm^{-1}$ in February.

A clear transition from non-heterocytic (*Microcystis* and *Planktothrix*) to heterocytic (*Anabaena*) dominance was observed (Fig. 6.7). *Microcystis aeruginosa* was the dominant non-heterocytic species in November ($1.35 \times 10^8$ $\mu m^3$). *Planktothrix* was also present in the November samples, but it only comprised 2.3% of total non-heterocytic volume. By January *M. flos-aquae* reached $8.83 \times 10^7$ $\mu m^3$. *Anabaena* was also present on the 18th December ($1.41 \times 10^6$ $\mu m^3$), although cell density did not reach bloom proportions until the 2nd January. The following week the bloom had subsided. By February, *Microcystis* was the only genus detected in the net samples, but was not present in the water samples.

### *6.3.4 Tomato Lake*

Tomato Lake was the only lake to show a decrease in both TP and TN over the sampling period (Fig. 6.8). The greatest decrease in TN was from November to December, corresponding to a decrease in $NH_3$-N (2.3-0.46 mg $L^{-1}$). The availability of DIN was greater in November (62% of TN). The $NH_3$-N concentrations recorded in Tomato Lake were the highest of all five sites, but not significantly (Scheffe's, $p > 0.05$). Both $NO_2$-N and $NO_3$-N were below the LOD until February. The concentration of SRP followed a similar pattern to TP, with a distinct decrease from November to January. Likewise, there was a greater availability of SRP in November, comprising 76% of TP, which decreased there after to 36% of TP by February. Similar to Blue Gum Lake, DIN:SRP showed a marked increase in February from 0.41 to 36.47. Water temperature ranged from 28.8 to 32 °C until January where it decreased to 25.6 °C. The EC increased over the months from 810 to 1397 μS $cm^{-1}$.

A distinct transition from heterocytic dominance in November and December to non-heterocytic dominance in January to February was observed (Fig. 6.9). In November, *Anabaena* density reached 1.98 × $10^6$ cells $mL^{-1}$ (2.30 × $10^8$ $\mu m^3$) and by January *Anabaena* was no longer present in the water samples. The non-heterocytic genera *Planktothrix* and *Microcystis* first appeared in December and January. *Planktothrix* dominated in December and January making up 85% and 100% of the total total biovolume, respectively. From the 23rd January, *Microcystis* was the only genus present.

### 6.3.5 Emu Lake

Emu Lake experienced an increase in both TN and TP (Fig. 6.10), with the greatest change from December to January. Interestingly, January and February recorded the same TN concentration of TN (1.7 mg $L^{-1}$). As TN increased, $NH_3$-N remained consistently low and $NO_3$-N and $NO_2$-N were below LOD. The SRP concentration also increased over the sampling period, with the availability of SRP being greater in November and December (SRP:TP; 0.26 and 0.28, respectively). Similar to Yangebup Lake, DIN:SRP was higher in November (3.75) and decreased over the four months of sampling. Water temperatures varied from 28.2 to 30.9 °C, decreasing to 24.5 °C in January. The highest water temperature was measured in November. Electrical conductivity ranged from 246 to 490 µS $cm^{-1}$.

Non-heterocytic species (*M. aeruginosa and M. flos-aquae*) dominated the water samples (Fig. 6.11). The highest cell count was reached on the 28th November with 6.7 × $10^7$ cells $mL^{-1}$. By December the *Microcystis* cell numbers had subsided and remained constant throughout January (9 × $10^7$ $\mu m^3$). The heterocytic species (*A. ovalisporum* and *A. affinis*) were present throughout the sampling period. On the 21st November, *Aphanizomenon* and *Anabaena* were the only species present in the water samples. Although *Aphanizomenon* was the dominant heterocytic species, it only comprised 15% of the total biovolume. By the 4th February there were minimal amounts of *Microcystis* and *Aphanizomenon* present in the water samples.

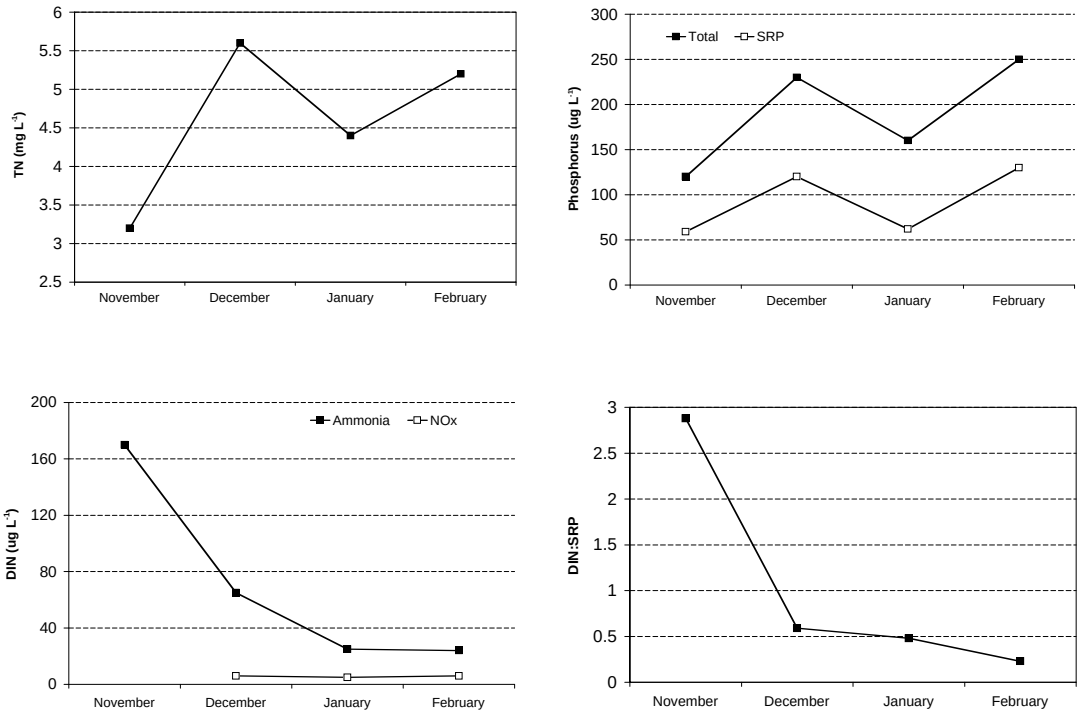



**Figure 6.2.** Monthly changes in nutrient concentrations at Yangebup Lake.

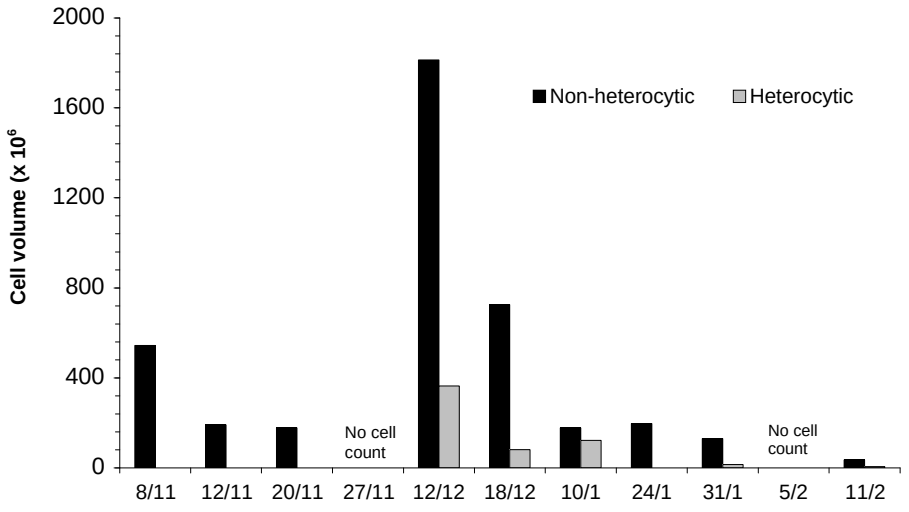



**Figure 6.3.** Monthly changes in biovolume of non-heterocytic (*M. aeruginosa* and *M. flos-aquae*) and heterocytic (*A. circinalis, A. elenkinii and N. spumigena)* species from Yangebup Lake. No cell count was performed on 27/11 and 5/2.

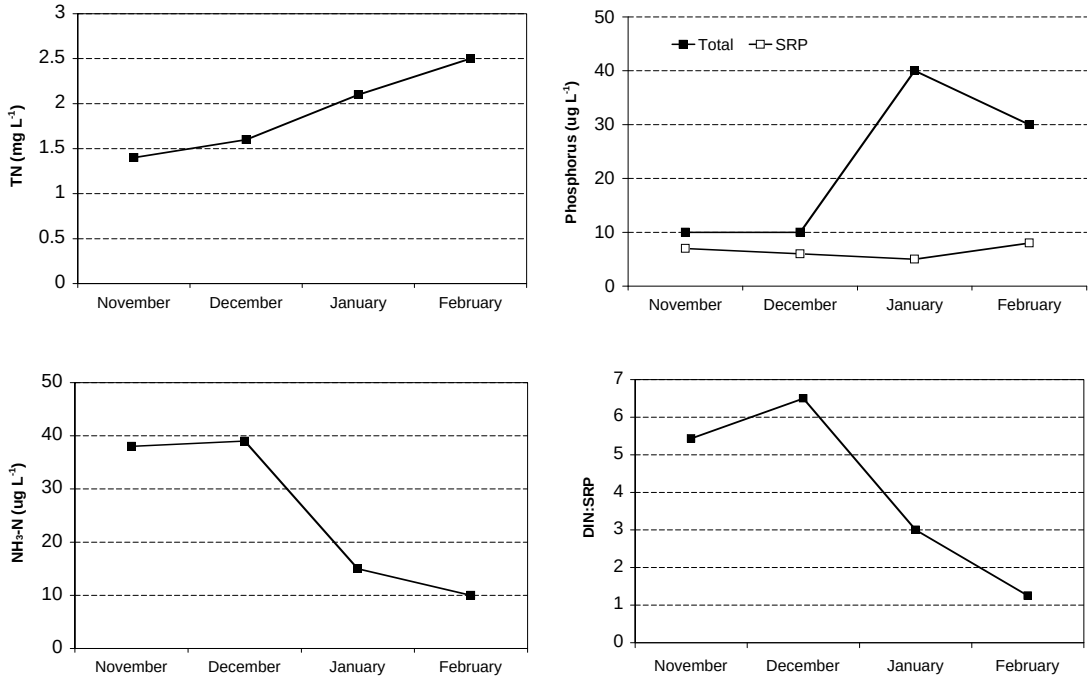



**Figure 6.4.** Monthly changes in nutrient concentrations at Bibra Lake.

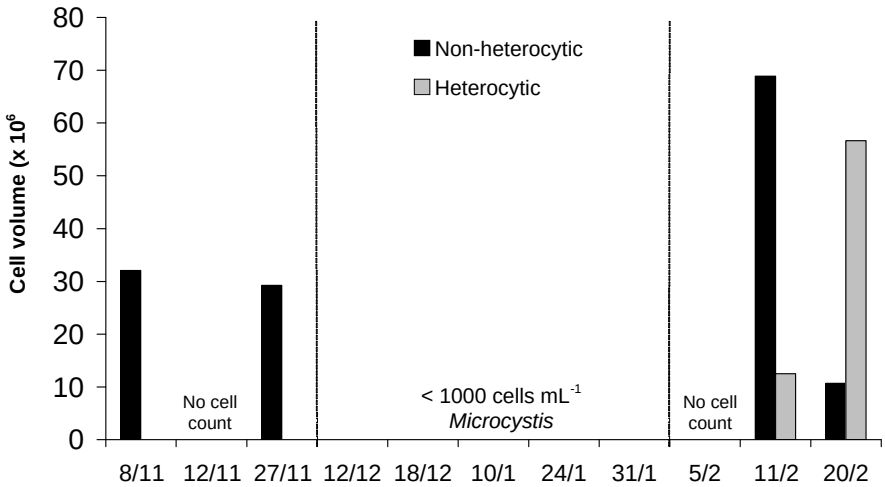



**Figure 6.5.** Monthly changes in biovolume of non-heterocytic (*M. aeruginosa* and *M. flos-aquae*) and heterocytic (*A.ovalisporum*) species from Bibra Lake. No water measurements recorded the 5/2 due to low water levels.

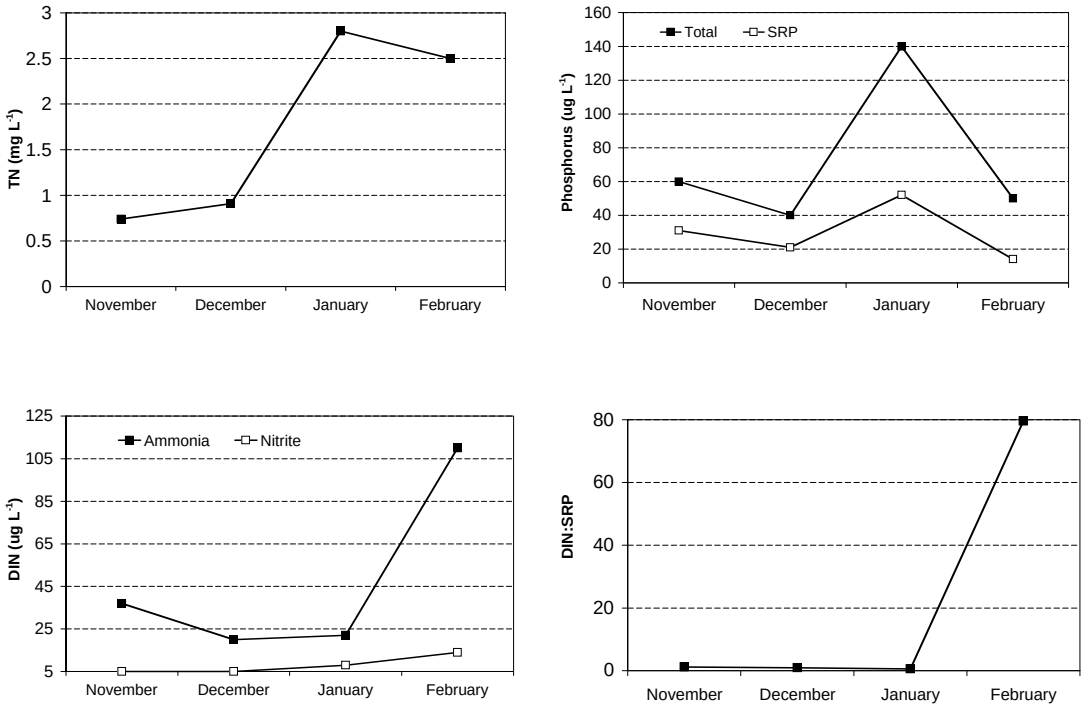



**Figure 6.6.** Monthly changes in nutrient concentrations at Blue Gum Lake.

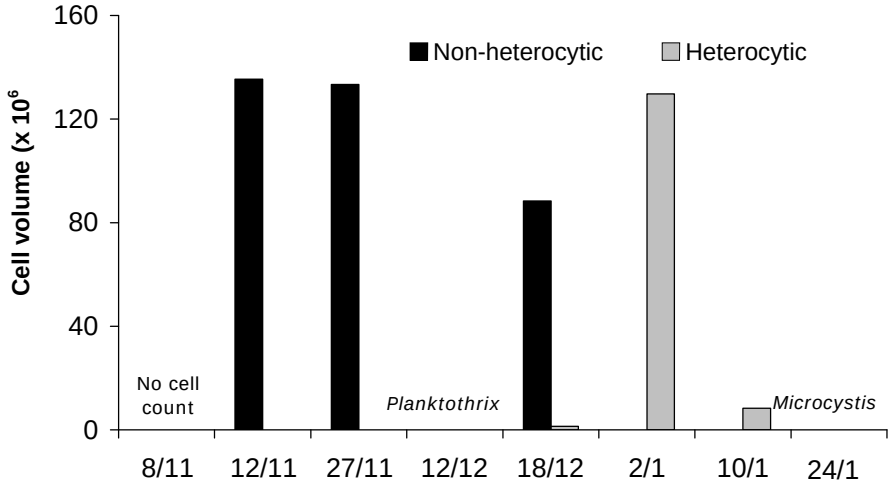



**Figure 6.7.** Monthly changes in biovolume of non-heterocytic (*M. aeruginosa*. *M. flos-aquae* and *P. mougeotii*) and heterocytic (*A. circinalis)* species from Blue Gum Lake. No cell count was performed on 8/11. *M. flos-aquae* present in net sample collected 8/11. *Planktothrix* present in 12/12 net sample. *Microcystis* present in 24/1 net sample.

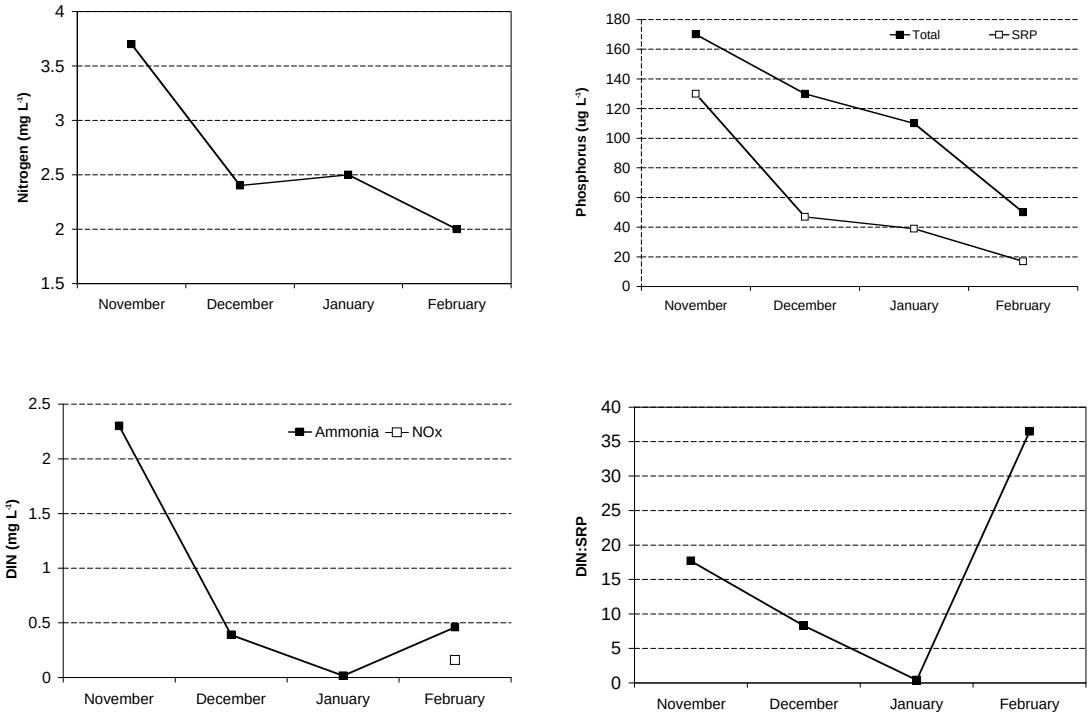



**Figure 6.8.** Monthly changes in nutrient concentrations at Tomato Lake.

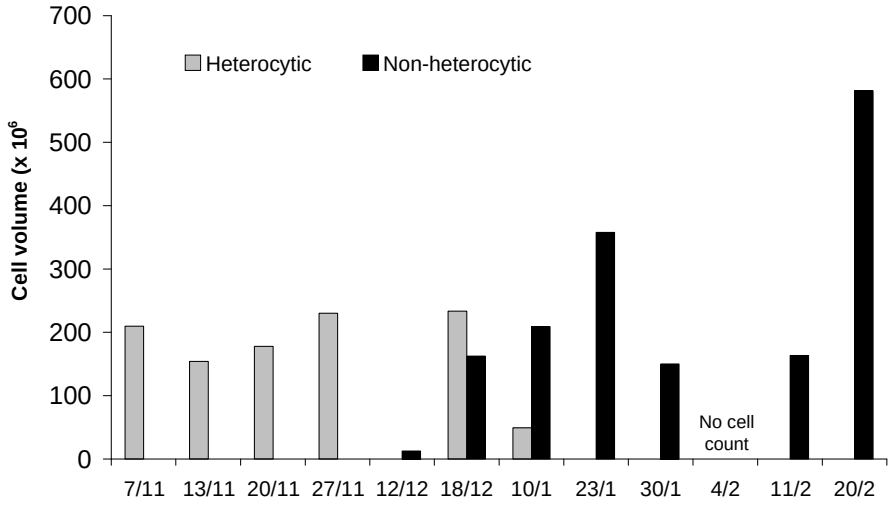



**Figure 6.9.** Monthly changes in biovolume of non-heterocytic (*M. aeruginosa*, *M. flos-aquae* and *P. mougeotii*) and heterocytic (*A. circinalis*) species from Tomato Lake. No cell count was performed on 4/2.

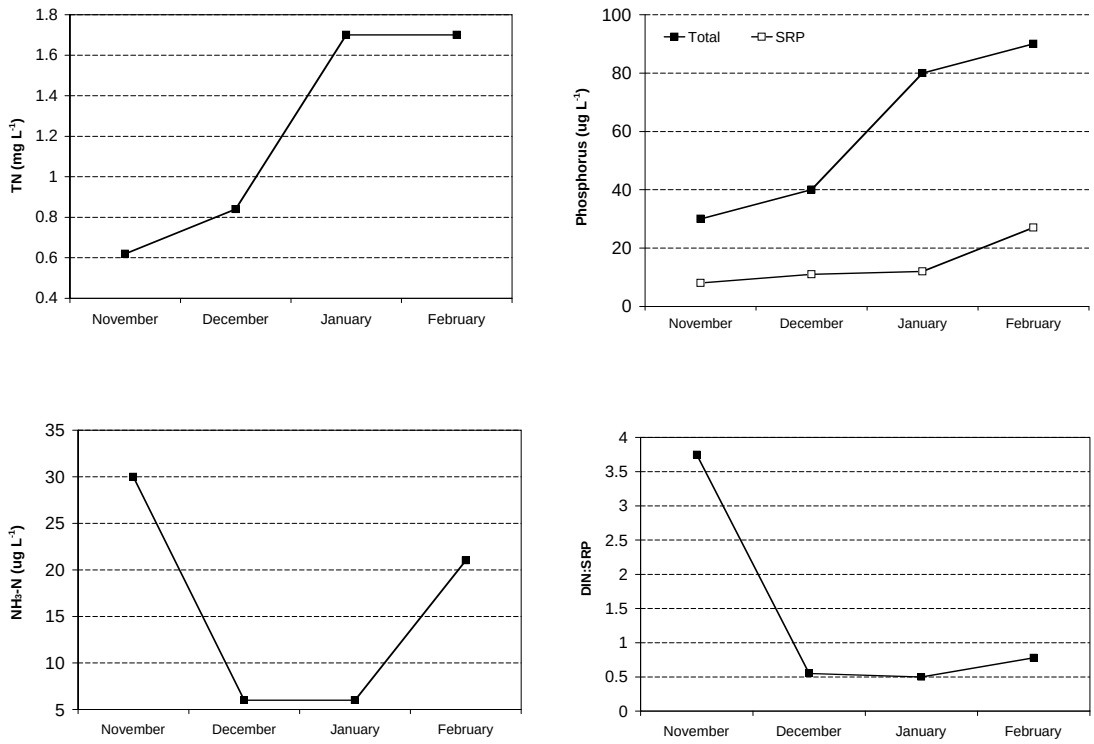



**Figure 6.10.** Monthly changes in nutrient concentrations at Emu Lake.

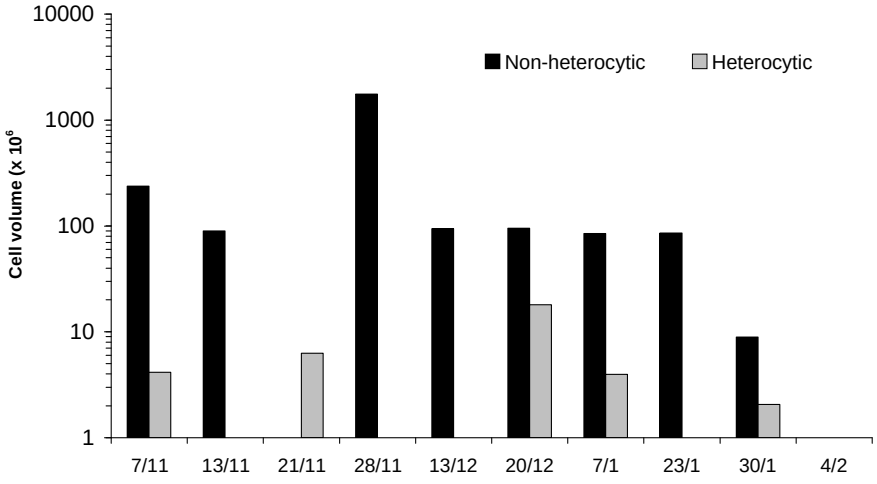



**Figure 6.11.** Monthly changes in biovolume of non-heterocytic (*M. flos-aquae* and *M. aeruginosa)* and heterocytic (*A. ovalisporum* and *A. affinis)* species from Emu Lake. Cell volume presented on a logarithmic scale.

## 6.4 Discussion

### *6.4.1 Nutrient status of the study lakes*

Throughout the study, the five lakes studied recorded TN and TP concentrations that exceeded the recommended ANZECC and ARMCANZ (2000) guideline for southwest lakes (100-500 μg TN $L^{-1}$ and 5-50 μg TP $L^{-1}$). Compared to spring 1998, Yangebup Lake showed a considerable increase in all nutrient concentrations (Table 6.6). More than a 90% increase in both DIN and SRP from 1998 to 2002 probably points to Yangebup Lake's status of having the worst water quality in Western Australia. The diversity of cyanoprokaryotes comprising the 2002-03 blooms also reflected the poor water quality, with five nuisance bloom-forming species identified in the water samples. However, Strano (2001) also reported large variation in the spring-summer nutrient data collected from Yangebup Lake from 1995 to 1999 (70-412 μg $L^{-1}$ TP and 1200-8260 μg $L^{-1}$ TN), therefore the large variation in nutrients measured from 1998 to 2002 may not be uncharacteristic of this lake.

In contrast, Tomato Lake and Bibra Lake showed minimal changes to TN and TP from 1998 to 2002. The DIN concentration measured in Tomato Lake in 2002 was more than 50 times higher than the DIN concentration in 1998, showing a greater availability of DIN during this spring. The dominance of heterocytic species (*Anabaena*) in Tomato Lake during November 2002 can imply N-fixation was contributing to the internal N load, although this was not examined. Nevertheless, $NH_3$-N was the only inorganic form of N present in November 2002, which is the end product of N-fixation. For Blue Gum Lake only TN and DIN had increased over the four years. However, TN was still below the overall average of 1950 μg $L^{-1}$ recorded from 1971 to 2003 (Fox and MacShane 2004a). For Blue Gum Lake and Bibra Lake, TP concentrations were low (10-60 μg $L^{-1}$), with a 50% decrease from 1998, but well within the ANZECC and ARMCANZ (2000) recommended range for TP.

The 1998 and 2002 results were compared with the OECD trophic classification, based on TP concentrations. Based on these results, Tomato Lake is hypertrophic,

Yangebup Lake is mesotrophic (1998) to hypertrophic (2002), Blue Gum Lake is hypertrophic to eutrophic and Bibra Lake is mesotrophic (OECD boundary values cited in Rast *et al.* 1989). The difference in trophic status between the years concurs with the view that a single trophic status cannot be assigned to the highly seasonal lakes of the SCP (Lund and Davis 2000). No 1998 nutrient data were available for Emu Lake, however the data collected during the current study classifies Emu Lake as mesotrophic in spring and eutrophic in summer.

**Table 6.6.** Comparison of spring N and P concentrations recorded in November 1998 and November 2002 as part of the current study. All concentrations presented as μg $L^{-1}$. Values < 5 μg $L^{-1}$ were below the limit of detection. No baseline nutrient data were obtained for Emu Lake.

| | 1998 | | | | 2002 | | | |
|---|---|---|---|---|---|---|---|---|
| | **TN** | **DIN** | **TP** | **SRP** | **TN** | **DIN** | **TP** | **SRP** |
| Tomato Lake | 3600 | 45 | 150 | 120 | 3700 | 2300 | 170 | 130 |
| Yangebup Lake | 950 | 15 | 20 | 4 | 3200 | 170 | 120 | 59 |
| Blue Gum Lake | < 5 | <5 | 130 | 39 | 740 | 37 | 60 | 31 |
| Bibra Lake | 1400 | 20 | 20 | <5 | 1400 | 38 | 10 | 7 |

The one-off sampling of nutrient data in November 1998 and once a month sampling undertaken from November 2002 to February 2003 do limit the interpretations that can be made about the nutrient dynamics in these SCP lakes. However within the constraints of this study, the 2002-03 data are valid in showing the vast fluctuation of nutrients in the five study lakes, and could were used to interpret the observed transition of N-fixing and non-N-fixing taxa in relation to changes in N and P concentrations.

### *6.4.2 Monthly changes in nitrogen and phosphorus*

No significant changes to any of the measured nutrient parameters from November 2002 to February 2003 were shown. The only variable to significantly differ between the months was water temperature. This complements the findings from Chapter 3 that the physico-chemical conditions of the SCP lakes do not differ significantly during spring and summer. However, dissimilarity between lakes within the same consanguineous classification is more apparent, evident in lakes Yangebup, Bibra

and Blue Gum, which recorded significantly different EC and TP, SRP and TN concentrations.

As expected, Yangebup Lake recorded significantly higher concentrations of TN and TP, the product of urban discharge and leaching of effluents from the wool scouring plant that operated until the 1990s. In addition, stratification and algal decomposition in Yangebup Lake during the warmer months can cause the bottom sediments to become anoxic (Davis *et al.* 1993; Arnold and Oldham 1997). This can cause substantial resuspension of nutrients stored in the sediment, contributing to the high nutrient concentrations observed throughout the study.

Tomato Lake was the only site to show an overall decrease in both TN and TP, which occurred concomitantly with the decrease in both DIN and SRP. These nutrient concentrations were adequate to sustain high cyanoprokaryote density throughout the sampling period. Conversely, both TP and TN levels increased in Emu Lake and Bibra Lake. Similarities between Bibra Lake and Emu Lake have been previously established in this research project (Chapter 4), and in this chapter no significant differences in nutrient concentrations from two lakes were shown. Both lakes recorded the lowest concentrations of DIN and SRP of the five study sites and Bibra Lake recorded significantly lower concentrations of TP and SRP, compared to neighbouring Yangebup Lake and Blue Gum Lake. Bibra Lake also produced the lowest cell counts throughout the study, with the dominant species (*Microcystis* and *Aphanizomenon*) not producing maximum biomass until late February, past the completion of the nutrient surveys. Although these cell counts were the lowest for the five lakes, the highest biovolume of *Microcystis* recorded ($3 \times 10^7$ $\mu m^3$) corresponded to $4.02 \times 10^5$ cells $mL^{-1}$, which is still above guideline values for recreational waters (WHO 2003).

### *6.4.3 Nutrient limitation and cyanoprokarota biomass*

The success of cyanoprokaryota blooms in temperate lakes has been associated with low TN:TP ratio that allows competition for nitrogen and the increase in occurrence of N-fixing species (Smith 1983). In some cases, TN:TP can be a poor predictor of

cyanoprokaryote dominance (McQueen and Lean 1987; Downing *et al.* 2001), especially in wetlands where N and P loadings are very large (Paerl 2008). The high concentrations of TN, and the epilimnetic TN:TP ratios never exceeding 10:1, indicate N was not limiting in the lakes chosen for this study (ANZECC and ARMCANZ 2000; Dokulil and Teubner 2000; Dodds 2003). This result is supported by the monthly TN and DIN concentrations not correlating with total cyanoprokaryota biovolume. However, TN is not often associated with cyanoprokaryota abundance, as total concentration does not reflect the availability of DIN, which is a better indicator of cyanoprokaryota dominance and the occurrence of N-fixing species (Pick and Lean 1987). Additionally, the dissolved organic fraction (DON) can be an important N source for cyanoprokaryotes either directly or indirectly contributing to the formation of blooms (Berman 1997; Berman and Chava 1999). In the study sites a large proportion of the TN was the dissolved organic fraction, accounting for more than 95% of TN in the majority of samples. In freshwater wetlands the DON constitute a considerable proportion of the total dissolved N pool, often derived from allochthonous sources such as terrestrial leaching and runoff (Berman and Bronk 2003; Willett *et al.* 2004).

Overall, lower concentrations of DIN did yield higher abundance of heterocytic species, as observed in Bibra Lake and Emu Lake. This was also evident in the significant negative correlation between heterocytic biovolume and DIN:SRP. However, a significant positive relationship between TN and heterocytic biovolume was also shown. This discrepancy in the results suggests that DON is important to the N-fixing species. Furthermore, heterocytic species were more dependent on TP and SRP, possible due to N being in high supply. At times throughout the study SRP limitation was observed in all sites except Yangebup Lake, therefore it is the variable that is most likely to influence biomass. Phosphorus bioavailability is a major constraint on the supportive capacity in shallow lakes (Reynolds 2006).

### *6.4.4 Species composition*

Moderately enriched wetlands are highly productive and tend to support mixed assemblages of N-fixing and non-N-fixing taxa (Paerl 2008). This was evident in all the study sites by the coexistence of *Microcystis, Anabaena, Aphanizomenon* and *Planktothrix*, although the dominance of either non-heterocytic or heterocytic taxa was apparent.

A transition from non-heterocytic to heterocytic dominance was seen in Blue Gum Lake and Bibra Lake. In Blue Gum Lake this succession influenced the availability of dissolved inorganic nutrients. Lower nutrient concentrations during November and December coupled with blooms of non-heterocytic taxa (*Microcystis* and *Planktothrix*). Throughout this period DIN:SRP remained low, possibly restricting the development of high cell densities as seen in lakes Yangebup, Emu and Tomato. The disappearance of *Microcystis* and *Planktothrix* in the December to January coincided with an increase in TN and TP, however TN and TP were comprised mostly of the dissolved organic forms. This implies the increase in TN and TP was the result of the *Anabaena* bloom that occurred in January. The concomitant increase in DIN:SRP during the period of *Anabaena* dominance shows N-fixation was probably contributing to the internal N load. By February the $NO_2$-N and $NH_3$-N concentrations were the highest recorded over the four months of sampling. These levels would be sufficient to support a late summer bloom of *Microcystis*, which was the only genus detected in the February net samples.

For Bibra Lake, the decreasing $NH_3$-N concentration and low DIN:SRP corresponded to low non-heterocytic biomass and a change in community structure. The *Microcystis* and *Aphanizomenon* blooms that are frequent in Bibra Lake did not occur until late February, past the duration of this study. Phosphorus limitation, indicated by the high TN:TP and very low concentrations of SRP, appeared to favour the dominance of *Microcystis* from November to early February, though biomass was low. The absence of *Microcystis* blooms has been linked to the low availability of P (Jacoby *et al.* 2000; Xie *et al.* 2002; 2003; Homma *et al.* 2008). This has also been previously described in Bibra Lake when heavy blooms of *M. aeruginosa*

during spring 1993 were positively correlated to the availability of organic and inorganic P (John, unpublished data). No nutrient data were collected on the 20th February when the *Aphanizomenon* bloom was recorded, but it can be assumed N was limiting as a result of the preceding *Microcystis* bloom. The appearances of blooms dominated by N-fixing genera can be associated with nitrogen depletion (Hadas *et al*. 1999; Nalewajiko and Murphy 2001; Vardaka *et al.* 2005; de Figueiredo *et al.* 2006; Paerl 2008).

Like Bibra Lake, the availability of DIN and low DIN:SRP appeared to influence community structure in Emu Lake. No species succession was observed as *Microcystis* dominated throughout the sampling period with *Aphanizomenon* appearing sporadically. The biovolume of *Microcystis* remained steady from the 13th December to 23rd January, most likely due to the low DIN concentrations measured during this time. Probably the low availability of DIN prevented *Microcystis* from attaining the high cell densities that were previously observed in November when DIN concentrations were five times higher. *Microcystis* would have benefited from the high DIN in November and outcompeted *Aphanizomenon* for SRP in P-limited conditions. The increase in both TN and TP in February, presumably from the release of intracelluar stores following the decline of the *Microcystis*, bloom supports this view.

Alternatively, a transition from heterocytic dominance to non-heterocytic dominance was observed in Tomato Lake. This transition to non-heterocytic dominance in January and February corresponded to the decrease in all nutrient concentrations and low DIN:SRP, suggesting ambient nutrients were being consumed to support growth. However the TN and TP concentrations in Tomato Lake throughout the sampling period were high and therefore may not exclusively regulate biomass and species composition. The same can be said for Yangebup Lake where N and P are in the excessive amounts and no species succession was observed. The increase in DIN in Tomato Lake in February, in the absence of N-fixing taxa, is most likely from decomposition. The high DIN:SRP may have fuelled the *Microcystis* bloom on the 20th February that followed the final nutrient sampling (4th February). *Microcystis*,

is considered an excellent P storage specialist (Oliver and Ganf 2000), which would have allowed growth to be maintained when P was limiting in Tomato Lake, likewise in Emu Lake.

In Yangebup Lake, *Microcystis* dominated with heterocytic taxa (*Anabaena, Anabaenopsis* and *Nodularia*) only observed in the later part of summer. Similar to Bibra Lake, the higher concentration of DIN in November would have established *Microcystis* as the dominant genus in Yangebup Lake. A decrease in DIN:SRP from November to December, most likely due to the *Microcystis* bloom recorded on the 12$^{th}$ December, coincided with the first observations of non-heterocytic species in the water samples. Therefore, as observed in Bibra Lake, N depletion in Yangebup Lake appears to support the occurrence of N-fixing taxa. By January the biomass of *Microcystis* were considerably lower compared to the November and December samples, possible due to the depleted DIN concentration.

Factors other than nutrient limitation could be influencing the biomass and composition of the cyanoprokaryota blooms in these study lakes. For example, the early *Microcystis* blooms, observed in all sites except Tomato Lake, may also be associated with the lower water temperatures and lower salinity experienced during spring. In highly eutrophic wetlands such as those used in this study, N and P are supplied in non-limiting amounts so factors such as light, vertical mixing, residence time, organic content and salinity may control the community structure (Paerl 2008). The lack of neither heterocytic nor non-heterocytic taxa in the February water samples collected from Yangebup Lake could relate to salinity and water temperature more than nutrients. Yangebup Lake recorded higher salinity than the other lakes used in the study and contained the salt tolerant genera *Anabaenopsis* and *Nodularia* in late summer. The low water levels presented in Bibra Lake in January would be a hindrance to summer bloom formation. Conversely, in Emu Lake the low EC (300-400 $\mu S\ cm^{-1}$), moderate water temperatures (24-29 °C) provided in December and January allowed *Microcystis* and *Aphanizomenon* to maintain bloom populations.

### *6.4.5 Functional associations*

The species assemblages observed in the five study lakes could be related to the freshwater phytoplankton associations defined by Reynolds (1997; 2006). The co-occurrence of Association-H and Association-M was revealed in these SCP sites. Nitrogen-fixing nostocalean genera comprise Association-H, which favour surface warming, good light levels and good nutrient supplies. Association-M includes *Microcystis* that tends to dominate the surface circulations of dielly-mixed, small, shallow, eutrophic lakes.

Yangebup Lake, Emu Lake and Bibra Lake showed a continual dominance of association M, with monogeneric *Microcystis* blooms occurring intermittently throughout the four months, although lower *Microcystis* biomass was recorded in Emu Lake and Bibra Lake. Such populations occur in continuously nutrient-rich, hypertrophic lakes (Reynolds 2006), which clearly define Yangebup Lake. Conversely, Blue Gum Lake showed a distinct transition from an Association-M to Association-H assemblage. Nitrogen deficiency is a selective factor for the occurrence of Association-H (Reynolds 2006), though this was not demonstrated in the Blue Gum Lake results. The assemblage observed in Tomato Lake transitioned from an Association-H to Association-M. The decreasing DIN:SRP in Tomato Lake would not benefit this non-N-fixing population, however ambient N and P concentrations show Tomato Lake to be consistently hypertrophic, which would support a high abundance of *Microcystis* during summer.

## 6.5 Conclusions

This study showed the eutrophic nature of the SCP lakes. For each lake a distinct cyanoprokaryote-nutrient relationship was revealed. Overall, high cyanoprokaryota biomass was associated with high ambient TN and TP concentrations, however for all sites except Emu Lake, there was an inverse relationship between total nutrient concentrations and cyanoprokaryote abundance. Higher cyanoprokaryote biomass combined with low DIN and SRP showed these fractions were probably assimilated by the phytoplankton. Low DIN appeared to support N-fixing Association-H

species in Bibra Lake and Blue Gum Lake, and suppress non-N-fixing Association-M species in Emu Lake and Yangebup Lake. In Tomato Lake, the transition to non-N-fixing dominance when DIN and SRP concentrations were low was most likely due to the breakdown of the preceding *Anabaena* blooms, and not ambient nutrient concentrations.

It is noted that the four months of nutrient sampling provided limited data to explain the formation of N-fixing and non-N-fixing blooms in the lakes studied. However, the data obtained does show nutrient concentrations in the SCP lakes to fluctuate greatly over the spring-summer months, which can directly contribute to the formation or decline of freshwater blooms. This study has also shown a bloom pattern can be predicted based on the physico-chemical properties of the wetland. For example, blooms containing multiple genera, capable of attain high cell numbers, tend to form in the hypertrophic lakes, as shown in Yangebup Lake, Blue Gum Lake and Tomato Lake. For these sites the dominant cyanoprokaryote, defined by biovolume, was *Microcystis*.

**Appendix 6.1.** Physico-chemical data collected from the East Beeliar wetlands sampled for the nutrient study (Nov 2002-Feb 2003).

| Site | Variable | Sampling Date | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **8/11** | **12/11** | **20/11** | **27/11** | **12/12** | **18/12** | **2/1** | **10/1** | **24/1** | **31/1** | **5/2** | **11/2** |
| Yangebup | Temp ºC | 28.6 | 27.8 | 25.9 | 28.9 | 31.2 | 29.9 | | 31.4 | 26.2 | 25.1 | 28.9 | 31.1 |
| | EC (µS $cm^{-1}$) | 2251 | 2272 | 2256 | 2293 | 2424 | 2533 | | 2686 | 2800 | 2830 | 2496 | 3230 |
| | Salinity (ppt) | 1.07 | 1.09 | 1.07 | 1.06 | 1.5 | 1.21 | | 1.42 | 1.49 | 1.51 | 1.32 | 1.74 |
| Bibra | Temp ºC | 26.4 | 25.5 | | 28.8 | 31.5 | 30.4 | | 30.7 | 24.4 | 23.5 | | 29 |
| | EC (µS $cm^{-1}$) | 1397 | 1418 | | 1520 | 1658 | 1735 | | 2101 | 2382 | 2544 | | 2890 |
| | Salinity (ppt) | 0.65 | 0.66 | | 0.71 | 0.77 | 0.81 | | 1.1 | 1.26 | 1.35 | | 1.53 |
| Blue Gum | Temp ºC | 30.2 | 29.1 | | 33.1 | 31.7 | 31.1 | 30.9 | 31.9 | 27.2 | 27.8 | 29.5 | |
| | EC (µS $cm^{-1}$) | 887 | 917 | | 1031 | 1188 | 1263 | 1432 | 1533 | 1752 | 1855 | 1888 | |
| | Salinity (ppt) | 0.4 | 0.42 | | 0.47 | 0.55 | 0.58 | 0.74 | 0.79 | 0.92 | 0.97 | 0.98 | |

**Appendix 6.2.** Physico-chemical data collected from Tomato Lake (Nov 2002-Feb 2003).

| Site | Variable | Sampling Date | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **7/11** | **13/11** | **20/11** | **27/11** | **12/12** | **18/12** | **10/1** | **23/1** | **30/1** | **4/2** | **11/2** |
| Tomato | Temp ºC | 28.8 | 30.9 | 30.5 | 31.5 | 31.2 | 30.9 | 32 | 31.4 | 25.6 | 26.9 | 29.4 |
| | EC (µS $cm^{-1}$) | 810 | 833 | 874 | 914 | 961 | 993 | 1134 | 1312 | 1355 | 1361 | 1397 |
| | Salinity (ppt) | 0.37 | 0.38 | 0.4 | 0.42 | 0.44 | 0.45 | 0.58 | 0.68 | 0.7 | 0.7 | 0.73 |

**Appendix 6.3.** Physico-chemical data collected from Emu Lake (Nov 2002-Feb 2003).

| Site | Variable | Sampling Date | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **7/11** | **13/11** | **21/11** | **28/11** | **13/12** | **20/12** | **7/1** | **23/1** | **30/1** | **4/2** | **11/2** |
| Emu | Temp ºC | 28.2 | 28.4 | 27.2 | 30.9 | 29.6 | 29.83 | 29.2 | 28.7 | 24.5 | 26.4 | 28.2 |
| | EC (µS $cm^{-1}$) | 246 | 257 | 276 | 289 | 310 | 328 | 391 | 454 | 443 | 405 | 490 |
| | Salinity (ppt) | 0.11 | 0.11 | 0.12 | 0.13 | 0.14 | 0.14 | 0.19 | 0.22 | 0.22 | 0.2 | 0.24 |

# Chapter 7: Identification of the common bloom-forming cyanoprokaryota in Western Australian freshwaters

## 7.1 Introduction

In Australia, the common cyanoprokaryotes impairing water quality through toxin production or taste and odour compounds have been well documented (Hayes 1989; Baker and Humpage 1994; Jones and Korth 1995; Bowling and Baker 1996; Baker 1991; Baker 1992; Steffensen *et al.* 1999; Falconer 2001; Falconer 2005). However, only a few taxonomic guides describing the morphology and distribution of Australian freshwater cyanoprokaryota mostly based on Eastern Australia have been published (Baker 1991; Baker 1992; McGregor and Fabbro 2001; Baker and Fabbro 2002; McGregor 2007). These do not represent Western Australia. For example, *Phormidium* is known to form toxic blooms throughout reservoirs and shallow lakes in temperate Australia (Baker and Fabbro 2002), yet it has not been described from the wetlands of the Swan Coastal Plain (SCP).

Even though a large number of SCP wetlands are experiencing frequent spring-summer blooms of multiple species (refer to chapters 4 and 5), detailed descriptions of the common species associated with these blooms have never been investigated. It is essential to produce regional taxonomic guidelines to facilitate the accurate identification of common taxa (McGregor and Fabbro 2001; Baker and Fabbro 2002). Therefore, to bridge the gap in knowledge relating to the common bloom-forming species in Western Australian waters, a taxonomic survey was completed during this four-year study. In total, 24 species were observed belonging to the orders Chroococcales, Nostocales and Oscillatoriales. Of these, 16 species were commonly observed in the SCP wetlands, with nine species not previously identified in the area.

### *7.1.1 Classification and identification of the cyanoprokaryotes*

The current taxonomic schemes divide Cyanophyceae into non-filamentous and filamentous forms, then into the presence or absence of specialised cells (heterocytes

and akinetes) and branching to form the four orders Chroococcales, Nostocales, Oscillatoriales and Stigonematales. Phenotypic identification of these groups is based on the morphological characteristics of the filaments or colonies, the positions of specialised cells and the cell shape and size (Baker 1992; Skulberg *et al.* 1993; Cronberg and Annadotter 2006). Order Chroococcales is comprised of unicellular or colonial-forming cells surrounded by common gelatinous mucilage with cell size ranging from 1 to 20 μm in diameter (Komárek 2003). Cell division by one, two or three perpendicular plane divides the genera, then cell size, mucilage width and colony arrangements, identifies the species.

In contrast, the representatives of the filamentous orders Nostocales and Oscillatoriales have their vegetative cells arranged to form a trichome. Cell division occurs on a single plane, perpendicular to the main axis of the filament, or in all directions (Komárek and Komarkova 2003). Representatives of the Order Oscillatoriales have simple straight unbranched trichomes, with cells identical in appearance and lacking heterocytes and akinetes. Cell size, constrictions at the crosswalls and cell shape, especially for the apical cells, are the main taxonomic criteria (Komárek and Anagnostidis 2005) for Oscillatoriales. Reproduction is by necridia (dead cells) that permit fragmentation of the trichome into segments called hormogonia.

The differentiation of the vegetative cells into heterocytes (nitrogen fixing cells) and akinetes (resting spores) is a characteristic feature of the order *Nostocales* with the shape, size and position of these cells used for species identification. For example, heterocytes can vary from spherical to oval in shape and can be located between cells (intercalary) or terminal on the trichome. Akinetes display a greater range of shapes, from spherical to cylindrical, and can occur in a solitary, paired or series arrangement. The position of the akinete in relation to the heterocyte is another identifying characteristic as they can be either next to the heterocyte, or remote.

Cyanoprokaryotes are a taxonomically diverse group where morphology can differ in response to environmental stimuli (Skulberg *et al.* 1993; Zapomelová *et al.* 2008).

For this reason, phenotypic analysis becomes difficult when there is the absence or limitation of characteristics expressed due to different environmental conditions (Skulberg *et al.* 1993, Hiroki *et al.* 1998; Rasmussen and Johansson 2002). Therefore, *in vitro* cultures are important when natural samples lack taxonomically distinguishing features. For example, *Microcystis flos-aquae* was once considered an environmentally induced form of *M. aeruginosa*, representing a different developmental stage (Doers and Parker 1988). Long-term cultivation has proven *M. aeruginosa* to be distinct from *M. flos-aquae* by cell size, cell aggregation pattern, width and sharpness of mucilage boundary (Doers and Parker 1988). However, isolation into culture conditions can also influence morphology, as seen in *Anabaena* (Hiroki *et al.* 1998). Hiroki *et al.* (1998) showed cell size, coil distance and diameter to change in *Anabaena* cultures and trichome form, trichome shape and akinete morphology should be given importance when identifying *Anabaena* to species level. It is for this reason that composite field and cultured populations are examined, rather than individual specimens, as this will represent a range of attributes (Baker 1992).

Beyond microscopic assessment, molecular biology and biochemistry are becoming widely used for the identification of harmful algal blooms. In Australia DNA-based tests have been developed to identify the main toxic species *Anabaena circinalis*, *Cylindrospermopsis raciboskii*, *Microcystis aeruginosa* and *Nodularia spumigena* (Neilan 2002). DNA fingerprinting has the potential to resolve fine-scale relationships between closely related individuals within a broad range of taxa (Bolch *et al.* 1999). The sequencing compares the nucleotide sequences of DNA fragments to isolate the genetic variation between strains. The sequence determination of 16S rDNA gene has been used in determining genotypes of *Microcystis*, *Anabaena*, *Planktothrix* and *Nodularia* species (Neilan *et al.* 1994; Janson *et al.* 1999; Barker *et al.* 1999; Pomati *et al.* 2000; Orcutt *et al.* 2002), while sequencing the DNA dependent RNA polymerase gene (*rpoCl*) can identify isolates of *Cylindrospermopsis raciborskii* and *Anabaena circinalis* (Saint and Fergusson 2002).

Molecular taxonomy can complement or supplement the phenotypic characterisation, but should never replace it (Neilan *et al.* 1994; Palinska *et al.* 1996). Phenotypic analysis is widely utilised and accepted, as many laboratories do not have the facilities or finances for genetic identification. Morphological descriptions derived from natural populations are essential for determining phenotypic variation and studying the ecology of particular species (Komárek 2005). For example, *Cylindrospermopsis raciborskii* produces two distinct morphotypes, straight and coiled, which DNA sequencing cannot distinguish (Saint and Fergusson 2002; Chonudomkul *et al.* 2004). Saker *et al.* (1999a) found these two morphotypes exhibit physiological differences, while McGregor and Fabbro (2000) recorded lower toxin content to cell number ratio in the coiled trichomes. Therefore, the two trichome forms should be recognised as distinct morphotypes (Saker *et al.* 1999). Precise genetic information must be obtained to establish a more reliable classification system (Rasmussen and Johansson 2002).

The aim of this chapter is to derive a guide from the morphological data collected that facilitates the identification of local cyanoprokaryota. This will benefit those that routinely monitor for harmful algal blooms. It does not attempt to describe all taxa common in Western Australia, but those observed during the duration of the current project. For each species collected, a description of morphology and distribution is presented.

## 7.2 Materials and Methods

### *7.2.1 Sample collection and identification*

Cyanoprokaryote samples were collected using the procedures described in section 4.2.1. The samples were viewed using an Olympus light microscope (400× to 1000×) for the features presented in Table 7.1. For each specimen, cells were collectively measured using a calibrated eyepiece, from all samples obtained throughout that sampling period. Photographs were taken using a Vanox photomicroscope. Samples were identified to the species level using Australian and International taxonomic guidelines and reviews (Bourrelly 1970; Geitler 1985; Baker 1991; Baker 1992;

Komárek and Anagnostidis 1998; Hindák 2000; Li *et al.* 2000; McGregor and Fabbro 2001; Baker and Fabbro 2002; Komárek 2003; Komárek *et al.* 2003; Watanabe *et al.* 2004; Komárek and Anagnostidis 2005; Cronberg and Annadotter 2006; L. Hoffmann 2007, personal communication, June 2007; Komárek and Zapomelová 2007; McGregor 2007).

### *7.2.2 Cyanoprokaryota cultures*

Colonies and individual filaments were isolated from field samples using a Leica dissecting microscope. They were then placed into 20 mL of BG-11 liquid medium (Appendix D), within Pyrex 100 mL beakers. The BG-11 liquid medium without $NaNO_3$ was used for culturing nitrogen-fixing species. The cultures were kept around 35 °C under fluorescent lights. Once established, they were transferred into 50 to 100 mL of BG-11, medium within 250 mL conical flasks. The colonies and filaments were harvested after four weeks. No attempt was made to make these cultures axenic. Cells were observed and measured as described in 7.2.1. Cultured species were used to confirm morphological features required for identification.

### *7.2.3 Statistical analysis*

Multivariate analysis was used to determine the similarities between three species groups: 1) *Anabaena* with circinate or spiralled trichomes, 2) *Anabena* and *Aphanizomenon* with straight trichomes, and 3) *Anabaenopsis*. Unweighted pair-group method using arithmetic means (UPGMA) was used to produce dendrograms that illustrated closely resembled species obtained from this study and a reference study (Baker 1991). Similarity matrices were generated using the Euclidean distance measure. Measurements were standardised by z scores to overcome unequal contribution of some characters. Separate tests were performed on samples with missing akinetes, or samples with less than five akinetes measured. All analyses were performed using SPSS version 11 for MacIntosh.

**Table 7.1.** Morphological characters used for species identification. The terms and definitions were adapted from Baker (1991,1992) and Baker and Fabbro (2002).

| Character | Description |
|---|---|
| Trichome form | Bundle, solitary |
| Trichome shape | Straight, attenuated (tapered towards ends), circinate (rounded), flexuous (wavy), regularly spiralled, irregularly spiralled. |
| Vegetative cell shape | Spherical, oval, oblong, ellipsoidal, barrel-shaped, cylindrical, discoid, depressed-globuse (subspherical), quadrate |
| Vegetative cell length (μm) | |
| Vegetative cell width (μm) | |
| Heterocyte shape | Spherical, barrel-shaped, oval, oblong, conical, rounded-conical |
| Heterocyte length (μm) | |
| Heterocyte width (μm) | |
| Akinete position | Solitary, paired, in series |
| Akinete location | Remote from the heterocyte, adjacent to one side of the heterocyte, adjacent to both sides of the heterocyte, rarely far from the heterocyte, rarely adjacent from the heterocyte |
| Akinete shape | Spherical, sausage-shaped, reniform, oblong, oval, ellipsoidal, pyriform (pear-shaped) |
| Akinete length (μm) | |
| Akinete width (μm) | |
| Spiral width (μm) | |
| Apical cell shape | Rounded, rounded-conical, bulbous |
| Apical cell length (μm) | |
| Apical cell width (μm) | |
| Spiral height (μm) | |

## 7.3 Results and Discussion

In total, 24 species were identified and photographed. For all species identified, the mean, standard deviation and range of cell diameters are presented in Appendix E. A descriptive summary for species with flexuous or straight trichomes is presented in Appendix F. The term “common” has been used to define species that were observed during more than one sampling period.

### *7.3.1 Order Chroococcales*

**Genus *Microcystis*** Kützing ex Lemmermann 1907

In Australia, 11 species of *Microcytis* have been identified with many found mainly in Central Queensland (McGregor and Fabbro 2001; Baker and Fabbro 2002). Three species, *Microcystis aeruginosa*, *M. flos-aquae* and *M. wesenbergii* were collected during this study, each showing distinctly different cell diameters and colony structure (Fig. 7.1; Plate 7.1). For all three species the colonial mucilage was hyaline and only visible using Indian ink.

*Microcystis aeruginosa* and *M. flos-aquae* are cosmopolitan in distribution and toxigenic (Baker and Fabbro 2002), whereas *M. wesenbergii*, yet to be recorded from Western Australia, has a predominantly temperate distribution (McGregor and Fabbro 2001). *Microcystis* is the most widely reported genus in the wetlands of the SCP (Congdon 1986; Walsh *et al.* 1992; Davis *et al.* 1993, Hosja and Deeley, 1994, Vincent 1995; Fox and MacShane 2004a,b). However, there is uncertainty regarding which species is responsible for the blooms reported, as samples were only identified to genus level. It is assumed these species were collectively grouped as *Microcystis sp*., which most often refers to both *M. aeruginosa* and *M. flos-aquae*.

***Microcystis aeruginosa*** **(**Kützing) Kützing 1846
(Plate 7.1: 1-3; Table E1)
Cells spherical, oblong before division, 3-7 μm in diameter. Cells either densely packed within the colony or widely dispersed and randomly arranged. Colonies of two distinct morphologies; clathrate with many fenestrations, or a compositon of many subcolonies joined together. Both arrangements existed together. Mucilage broad, colourless and indistinctly defined; extending 8-31 μm beyond cells. Larger colonies were clathrate with densely packed cells, and smaller colonies irregularly outlined with randomly arranged cells.

Common to nearly all lakes studied, except Lake Coogee, Lake Claremont and Shenton Park. Previous records include Loch McNess, Lake Joondalup and Lake Monger (Gordon *et al.* 1981; Lund and Davis 2000), North Lake (Bayley *et al.*

1989), the Swan River (Atkins *et al.* 2001) and the Peel-Harvey estuary (Huber 1980). *Microcystis aeruginosa* is the most abundant bloom-forming colonial species in Australia capable of producing the hepatotoxin, microcystin (Baker 1992; Steffensen *et al.* 1999).

***Microcystis flos-aquae*** (Wittrock) Kirchner 1898
(Plate 7.1: 4-6, Table E2)
*Microcystis flos-aquae* was previously known as *Microcystis aeruginosa* f. *flos-aquae* (Wittrock) Elenkin 1938 (Baker 1992). It is now classified as a separate species (Komárek and Anagnostidis 1998). Cells were spherical to subspherical, 2-5 μm in diameter, either compacted or evenly distributed throughout the colony. Cells were smaller than those observed in *M. aeruginosa* and *M. wesenbergii* (Fig. 7.1). Colonies either large, globular, irregularly shaped; or small, spherical, or rounded in outline. Mucilage indistinct or narrow. Colonies without fenestrations, as seen with *M. aeruginosa*, or lobed protrusions, as with *M. wesenbergii*.

*Microcystis flos-aquae* was the most common bloom forming colonial species the SCP wetlands. It coexisted with *M. aeruginosa*, forming water-blooms in nearly all lakes studied, but tended to dominate only at higher salinity levels. Perry Lakes was the only freshwater site not to contain *M. flos-aquae.* This species was responsible for producing a massive bloom in the Swan River estuary in February 2000.

***Microcystis wesenbergii*** (Komárek) Komárek in Kondratevá 1968
(Plate 7.1: 7-9, Table E3)
Cells spherical to oval, 4.44-8 μm in diameter. Cells either sparsely distributed or evenly distributed within the colony; or concentrated on the colony boundary. Colonies either spherical, ellipsoidal or intensely lobate in shape. Smaller colonies circular in shape; diameter 80-605 μm; larger colonies irregularly shaped and lobate. Mucilage and boundary of colony sharply defined, extending 2-6 μm beyond cells.

This is the first report of *M. wesenbergii* in a Western Australian wetland. Commonly observed in Hyde Park within a mixed population containing

*M. aeruginosa* and *M. flos-aquae*. Cell numbers exceeded $1 \times 10^5$ cells $mL^{-1}$ during both the spring-summer and autumn-winter collections. Also observed in Shenton Park, Tomato Lake and Neil McDougall Park during summer and in Lake Claremont and Perry Lakes during autumn-winter 2001.

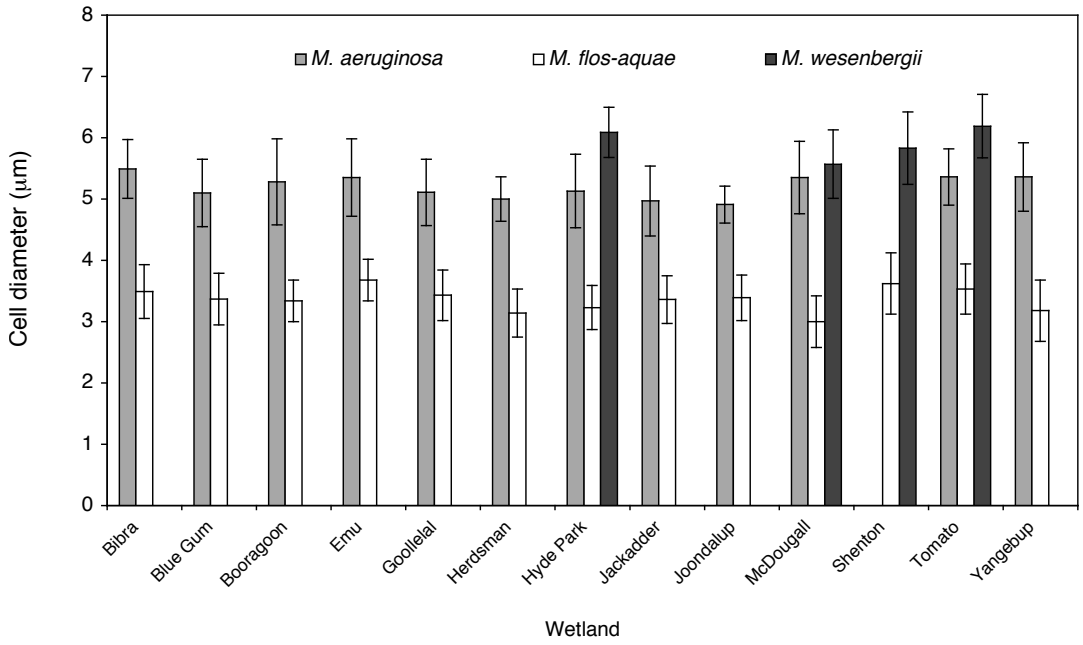



**Figure 7.1.** Mean diameter of cells (n = 50) of *M. aeruginosa, M. flos-aquae* and *M. wesenbergii,* collected from 13 wetlands, 2000 to 2003, with standard deviation.

### *7.3.2 Order Nostocales*

**Genus *Anabaena*** Bory ex Bornet et Flahault

The genus *Anabaena* is comprised of 80 species distributed worldwide (Cronberg and Anadotter 2006), with only 16 species identified in Australia (Baker and Fabbro 2002). Seven of these species were identified during the current study. All seven species were commonly observed in the SCP lakes except for *A. torulosa* var. *tenuis*, which was observed once in Mary Carroll Park.

***Anabaena affinis*** Lemmermann 1897

(Plate 7.2: 10-11, Table E4)

Trichomes straight or flexuous, not attenuated. Vegetative cells spherical with distinct cell connections; length 4-7 μm; breadth 5-7 μm. Heterocytes intercalary and sometimes terminal, spherical in shape; length 6-9 μm; breadth 6-8 μm. Akinetes solitary, oblong, adjacent to the heterocyte on one side; length 17-18 μm and breadth 7-10 μm. Apical cells indistinct. *Anabaena affinis* was unique to Emu Lake, recorded in both 2001-02 and 2002-03.

***Anabaena aphanizomenoides*** Forti 1912

(Plate 7.2: 12-14, Table E5)

Trichome straight. Vegetative cells quadrate or short cylindrical; constricted at the cross-walls; length 2-6 μm; breadth 2-5 μm. Heterocytes intercalary, spherical to quadrate; length 3-7 μm; breadth 2-8 μm. Akinete oval, solitary and adjacent to one side or both sides of the heterocyte; length 6-11 μm; breadth 5-10 μm. Apical cells elongated and narrower; length 3-6.5 μm; breadth 2-4 μm.

*Anabaena aphanizomenoides* was common in Mary Carroll Park and was recorded in Lake Joondalup only once. The UPGMA dendrogram comparing *Nostocales* species with straight trichomes showed a high level of similarity between the Joondalup Lake and Mary Carroll Park samples in relation to cell dimensions (Fig. 7.6a).

***Anabaena bergii* var. *limnetica*** Coute et Preisig 1978

(Plate 7.2: 15-17 and 7.5: 39: Table E6)

Trichome solitary, straight and slightly attenuated. Vegetative cells depressed globuse, discoid or long quadrate; length 2-7 μm; width 3-7 μm. Heterocytes, intercalary, spherical, 1-3 per trichome; length 3-10 μm; width 2.78-11 μm. Akinetes oval to oblong-ovate, single or in series, remote from the heterocyte, more than 1 per trichome, epispore dark in colour; length 6-23 μm; width 5-19 μm. Apical cells conical, hyaline, tapered at one end club-shaped at the other.

*Anabaena bergii* var. *limnetica* was common in lakes Joondalup, Brearley, Bibra, Emu and Mary Carroll Park, and only recorded once in Lake Coogee. All except Lake Brearley and Lake Coogee also contained either the straight filamentous species *A. aphanizomenoides* (Joondalup and Mary Carroll) or *Aphanizomenon ovalisporum* (Joondalup, Bibra and Emu). Clustering analysis showed the difference in cell dimensions between *A. bergii* var. *limnetica*, *A. aphanizomenoides* and *A. ovalisporum* from these sites (Fig. 7.6a and 7.6b). *Anabaena bergii* var. *limnetica* displayed a wider trichome with tapered and hyaline apical cells, distinguishing it from *A. aphanizomenoides* and *A. ovalisporum* (Plate 7.2 and 7.5).

Within the *A. bergii* var. *limnetica* cluster presented in Figure 7.6a and 7.6b, Bibra Lake grouped with Emu Lake, Mary Carroll Park with Lake Brearley and Lake Joondalup with Lake Coogee. Although the lakes in each pair are geographically separated and not within the same consanguineous groups, there is connection between these sites in relation to salinity. Bibra Lake and Emu Lake are both freshwater, while Mary Carroll Park and Lake Brearley are hyposaline. Lake Joondalup, a seasonal freshwater lake, contained A. *bergii* var. *limnetica* in late summer when water levels were lower and hyposaline, while Lake Coogee is saline. This shows Western Australian populations of *A. bergii* var. *limnetica* to have a wide distribution in varied salinities.

***Anabaena circinalis*** Rabenhorst ex Bornet et Flahault 1888
(Plate 7.3: 18-21, Table E7 and E8)
Trichome, solitary or bundled; circinate, flexuous or regularly spiralled. Spirals 15-47.5 μm in height and 25-85 μm in width. Vegetative cells spherical to subspherical, compressed at the crosswalls; length 3-7 μm; breadth 4-10 μm. Heterocytes spherical and intercalary; length 4-11 μm; breadth 4-12 μm wide. Akinete oblong-ovate to pear-shaped (pyriform), solitary or paired, remote from the heterocyte; length 10-35 μm; breadth 7-18 μm.

*Anabaena circinalis* is a common bloom-forming cyanobacterium in Western Australian wetlands with previous records in the metropolitan lakes and the Canning

and Serpentine rivers (Davis *et al.* 1993; Hosja and Deeley 1994; Vincent 1995). In the present study, *A. circinalis* was common in the Canning River, Yangebup Lake, North Lake, Tomato Lake, Blue Gum Lake, Herdsman Lake and Neil McDougall Park. Elsewhere, the genus *Anabaena* has been reported in lakes Joondalup, Goollelal, Bibra, Yangebup, Herdsman, North and Forrestdale (Gordon *et al.* 1981; Davis *et al.* 1993). *Anabaena circinalis* is a producer of the neurotoxin, saxitoxin (Humpage *et al.* 1994).

Hierarchical classification of samples identified as *A. circinalis, A. flos-aquae* or *A. spiroides f. spiroides* are presented in Figure 7.2 and 7.3. *Anabaena circinalis* samples, from Neil McDougall Park, Yangebup Lake, North Lake, Herdsman Lake and Canning River were shown to be closely related, whereas samples from Tomato Lake and Blue Gum Lake grouped with *A. flos-aquae* and *A. spiroides* f. *spiroides* based on smaller akinete dimensions. A separate analysis performed only on the sites containing both *A. circinalis* and *A. flos-aquae*, with akinetes excluded produced a similar result (Fig. 7.4). Again, *A. circinalis* samples from Tomato Lake and Blue Gum Lake grouped with the *A. flos-aquae* samples. The UPGMA analysis was only based on cell size and did not consider trichome morphology and cell shape, which distinguishes *A. circinalis* from *A. flos-aquae* and *A. spiroides* f. *spiroides*.

Confusion does exist in the morphological differentiation between *A. circinalis* and *A. flos-aquae*, especially in the absence of akinetes (Baker 1991). However, trichome morphology and vegetative cell shape should suffice in identifying these two species. *Anabaena flos-aquae* differs from *A. circinalis* by having smaller more spherical vegetative cells and forms solitary contorted or tight spiralled trichomes, which is also shown in *A. spiroides* f. *spiroides,* while *A. circinalis* produces large open spirals (Baker and Fabbro 2002). This was observed in the samples collected during this study (Plate 7.3). Furthermore the presence of *A. circinalis* and *A. flos-aquae* together in Herdsman Lake, North Lake and the Canning River allows morphological comparison to be made. *Anabaena flos-aquae* is usually a minor component in mixed blooms dominated by *A. circinalis* and *A. spiroides* (Baker 1991), so there will be visual differences in trichome morphology (circinate, flexuous or spiralled).

***Anabaena flos-aquae*** (Lyngbye) Brébisson ex Bornet et Flauhault 1888
(Plate 7.3: 22-24, Table E9)
Trichome irregularly twisted, entangled or forming loose spirals. Vegetative cells spherical, compressed at the ends; length 4-6 μm; breadth 5-6.5 μm. Heterocytes intercalary, spherical; length 5-9 μm; breadth 5-9 μm. Akinetes oblong, solitary or paired, remote from the heterocyte; length 14-20 μm; breadth 8-12 μm.

*Anabaena flos-aquae* was recorded in North Lake, Herdsman Lake and the Canning river. Recently, this species has been observed in Herdsman Lake showing a curved cylindrical shaped akinete (J. John, personal communication, September 2007). Oblong shaped akinetes were only observed in the North Lake and Canning River samples. The shape shows these akinetes to be immature. Mature akinetes of *A. flos-aquae* are cylindrical in shape, more than one and a half times longer than broad (Baker 1991). North Lake and the Canning River showed a high level of similarity (Fig. 7.2, group 2). Based only on the dimension of the vegetative cells and heterocytes, the two Herdsman Lake samples united in the same cluster (Fig. 7.3, group 1). In the absence of akinetes, recognizing *A. circinalis* from *A. flos-aquae* can be difficult. Although both species are similar in appearance, the dimension of the vegetative cells and arrangement of the trichome will assist with identifying the sample to species level.

***Anabaena spiroides* f. *spiroides*** (Elenkin) Komárek
(Plate 7.3: 25-27, Table E10)
The two *Anabaena spiroides* f. *spiroides* morphotypes described by Baker (1991) were observed during this study. These two morphotypes differ only by akinete shape and positioning on the trichome, which makes identification difficult if akinetes are not present when both morphotypes are in the same population (Baker 1991). *Anabaena spiroides* f. *spiroides* (Morphotype 2) shows distinctly spherical akinetes, adjacent to one or both sides of a heterocyte (Baker 1991). In the current study no attempt was made to distinguish the two morphotypes, therefore they are both collectively called *Anabaena spiroides* f. *spiroides* in this thesis. However

Table F1 does show the akinete being located adjacent to the heterocyte in the A. *spiroides* f. *spiroides* samples, which suggests these to be Morphotype 2.

Due to the spherical akinete shape and position on the trichome, Baker and Fabbro (2002) considered *Anabaena spiroides* f. *spiroides* (Morphotype 2) to be an independent species and renamed it *Anabaena* sp. nova. *Anabaena* sp. nova has been recorded in all Australian states except the Northern Territory (Baker and Fabbro 2002). This thesis provides the first account of a similar species in WA. However, further research is needed to justify *Anabaena* sp. nova as a new Australian species. *Anabaena* sp. nova needs to be characterised, based on both phenotypic and genotypic traits of strain and natural population, and compared to similar lesser-known species found elsewhere in the world, for example *Anabaena oumiana*, *A. reniformis*, *Anabaena eucompacta,* and *Anabaena torques-reginae* (Li and Watanabe 1999; Li *et al*. 2000; Komárek 2005; Werner *et al.* 2007; Komárek and Zapomelová 2007).

Trichomes darkly pigmented solitary, sometimes in bundles; regularly and irregularly spiralled, spiral height 7-26 μm and width 11-33 μm. Vegetative cells spherical, subspherical when dividing, distinctly constricted at cross-walls; length 2-7 μm; width 3-8 μm. Heterocytes spherical to ovate; length 4-9 μm; width 5-10 μm. Akinete spherical to oval is shape, solitary, more than one per trichome, adjacent to one or both sides of the heterocyte; length 6-17 μm long; width 7-13μm.

Clustering analysis showed a clear separation of *A. spiroides* f. *spiroides* (group 1) from *A. circinalis* (group 5) based on smaller vegetative cells, heterocytes and akinetes (Fig. 7.2). The *Anabaena spiroides* f. *spiroides* samples in group 1 showed a level of similarity with the *Anabaena spiroides* f. *spiroides* (Morphotype 2) reference sample from Baker (1991). Only two *Anabaena spiroides* f. *spiroides* samples (Tomato and Joondalup) were not included in group 1. A sample from Lake Joondalup recorded smaller cell dimensions, so grouped with *A. spiroides* var. *minima* from Baker (1991) (group 3). A sample from Tomato Lake was shown to be

closely associated with *A. circinalis* (group 2) due to oval shaped akinetes, which would classify this sample as *A. spiroides* f. *spiroides* Morphotype 1.

*Anabaena spiroides* f. *spiroides* was common in North Lake, Tomato Lake and Lake Joondalup. Also collected from Lake Claremont and Shenton Park (February 2002). *Anabaena spiroides* Klebahn has been previously identified in the Canning River (Vincent 1995) and Lake Joondalup (Congdon 1986). However, a photograph presented by Vincent (1995) depicted a trichome with spherical akinetes next to the heterocyte, which is characteristic of *A.* sp nova. Therefore, the two morphotypes of *A. spiroides* f. *spiroides* may also be present in the Canning River.

***Anabaena torulosa* var. *tenuis*** (Lemmermann) Geitler 1932
(Plate 7.4: 28, Table E11)
Trichome straight. Vegetative cells quadrate or short cylindrical; constricted at the cross-walls, sometimes with distinct connections; length 2-6 μm; breadth 2.5-4 μm. Heterocytes intercalary, spherical; length 3-6 μm; breadth 3-4 μm. Akinete oval, solitary and adjacent to one side of the heterocyte; length 6-10 μm; breadth 5-8 μm. Apical cells elongated and narrower; breadth 2-3 μm. *Anabaena torlusa var. tenuis* was unique to Mary Carroll Park. It has previously been recorded in the Peel-Harvey estuary (Huber 1980).

**Genus *Anabaenopsis*** (Woloszynska) Miller

Only three species of *Anabaenopsis* have been identified in Australia (Baker and Fabbro 2002) and all were present in the SCP wetlands. Clustering analysis based on the dimensions of the vegetative cells and heterocytes generated three groups comprised of *A. elenkinii* (group 1), *A. tanganyikae* (group 2) and *A. arnoldii* (group 3) (Fig. 7.5).

***Anabaenopsis arnoldii*** Aptekarj 1926

(Plate 7.4: 29-30, Table E12)

Trichome coiled or regularly spiralled; spirals height 1-27.5 μm and width 32.5-50 μm. Vegetative cells short barrel-shaped to spherical, slightly constricted at the cross-walls; length 3-9 μm; breadth 7-11 μm. Heterocyte oval, intercalary in pairs; length 7-12 μm, breadth 7-12 μm; or terminal; length 8-12 μm; breadth 7-12 μm. Akinetes oblong-ovate, solitary, paired or in series, adjacent to one side of the heterocyte; length 11-17 μm; breadth 9-15 μm. *Anabaenopsis arnoldii* was observed only once during this study in Lake Claremont (February 2002) at $2 \times 10^6$ cells $mL^{-1}$.

***Anabaenopsis elenkinii*** Miller 1923

(Plate 7.4: 31-33, Table E13 and E14)

Trichomes solitary, loosely spiralled or circinate. Vegetative cells barrel-shaped and constricted at the crosswalls; length 3-12 μm; breadth 3-8μm. Heterocytes intercalary; length 3-10 μm and breadth 3-9 μm; and terminal; length 3-10 μm and breadth 3-9 μm. Terminal heterocytes oval; intercalary heterocyst spherical, single or paired, at times juvenille. Akinetes solitary, oblong-ovate; solitary, paired or in series, either remote from the heterocyte or adjacent to the intercalary heterocyte; length 8-17 μm; breadth 7-14 μm.

*Anabaenopsis elenkinii* was common to lakes Bibra, Joondalup, Neil McDougall Park, Shenton Park and the upper Serpentine River. Also present in Mary Carroll Park and Yangebup Lake. This species showed a wide salinity tolerance occurring in salinities as low as 0.15 ppt in Shenton Park, to 31.4 ppt in the Serpentine River. Rarely dominating the bloom, *A. elenkinii* occurred with other filamentous species, for example *A. spiroides* in Shenton Park and with *N. spumigena* in the Serpentine River. Previously reported in the Peel-Harvey Estuary and Canning River (Huber 1980; Vincent 1995).

***Anabaenopsis tanganyikae*** (G.S. West) Woloszynska et Miller 1923

(Plate 7.4:34-35, Table E15)

Trichome curled. Vegetative cells curved cylindrical, slightly constricted at the cross-walls, distinctly longer than wide; length 6-18 μm; breadth 3.5-6 μm. Heterocytes intercalary and terminal, spherical to oval in shape; length 4-9 μm; breadth 3-7 μm. Akinete kidney-shaped, solitary, remote from the heterocyte; length 8-18 μm; breadth 5-11 μm.

*Anabaenopsis tanganyikae* was common to Mary Carroll Park and Lake Joondalup. High cell numbers were most often observed in late summer (February) when salinity was high. For example, in February 2002, Mary Carroll Park recorded $3 \times 10^5$ cells $mL^{-1}$ in 13.53 ppt (22.67 mS $cm^{-1}$). During the same period, Lake Joondalup recorded $9 \times 10^5$ cells $mL^{-1}$ in 3.26 ppt (5.92 mS $cm^{-1}$).

**Genus *Aphanizomenon*** Morren ex Bornet et Flahault

***Aphanizomenon gracile*** Lemmermann 1907

(Plate 7.5: 36-38, Table E16)

Trichomes solitary, straight sometimes attenuated. Vegetative cells quadrate in shape not constricted at crosswalls; length 3-6 μm; breadth 2.5-4 μm. Heterocytes oblong-ovate; length 5-9 μm; breadth 3-6μm. Akinetes long and cylindrical, usually 1-3 per trichome, remote from the heterocyte; 10-30 μm; breadth 3-8 μm.

*Aphanizomenon gracile* was sampled only once in Bibra Lake, where *A. ovalisporum* is common. These two species can be distinguished by *A. gracile* having an elongated, cylindrical akinete, with no overlapping sheath. Clustering analysis also identified the difference in cell dimensions between *A. gracile* and *A. ovalisporum* (Fig. 7.6a, group 4). *Aphanizomenon gracile* has previously been reported in the Blackwood River (Hosja and Deeley 1994) and Peel-Harvey estuary (Huber 1980). No toxins have been shown in Australian populations (Baker and Humpage 1994), although paralytic shellfish toxins have been associated with *A. gracile* bloom in a Portuguese reservoir (Pereira *et al.* 2004).

***Aphanizomenon ovalisporum*** Forti 1912

(Plate 7.5:39-41, Table E17)

Trichomes solitary or bundled, straight and attenuated. Mucilage sheath not evident. Vegetative cells quadrate to cylindrical; length 4-8 μm; breadth 3-5 μm. Heterocytes oblong- ovate, intercalary and usually two per trichome. Akinetes remote from the heterocyte, solitary or paired, oblong in shape. Apical cells longer than vegetative cells; length 5-13 μm; breadth 3-5 μm.

*Aphanizomenon ovalisporum* has only recently been identified in Australian waters and is a producer of the hepatotoxin cylindrospermopsin (Shaw *et al.* 1999; Baker and Fabbro 2002). This species was common in Bibra Lake and Emu Lake, and was present in Lake Joondalup during the 2002-03 sampling period. *A. bergii var. limnetica* was also present in these sites. *Aphanizomenon ovalisporum* and *A. bergii var. limnetica* can be easily distinguished by cell dimensions and shape of the apical cell (Fig. 7.6a).

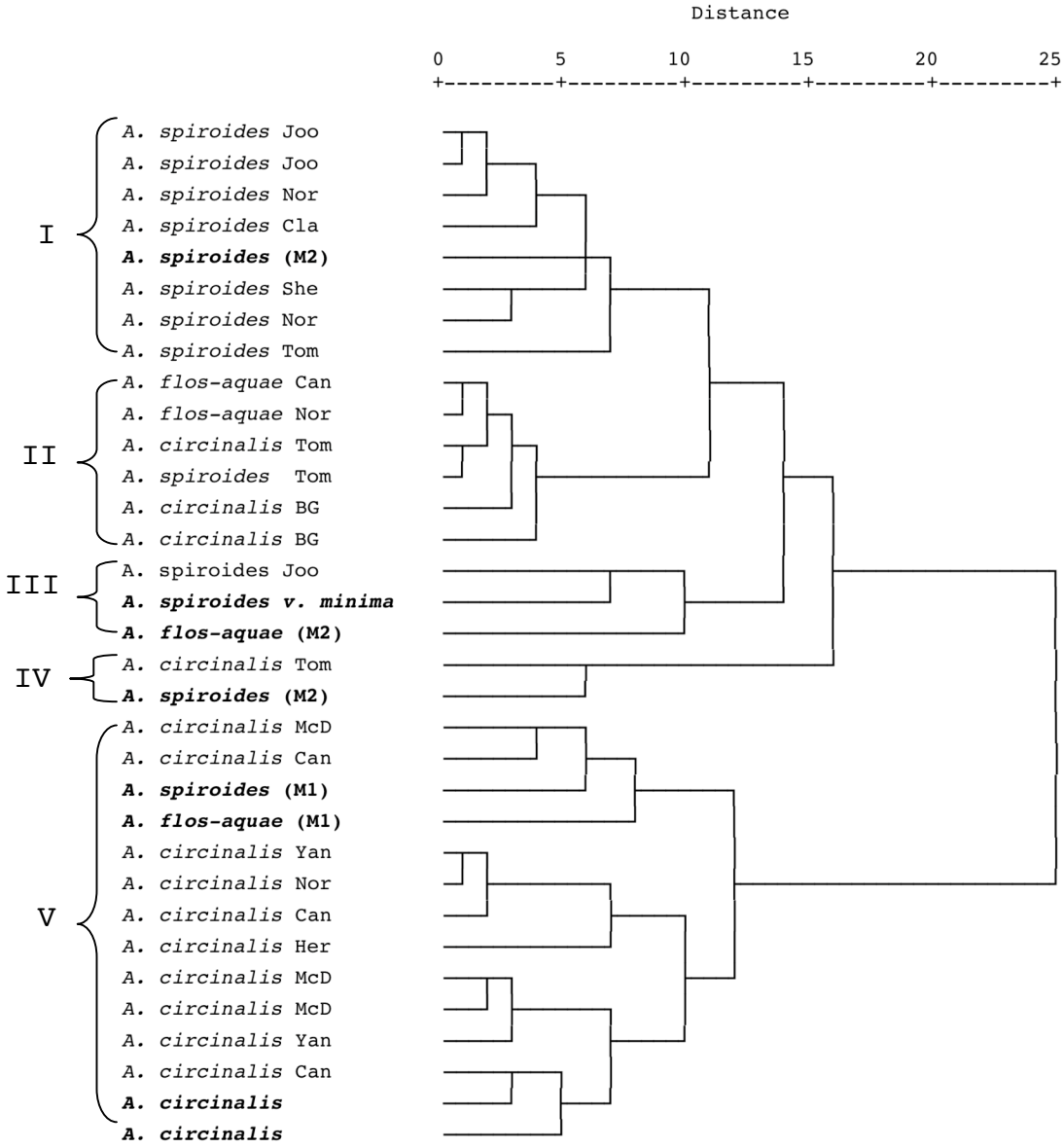



**Figure 7.2.** UPGMA distance dendrogram based on cell dimensions (akinetes included) measured from species of *Anabaena* with circinate or spiralled trichomes. Reference samples in bold.

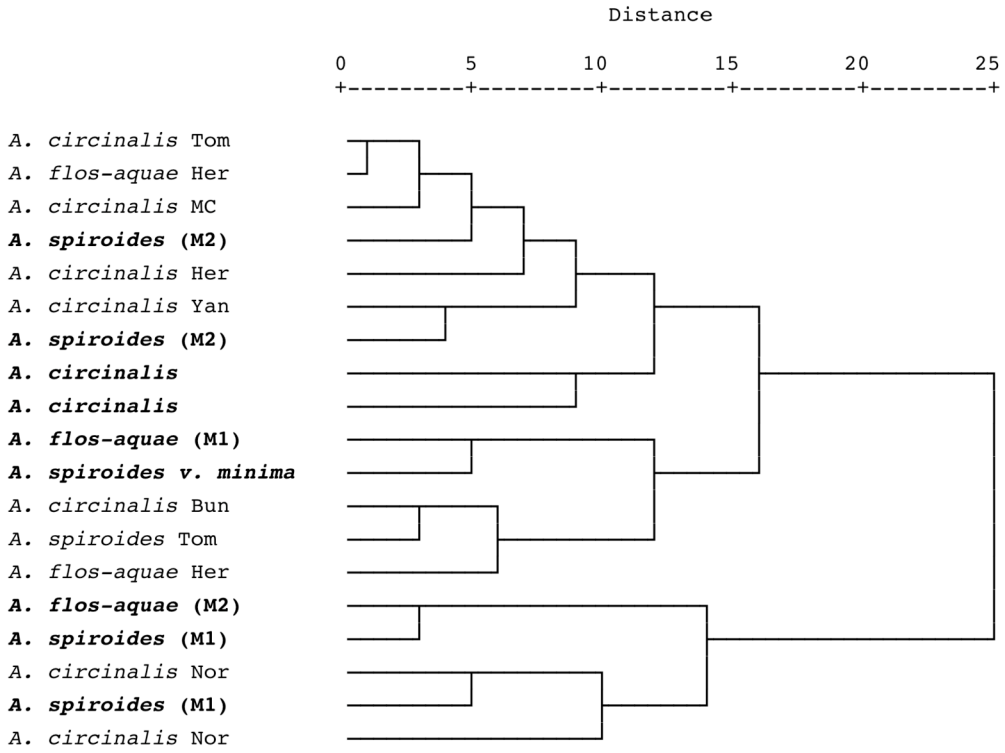



**Figure 7.3.** UPGMA distance dendrogram for *Anabaena* species with circinate or spiralled trichomes based on vegetative cell and heterocyte dimensions. Reference samples in bold.

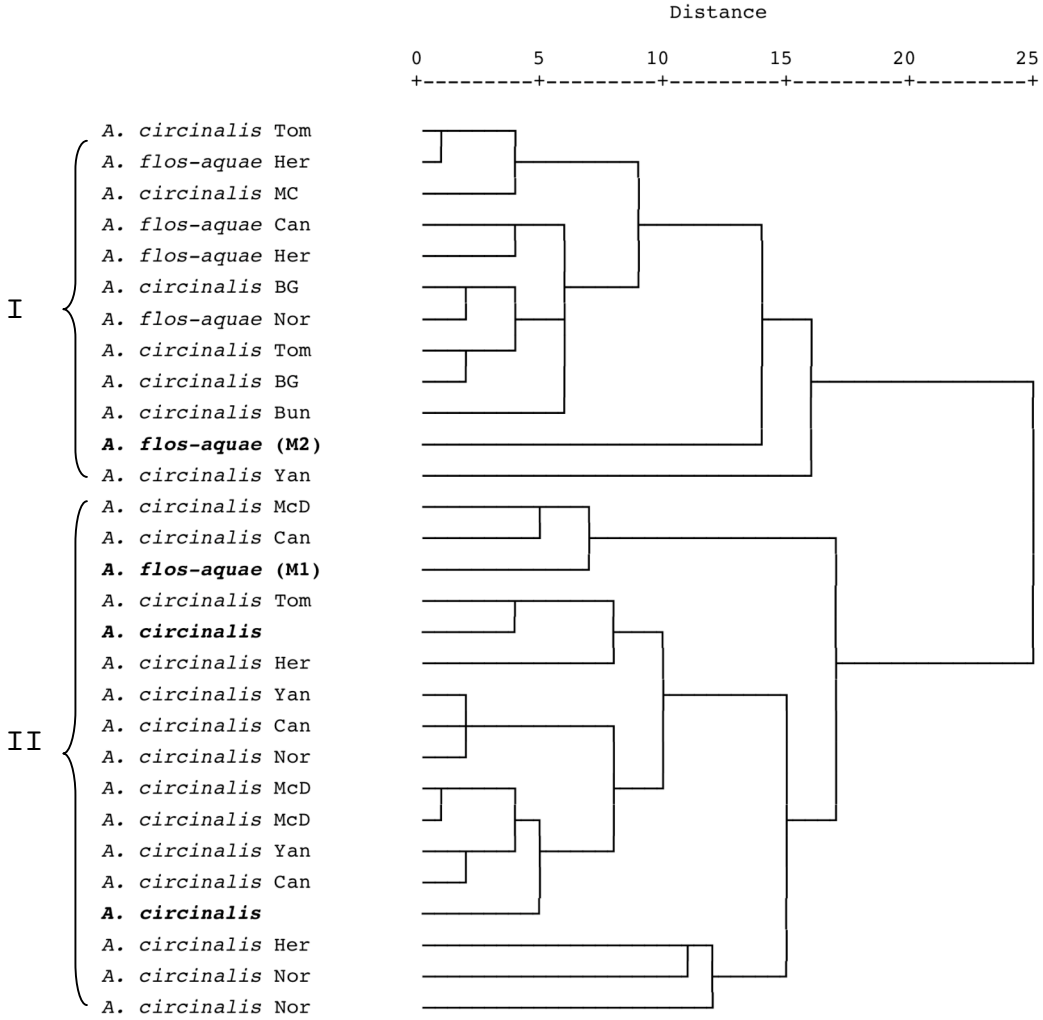



**Figure 7.4.** UPGMA distance dendrogram based on *A. circinalis* and A. *flos-aquae* samples collected from lakes Herdsman, North, Blue Gum, Tomato and the Canning River (akinetes not included). Reference samples in bold.

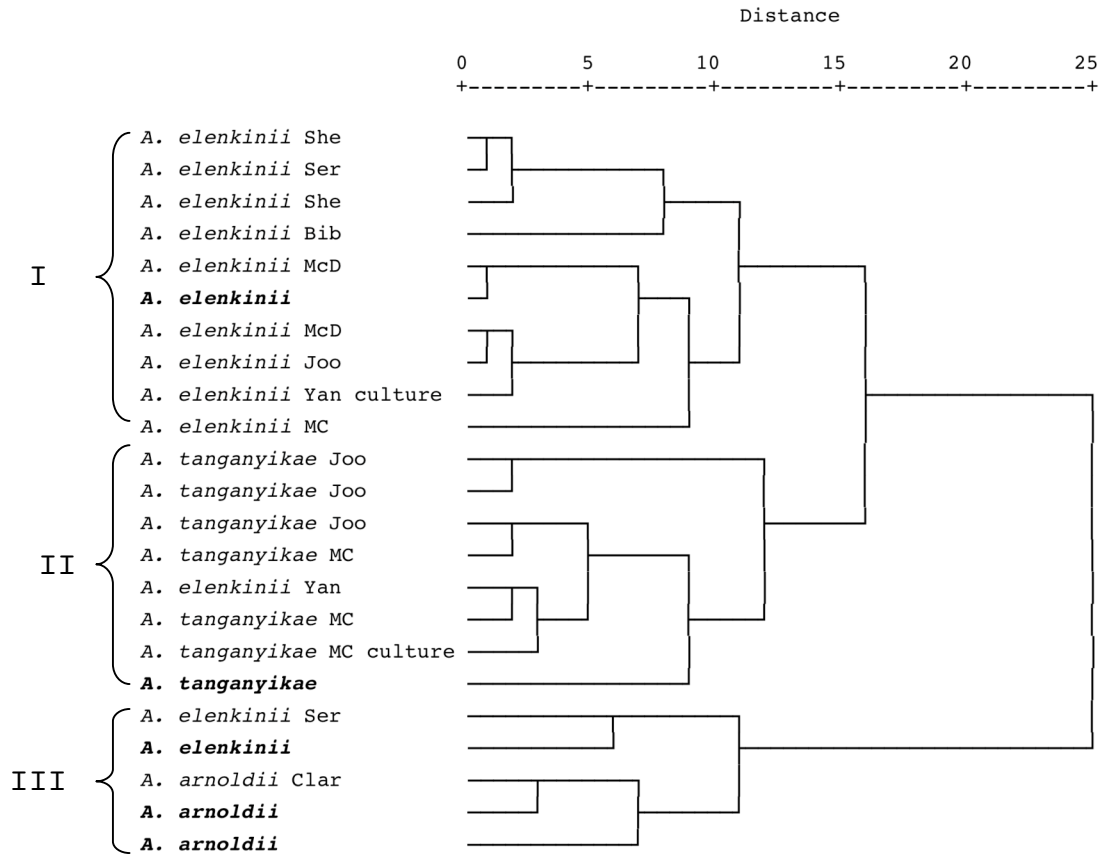



**Figure 7.5.** UPGMA distance dendrogram based on cell dimensions (akinetes included) measured from species of *Anabaenopsis*. Reference samples in bold.

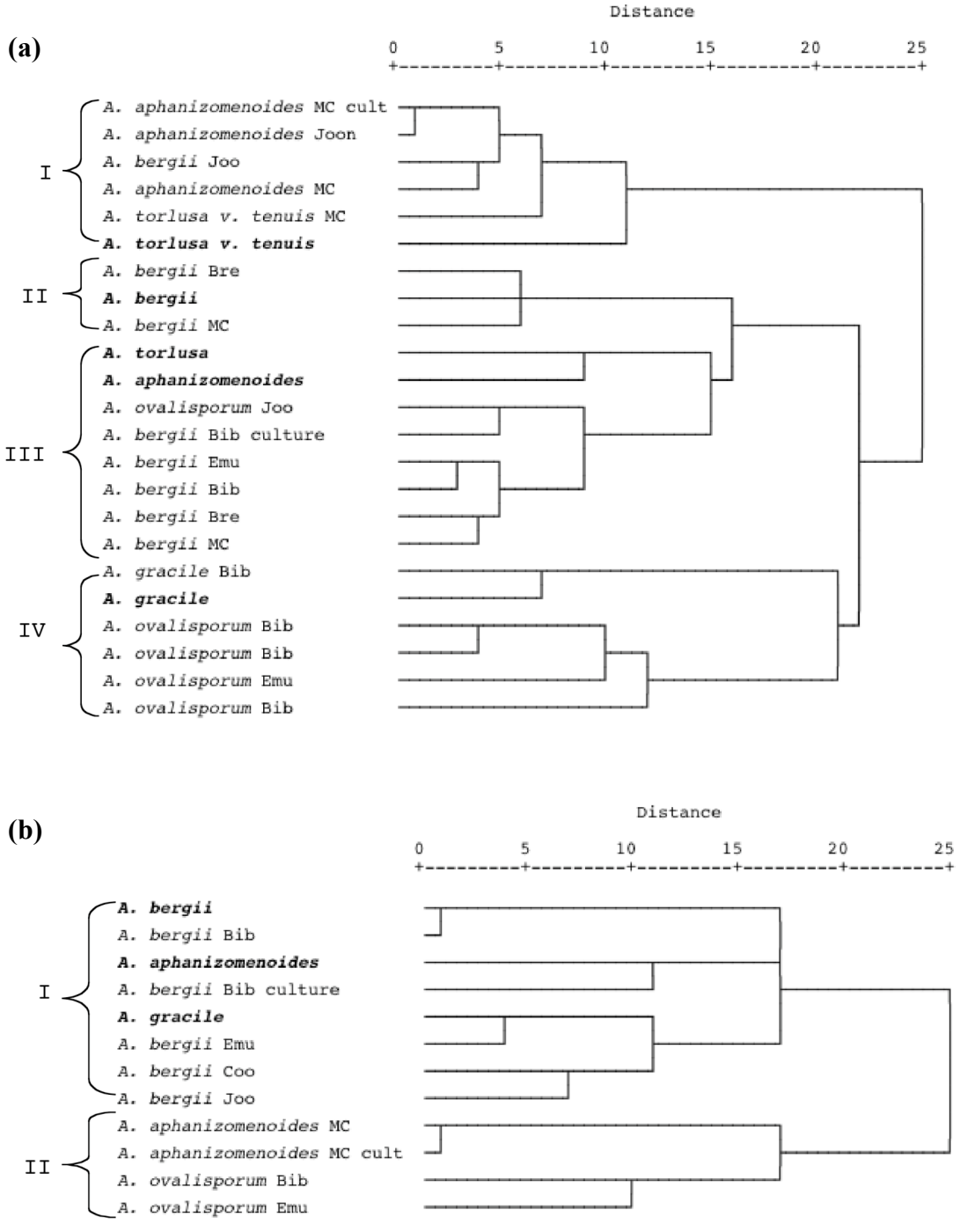



**Figure 7.6.** UPGMA distance dendrogram showing the relationship between Nostocales species with straight trichomes based on cell dimensions with **(a)** akinetes present and **(b)** akinetes absent. Reference samples from Baker (1991) in bold.

**Genus *Cylindrospermopsis*** Seenaya et Subba Raju

***Cylindrospermopsis raciborskii*** (Woloszynska) Seenaya et Subba Raju 1972
(Plate 7.5: 42-43, Table E18)
Trichomes solitary and straight. Vegetative cells long quadrate to cylindrical, with length greater than breadth, and crosswalls indistinct; length 5-11 μm; breadth 1.5-3 μm. The terminal heterocytes are conical in shape; length 4-9 μm; breadth 1.5-3 μm. Akinetes were not present in these samples. *Cylindrospermopsis raciborskii* was only collected from Bibra Lake in 2001. After the completion of this study *C. raciborskii* was reported in Emu Lake and Hyde Park (ARNAT 2004). To the best of the author's knowledge, the 2001 occurrence of *C. raciborskii* in Bibra Lake was the first record of this species in Western Australia.

Australian populations of *C. raciborskii* show three morphological forms; straight, coiled and sigmoid/irregular (McGregor and Fabbro 2000; Saker and Griffiths 2001; Baker and Fabbro 2002). All three forms are mostly observed in tropical populations (McGregor and Fabbro 2000; Saker and Griffiths 2001). Only the straight filaments were observed in Bibra Lake. Elsewhere, this straight trichome morphology has been collected from wetlands in France (Briand *et al.* 2002), New Zealand (Wood and Stirling 2003), Brazil (Lagos *et al.* 1999) and USA (Chapman and Schelske 1997). Irrespective, pure cultures show all morphological forms to be similar with regards to cylindrospermopsin content (Saker *et al.* 1999a).

**Genus *Nodularia*** Mertens ex Bornet et Flahault

***Nodularia spumigena*** Mertens ex Bornet et Flahault 1886
(Plate 7.2: 12 and 7.6: 44-46; Table E19)
Trichome solitary, long and straight, with sheath; breadth 8-13 μm. Vegetative cells discoid, length 2-4 μm. Heterocytes oblong, intercalary and evenly spaced along the trichome; length 3-8 μm; breadth 7-15μm. Akinete oblong, paired or in series, remote from the heterocyte; length 5-10 μm; breadth 9-14μm. Apical cell indistinctive.

*Nodularia spumigena* was common in the upper reaches of the Serpentine River during spring. It was also recorded in Yangebup Lake in 2001 and 2002. Elsewhere, *N. spumigena* has been reported in the Swan, Avon, Canning and Blackwood rivers (Hosja and Deeley 1994), and in the southern metropolitan lakes Forrestdale and Thomsons (John and Kemp 2006). In the current study, samples from Thomsons Lake and Yangebup Lake have tested positive for nodularin.

**Genus Nostoc** Vaucher ex Bornet et Flahault

***Nostoc linckia*** (Roth) Bornet ex Bornet et Flahault 1888
(Plate 7.6: 47, Table E20)
Trichome irregularly spiralled forming colonies within mucilage. Vegetative cells quadrate in shape with distinct connections; length 2-7 μm; breadth 2.5-5 μm. Heterocytes spherical, intercalary and terminal; length 3-7 μm; breadth 3-6 μm. Akinete oval, in series remote from the heterocyst; length 5-9 μm; breadth 4.5-8 μm. *Nostoc linckia* was collected only from North Lake

### *7.3.3 Order Oscillatoriales*

**Genus Oscillatoria** Vaucher ex Gomont

***Oscillatoria sancta*** Küetzing ex Gomont 1892
(Plate 7.6: 48-49, Table E21)
Trichome straight, sometimes forming an aggregated mass, surrounded by a distinct, unbranched sheath; total breadth 10-15 μm. Vegetative cells were broader in width and not constricted at the cross walls. Apical cells were hyaline and rounded; length 4.75-9 μm; breath 5-13.5 μm; sometimes with calyptra. *Oscillatoria sancta* was only observed in Thomsons Lake. *Oscillatoria* has previously been reported in the Avon River (Hosja and Deeley 1994).

**Genus *Phormidium*** Küetzing

***Phormidium* aff. *amoenum*** Küetzing 1843 ex Anagnostidis et Komárek 1988
(Plate 7.6: 50-51, Table E22)
Trichome straight, not constricted at the cross walls; breadth 5-7 μm. Slightly tapered towards the apex, apical cell arched; length 2-5 μm and breadth 2-6 μm. *Phormidium.* aff. *amoenum* was recorded in Neil McDougall Park and Blue Gum Lake.

In the current study, *P.* aff. *amoenum* was collected as solitary filaments. However, it commonly forms benthic mats that can dislodge and become free floating in the water column (Baker 1992). Recently, samples from South Australia were proven toxic, with the highest health risk occurring when filaments detach and become suspended in the water column (Baker *et al.* 2001).

**Genus *Planktothrix*** Anagnostidis et Komárek

***Planktothrix mougeotii*** (Bory ex Gomont) Anagnostidis et Komárek 1988
(Plate 7.6: 52-53, Table E23)
Trichome solitary, straight, not attenuated, 5.5-6.5 μm wide. Cells not constricted at cross-walls. Apical cell indistinct. *Planktothrix mougeotii* was common in Tomato Lake and also collected from Hyde Park, Neil McDougall Park and Blue Gum Lake.

***Planktothrix perornata* f. *attenuata*** (Skuja) Anagnostidis et Komárek 1988
(Plate 7.7: 54-55, Table E24)
Trichome solitary, straight, 8-11 μm in width. Cells slightly constricted at cross-walls, shorter than wide, 2-5 μm in length. Apical cell lighter in colour and bent only at one end; length 3-13 μm; breadth 6-10 μm. Collected only from Lake Coogee.

***Planktothrix planctonica*** (Elenkin) Anagnostidis et Komárek 1988
(Plate 7.7: 56-57, Table E25)
Trichomes solitary, straight, dark blue-green in colour, 13-19 μm in width. Trichomes not attenuated. Vegetative cells much shorter than broad, not constricted at cross walls, length 1-4 μm. Apical cells sometimes lighter in colour with a

calyptra only at one end; not capitate; length 2-7 μm; breadth 12.5-17 μm. *Planktothrix planctonica* was common in the Canning River and Blue Gum Lake. It was also sampled from Tomato Lake and Forrestdale Lake.

**Genus *Trichodesmium*** Ehrenberg ex Gomont

***Trichodesmium iwanoffianum*** Nygaard 1926

(Plate 7.7; 58-59, Table 26)

Trichomes solitary, straight, sheath not evident; breadth 4.5-7 μm. Vegetative cells discoid and slightly constricted at the cross walls with length ranging from 1.5-5.5 μm. Apical cells either indistinct, rounded conical or taper into hyaline quadrate cells.

The most frequently observed Oscillatoriales. Common to Neil McDougall Park and also observed in Tomato Lake, Blue Gum Lake, Lake Goollelal and Lake Bungana. The marine species *Trichodesmium erythraeum* has been known to form blooms off the coast of Cockburn Sound and the northwest coast of Western Australia (Creagh 1985; Hosja and Deeley 1994).

*Trichodesmium iwanoffianum* forms free-floating colonies with radially arranged fascicles (parallel bundles) or flocculent masses, and rarely occurs in solitary trichomes (Komárek and Anagnostidis 2005). However, in Australia, *Trichodesmium iwanoffianum* has been collected as solitary trichomes, occasionally forming colonies (Baker 1991). In the current study, *T. iwanoffianum* occurred as single strands, as defined by (Baker 1991).

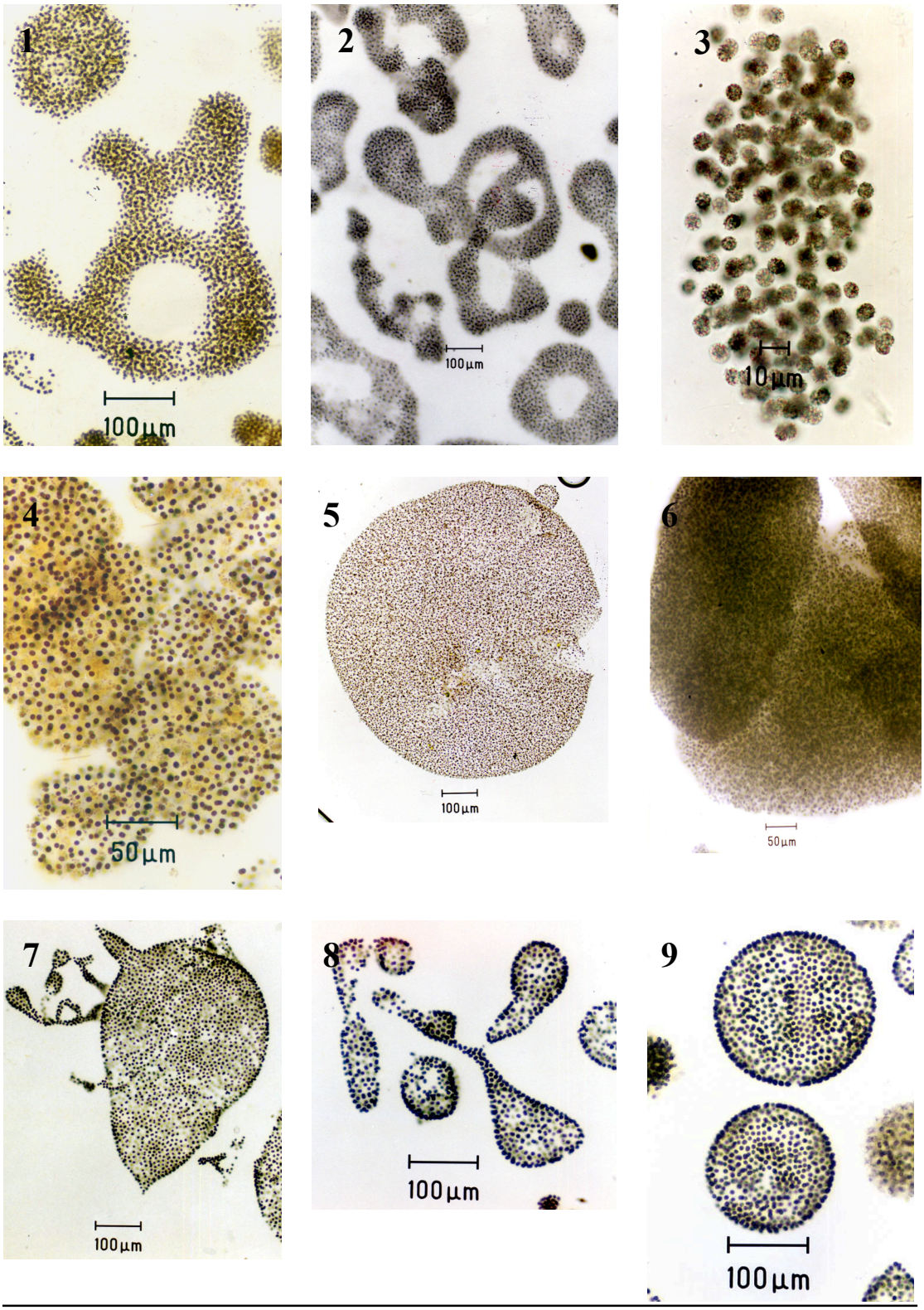



**Plate 7.1.** (1-3) *Microcystis aeruginosa*; (4-6) *Microcystis flos-aquae*; (7-9) *Microcystis wesenbergii*.

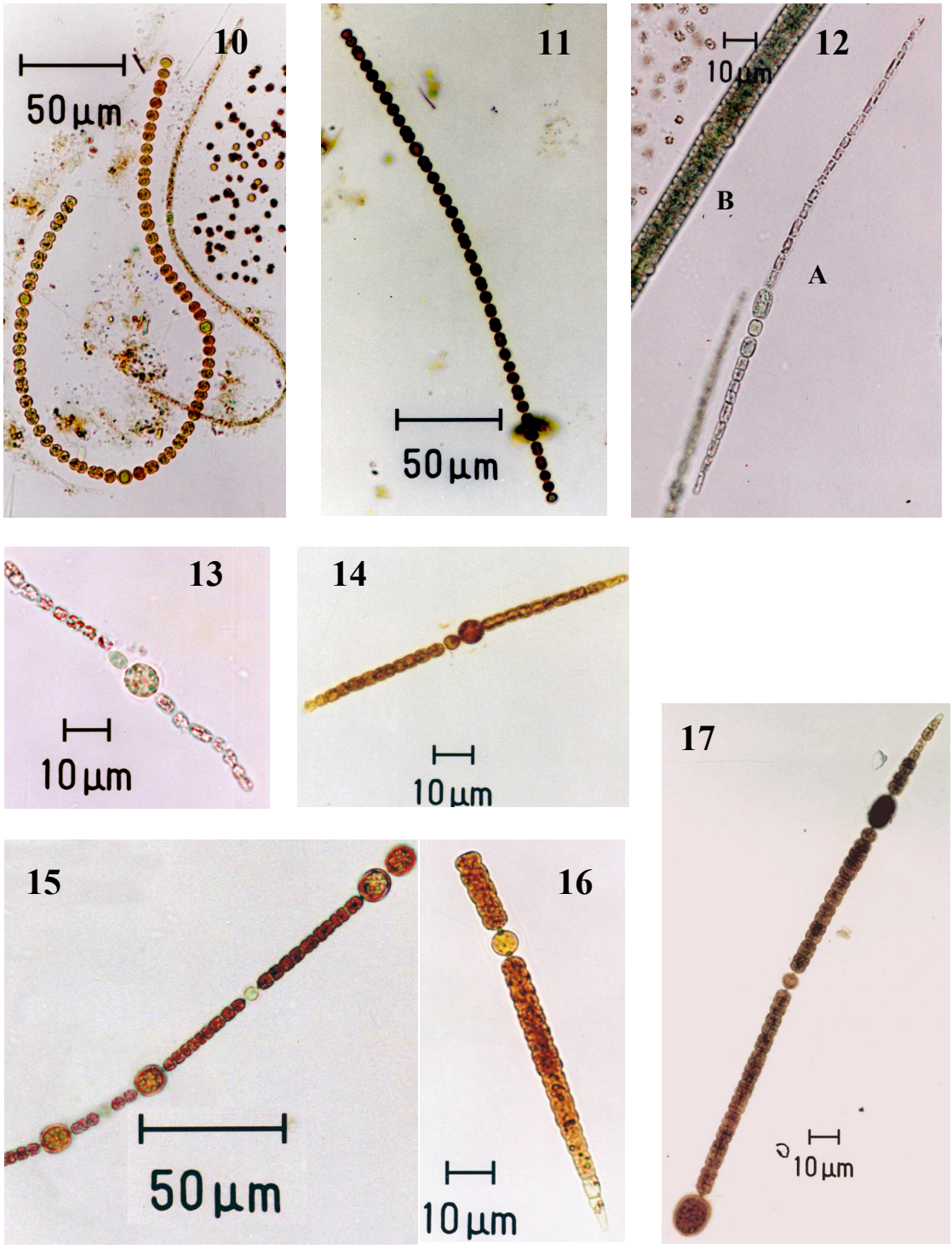



**Plate 7.2.** (10-11) *Anabaena affinis*; (12) *Anabaena aphanizomenioides* (A) and *Nodularia spumigena* (B); (13-14) *Anabaena aphanizomenioides*; (15-17) *Anabaena bergii* var. *limnetica*.

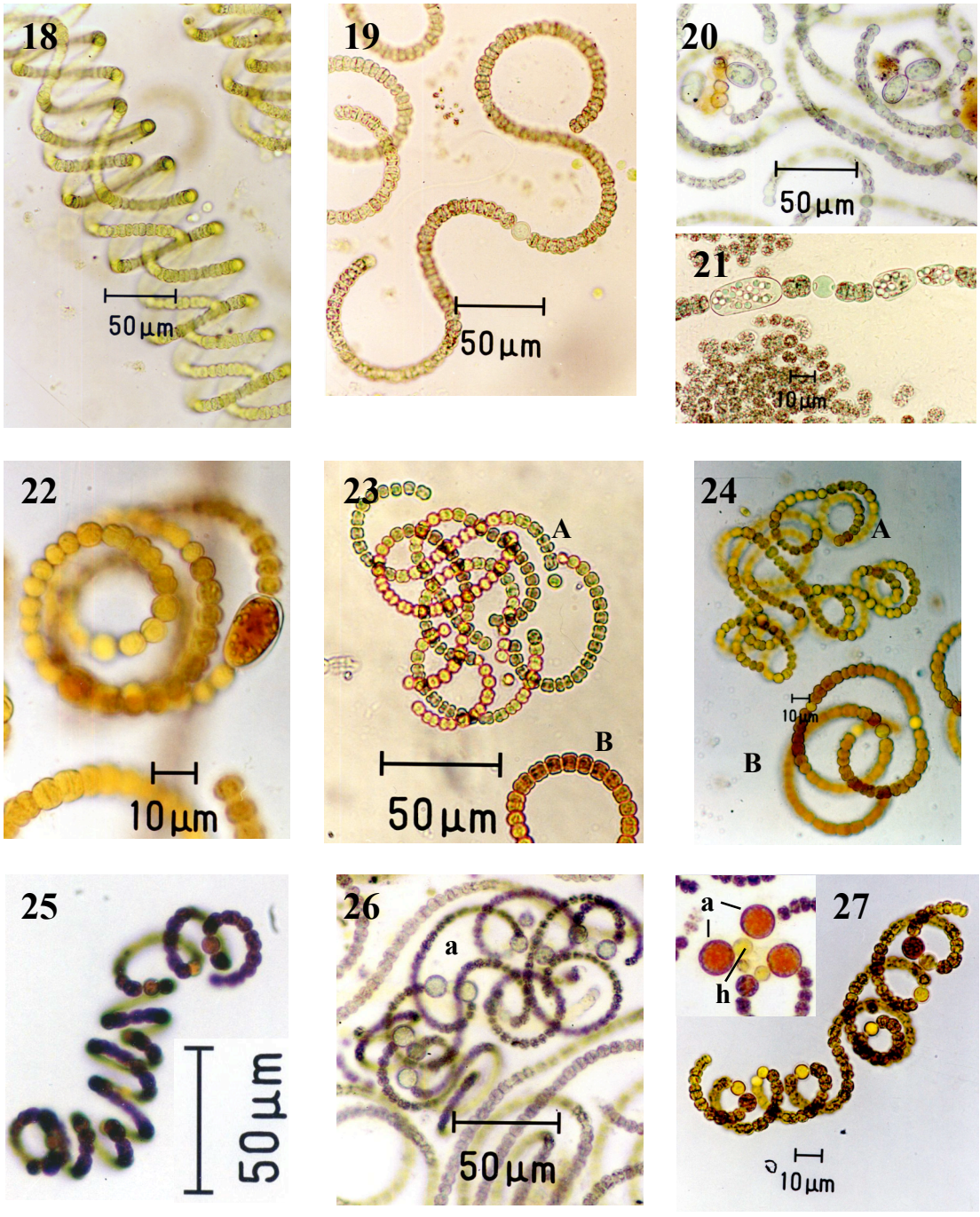



**Plate 7.3.** (18-19) *Anabaena circinalis*; (20-21) *Anabaena circinalis* akinetes; (22-24) *Anabaena flos-aquae* (A) with *A. circinalis* (B); (25) *Anabaena spiroides* f. *spiroides* (26-27) *Anabaena spiroides* f. *spiroides* (Morphotype 2) (Syn. *Anabaena* sp. nova) showing the spherical akinete (a) adjacent to the heterocyte (h).

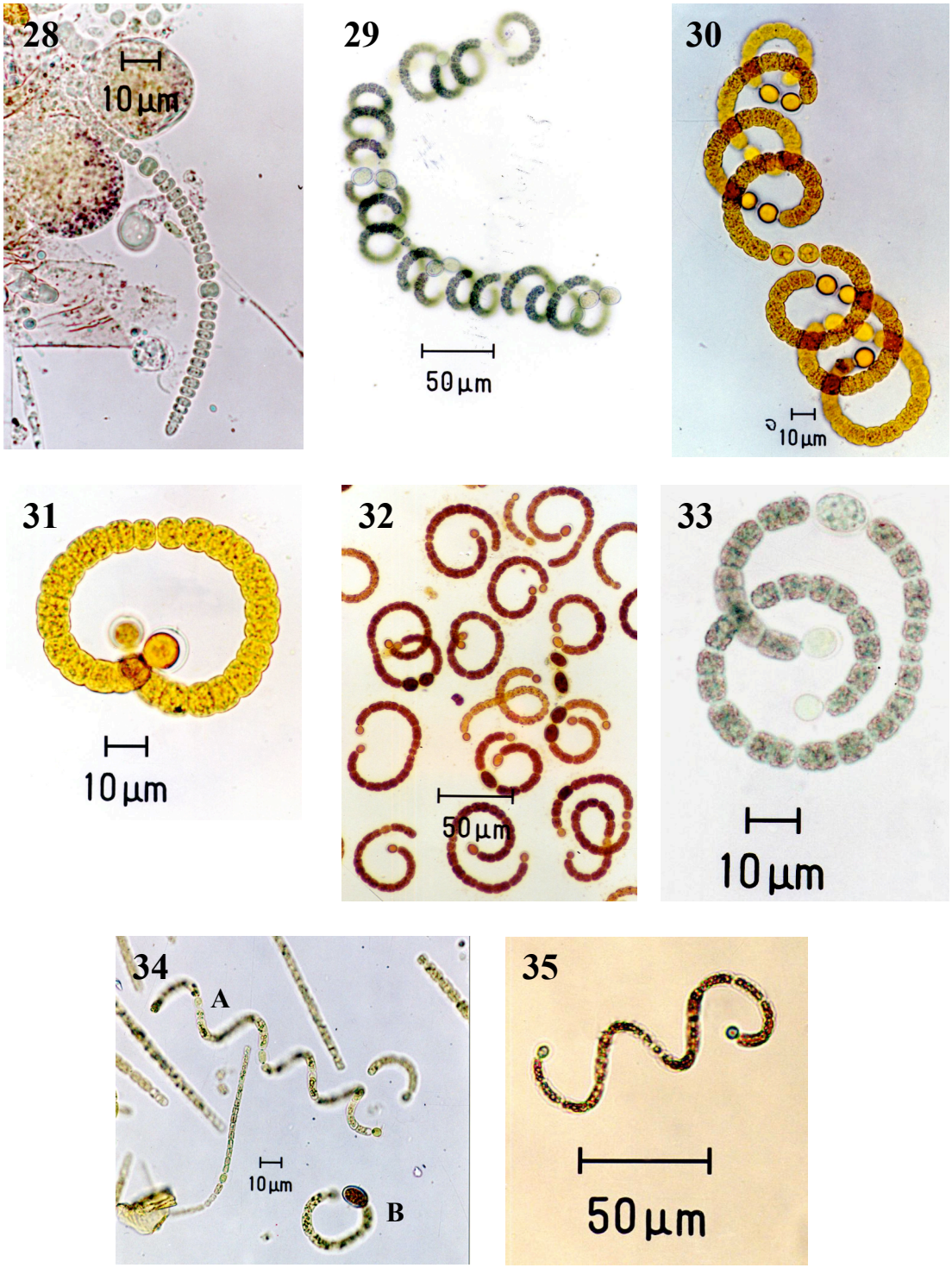



**Plate 7.4.** (28) *Anabaena torlusa* var. *tenuis*; (29-30) *Anabaenopsis arnoldii*; (31-33) *Anabaenopsis elenkinii*; (34-35) *Anabaenopsis tanganyikae* (A) and *Anabaenopsis elenkinii* (B).

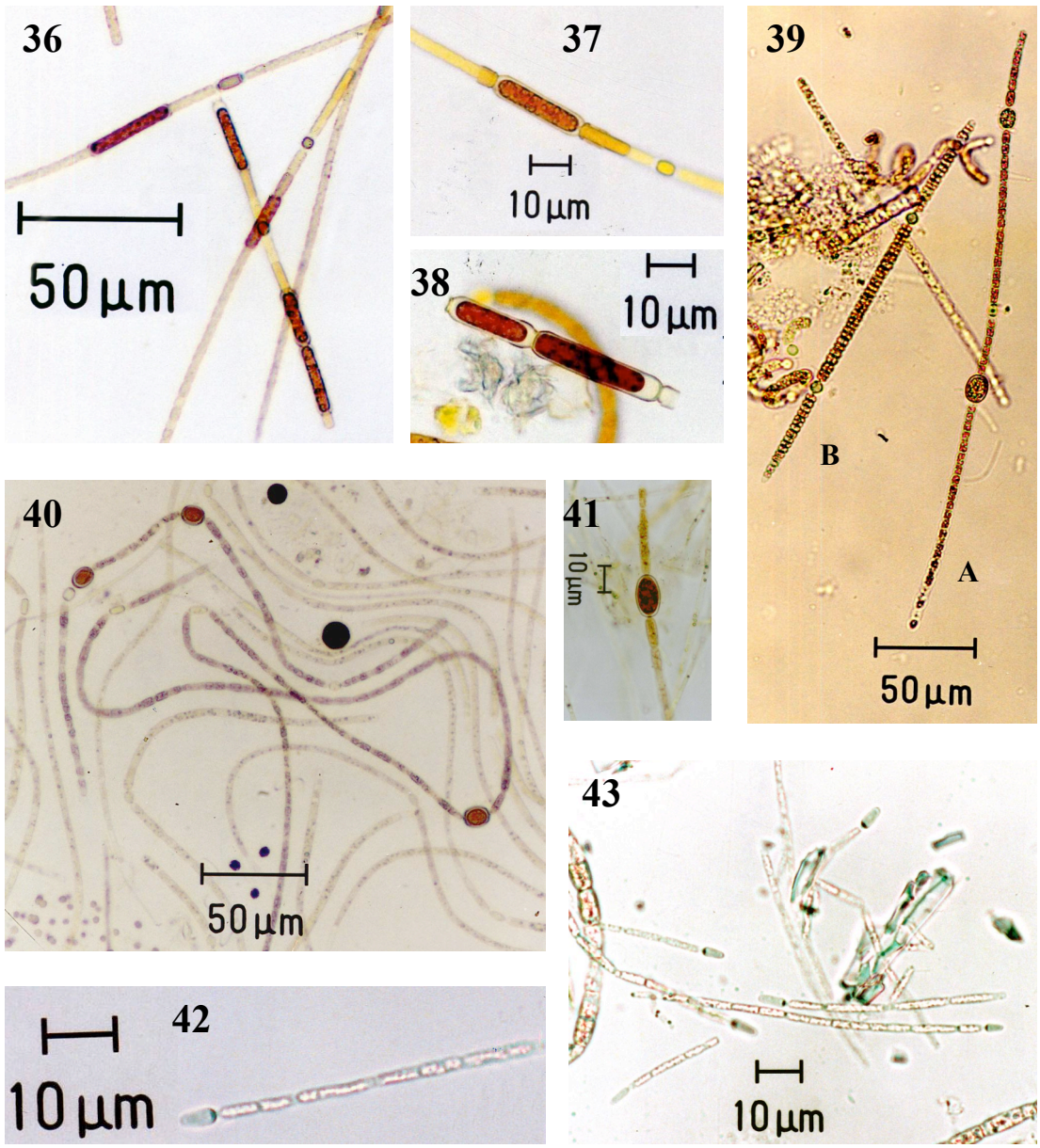



**Plate 7.5.** (36) *Aphanizomenon gracile*; (37-38) *Aphanizomenon gracile* akinete; (39) *Aphanizomenon ovalisporum* (A) and *Anabaena bergii* var. *limnetica* (B); (40-41) *Aphanizomenon ovalisporum*; (42-43) *Cylindrospermopsis raciborskii*.

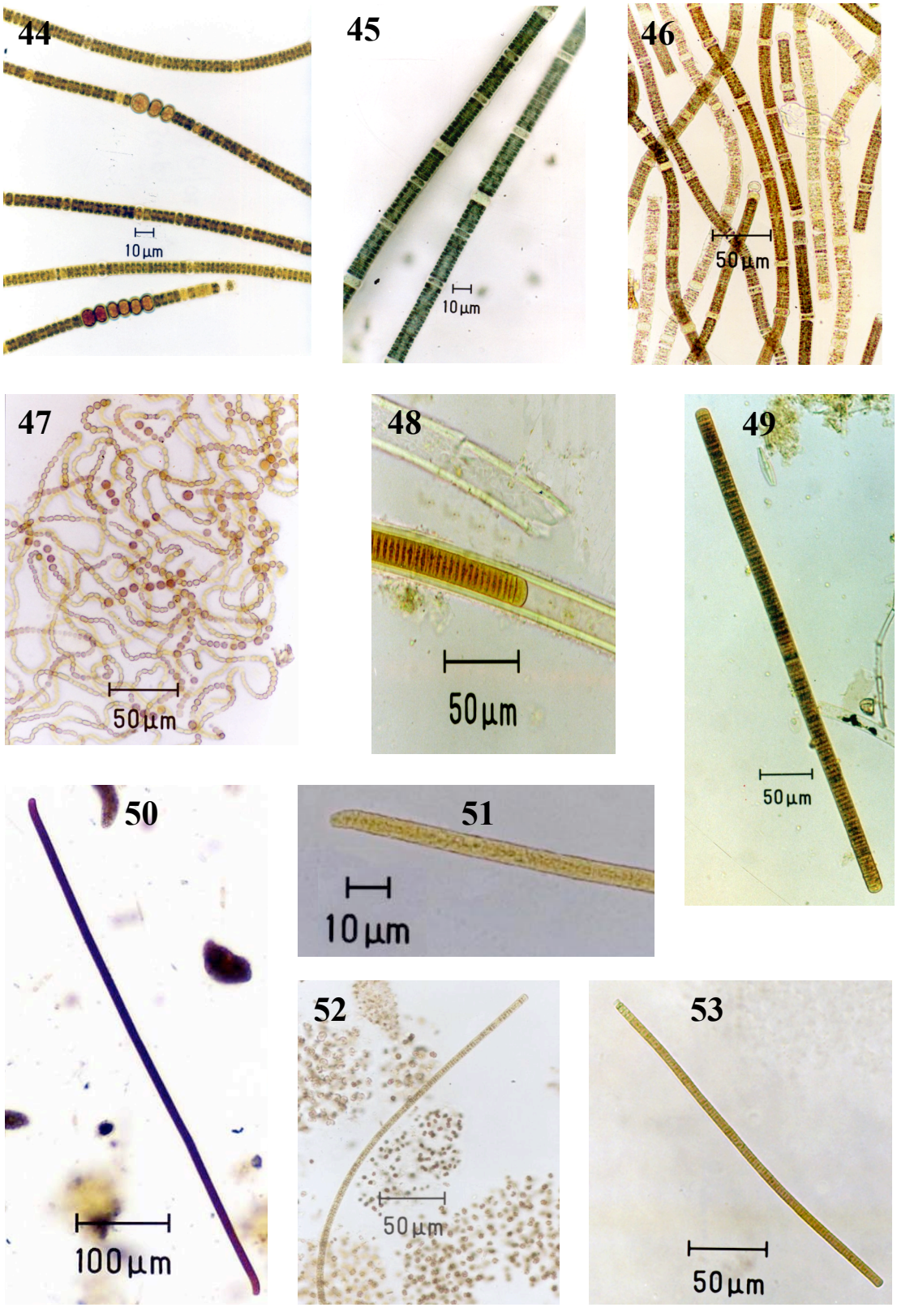



**Plate 7.6.** (44-46) *Nodularia spumigena*; (46) *Nostoc linckia*; (48-49) *Oscillatoria sancta*; (50-51) *Phormidium* aff. *amoenum*; (52-53) *Planktothrix mougeotii*.

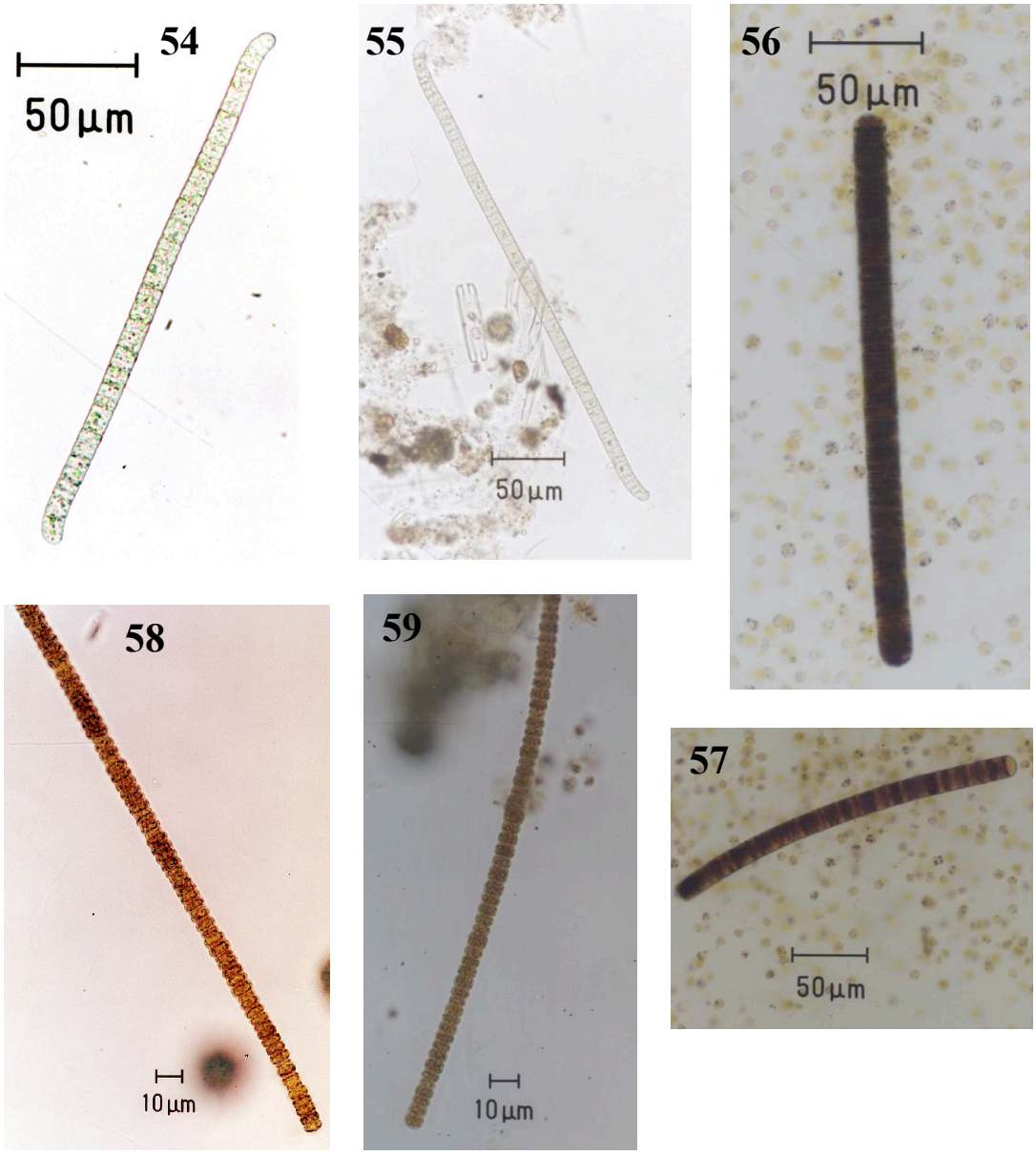



**Plate 7.7.** (54-55) *Planktothrix perornata* f. *attenuata*; (56-57) *Planktothrix planctonica*; (58-59) *Trichodesmium iwanoffianum*.

## 7.4 Conclusions

The most widely distributed and recognisable genus was *Microcystis*. Cell diameter, colony structure and mucilage were effective in identifying the three main species – *M. aeruginosa*, *M. flos-aquae* and *M. wesenbergii*. This is essential as *M. flos-aquae* and *M. aeruginosa* can coexist in freshwater blooms, however *M. flos-aquae* will produce smaller vegetative cells in large colonies that lack fenestrations or lobed protrusions.

Dendrograms generated using qualitative data visually displayed the high level of similarity between the Western Australian populations of *Anabaena*, *Aphanizomenon* and *Anabaenopsis* and the reference samples collected from South Australia, Victoria, New South Wales and southeastern Queensland. Of the eight *Anabaena* species identified, five had never been reported in the SCP wetlands. Both morphotypes of *A. spiroides* f. *spiroides*, including *Anabaena* sp nova, were identified, although it was difficult to distinguish both forms when in the absence of akinetes. Further research that combines samples from multiple sources around Australia is needed to classify *Anabaena* sp nova as a new Australian taxon. At present, identification guides that provide descriptions of cyanoprokaryote taxa in Australia, such as *Anabaena* sp nova, do not include Western Australian populations.

This study illustrates the diversity of species common to the SCP wetlands and the presence and distribution of species known to be toxic in Australian waters (*M. aeruginosa*, *A. circinalis*, *N. spumigena*, *C. raciborskii* and *A. ovalisporum*). Nine species had not been previously recorded in the area so there is little known about their distribution and ecology. For example, *A. ovalisporum* and *C. raciborskii*, two toxic species recently identified in temperate waters, are reported for the first time in Western Australia. Therefore, the morphological descriptions provided in this chapter will benefit future research into freshwater cyanoprokaryota blooms in Western Australia, especially molecular characterisation of toxic species. The next stage in this research project is to determine which species are associated with toxic blooms in the SCP wetlands.

# Chapter 8: Microcystins in the wetlands of the Swan Coastal Plain

## 8.1 Introduction

In this section, cyanoprokaryote samples were analysed for cyanotoxins using the most common analytical procedure, high performance liquid chromatography (HPLC). In Chapters 4 and 5 the distribution of cyanoprokaryota blooms in the wetlands of the Swan Coastal Plain (SCP) identified *Microcystis flos-aquae* and *M. aeruginosa* as the dominant species present in over 80% of the lakes surveyed. Both are the foremost producers of the hepatotoxin microcystin (MC). Therefore, in this chapter the detection of MCs in natural bloom samples collected from 15 study sites are investigated.

Throughout the world cyanoprokaryota blooms are causing adverse problems due to many freshwater species being able to synthesise cyantoxins, secondary metabolites that produce hepatotoxic or neurotoxic toxicoses in organisms that come in contact with bloom material. Cyanotoxins are characterised by their chemical structure and mode of action, for example, hepatotoxins (microcystin and nodularin) are the cyclic peptides that target the liver, while neurotoxins (anatoxin and saxitoxin) are alkaloids that interfere with the functioning of the nervous system (Sivonen and Jones 1999). Of these, hepatotoxicity is the most common form of toxicity associated with cyanoprokaryota blooms mainly due to the widespread occurrence of MCs (Sivonen and Jones 1999). In Australia, toxic blooms are a long-standing problem in agricultural, recreational and drinking water supplies (Falconer 2001). Most current research is aimed at identifying the main toxicogenic species, the factors that support their growth and toxin production to effectively monitor and manage Australia's water resources (Johnstone 1994; Steffensen *et al.* 1999).

Hepatic MCs are frequently detected in ponds, lakes, rivers and drinking water supplies in association with *Microcystis aeruginosa* (Sivonen and Jones 1999), which has been identified as the main species producing hepatotoxic blooms in

Australian waters (Steffenson *et al.* 1999). *Microcystis aeruginosa* with *M. flos-aquae* are the two most common cyanoprokaryotes in the SCP wetlands, Western Australia (John and Kemp 2006), with most blooms exceeding 20 000 cells $mL^{-1}$, which is the recommended guideline for safe recreational waters (WHO 2003). However, the presence of MC in connection with a *Microcystis* bloom has only been previously confirmed in the Swan-Canning Estuary (Hosja and Deeley 1994; Atkins *et al.* 2001). No toxicity testing has been carried out on cyanoprokaryota blooms in the urban lakes of Western Australia until now.

The current knowledge reports MC production by species of *Microcystis*, *Anabaena*, *Planktothrix*, *Phormidium*, *Nostoc* and *Anabaenopsis* (Sivonen and Jones 1999; Metcalf and Codd 2004), all of which have been identified in Western Australia. Experimental evidence using mice has shown both *Microcystis* extracts and purified MCs cause blood accumulation in the liver with extensive structural damage with hepatocyte degeneration and necrosis (Elleman *et al.* 1978; Falconer *et al.* 1981). Death occurs within hours due to circulatory failure as a result of blood loss from circulation (Falconer *et al.* 1981). Due to these toxic effects and the high incidence of summer cyanoprokaryota blooms, health warning signs have become a regular sight around many of the SCP wetlands.

To date, there are four common toxic species reported from Australia: *M. aeruginosa*, *Anabaena circinalis, Nodularia spumigena* and *Cylindrospermopsis raciborskii* (Steffensen *et al.* 1999). In addition, Shaw *et al.* (1999) has confirmed cylindrospermopsin, a distinct alkaloid hepatotoxin, in blooms of *Aphanizomenon ovalisporum* in Queensland and Baker *et al.* (2001) reported the production of an unknown toxin in *Phormidium* aff. *formosum* in South Australia. Microcystins have also been associated with blooms of *M. panniformis* in Lake Elphinstone, Queensland (White *et al.* 2003). However, *M. aeruginosa* and *N. spumigena* are the main species causing hepatoxicity in Australian waters with *Anabaena* producing acute neurotoxicity only at high cell densities (Steffenson *et al.* 1999). Australian populations of *A. circinalis* produce saxitoxins (STX) (Beltran and Neilan 2000), sodium-channel blocking alkaloids that inhibit nerve impulse transmission

(Humpage *et al.* 1994). The production of MCs by *Anabaena* is yet to be reported, although there has been evidence of hepatotoxicity from some samples from the Murray-Darling Basin, southeastern Australia, and Lake Cargelligo, New South Wales (Baker and Humpage 1994; Bowling 1994).

In Western Australia, toxicity analyses for cyanotoxins have been sporadic and focused on the major river systems. From 1993 to 1994 there were significant new outbreaks of toxic cyanoprokaryota blooms in Western Australia, with the main toxic species identified as *M. aeruginosa*, *A. circinalis* and *N. spumigena* (Hosja and Deeley 1994). A massive bloom of *A. circinalis* and *A. spiroides* in the Canning River in 1994 was toxic by mouse bioassay. In February 2003, another *Anabaena* bloom closed the upper sections of Canning River for six weeks because cell counts exceeded the national recreational contact guidelines. The hepatotoxic *Microcystis* also blooms in this area, yet current toxicity testing has not been completed. *Nodularia spumigena* blooms, which have occurred frequently in the Peel Harvey Inlet since the 1960s, were proven hepatotoxic in 1982 (Hosja and Deeley, 1994). *Nodularia* blooms still occur in the upper Serpentine River and present a potential health risk as swimming, crabbing and canoeing occur in this area. In February 2000, a *M. aeruginosa* bloom in the Swan-Canning Estuary caused the closure of the estuary for more than two weeks as MC concentrations in water samples reached 8 µg $L^{-1}$ (Atkins *et al.* 2001). Yet, despite this evidence of toxic blooms in the rivers, there has been little documentation about cyanotoxins in the surrounding freshwater lakes.

The current alert level framework recognized for potentially toxic blooms of cyanoprokaryota in public waters is based on cell density (WHO 2003). In temperate climates cyanoprokaryota blooms occur during the spring and summer months, when the demand for recreational water is highest (Falconer *et al.* 1999). However, actual toxicity testing is not required, if significant cell numbers of a potentially toxic species are detected. For this reason, no toxicity testing has been carried out on cyanoprokaryota blooms in the lakes of the SCP. There have been additional reports of potentially toxic blooms in other parts of Western Australia, for example, the

Blackwood River, Balingup Brook and Vasse River, with cell density exceeding 20 000 cells $mL^{-1}$. This level conveys a relatively mild or low probability of adverse health effects (WHO 2003). At 100 000 cells $mL^{-1}$ there is a moderate probability for adverse health effects with a MC concentration of 20 μg $L^{-1}$, if *Microcystis* is dominant (WHO 2003). Shoreline scum formation presents a greater threat and poses an immediate public safety risk. It represents a thousand-fold to million-fold concentration of cyanoprokaryote cells with high MC concentrations (24 mg $L^{-1}$) being encountered over a short period of time (Falconer *et al.* 1999).

The aim of this chapter was to investigate the occurrence and diversity of MCs in the SCP lakes in relation to species composition and cell density. A total 32 samples, representing 13 lakes, were analysed for MCs to produce the first evidence of toxic blooms in the freshwater lakes of the city of Perth. There are at least 60 known structural MC variations with MC-LR, MC-RR and MC-YR the three most commonly identified (Sivonen and Jones 1999). Quantification of variants MC-LR, MC-YR, MC-RR was determined using HPLC performed at the Australian Water Quality Centre, a NATA (National Association of Testing Authorities) accredited facility used by water authorities throughout Australia. No study like this has been previously undertaken in Western Australia, with the aim of identifying sites of toxic *Microcystis* blooms.

## 8.2 Materials and Methods

### *8.2.1 Enumeration*

Cyanoprokaryote samples were collected using a 25-μm phytoplankton net and identified to the species level using universally accepted taxonomic guides (refer to section 7.2.1 for complete reference list). Algal cell concentrations (cells $mL^{-1}$) were determined from water samples using a Lund Cell at 400x magnification. Cell counts were then converted into volumetric biomass ($\mu m^3$ $mL^{-1}$) by multiplying the mean cell biovolume ($\mu m^3$) by the total cell count (cells $mL^{-1}$). Mean cell biovolume was determined by finding the nearest geometric shape and measuring the appropriate

cell dimensions according to Sun and Lui (2003). A complete description of this procedure is provided in section 6.2.3.

### *8.2.2 Toxin analysis*

One litre of concentrated algal samples was collected using the phytoplankton net from areas showing surface accumulations. A total of 32 samples, representing 13 lakes, were collected (Fig. 8.1). In addition, five samples were collected from the Swan River in February 2000 and one sample from Canning River in February 2001 (refer to section 5.2). Samples were frozen and sent to the Australian Water Quality Centre (Bolivar) to be analysed for MC-LR, MC-RR, MC-YR and MC analogues by HPLC. Samples of *N. spumigena* were analysed for nodularins (NODs). Total MC concentration is defined as the sum of the extracellular and intracellular fractions.

### *8.2.3 Statistical analysis*

Spearman's rank correlation was used to explore the relationship between total MC concentrations, individual variant concentrations and *Microcystis* biovolume. These data were tested for normality then total MC and MC-LR concentrations were log transformed, while the *Microcystis* cell counts (cells $mL^{-1}$), calculated biovolume ($\mu m^3$ $mL^{-1}$) and remaining MC variants were log (n+1) transformed. Samples where cell counts were not available were omitted from the statistical analysis. For graphical representation of cell abundance, cell counts were used from the nearest date.

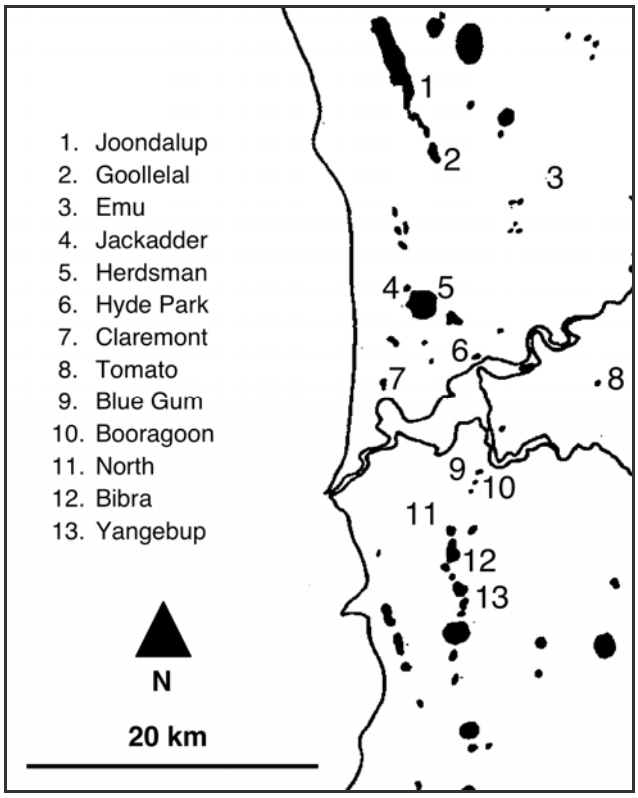



**Figure 8.1.** Reference map showing the location of the 13 lakes. Sampling dates are shown in Table 8.1.

## 8.3 Results

### *8.3.1 Microcystin concentration*

Microcystins were detected in 28 of the 32 samples analysed (Table 8.1). Highest MC concentrations were recorded in Herdsman Lake (8428.6 μg $L^{-1}$), Hyde Park (3261.8 μg $L^{-1}$), Yangebup Lake (2335.25 μg $L^{-1}$), Jackadder Lake (2381.6 μg $L^{-1}$) and Bibra Lake (1645 μg $L^{-1}$). Yangebup Lake produced three samples with MC concentrations greater than 1000 μg $L^{-1}$ and NOD was detected in November 2001 (1664.4 μg $L^{-1}$) in this lake as well. Only 10 samples produced MC concentrations greater than 100 μg $L^{-1}$. Low amounts of MCs concentrations were detected in Lake Goollelal (0.5-0.9 μg $L^{-1}$), Tomato Lake (1.3-5.7 μg $L^{-1}$) and Bibra Lake (2.3-6 μg $L^{-1}$). No MCs were detected in Blue Gum Lake, North Lake and Lake Claremont due to results below the HPLC detection limit (< 0.05 μg $L^{-1}$).

Total MC was fractionated into intracellular and extracellular concentrations. As the concentrated net samples were frozen and stored prior to analysis, higher

concentrations of extracellular MCs were measured in a large proportion of samples (Fig. 8.2). Dissolved MCs formed 86 to 100% of the total MC concentration. Extracellular MC-LR was detected in all the lake samples at a higher concentration than MC-RR and MC-YR. The MC analogues were extracellular in 79% of the samples at maximum concentration similar to MC-LR.

The MC concentration in the Swan River ranged from 1.05 to 124.16 μg $L^{-1}$ (Table 8.2). The highest amounts were associated with scum formation in the sheltered locations of the Old Swan Brewery (124.16 μg $L^{-1}$), Tranby House (12.59 μg $L^{-1}$) and Matilda Bay (10.22 μg $L^{-1}$). Swan River samples were dominated by *Microcystis flos-aquae* (> $2 \times 10^7$ cells $mL^{-1}$) represented by colonies made up of large masses of cells, without fenestrations or protrusions, with cells generally 2 to 4 μm in diameter (refer to section 7.3.1). The Kent Street Weir, the only sample from the Canning River, contained a low concentration of MCs (4.7 μg $L^{-1}$), and contained both *A. circinalis* ($2.81 \times 10^6$ cells $mL^{-1}$) and *M. flos-aquae* ($1.52 \times 10^7$ cells $mL^{-1}$).

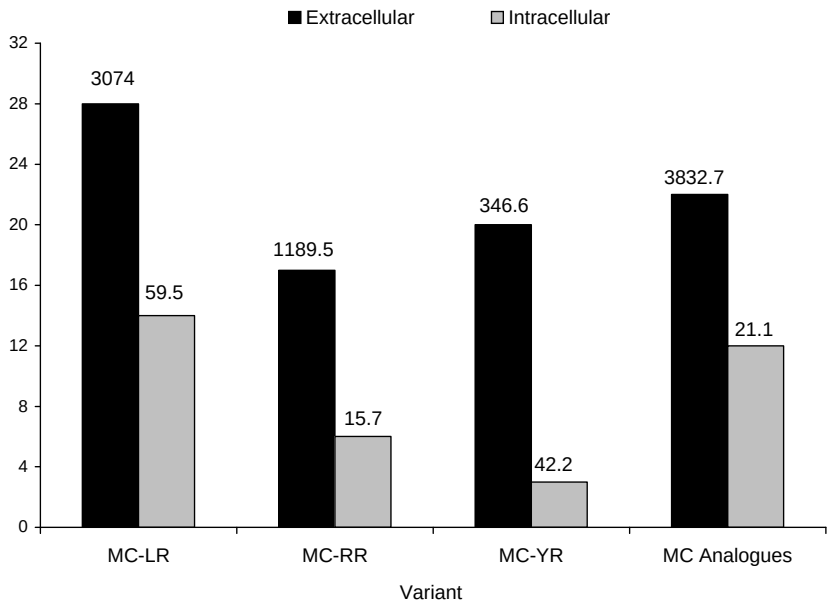



**Figure 8.2.** Number of samples (total = 28) containing extracellular and intracellular MCs with the maximum concentration (μg $L^{-1}$) shown for each structural variant.

**Table 8.1.** The lake, sampling date, sample number, combined intracellular and extracellular concentration for each detectable variant (-LR, -RR, -YR, Analogues) and the total MC concentration. All concentrations measured as μg $L^{-1}$.

| Site[a] & Date | Sample | MC-LR | MC-RR | MC-YR | Analogues | Total MC |
|---|---|---|---|---|---|---|
| **Herdsman** | | | | | | |
| 4/12/00 | 1 | 6.1 | 27.5 | 29.1 | 18.2 | 80.9 |
| 9/5/01 | 2 | 2997 | 1189.5 | 388.8 | 3853.8 | 8429.1 |
| 14/11/01 | 3 | 35.9 | 7.5 | 2.1 | 10.7 | 56.2 |
| **Yangebup** | | | | | | |
| 31/10/99 | 4 | 1.1 | — | — | 20.4 | 21.5 |
| 21/1/00 | 5 | 4 | — | — | 18 | 22 |
| 3/8/00 | 6 | 85.9 | 15.9 | 6.6 | 550.5 | 658.9 |
| 22/10/01 | 7 | 1008.9 | 105 | 74.4 | 129.2 | 1317.5 |
| 14/11/01[b] | 8 | 1452.1 | 38.8 | 43.8 | 241.3 | 1776 |
| 27/11/02 | 9 | 880.15 | 563.6 | 210.8 | 680.7 | 2335.25 |
| **Bibra** | | | | | | |
| 31/10/99 | 10 | 1534 | — | — | 111.03 | 1645.03 |
| 24/1/00 | 11 | 3 | — | — | 3 | 6 |
| 15/9/00 | 12 | 2.2 | 0.1 | — | — | 2.3 |
| 21/6/01 | 13 | 17.6 | 37 | 15.1 | 18.6 | 88.3 |
| 21/1/02 | 14 | 45.3 | 2.5 | 0.9 | 15.5 | 64.2 |
| **Goollelal** | | | | | | |
| 4/12/00 | 15 | 0.1 | 0.4 | 0.2 | 0.2 | 0.9 |
| 15/11/01 | 16 | 0.3 | — | 0.2 | — | 0.5 |
| **Hyde Park** | | | | | | |
| 13/11/00 | 17 | 172.2 | — | 4.4 | 23.05 | 199.6 |
| 22/6/01 | 18 | 3108 | — | 86.9 | 66.9 | 3261.8 |
| 15/11/01 | 19 | 55.3 | — | 4.9 | — | 60.2 |
| **Claremont** | | | | | | |
| 5/12/00 | 20 | 1.5 | 6.5 | 1.4 | 5.15 | 14.55 |
| **Tomato** | | | | | | |
| 11/12/00 | 21 | 0.2 | 0.4 | 0.7 | — | 1.3 |
| 14/11/01 | 22 | 5.7 | — | — | — | 5.7 |
| 14/2/02 | 23 | 8.49 | 17.9 | — | — | 26.39 |
| **Jackadder** | | | | | | |
| 22/6/01 | 24 | 1824 | 14.1 | 82.1 | 461.4 | 2381.6 |
| 15/11/01 | 25 | 9.8 | — | — | 3.7 | 13.5 |
| **Joondalup** | | | | | | |
| 4/12/00 | 26 | 2 | — | 1.2 | 10.15 | 13.35 |
| **Emu** | | | | | | |
| 22/1/02 | 27 | 47 | 400.2 | 35.2 | 151.8 | 634.2 |
| **Booragoon** | | | | | | |
| 11/1/01 | 28 | 9.2 | 12.1 | 0.6 | 4.2 | 26.1 |

[a] Four samples below the detection limit (< 0.05 μg $L^{-1}$) omitted; Blue Gum, Feb 2000, Sept 2000; North, Nov 2000; Claremont, Feb 2002. Total number of samples, 32. [b] NOD also detected in sample. — Indicates MC concentration below detection level of 0.05 μg $L^{-1}$.

**Table 8.2.** The combined intracellular and extracellular concentration for each detectable variant (-LR, -RR, -YR, analogues) and the total MC concentration (μg $L^{-1}$) for samples collected from the Swan-Canning Estuary.

| Site | MC-LR (μg $L^{-1}$) | MC-RR (μg $L^{-1}$) | MC-YR (μg $L^{-1}$) | Analogues (μg $L^{-1}$) | Total MC (μg $L^{-1}$) |
|---|---|---|---|---|---|
| Narrows Bridge | 0.31 | — | — | 0.74 | 1.05 |
| Old Swan Brewery | 23.88 | — | — | 100.28 | 124.16 |
| Tranby House | 0.17 | — | — | 12.41 | 12.59 |
| Maylands Yacht Club | — | — | — | 4.43 | 4.53 |
| Matilda Bay | 2.33 | — | — | 7.89 | 10.22 |
| Kent Street Weir | 2.9 | — | 1.8 | — | 4.7 |

Swan River samples collected 11/2/00. Canning River sample collected 2/2/01.
— Indicates MC concentration below detection level of 0.05 μg $L^{-1}$.

### *8.3.2 Microcystin diversity*

All samples that were positive for MCs contained MC-LR (Table 8.3; Fig. 8.3). Microcystin-YR was present in 71.4% of the samples, MC-RR in 60.7% and the MC analogues in 78.6%. The percentage of MC-LR in the total MC concentration ranged from 5% to 100% (mean 49%), for MC-RR it ranged from 0% to 68% (mean 16%) and for MC-YR it ranged from 0% to 54% (mean 9%) (Fig. 8.3). The remaining analogues comprised 1% to 95% (mean 26%) of the total MC concentration. The samples with the highest MC concentrations contained all three isolated variants, with MC-LR making up a large percentage of the total (Fig. 8.3). A high percentage of the next common variant, MC-RR, was observed in lakes with less than 40% MC-LR. Together MC-LR, MC-RR, MC-YR and MC analogues were detected in 50% of the samples (Table 8.3). The next combination common throughout the lake samples was MC-LR with the MC analogues, which was mostly seen in samples from Yangebup Lake and Bibra Lake.

Total MC concentration did not correlate with the total number of MC variants present in the lake samples (r = 0.301). However, total MC did show a very strong positive correlation with MC-LR (r = 0.935, $p > 0.01$, n = 28) and MC analogues (r = 0.869, $p > 0.01$, n = 28), which were the principal MC variants in the samples. From viewing Table 8.1 no relationship between site and variant composition is seen,

except for absence of MC-RR in Hyde Park and absence of MC analogues in Tomato Lake. Also, there were no analogues detected in samples 12, 16 and 19.

Microcystin-LR was the dominant variant recording a maximum concentration of 3108 μg $L^{-1}$. However, MC-RR concentration did exceed that of MC-LR in samples 1, 13, 15, 20, 21, 23 and 27. Conversely, the concentration of MC-YR only exceeded MC-LR in three samples (1, 15 and 22). In relation to species, the samples with a higher percentage of MC-LR were dominated by *M. aeruginosa* by cell numbers (samples 14, 17, 18, 19, and 22). The MC-LR concentration showed a significant correlation with the *M. aeruginosa* cell counts ($r = 0.657$, $p < 0.01$, $n = 20$). In contrast, samples with a higher percentage of MC-RR were dominated by *M. flos-aquae* (sample number 1, 13, 15, 20, 23, 27) ($r = 0.816$, $p < 0.01$, $n = 20$).

Only MC-LR and the MC analogues were present in the Swan River samples, with the analogues at a greater concentration in every sample (Table 8.2). No MC-LR was detected at the Maylands Yacht Club, only the MC analogues. The Kent Street Weir sample consisted only of the variants MC-LR and MC-YR.

**Table 8.3**. Summary of the MC variant combinations recorded for the 28 lake samples testing positive for MCs.

| MC variant composition | Number of samples |
|---|---|
| -LR, -RR, -YR, analogues | 14 |
| -LR, -YR, analogues | 3 |
| -LR, analogues | 5 |
| -LR, -RR, -YR | 1 |
| -LR, -RR | 2 |
| -LR, -YR | 2 |
| -LR | 1 |
| | **n = 28** |

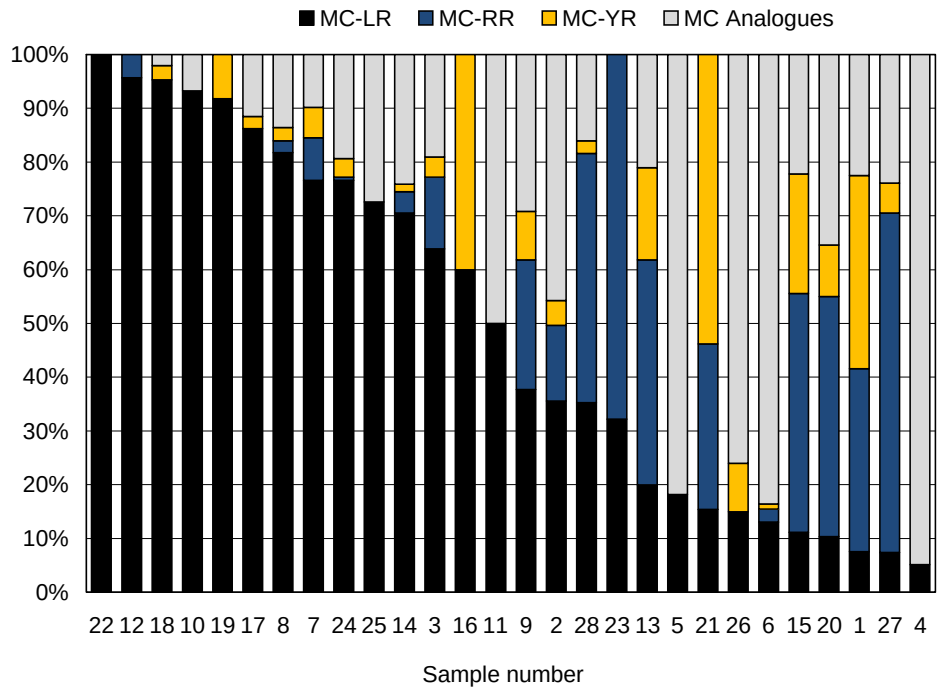



**Figure 8.3.** The percentage that each variant; MC-LR, MC-RR and MC-YR, contributes to the total MC concentration.

### *8.3.3 Species composition*

The relationship between species composition and total MC content was explored. *Microcystis* was the dominant genus (> 40 %) in all the lake samples, except for Claremont Lake. The Claremont Lake sample contained only *A. elenkinii* and recorded no MCs. Samples containing *Anabaena* (Tomato Lake, North Lake and Canning River) recorded low amounts of MC (0.05-26.39 μg $L^{-1}$).

The predominant species was *M. aeruginosa* with a relative abundance ranging from 7 % to 100 % of the total biovolume (Fig. 8.4a). Total MC showed a moderate but significant correlation to total *Microcystis* biovolume (r = 0.644, $p > 0.01$, n = 21) and *M. aeruginosa* biovolume (r = 0.618, $p > 0.01$, n = 21). *Microcystis aeruginosa* was prevalent in the samples that produced high concentrations of MCs (Fig. 8.4a and 8.5). The Blue Gum Lake 2000 sample recorded no MCs and contained a low abundance of *M. aeruginosa* (9% total volume) (Fig. 8.4b). Likewise, in sample 1

from Herdsman Lake, *M. aeruginosa* comprised only 17% of the total biovolume with 80.9 μg $L^{-1}$ of MC.

Low abundances (< 10% total biovolume) of *M. aeruginosa* were observed in samples dominated by *M. flos-aquae* (1, 11, 12, 13, 15, 20, 24, 26, 27 and Blue Gum Lake). The poor correlation (r = 0.278, n = 21) between total MC concentration and *M. flos-aquae* biovolume was the result of the total MC concentration ranging from < 0.05 to 634.2 μg $L^{-1}$ in the before mentioned samples. Samples 15, 16, 20 and 26 containing only *M. flos-aquae* produced some of the lowest concentrations of MCs recorded in this study. The median total MC concentration for these samples was 13.95 μg $L^{-1}$, lower than the median value of 132.1 μg $L^{-1}$ for samples dominated by *M. aeruginosa* (by biovolume) (Table 8.4; Fig. 8.4a).

Regarding the cell counts, all except Lake Joondalup (sample 26) had *Microcystis* in excess of 100 000 cells $mL^{-1}$ (Fig. 8.5). The combined cell count of *M. aeruginosa* and *M. flos-aquae* had a significant but only moderate correlation with total MC concentration (r = 0.549, $p < 0.01$). High cell counts did correspond to higher total MC concentrations (> 1000 μg $L^{-1}$) for samples 2, 7, 9, 18, 24 and 27, and the presence of both species (Fig. 8.5). Lower *Microcystis* cell counts (samples 12, 21 and Blue Gum), or the presence of only *M. flos-aquae* (samples 11, 15, 20, 26), did relate to lower concentrations of MCs.

**Table 8.4.** The allocated sample number and species composition based on biovolume ($\mu m^3$ $mL^{-1}$).

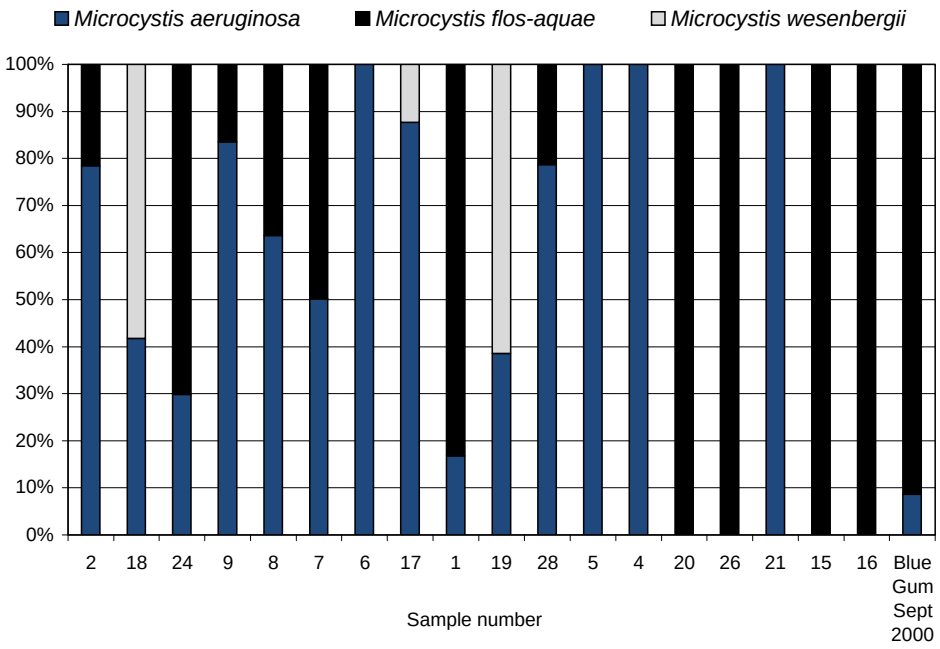
| Lake | Sample No. | Dominant Species[a] | Subdominant Species |
|---|---|---|---|
| Herdsman | 1 | *M. flos-aquae* | *M. aeruginosa* |
| | 2 | *M. aeruginosa* | |
| | 3[c] | *M. aeruginosa, M. flos-aquae, A. circinalis* | |
| Yangebup | 4 | *M. aeruginosa* | |
| | 5 | *M. aeruginosa* | |
| | 6 | *M. aeruginosa* | |
| | 7 | *M. flos-aquae* | *M. aeruginosa, N. spumigena* |
| | 8[c] | *M. flos-aquae* | *M. aeruginosa* |
| | 9 | *M. aeruginosa, M. flos-aquae* | |
| Bibra | 10[c] | *M. aeruginosa* | |
| | 11 | *M. flos-aquae* | *A. gracile* |
| | 12 | *M. flos-aquae, M. aeruginosa* | *A. elenkinii* |
| | 13 | *M. flos-aquae* | *M. aeruginosa, C. raciborskii, A. ovalisporum* |
| | 14 | *M. aeruginosa* | *M. flos-aquae, A. ovalisporum, A. bergii* var. *limnetica* |
| Goollelal | 15 | *M. flos-aquae* | |
| | 16[c] | *M. flos-aquae* | |
| Hyde | 17 | *M. aeruginosa* | *M. wesenbergii* |
| | 18 | *M. aeruginosa, M. wesenbergii* | |
| | 19[c] | *M. aeruginosa, M. wesenbergii* | |
| Claremont | 20 | *M. flos-aquae* | |
| | Feb 2002 | *A. elenkinii* | |

**Table 8.4** (*Continued*)

| Lake | Sample No. | Dominant Species[a] | Subdominant Species |
|---|---|---|---|
| Tomato | 21 | *M. aeruginosa* | |
| | 22[c] | *M. aeruginosa, A. circinalis* | |
| | 23[b] | *M. flos-aquae, M. aeruginosa, A. circinalis* | |
| Jackadder | 24 | *M. aeruginosa, M. flos-aquae* | |
| | 25[c] | *M. aeruginosa, M. flos-aquae* | |
| Joondalup | 26 | *M. flos-aquae* | |
| Emu | 27 | *M. flos-aquae* | *M. aeruginosa, A. ovalisporum* |
| Booragoon | 28 | *M. aeruginosa, M. flos-aquae* | |
| North | Nov 2000[c] | *A. circinalis, A. spiroides* f. *spiroides, M. flos-aquae* | |
| Blue Gum | Feb 2000[c] | *M. aeruginosa, M. flos-aquae* | |
| | Sept 2000 | *M. flos-aquae* | *M. aeruginosa* |

[a] The dominant species constituted > 40 % of the total cyanoprokaryota volume, except where stated [b].
[b] *M. flos-aquae* (31%), *M. aeruginosa* (31%), *A. circinalis* (38%).
[c] No cell count performed for that date. *Microcystis* and *Anabaena* detected in net sample.
Samples allocated a date rather than a sample number recorded MC concentration below the detection limit of 0.05 μg $L^{-1}$.

**(a)**




**(b)**

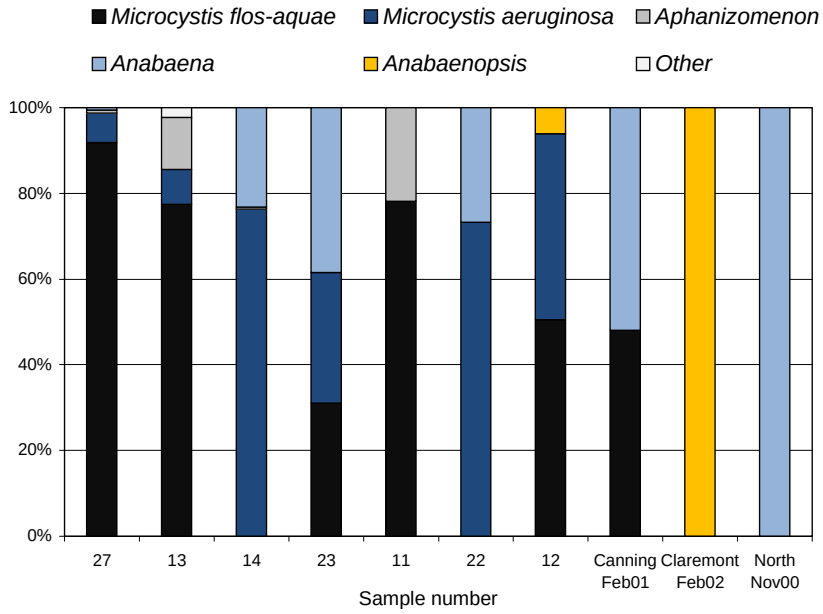



**Figure 8.4.** Relative abundance of cyanoprokaryotes in samples with **(a)** *Microcystis* only and **(b)** with multiple genera in descending order of total MC concentration. Samples from Blue Gum Lake and Lake Claremont below detection limit (0.05 μg $L^{-1}$).

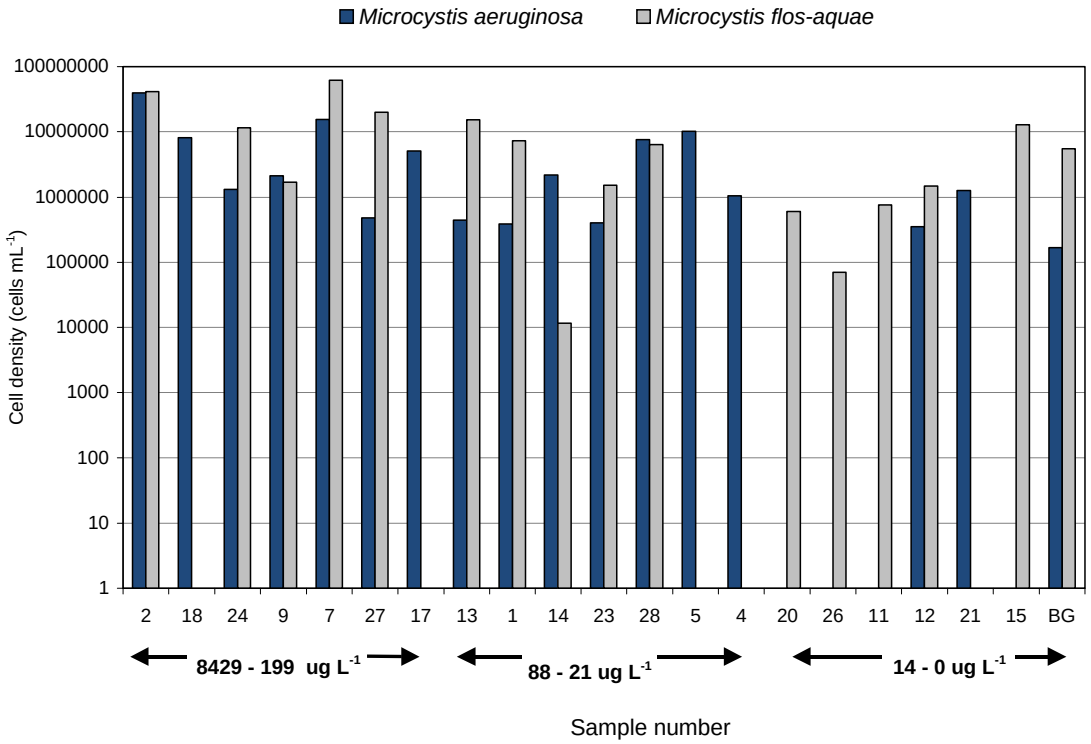



**Figure 8.5.** Cell counts of the two dominant species, *M. aeruginosa* and *M. flos-aquae*, arranged in descending order by total MC concentration. Blue Gum Lake (BG) September 2000 sample below MC detection limit (0.05 μg $L^{-1}$).

## 8.4 Discussion

### *8.4.1 Occurrence of MC-producing blooms*

In this study, MCs were detected in over 80% of the lake samples tested, which is a greater percentage compared to results of other surveys. A global review by Sivonen and Jones (1999) found that on average 59% of samples containing cyanoprokaryota were toxic, with a low percentage being the result of a low frequency of toxic species. Baker and Humpage (1994) reported acute toxicity in only 42% of samples from the Murray-Darling Basin, with 56% of the samples dominated by the hepatotoxic *Microcystis aeruginosa*. Henriksen and Moestrup (1997) found MCs in 67% of samples from Denmark, while Park *et al.* (1998b) reported MCs in 72% of samples from Korea. However, all samples were found to be hepatotoxic in the

studies by Vasconcelos *et al.* (1996) and Gkelis *et al.* (2005). In both cases *Microcystis* were also the dominant genera.

The high percentage of positive samples presented in the current study reflects the decision to analyse bloom material dominated by *Microcystis*, the main producer of MC. Samples testing not positive for MCs were either without *M. aeruginosa*, for example Lake Claremont and North Lake, or had a higher cell density of *M. flos-aquae*, as observed in samples from lakes Bibra, Blue Gum, Claremont, Goollelal and Joondalup. The presence of multiple species also corresponded to lower MC concentration (e.g. Bibra Lake). Park *et al*. (1998a), Fastner *et al.* (1999) and Henriksen (2001) reported similar results with lower MC content in samples that were either dominated by mixed genera or by species of *Anabaena*, compared to the *Microcystis* dominated samples.

Blooms that produced high concentrations of MCs (> 1000 μg $L^{-1}$) occurred in Herdsman Lake, Hyde Park, Yangebup Lake, Jackadder Lake and Bibra Lake. All experience recurring cyanoprokaryota blooms with visible shoreline scum accumulation. Very high concentration of MCs per litre of water is expected in scum material with the highest value published being 25 000 μg $L^{-1}$ from the shores of the Havel River, Berlin (Fastner *et al.* 1999) (Table 8.5). Other studies have additionally reported high concentrations from Germany (11410 μg $L^{-1}$, Frank 2002; 1200 μg $L^{-1}$, Welker *et al.* 2001), especially in association with fringing vegetation where 10 to 15 cm thick scums had accumulated (Welker *et al.* 2001). However, the concentration of MCs in the pelagic zone outside the scum accumulation are usually reported to be lower (Chorus 2001; Frank 2002; Nasri *et al.* 2004; Dyble *et al.* 2008) as shown in Table 8.5

People are more likely to be in contact with these shoreline scum accumulations (Falconer *et al.* 1999), therefore the high MC content in the above-mentioned sites are a potential health risk. The SCP wetlands are predominantly used for passive forms of recreation (walking and picnicking), although swimming, wadding and the use of watercraft are discouraged, but they are not prohibited. So there is the

likelihood of body contact with scum material. There have been reports of rapid neurotoxicosis and death of domestic dogs after swimming in scum accumulation along shorelines in Scotland (Codd *et al.* 1995) and the mass deaths of wild birds after the sudden appearance of *M. aeruginosa* bloom in Japan (Matsunaga *et al.* 1999). In 2002, the first account of human death following exposure to a heavy scum of *Anabaena flos-aquae* (Behm 2003) was reported. Although this was an extreme case, allergic reactions from swimming in cyanoprokaryota blooms are more commonly reported (Pilotto *et al.* 1997).

The highest MC concentration recorded in this study was from Herdsman Lake (8428 μg $L^{-1}$), although Yangebup Lake produced consecutive samples greater than 1000 μg $L^{-1}$. In comparison with MC concentrations published in worldwide surveys, these results prove the *Microcystis* blooms in the urban lakes of the SCP are highly toxic (Table 8.5). Even the median MC concentration (58.2 μg $L^{-1}$), determined from the 28 positive samples, exceeding those reported Japan, United States, Germany, Zimbabwe and Brazil, as well as the samples that were collected from the Swan and Canning rivers.

It appears that Emu Lake has the potential to produce highly toxic blooms. Only one sample was analysed from this site, which produced a MC concentration that was similar to a spring sample from Yangebup Lake (634.2 μg $L^{-1}$ and 658.9 μg $L^{-1}$, respectively). Similar to most lakes used in this study, water blooms in Emu Lake generally develop in early spring, whereas this sample was collected mid-summer in late January. Therefore a higher MC concentration may well be observed earlier in the season. Emu Lake also shows extensive scum accumulation along the shoreline, which is a concern as the shoreline is proximal to houses in the Lakes Estate, Ballajura. *Aphanizomenon ovalisporum* regularly blooms in this lake and recently there have been reports of *Cylindrospermopsis raciborskii* (Chapter 4). Similar to Emu Lake, the winter sample from Bibra Lake also contained *A. ovalisporum* and *C. raciborskii*. Both species are producers of the hepatotoxin CYL, which affects not only the liver, as shown by MC, but also the kidneys and intestine (Falconer 2005). This cyanotoxin is yet to be identified in Western Australian wetlands.

Of interest were the toxic *Microcystis* blooms observed in autumn-winter 2001 in Herdsman Lake, Hyde Park and Jackadder Lake because MC concentrations were higher than the summer and spring samples of that same year. The toxicity of Herdsman Lake was more than 100 times greater in winter 2001 than summer 2000 and spring 2001. This difference in MC content can be related to the abundance of MC-producing species (Vasconcelos and Pereira 2001), which in this case is *M. aeruginosa*. However, this was only seen in the results for Herdsman Lake as the other winter samples showed dominance of *M. flos-aquae* by biovolume. Cyanotoxin production is strain specific and genetically determined. Although the relationship between environmental conditions and toxin production is complex, individual strains have different environmental optima that will influence toxin content (Sivonen and Jones 1999). Temperature and nutrient availability have been shown to determine MC biosynthesis in cultured strains of *Microcystis* (Vezie *et al.* 2002), *Anabaena* (Rapala *et al.* 1997) and *Oscillatoria* (Sivonen 1990), and in natural populations of *Microcystis* (Wang *et al.* 2002; Izydorczyk *et al.* 2008; Wu *et al*. 2008). The three MC variants respond differently to growth stimuli (Rapala *et al.* 1997) but all are positively correlated with nitrogen and phosphorus (Wang *et al.* 2002; Wu *et al*. 2008), which are in abundant supply in lakes Herdsman, Hyde and Jackadder.

However, the main difference between autumn-winter and spring-summer are the lower water temperatures in the colder months (14-20 °C; mean 16 °C). Rapala *et al.* (1997) found MC-LR negatively correlated to temperatures ranging from 12.5 to 25 °C, whereas MC-RR demonstrated a higher temperature range (> 25 °C). Wang *et al.* (2002) also observed the presence of MC-LR at lower temperatures, compared to MC-RR, while a recent study by Izydorczyk *et al.* (2008) reported higher MC content in a *Microcystis* bloom coincided with water temperatures less than 18 °C. Although this study does not provide conclusive reason for the high winter MC concentrations, it does illustrate the health risk associated with "unseasonable" blooms.

**Table 8.5.** Review of maximum MC concentrations reported in the literature from water bodies throughout the world and within Australia, including the present study.

| Location | Max MC µg $L^{-1}$ | Max MC µg $g^{-1}$ | Sample | Method | Reference |
|---|---|---|---|---|---|
| Germany | 1200 | | s | 1 | Welker *et al.* 2001 |
| | 15 | | s | 1 | Welker *et al.* 2003 |
| | 3 | | w | 1 | Welker *et al.* 2003 |
| Germany | 11 410 | | s | 1 | Frank 2002 |
| | 7020 | | w | 1 | Frank 2002 |
| Germany | 25 000 | | s | 1 | Fastner *et al.* 1999 |
| France | | 230 | s | 1 | Vezie *et al.* 1998 |
| Greece | | 2564.3 | s | 1 | Gkelis *et al.* 2005 |
| Greece | 3186 | | w | 1 | Cook et al. 2004 |
| Poland | | 1687 | s | 1 | Jurczak *et al.* 2004 |
| Czech Republic | | 5804 | s | 1 | Marsálek *et al.* 2001 |
| Czech Republic | 37 | | w | 2 | Bláhová *et al.* 2007 |
| Belgium | | 2231 | s | 1 | Williame *et al.* 2005 |
| Turkey | 24.2 | | w | 1 | Albay *et al.* 2005 |
| China | 33.2 | | w | 1 | Zheng *et al.* 2004 |
| Korea | | 1489 | s | 1 | Park *et al.* 1998b |
| Japan | 3.62 | | s | 1 | Ozawa *et al.* 2005 |
| Philippines | | 4049 | s | 1 | Cuvin-Aralar *et al.* 2002 |
| Sri Lanka | 81 | | s | 1 | Jayatissa *et al.* 2005 |
| Zimbabwe | 22.48 | | c | 1 | Ndebele & Magadza 2006 |
| Kenya | | 4593 | w | 1 | Ballott *et al.* 2004 |
| Tanzania | 0.99 | | w | 1 | Sekadene *et al.* 2005 |
| Morocco | | 1777.64 | s | 1 | Oudra *et al.* 2002 |
| Algeria | 29 163 | | s | 3 | Nasri *et al.* 2004 |
| | 28.89 | | w | 3 | Nasri *et al.* 2004 |
| U.S.A | 43 | | w | 2 | Johnston & Jacoby 2003 |
| U.S.A | 4.5 | | s | 2 | Graham *et al.* 2004 |
| U.S.A | 2.5 | | w | 2 | Hotto *et al.* 2008 |
| U.S.A | 58 | | s | 1 | Dyble *et al.* 2008 |
| | 5 | | w | 1 | Dyble *et al.* 2008 |
| Brazil | 45 | | s | 2 | Sotero-Santos *et al.* 2008 |
| Argentina | 923 | | w | 2 | Conti *et al.* 2006 |
| Argentina | | 2400 | s | 1 | Amé *et al.* 2003 |
| Uruguay | | 1074 | w | 2 | De Leon & Yunes 2001 |
| Western Australia | 8 | | w | 1 | Atkins *et al.* 2001 |
| Queensland | 8 | | w | 1 | Hoeger *et al.* 2004 |
| Queensland | 2500 | | s | 1 | White *et al.* 2003 |
| New South Wales | 40 | | b | 3 | Dasey *et al.* 2005 |
| Herdsman Lake | 8428.6 | | s | 1 | current study |
| Median MC conc | 58.2 | | | | current study |

**Sampling method:** (s) represents a near-shore surface accumulation (scum) or concentrated sample collected by plankton net; (w) represents open water sample or integrated depth sample; (c) cultured material; (b) benthic. **Analytical method:** (1) HPLC; (2) enzyme-linked immunosorbent assay; (3) protein phosphatase inhibition assay.

### *8.4.2 Diversity of the MC variants*

The three MC structural variants differ in toxicity as shown in biochemical and histological evaluations using both mice and fish (Gupta *et al.* 2003; Atencio *et al.* 2008). The variants containing more hydrophobic L-amino acids (e.g. MC-LR and MC-YR) are more potent than those containing hydrophilic amino acids (e.g. MC-RR) (Sivonen and Jones 1999; Falconer 2005). However, all three variants are highly toxic and pose a major environmental health hazard (Gupta *et al.* 2003). The lake samples demonstrating a high prevalence of MC-LR, at mean concentration of 506.9 μg $L^{-1}$ (median 8.85 μg $L^{-1}$, maximum 3108 μg $L^{-1}$) should cause the need for further action in monitoring and managing cyanoprokaryota blooms in the SCP wetlands. A MC-LR concentration of 20 μg $L^{-1}$ does present a moderate probability of adverse health effects (based on WHO provisional guideline), with higher concentrations potentially producing severe health problems (WHO 2003).

Microcystin-LR, -YR and -RR will dominate *Microcystis* blooms although different combinations can exist (Sivonen and Jones 1999). For example, MC-LR and MC-RR are the main variants associated with *M. aeruginosa* (Kotak *et al.* 1995; Park *et al.* 1998a,b; Vezie *et al.* 1998; Cuvin-Arala *et al.* 2002; Gkelis *et al.* 2005). This was evident in the current study by MC-LR comprising a higher percentage of the total MC in samples dominated by *M. aeruginosa.* Conversely lake samples with a higher percentage of MC-RR were dominated by *M. flos-aquae*. Both variants were also positively correlated to *Microcystis* density. This result is in agreement with the findings of Lahti *et al.* (1997) and Vezie *et al.* (1998). Total MC concentration was positively correlated with the biomass of *M. aeruginosa*, *M. wesenbergii* and *M. viridis* (Lahti *et al.* 1997) and MC-LR, MC-RR, total MC concentrations were positively correlated and chlorophyll *a* (Vezie *et al.* 1998). Therefore, regarding the potency of these two variants and the results of this study, it may be inferred that *M. aeruginosa* blooms will be more toxic than blooms dominated by *M. flos-aquae*.

The absence of MC-RR and MC-YR in the Swan River samples may be due to the HPLC analysis being completed at an earlier date and the samples being dominated by *M. flos-aquae*. The October 1999 and January 2000 samples from Yangebup

Lake and Bibra Lake were analysed at the same time and also contained only MC-LR and the analogues, even though samples analysed at a later date contained MC-RR and MC-YR.

### *8.4.3 Species associated with toxic blooms*

The variability of MC concentration between sites is related to the biovolume of MC-producing species and the presence of toxic strains. However, in this study there was no attempt to determine which species were responsible for the MC production. *Microcystis* dominated all samples that were positive for MCs, with *M. aeruginosa* being the dominant species. The other *Microcystis* species identified were *Microcystis flos-aquae* and *M. wesenbergii*. Lake Goollelal samples contained only *M. flos-aquae*, which was also the dominant species in Emu Lake and Lake Claremont. Hyde Park was the only site to contain *M. wesenbergii*. The ability of *M. flos-aquae* and *M. wesenbergii* to produce MCs is yet to be confirmed (Steffensen *et al.* 1999), although both regularly occur with *M. aeruginosa* with which MCs are associated. In these results samples where *M. flos-aquae* are predominant showed a variety of MC concentrations that ranged from 0.5 µg $L^{-1}$, in Lake Goollelal, to 634.2 µg $L^{-1}$, in Emu Lake. Therefore, the presence of *M. flos-aquae* exclusively in the metropolitan lakes does not imply absence of MCs.

Variation in the morphology of *M. aeruginosa* exists with both morphological forms being distinctly different in MC production. Scott *et al.* (1981) found the most toxic natural bloom samples were comprised entirely of *M. aeruginosa f. aeruginosa* and *M. aeruginosa f. flos-aquae* were less toxic or non-toxic. Orr *et al.* (2004) made a similar observation. *Microcystis flos-aquae* has also exhibited low toxicity in water bloom samples from the Czech Republic (Marsálek *et al.* 2001). This is further justified by the variation in toxicity reported for the Swan River *Microcystis* bloom. Atkins *et al.* (2001) detected 8 µg $L^{-1}$ of MC in water samples while concentration reached 124.16 µg $L^{-1}$ in this study. Orr *et al.* (2004) identified the Swan River bloom as a mixed population of *M. aeruginosa* f. *aeruginosa* and *M. aeruginosa* f. *flos-aquae* with batch cultures producing a melange of genotypes with differing toxigenicities. At least 11 MCs were present in bloom material, although baseline

resolution could not separate the HPLC peaks. Orr *et al.* (2004) concluded *Microcystis aeruginosa* f. *aeruginosa* was toxigenic while *M. aeruginosa* f. *flos-aquae* was not.

Similarly, *Microcystis wesenbergii* contains little or no MCs (Marsálek *et al* 2001; Komárek *et al.* 2003; Xu *et al.* 2008). Traces of MCs below the detection limit for HPLC were found for *M. wesenbergii* samples from Lake Biwa, Japan (Ozawa *et al.* 2005). Likewise the samples from Hyde Park (samples 18 and 19) showed a codominance of *M. aeruginosa* and *M. wesenbergii*, at similar percentage (Fig. 8.4a), yet produced considerably different concentrations of MC (3216.8 and 60.2 µg $L^{-1}$). In fact, a low MC concentration was recorded when the percentage biovolume was 61% *M. wesenbergii* and 39% *M. aeruginosa*.

The lakes with low MC content were those dominated by filamentous species of *Anabaena*, *Aphanizomenon* or *Anabaenopsis*, which could potentially be producing STX or CYL. These sites also recorded a higher abundance of *M. flos-aquae*. For example, low toxicity was associated with *Anabaena* in Tomato Lake and M. flos-aquae in Lake Goollelal, while no MCs were detected in Blue Gum Lake (*M. flos-aquae*), North Lake (*Anabaena*) and Lake Claremont (*Anabaenopsis*). The negative result from Blue Gum Lake, even though the sample did contain both *M. aeruginosa* and *M. flos-aquae*, indicates the presence of non-toxic strains. For *Anabaena*, the production of MCs has not been confirmed (Steffensen *et al.* 1999), although, hepatotoxicity has been associated with samples from the Murray-Darling Basin (Baker and Humpage 1994). However, those samples also contained considerable amounts of *Nodularia*, which produces the hepatotoxin NOD. Finally, *Anabaenopsis* has not been proven toxic in Australia.

The occurrence of *Nodularia spumigena* with a high concentration of NOD in Yangebup Lake deserves immediate attention. In November 2001 a total NOD concentration of 1664.4 µg $L^{-1}$ was recorded, which in addition to a total MC content of 1776 µg $L^{-1}$, makes this sample from Yangebup Lake one of the most toxic in this study. Nodularin was present in the November 2001 concentrated net

sample, yet *Nodularia* cells were not identified in the cell counts performed from the water samples. Cell counts from integrated water samples are used for risk assessment. The highest *Nodularia* cell count was reached in late October 2001 (27000 cells $mL^{-1}$) although NOD was not detected. Younger blooms tend to have a higher percentage of toxin contained within the cells compared to older blooms, that have a higher percentage of toxin in the water column (Chiswell *et al.* 1999; Welker *et al.* 2001). Following the decline of a *C. raciborskii* bloom, Chiswell *et al.* (1999) found approximately 92 to 98% of total CYL was present in the water column. Therefore, the November 2001 sample from Yangebup Lake may have marked the decline of the *Nodularia* bloom. Of the total NOD concentration, 97.6% was extracellular, which support this explanation. Even though cell numbers had decreased, there was still a risk of exposure to dissolved toxins.

Previously *Nodularia* blooms had been recorded in Forrestdale Lake and Thomson Lake in spring 1993 (John and Kemp 2006). In November 1999 a NOD concentration of 2.11 μg $L^{-1}$ was measured in concentrated algal samples collected from Thomsons Lake (Kemp, unpublished data). Yangebup Lake and Thomsons Lake are part of the Bibra suite of consanguineous wetlands (Semeniuk 1996), with the South Jandakot Drain diverting excess water away from Thomsons Lake to Yangebup Lake (Thompson Palmer Pty. Ltd. 2005). The presence of toxic *Nodularia* blooms in these wetlands needs further investigation. The recurrence of *Nodularia* blooms for prolonged periods is likely as salinity and phosphorus levels increase (John and Kemp 2006).

### *8.4.4 Cell numbers in relation to MC content*

Cell counts for this study were taken from water samples collected outside scum formation, so were not an indication of cyanoprokaryote biomass in the HPLC samples. However, many were in excess of the 100 000 cells $mL^{-1}$ guideline value for a moderate health alert in recreational waters, which is equivalent to 20 μg $L^{-1}$ if the bloom consists of *Microcystis* (WHO 2003). However, it has been reported by Pilotto *et al.* (1997), based on a survey assessing health problems resulting from recreational exposure, that the threshold cell count for exposure (20 000 cells $mL^{-1}$)

is too high. Symptoms of toxin exposure (e.g. diarrhoea, vomiting, skin rashes, mouth ulcers) occur at a cell density as low as 5000 cells $mL^{-1}$ (Pilotto *et al.* 1997).

Cell numbers do not always reflect the total toxin concentration, as demonstrated by the subdominance of *Nodularia* in Yangebup Lake. Heresztyn and Nicholson (1997) reported the total NOD concentration follows the general trend of *Nodularia* cell numbers, while Kotak *et al.* (1995) observed changes in MC concentration to be related to the biomass of *Microcystis* spp. Other studies found no relationship between *Microcystis* cell numbers and MC concentration with changes in MC concentration related to which *Microcystis* species dominated (Jacoby *et al.* 2000; Hoeger *et al.* 2004; Ozawa *et al.* 2005). Furthermore Jacoby *et al.* (2000) reported the presence of MCs even when conditions were not favourable for *M. aeruginosa* dominance. Microcystin can also be present in the water at low concentrations, even after the visible bloom has disappeared (Lahti *et al.* 1997). In the current study cell counts exceeded recommended guideline values because the samples used for HPLC analysis were chosen based on the high abundance of *Microcystis*. Conversely, non-toxic to low toxic samples were the result of non-toxic species being present within the samples.

## 8.5 Conclusions

The results of this study revealed that substantial amounts of MCs occur in the lakes throughout the city of Perth with the high content of MCs in some of the scum samples signifying a serious public health safety risk. The large variation in total MC content showed some lakes to be experiencing blooms that were not highly toxic. However**,** this study did not address seasonal variation or the presence of STXs in samples of *Anabaena*. The occurrence of NOD in Yangebup Lake shows the ability for blooms of multiple species to produce different cyanotoxins simultaneously. With the common MC identified as MC-LR, competitive enzyme-linked immunosorbent assays have the potential to offer a more rapid and effective method for the routine monitoring of MCs in wetlands of the SCP.

# Chapter 9: The use of primary screening techniques for detecting microcystins in bloom samples

## 9.1 Introduction

In the previous chapter natural bloom samples from 15 Perth wetlands were analysed for microcystins (MCs) using high performance liquid chromatography (HPLC). Results showed the prevalence of the structural variant microcystin-LR (MC-LR) in the majority of samples. Therefore, the present chapter evaluates two screening methods currently used for hepatotoxin determination, enzyme-linked immunoassay and brine shrimp bioassay, in comparison with the HPLC results obtained, as alternative methods for the detecting toxic cyanoprokaryota blooms in the wetlands of the Swan Coastal Plain (SCP).

Various analytical procedures have been developed for detecting and measuring the concentration of cyanotoxins in bloom samples. Choosing which technique to use depends upon the facilities and expertise available coupled with the type of information required (Harada *et al.* 1999). In recent years, primary screening techniques have been developed as a rapid, low cost method for determining the potential hazard of a cyanoprokaryota bloom. Such techniques include invertebrate assays (*Artemia* bioassay), biochemical assay (inhibition assay) and immunological assays. They allow for the initial indication of toxicity, assisting laboratories that lack resources to carry out more elaborate analyses such as the widely used HPLC. Furthermore, screening provides a low cost method for analysing large quantities of samples, making it suitable for continuous surveys.

In Chapter 8, the SCP wetlands were shown to produce hepatotoxic blooms that pose a potential public safety risk. However, facilities for the quantification of cyanotoxins are not generally available in Western Australia. In the past, mouse bioassay was used to screen for toxins in *Anabaena* and *Microcystis* blooms in Canning River, *Nodularia* blooms in the Serpentine River and the 2000 *Microcystis* bloom in the Swan-Canning estuary (Hosja and Deeley 1994; Atkins *et al*. 2001).

Although there is evidence of toxic blooms in the SCP wetlands, research into bloom toxicology in Western Australia has been limited due to the high cost of analyses and the lack of facilities.

Traditionally, intraperitoneal mouse bioassay has been relied on for toxicity assessment of cyanoprokayota (Elleman *et al.* 1978; Falconer *et al.* 1981). In this bioassay a volume of cyanoprokaryote extract or purified toxin is injected into Swiss albino mice that are then observed over a 24-hour period for progressive organ failure before being killed for post mortem examination (Falconer 1993). A lethal concentration of a hepatotoxin would typically kill the mice within five minutes to four hours, while neurotoxins can take about 15 minutes (Falconer 1993). Although the mouse bioassay provides a response within a few hours, it was not very sensitive or specific in what it detects (Harada *et al.* 1999). Faster acting neurotoxins tend to mask the effect of slower acting hepatotoxins (Meriluoto *et al.* 1996). The detection limit for MC-LR is 1 μg $mL^{-1}$, but this limit is higher for most other MCs (Meriluoto *et al*. 1996). Furthermore there are the ethical and moral implications when using live animals. The strict guidelines require ethical committee approval with lethal dose studies only approved after an exploration of all alternative procedures (Falconer 1993). Nevertheless, in the past mouse bioassay has been effective in the initial screening of highly concentrated samples of unknown toxicity.

Currently the most extensively used technique for the purification and quantification of cyanotoxins in cyanoprokaryotes is HPLC coupled with UV or photodiode array detection. This procedure allows for the accurate determination of intra- and extracellular components, providing both quantitative and qualitative data (McElhiney and Lawton 2005). Advancements have also seen the development of Thin Layer Chromatography a simple, cost-effective method, while Liquid Chromatography-Mass Spectrometry provides a more accurate technique enabling the simultaneous separation and identification of MCs (Pelander *et al.* 2000; Poon *et al.* 1993). However, these analytical methods can be expensive and time consuming with lengthy sample processing to concentrate the MCs (McElhiney and Lawton 2005). Newer technologies using invertebrate bioassays and enzyme immunoassays

have been developed to replace the traditional mouse bioassay and provide a rapid, simple and inexpensive alternative to HPLC. They allow for the initial indication of toxicity (toxic and non-toxic), after which positive samples can then be sent to a qualified laboratory for full analytical investigation, if required.

Bioassays for MCs have been developed using invertebrates such as the brine shrimp *Artemia salina* (Kiviranta *et al.* 1991), the bacteria *Pseudomonas putida* (Volterra *et al.* 1992) and the freshwater crustacean *Thamnocephalus platyurus* (Torokne 1999). Of these, the most developed and assessed technique is the brine shrimp bioassay, also known as the Brine Shrimp Lethality Test (BSLT). One-day-old brine shrimp larvae are exposed to different concentrations of a test solution and after 24 hours, the numbers of dead or atypically moving larvae are counted and the concentration that is lethal to 50% of the population ($LC_{50}$) can be calculated (Kiviranta *et al.* 1991). From these values the he BSLT can successfully detect MCs in both natural bloom samples and isolated strains (Kiviranta *et al.* 1991), with results that correlate well with mouse bioassay (Vezie *et al.* 1996; Lee *et al*. 1999).

In recent years, immunological assays have become widely used, especially for routine water supply monitoring. These assays are as sensitive as HPLC with a detection limit of 0.1μg $L^{-1}$ and are convenient for screening a large number of environmental samples (Harada *et al.* 1996; Carmichael and An 1999). For these reasons immunological assays are capable of monitoring MCs in drinking water. Immunological assays are based on a reaction between a target analyte and a specific antibody (Van Emon and Lopez-Avila 1992). The enzyme linked immunosorbent assay (ELISA) uses both polyclonal and monoclonal antibodies and enzyme-labelled antigens to measure the quantity of MC in a sample (Monroe 1984). There are commercially available ELISA kits based on the polyclonal antibodies developed by Chu *et al.* (1989). They produce semi-quantitative results depending on the MCs present in the algal sample and their cross-reactivities relative to the MC-LR (Nicholson and Burch 2001). Such kits have been successfully used to identify the presence of MCs in both water and bloom samples (Oudra *et al.* 2001; Frank 2002,

Fromme *et al.* 2000, Vieira *et al.* 2005), and is the most promising for the rapid screening of environmental samples for MCs (Harada *et al*. 1999).

The aim of this study was to evaluate the use of ELISA kits and BSLT for screening cyanoprokaryote samples collected from SCP wetlands for cyanotoxins. This trial will compare both methods to HPLC, which confirmed the presence of MCs in 28 samples. The presence of MC-LR in most of these samples should allow ELISA to be an effective detection method. Furthermore, as the brine shrimp can be affected by both hepatotoxins and neurotoxins, the BSLT has the advantage in detecting additional toxins in samples where MC concentration is low but potentially neurotoxic species are present.

## 9.2 Materials and Methods

### *9.2.1 Sample preparation*

A total of 44 phytoplankon samples were collected using a 25-µm mesh net and frozen. This represented 14 study sites. Due to financial constraints, only 28 samples had their MC concentrations predetermined by HPLC at the Australian Water Quality Centre (Bolivar) (refer to section 8.3.1). The samples were identified to species. All except one sample contained hepatotoxin-producing species (*Microcystis* or *Nodularia*). Cyanotoxins were extracted by freezing then thawing the phytoplankon samples for three cycles. Samples were then sonicated and filtered using Whatman GF/C Glass Microfibre filters to remove cell material.

### *9.2.2 Enzyme-linked immunosorbent assay procedure*

The EnviroGard® Microcystins Plate Kit from Strategic Diagnostics Inc. and the Envirologix QuantiPlate™ Kit for Microcystins were used. All analyses were performed according to the manufacturer's instructions with MC determinations compared with the supplied calibrators within the kits. Envirologix can detect MCs in the range of 0.16 to 2.5 µg $L^{-1}$, while EnviroGard® had a lower detection range of 0.1 to 1.6 µg $L^{-1}$. Twenty-eight samples, which had MC concentrations

predetermined by HPLC, were diluted with distilled water to bring the MC concentration within the detection range specified for the kits. For the remaining 14 samples, where the concentration of MC was unknown, the algal extracts were diluted based on species composition and the abundance of MC-producing species. Prior to using the Envirologix kit, all extracts were further diluted by a factor of 1:8.

Both kits consisted of 96 antibody-coated microwells, assay diluent, negative control, MC-LR calibrators, MC-enzyme conjugate, substrate, stop solution and wash salts. Using a multi-channel pipette, the assay diluent was added to each well. The negative control, calibrators and unknown samples were added to duplicate wells, and incubated for 30 minutes at ambient temperature. An aliquot of MC enzyme conjugate was then added and the wells incubated for another 30 minutes. The contents of the wells were emptied and then flooded with the prepared wash solution (phosphate-buffered saline). This was repeated four times. Then substrate was added to each well and incubated for a further 30 minutes. Finally, the stop solution was added. The optical density (OD) for both wells was measured using a microplate reader set to 405 nm. The spectrophotometer was blanked using deionised water. The enzymatic reaction causes a colour change, which is inversely proportional to MC concentration.

The ELISA response ($\%B_O$) was determined using the following equation;

$$\%B_O = \frac{\text{average OD of sample or standard}}{\text{average OD of negative control}} \times 100$$

The standard curve was generated by plotting $\%B_O$ of each calibrator against its MC-LR equivalent on a semi-logarithmic scale. The $\%B_O$ of the unknown samples were converted to MC-LR equivalents using the standard curve. Interpolation of the unknown sample concentration is only possible if the $\%B_O$ falls within the range set by the calibrators. The %CV (coefficient of variation = standard deviation/mean in percentage) for the calibrators should not exceed 15%. As MC-LR is used as the calibrator, all amounts are reported as microcystin-LR equivalents (MC-LR eq).

### *9.2.3 Brine shrimp lethality test*

Bloom samples were screened for chronic toxicity (24-hour exposure) using BSLT. Dried *Artemia salina* cysts were obtained from Dr M. Payne and Dr R. Rippingale, Curtin University of Technology, Western Australia. Cysts were hydrated by aeration in seawater for one to two hours with exposure to bright light. They were then washed in 2.5% bleach solution and rinsed until all traces of bleach were removed. Decapsulated cysts were then aerated in saltwater for 24 hours at 25°C with exposure to bright light. Once hatched, 10 shrimps were isolated and placed into vials containing 3 mL saltwater and 1 mL bloom extract. The dosage was a series of dilutions (0, 10, 100 and 1000) of the total MC concentration as determined by HPLC or ELISA. Trials were run in triplicate. The negative control comprised of brine shrimp placed in vials containing 20% seawater only. The results are considered acceptable if no mortality was observed in the negative control (Sam 1993). Potassium dichromate ($K_2Cr_2O_7$), a standard heavy metal salt toxicant, was used as the positive control at concentrations of 1, 10, 100 and 1000 ppm. The vials were incubated in a 25 °C waterbath for 24 hours, after which, the number of dead or immobilised nauplii were counted and the mortality, accumulated deaths and accumulated live numbers were calculated.

The median lethal concentration ($LC_{50}$) was derived by the Reed-Muench procedure plotting accumulated deaths against accumulated survivors (Fig. 9.1). The standard error and range were determined using the logarithmic-probit method, which plots percentage mortality against dosage (Fig. 9.2). A total of 20 trials were conducted, representing 11 sites (Table 9.1).

**Table 9.1.** The samples and trial numbers for the algal extracts used in the BSLT.

| Trial | Site | Date |
|---|---|---|
| 1 | Yangebup Lake | Nov 02 |
| 2 | | Oct 01 |
| 3 | | Nov 01 |
| 4 | Hyde Park | Nov 00 |
| 5 | | Jun 01 |
| 6 | Herdsman Lake | Dec 00 |
| 7 | | May 01 |
| 8 | | Feb 03 |
| 9 | Jackadder Lake | June 01 |
| 10 | | Nov 01 |
| 11 | Lake Claremont | Dec 00 |
| 12 | Emu Lake | Jan 01 |
| 13 | | Nov 02 |
| 14 | Tomato Lake | Nov 01 |
| 15 | | Feb 03 |
| 16 | | Feb 02 |
| 17 | Lake Joondalup | Dec 00 |
| 18 | Lake Goollelal | Dec 00 |
| 19 | Canning River | Feb 01 |
| 20 | Serpentine River | Nov 00 |

### *9.2.4 Statistical analysis*

The MC concentrations obtained by both ELISA and HPLC were examined for normality and log transformed. The differences in the amount of MCs detected by both methods were investigated using a single factor ANOVA, testing the hypotheses that the mean results are statistically the same at the 95 % confidence level. If the calculated F value (F calc) was less than the critical F value (F crit) then the hypothesis was accepted. For the BSLT the relationship between the percentage mortality (log n+1 transformed) and dosage (log MC concentration) was determined using Spearman's Ranked Correlation. Statistical analysis was performed using SPSS Version 11 for Mac OS X. The trials (n = 3) which had their MC concentration determined by ELISA were omitted from the analyses as total MC concentration was not confirmed or clarified using HPLC.

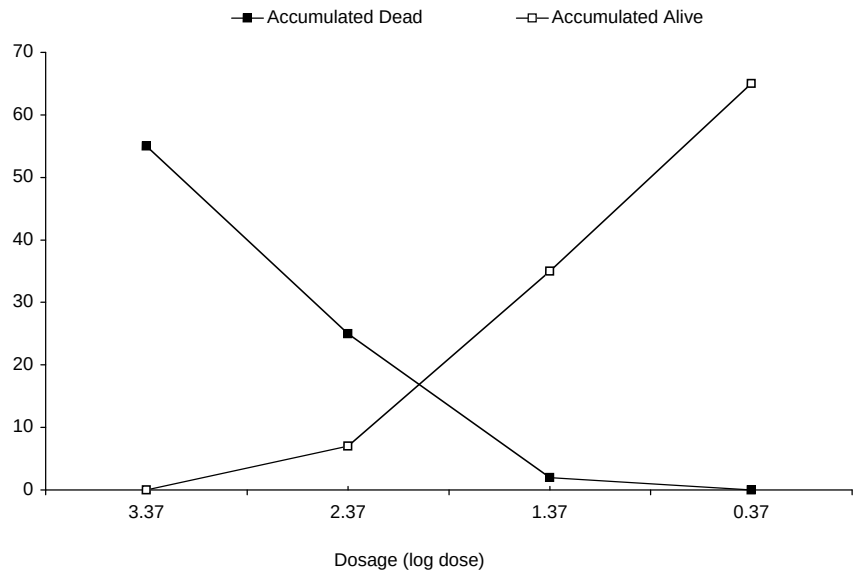



**Figure 9.1.** Estimation of the median lethal concentration ($LC_{50}$) using the Reed-Muench method as described by Sam (1993). The two lines intercept at the 50% lethal dose.

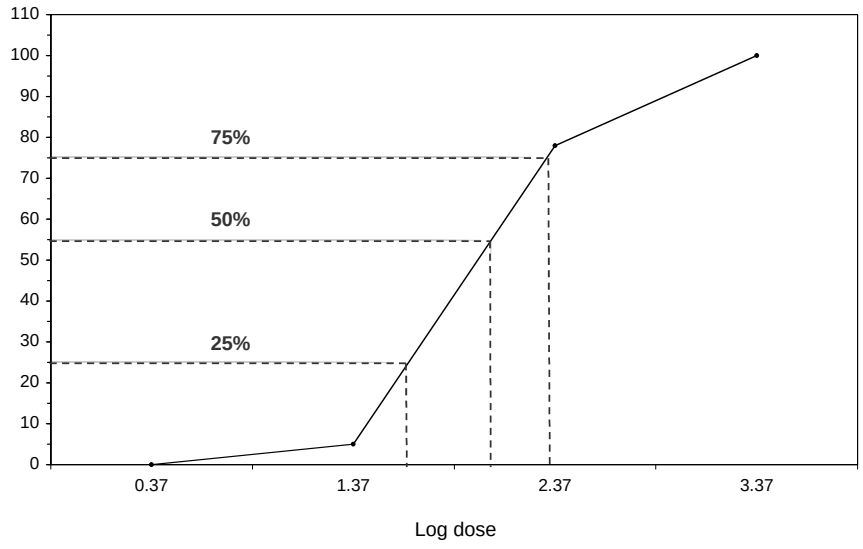



**Figure 9.2.** Estimation of $LD_{25}$, $LD_{50}$ and $LD_{75}$ for calculating the standard error and 95% confidence limits by log-probit method, as described by Sam (1993).

## 9.3 Results

### *9.3.1 Detection of MCs using ELISA*

The first assay did not produce acceptable concentrations due to inconsistent calibration results, based on the lower than recommended $R^2$ value (0.7198) (Table 9.2). The $\%B_O$ for each calibrator fell below the guideline range stated in the manufacturer's instructions. However the first assay did indicate the need for further dilution of samples, especially those with concentrations not predetermined by HPLC, as the MC-LR eq exceeded the 2.5 μg $L^{-1}$ detection limit. The second assay produced acceptable results, based on the high $R^2$ value (0.997) (Fig. 9.3). These are the results that will be used in this study.

Microcystin-LR equivalents were determined in 31 of the 42 samples (Table 9.3 and 9.4). The remaining eleven samples produced readings below the level of detection (0.1 μg $L^{-1}$). Eight of these undetermined samples did not have their MC concentration predetermined by HPLC and were diluted based on the presence of MC-producing species. Therefore it is likely these samples were diluted below the range of the kit. Microcystin-LR equivalent concentrations in the range of 0.1 to 1.6 μg $L^{-1}$ were recorded in 43% of samples and greater than 1.6 μg $L^{-1}$ in 31% of samples.

All three samples collected from Emu Lake had concentrations exceeding the detection limit of 1.6 μg $L^{-1}$, showing the need for further dilution. Samples from the Canning River, Bibra Lake, Lake Claremont and Tomato Lake were below the detection limit, requiring a lower dilution factor. Two samples from the upper Serpentine River, both containing *Nodularia spumigena*, produced results of < 0.1 μg $L^{-1}$ and 0.64 μg $L^{-1}$ MC-LR eq, the latter being used for the BSLT.

**Table 9.2**. Calculated $\%B_O$ and determined MC concentration for the two ELISA kits.

| Kit | Calibrator ($\mu g\ L^{-1}$) | % $B_O$ | MC conc. ($\mu g\ L^{-1}$) | $R^2$ |
|---|---|---|---|---|
| EnviroGard® | 0.16 | 54.95 | < 0.16 | 0.7198 |
| | 0.6 | 22.99 | 1.44 | |
| | 2.5 | 8.39 | 2.34 | |
| Envirologix | 0.1 | 73.44 | 0.13 | 0.997 |
| | 0.4 | 68.20 | 0.36 | |
| | 1.6 | 39.21 | 1.61 | |

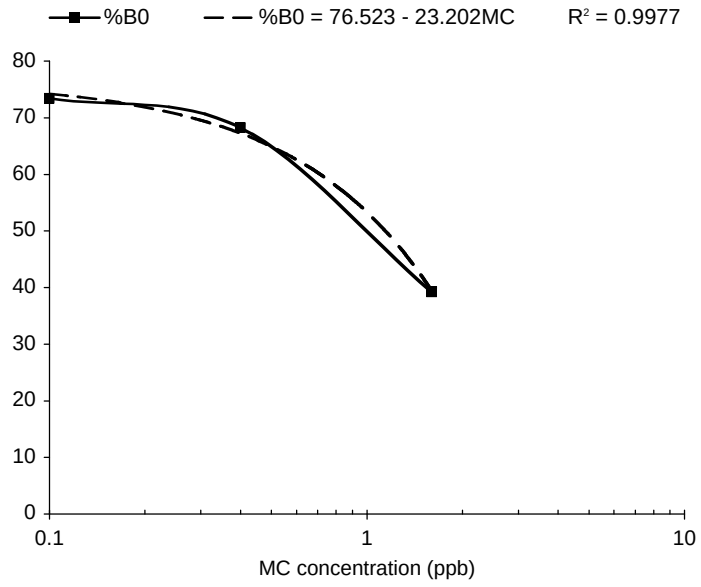



**Figure 9.3.** The standard curve for the ELISA results (Envirologix kit).

Considering only the samples with MC-LR eq concentrations above 0.01 μg $L^{-1}$, the concentration of MC (comparing total MC, MC-LR and MC-LR eq) determined by HPLC and ELISA were significantly different (F calc = 21.06, F crit = 3.12, P < 0.05, n = 24). Concentrations determined by ELISA usually exceeded the predicted HPLC total MC concentration by 10 to 98% (Fig. 9.4). This was regardless of MC-LR concentration. In samples 2, 6, 7, 10, 19 and 29 where MC-LR comprised more than 70% of the total MC, ELISA had overestimated the MC concentration by 66 to 96%. Overestimation by ELISA was also observed in samples containing lower percentage of MC-LR (< 20 % total MC) (samples 5, 17, 31 and 36). Of these, samples 5, 12 and 38 had a greater percentage of the unidentified analogues (46%, 84% and 76%, respectively), with no MC analogues detected in samples 8, 22, 25 and 29.

ELISA produced lower concentrations that predicted in samples 14, 23, 26 and 38. For samples 23 and 38, MC-LR eq was below the detection limit of 0.1 μg $L^{-1}$, showing the samples were too diluted. Samples 14 and 26 both had lower concentrations of MC-LR (< 20% MC total), which would explain the low MC-LR eq concentration. Also the predominant variant in these two samples were MC-RR and MC-YR in sample 14, and MC-RR in sample 26 (Table 9.4).

**Table 9.3.** Summary of the ELISA results showing the number of samples (n = 42) within each MC-LR equivalent concentration range.

| MC-LR equivalent (μg $L^{-1}$) | Number of samples |
|---|---|
| < 0.1 | 11 |
| 0.1 – 1.6 | 18 |
| > 1.6 | 13 |

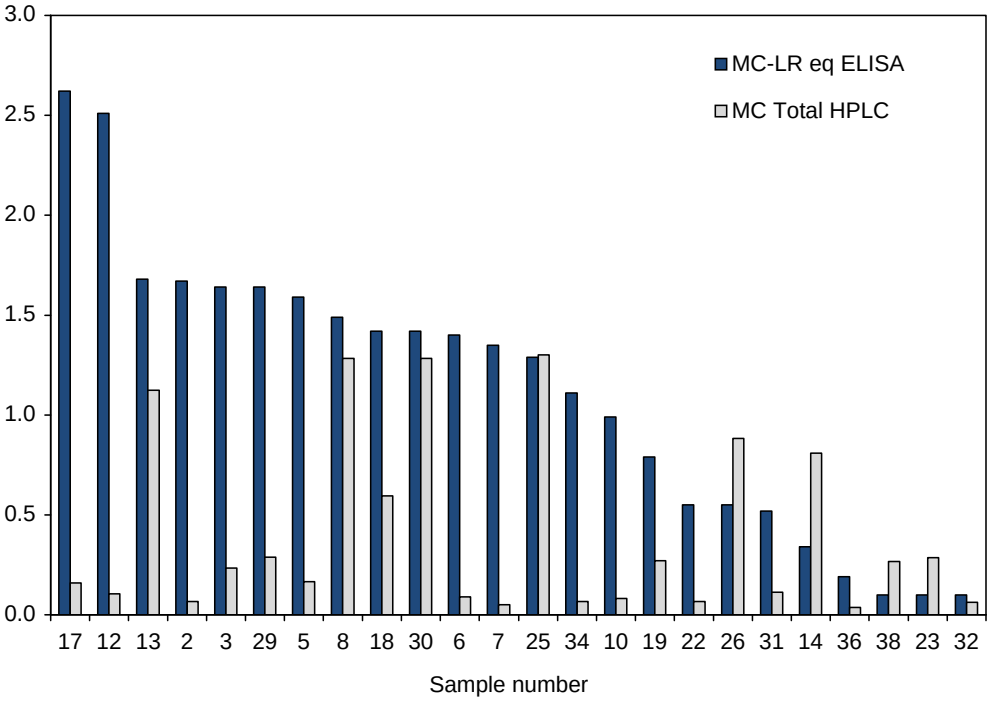



**Figure 9.4.** Comparison of the total MC concentration (μg $L^{-1}$) as determined by HPLC and ELISA. Samples arranged in descending order based on MC-LR eq concentration.

**Table 9.4.** Concentration of microcystins (MC-LR equivalents) in algal samples as determined by HPLC and ELISA.

| Site | Date | Sample number | Dilution of extracts | Species identified[a] | HPLC (μg $L^{-1}$) | | | | | ELISA MC-LR eq (μg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | MC-LR | MC-RR | MC-YR | Analogues | MC total | |
| Herdsman | Dec 00 | 14 | 100 | Mf, Ma | 0.06 | 0.28 | 0.29 | 0.18 | 0.81 | 0.34 |
| | May 01 | 12 | 80000 | Mf, Ma | 0.04 | 0.02 | 0.005 | 0.05 | 0.11 | >1.6 (2.51) |
| | Nov 01 | 13 | 50 | Mf, Ma, Ac | 0.72 | 0.15 | 0.04 | 0.21 | 1.12 | >1.6 (1.68) |
| | Feb 03 | 11 | 2500 | Mf, Ma, Ac | | | | | | 1.44 |
| Yangebup | Aug 00 | 5 | 4000 | Ma | 0.02 | 0.004 | 0.002 | 0.14 | 0.16 | 1.59 |
| | Jan 01 | 4 | 2500 | Mf, Ma | | | | | | >1.6 (1.81) |
| | May 01 | 1 | 40000 | Mf, Ma | | | | | | >1.6 (1.75) |
| | Oct 01[b] | 2 | 20000 | Mf, Ma, Ac, Ns | 0.05 | 0.005 | 0.004 | 0.01 | 0.07 | >1.6 (1.67) |
| | Nov 01 | 6 | 20000 | Mf, Ma | 0.07 | 0.002 | 0.002 | 0.01 | 0.09 | 1.4 |
| | Nov 02 | 3 | 20000 | Mf, Ma | 0.04 | 0.03 | 0.01 | 0.03 | 0.23 | >1.6 (1.64) |
| Bibra | Sep 00 | 29 | 8 | Mf, Ma, Ae | 0.28 | 0.01 | | | 0.29 | >1.6 (1.64) |
| | Dec 00 | 27 | 50 | Mf, Ma, Ao, Ab | | | | | | <0.1 |
| | Jan 01 | 28 | 50 | Mf, Ma, Ao, Ab | | | | | | <0.1 |
| | Jun 01 | 26 | 100 | Mf, Ma, Ao, Cr | 0.18 | 0.37 | 0.15 | 0.19 | 0.88 | 0.55 |
| | Jan 02 | 30 | 50 | Mf, Ma, Ao, Ab | 0.91 | 0.05 | 0.2 | 0.31 | 1.28 | 1.42 |
| Booragoon | Oct 00 | 34 | 400 | Ma, Mf | 0.02 | 0.03 | 0.002 | 0.01 | 0.07 | 1.11 |
| | Jan 01 | 33 | 50 | Ma, Mf | | | | | | >1.6 (3.01) |
| Goollelal | Dec 00 | 31 | 8 | Mf | 0.01 | 0.05 | 0.03 | 0.03 | 0.11 | 0.52 |
| | Nov 01 | 32 | 8 | Mf | 0.04 | | 0.03 | | 0.06 | <0.1 |

**Table 9.4.** (*Continued*)

| Site | Date | Sample number | Dilution of extracts | Species identified[a] | HPLC (μg $L^{-1}$) | | | | | ELISA MC-LR eq (μg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | MC-LR | MC-RR | MC-YR | Analogues | MC total | |
| Hyde Park | Nov 00 | 7 | 4000 | Ma, Mw | 0.04 | | 0.001 | 0.01 | 0.05 | 1.35 |
| | Jan 01 | 9 | 4000 | Ma, Mw | | | | | | >1.6 (1.97) |
| | Jun 01 | 10 | 40000 | Ma, Mw | 0.08 | | 0.002 | | 0.08 | 0.99 |
| | Nov 01 | 8 | 50 | Ma, Mw | 1.11 | | 0.1 | | 1.21 | 1.49 |
| Joondalup | Dec 00 | 38 | 50 | Mf, Ma | 0.04 | | 0.02 | 0.2 | 0.26 | <0.1 |
| | Jan 01 | 37 | 400 | Mf, As, Ab, Ae | | | | | | <0.1 |
| | Dec 02 | 39 | 400 | Mf, As | | | | | | >1.6 (2.36) |
| Claremont | Dec 00 | 36 | 400 | Mf | 0.004 | 0.02 | 0.004 | 0.01 | 0.04 | 0.19 |
| | Jan 01 | 35 | 400 | Mf | | | | | | <0.1 |
| Tomato | Dec 00 | 25 | 0 | Ma | 0.2 | 0.4 | 0.7 | | 1.3 | 1.29 |
| | Nov 01 | 23 | 20 | Ma, Mf, Ac | 0.29 | | | | 0.29 | <0.1 |
| | Feb 02 | 22 | 400 | Ma, Mf, Ac | 0.02 | 0.05 | | | 0.07 | 0.55 |
| | Feb 03 | 24 | 50 | Ma, Mf, Ac | | | | | | <0.1 |
| Jackadder | Dec 00 | 20 | 50 | Ma, Mf | | | | | | 0.53 |
| | Jun 01 | 18 | 4000 | Ma, Mf | 0.46 | 0.004 | 0.02 | 0.12 | 0.6 | 1.42 |
| | Nov 01 | 19 | 50 | Ma, Mf | 0.2 | | | 0.07 | 0.27 | 0.79 |
| Emu | Jan 02 | 17 | 4000 | Mf, Ma, Ao | 0.01 | 0.1 | 0.01 | 0.4 | 0.16 | >1.6 (2.74) |
| | Nov 02 | 16 | 4000 | Mf, Ao | | | | | | >1.6 (2.66) |
| | Jan 03 | 15 | 4000 | Mf | | | | | | >1.6 (2.74) |
| Neil McDougall | Aug 00 | 21 | 500 | Mf, Ma, Ac | | | | | | <0.1 |

**Table 9.4.** (*Continued*)

| Site | Date | Sample number | Dilution of extracts | Species identified[a] | HPLC (μg $L^{-1}$) | | | | | ELISA MC-LR eq (μg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | MC-LR | MC-RR | MC-YR | Analogues | MC total | |
| Canning River | 2nd Feb 01 | 40 | 20 | Mf, Ac | 0.07 | | 0.04 | | 0.12 | 1.04 |
| | 23rd Feb 01 | 41 | 50 | Mf | | | | | | <0.1 |
| | Jan 03 | 42 | 50 | | | | | | | <0.1 |
| Serpentine River | Nov 2000 | 43 | 40000 | Ns, Ae | | | | | | 0.64 |
| | Dec 2001 | 44 | 40000 | Ns, Ae | | | | | | <0.1 |

[a] Ab, *Anabaena bergii* var. *limnetica*; Ac, *A. circinalis*; As, *Anabaena spiroides* f *spiroides*; Ae, *Anabaenopsis elenkinii*; Ao, *Aphanizomenon ovalisporum*; Cr, *Cylindrospermopsis raciborskii*; Ma, *Microcystis aeruginosa*; Mf, *M. flos-aquae*; Mw, *M. wesenbergii*; Ns, *Nodularia spumigena*.
Value in parenthesis calculated using standard curve.

### *9.3.2 Response of* Artemia *to the algal extracts*

Of the 20 trials conducted, the median lethal concentration ($LC_{50}$) could be determined for 14 of the trials (Table 9.5). Following 24-hour exposure to the algal extracts there were no deaths recorded in trials 6, 10 and 18 and trials 11, 14 and 17 showed low mortality at the higher doses (14.5, 5.7 and 13.3 μg $L^{-1}$, respectively). The % mortalities of each trial are displayed in Figure 9.5. The dosage administered was considered ineffective if only a few (<10%) of the larvae died. This point was used to determine the lowest observed effect concentration (LOEC). The % mortality was significantly correlated with dosage (log MC concentration) ($r = 0.718$, $p > 0.01$, $n = 63$). After 24 hours, 100% mortality was observed in the samples with MC concentration in excess of 1000 μg $L^{-1}$ (trials 1, 2, 3, 7 and 9), as well as trials 4 (199.6 μg $L^{-1}$), 12 (634.2 μg $L^{-1}$) and 19 (4.7 μg $L^{-1}$). Samples used in the trials were classified as toxic, if the highest dose affected over 50% of the *Artemia* population.

Highly toxic trials were defined as those that produced a response at the lower doses and had a small percentage (< 10%) of the total MC concentration causing mortality to 50% of the *Artemia* population. Therefore, the most highly toxic samples were from trials 2, 15, 16 and 19. Trial 2 was a sample collected from Yangebup Lake ($LC_{50}$ 115.13 μg $L^{-1}$; LOEC 2.3 μg $L^{-1}$). Trials 15 and 16 were both samples from Tomato Lake and produced $LC_{50}$ values of 4.53 μg $L^{-1}$ and 7.63 μg $L^{-1}$, respectively. They also showed a response at the lower doses (1:1000), which were both less than 0.05 μg $L^{-1}$. The *Artemia* were atypically swimming. The LOEC was 0.29 μg $L^{-1}$ for trial 15, and 0.34 μg $L^{-1}$ for trial 16. The sample used for trial 19 was collected from the Canning River and produced the lowest $LC_{50}$ for all trials (0.25 μg $L^{-1}$). For this sample, 73% mortality was recorded at 0.47 μg $L^{-1}$.

Moderate toxicity was observed in trials 1, 9 and 20, where the $LC_{50}$ comprised a low percentage of the total MC concentration (5%, 9% and 6% respectively). However, at the lower doses (1:100 or 1:1000 dilution) there was less than 10% mortality (Fig. 9.5). Trial 9 recorded no deaths at 23.8 μg $L^{-1}$ but three deaths at 2.38 μg $L^{-1}$. It can be assumed that this may be attributed to natural mortality at 2.38 μg

$L^{-1}$. Trial 20, the *N. spumigena* sample collected from the upper Serpentine River, produced a $LC_{50}$ value of 1643.21 μg $L^{-1}$, which was 6% of the MC-LR eq concentration determined by ELISA. However, no to low mortality was observed at the lowest doses (256 μg $L^{-1}$ and 25.6 μg $L^{-1}$).

Samples classified as low in toxicity showed low mortalities at the higher doses and the $LC_{50}$ could not be determined. This was observed in trials 11, 14 and 17. Trial 11 with only 9% mortality at 14.5 μg $L^{-1}$, trial 14 with 27% at 5.7 μg $L^{-1}$ and trial 17 with 13 % mortality at 13.3 μg $L^{-1}$ (Fig. 9.5). Although trial 14 failed to kill 50% of the population, it did produce a response at 0.57 μg $L^{-1}$ with 5% mortality. Low toxicity was also observed in trials 8 and 13. Both required a high concentration (1807.05 and 5519.95 μg $L^{-1}$) to kill 50 % of the population with low mortalities observed at the lower doses.

**Table 9.5.** The chronic median concentration ($LC_{50}$) and relative toxicities (RT) of natural cyanoprokaryota samples compared to $K_2Cr_2O_7$ (Sam 1993).

| Trial | Site | MC (μg $L^{-1}$) | $LC_{50}$ (μg $L^{-1}$) | 95% confidence limit[b] (μg $L^{-1}$) |
|---|---|---|---|---|
| 13 | Emu | 10000[a] | 5519.95 | 2691.53 - 5370.32 |
| 7 | Herdsman | 8428.6 | 1816.91 | 870.96 – 3235.94 |
| 8 | Herdsman | 3600[a] | 1807.05 | 1122.02 – 4073.8 |
| 20 | Serpentine | 25600 [a] | 1643.21 | 616.59 – 2884.03 |
| 3 | Yangebup | 3440.4 | 1156.95 | 1543.62 – 3325.26 |
| 5 | Hyde | 3261.8 | 954.26 | 512.86 – 1862.09 |
| 9 | Jackadder | 2381.6 | 215.54 | 91.2 – 416.87 |
| 12 | Emu | 634.2 | 165.45 | 95.5 – 295.12 |
| 2 | Yangebup | 1317.5 | 113.07 | 45.71 – 239.88 |
| 1 | Yangebup | 2335.25 | 105.4 | 47.86 – 204.17 |
| 4 | Hyde | 199.6 | 35.56 | 21.88 – 79.43 |
| 15 | Tomato | 26.39 | 7.63 | 5.26 – 9.61 |
| 16 | Tomato | 29 | 7.62 | 4.94 – 10.79 |
| 19 | Canning | 4.7 | 0.25 | 0.15 – 0.61 |
| 11 | Claremont | 14.5 | | |
| 14 | Tomato | 5.7 | | |
| 17 | Joondalup | 13.35 | | |
| 10 | Jackadder | 13.5 | | |
| 6 | Herdsman | 80.9 | | |
| 18 | Goollelal | 0.9 | | |

[a] MC-LR equivalents determined by ELISA immunoassay.
[b] 95% Confidence limit, log $LD_{50} \pm 2$ SE $LD_{50}$

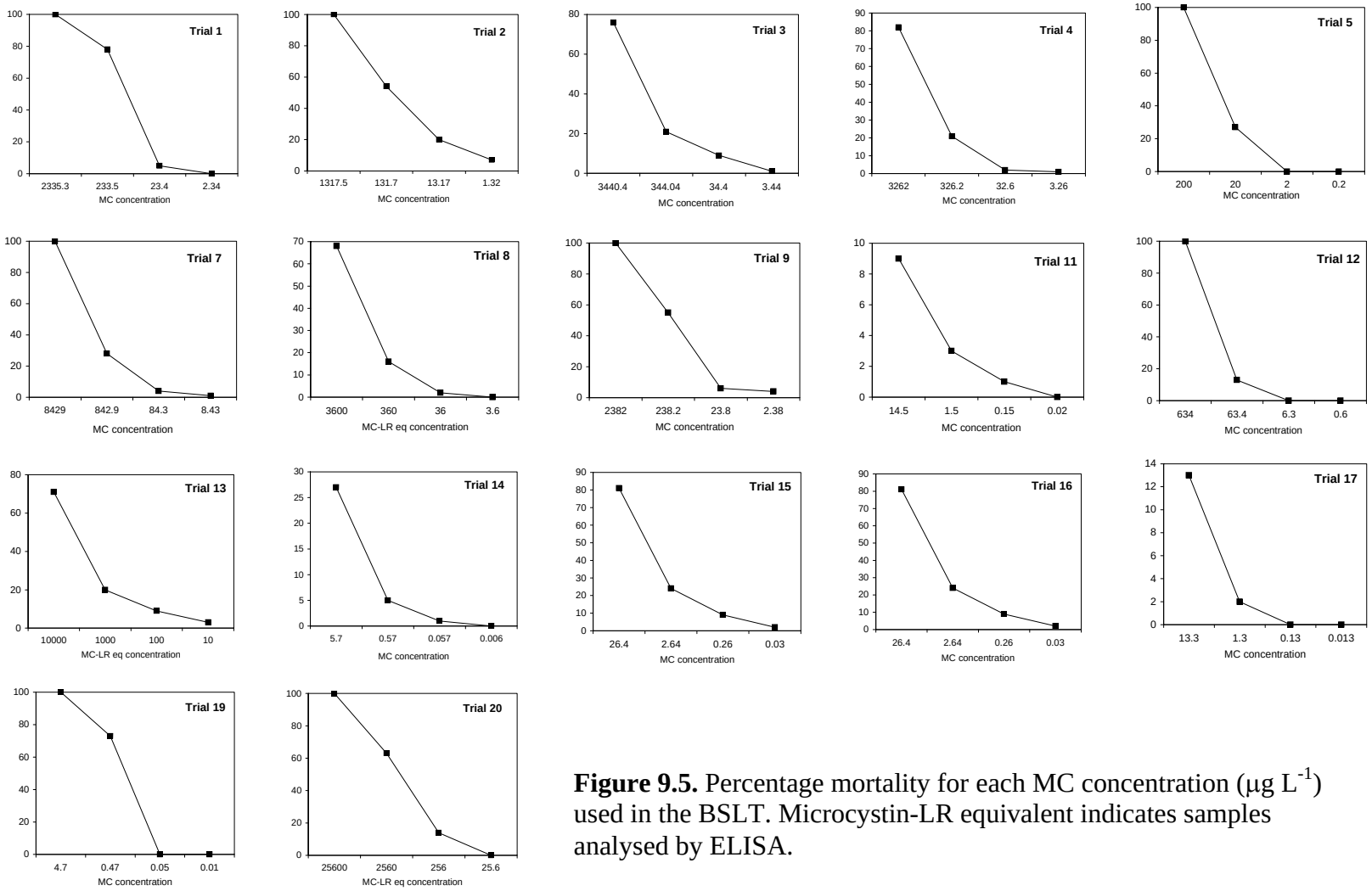



**Figure 9.5.** Percentage mortality for each MC concentration (μg $L^{-1}$) used in the BSLT. Microcystin-LR equivalent indicates samples analysed by ELISA.

## 9.4 Discussion

### *9.4.1 Comparison of ELISA with HPLC*

Immunoassays are becoming widely used as an analytical procedure for the determination of MCs in natural bloom and water samples. As HPLC facilities for the determination of MCs are not available in Western Australia, the use of the commercially available kits, such as the two kits used in this study, are useful alternatives. ELISA can be used for preliminary screening, with the identity of the individual MCs later confirmed by HPLC. In recent times, this procedure has been used to confirm toxicity in samples from public water supply reservoirs (Conti *et al.* 2005; Vieira *et al.* 2005) and recreational waterbodies (Sabour *et al*. 2002; Sabour *et al*. 2005). With a detection limit of 0.2 μg $L^{-1}$, ELISA is sufficient to detect MCs in unconcentrated lake water (Falconer 2005), and for the mass monitoring of MCs in environmental waters, it is considered beneficial for continuous surveys (Ueno *et al.* 1996b).

The MC-LR eq concentration was determined in 74% of the samples tested. High concentrations (> 1.6 μg $L^{-1}$) were detected in 31% of the samples, including several samples from lakes Herdsman, Yangebup and Emu. Herdsman and Yangebup are two lakes that produce toxic *Microcystis* blooms, as shown in the results from the previous chapter. Furthermore, the unknown samples from Herdsman Lake and Yangebup Lake (samples 1 and 11, respectively) were both highly toxic according to ELISA. As discussed in Chapter 8, Emu Lake has the potential to produce highly toxic blooms due to the presence of multiple hepatotoxin-producing genera (*Microcystis* and *Aphanizomenon*). This is further supported the ELISA and BSLT results (Table 9.6). These three lakes require serious attention during spring and summer when these toxic blooms develop and scum formation is visible.

Moderate to high toxicity was also demonstrated in samples from Bibra Lake, Hyde Park and Jackadder Lake, although there were discrepancies compared to HPLC or BSLT. The November 2001 sample from Jackadder Lake, although classified as moderately toxic by ELISA, demonstrated low toxicity by HPLC (13.5 μg $L^{-1}$) and was deemed non-toxic to the *Artemia* (Table 9.6). However, ELISA overestimated

the concentration by only 34%, which still showed the concentration of MCs in this sample to be low (39.5 μg $L^{-1}$). Likewise, sample 29 from Bibra Lake showed ELISA overestimated the concentration compared to HPLC. Unfortunately, this sample was not used in the BSLT, which would have further verified the result.

**Table 9.6.** Comparison of toxicity for the algal extracts that were evaluated by the two screening methods.

| Site | Date | Species recorded[a] | ELISA[b] | BSLT[c] | HPLC[d] |
|---|---|---|---|---|---|
| Herdsman Lake | Dec 2000 | Mf, Ma | M | nT | L |
| | May 2001 | Mf, Ma | H | T | H |
| | Feb 2003 | Mf, Ma, Ac | H | T | |
| Yangebup Lake | Aug 2000 | Ma | H | | M |
| | Oct 2001 | Mf, Ma, Ac, Ns | H | hT | H |
| | Nov 2001 | Mf, Ma | M | T | H |
| | Nov 2002 | Mf, Ma | H | T | H |
| Bibra Lake | Sep 00 | Mf, Ma, Ae | H | | L |
| | Jun 01 | Mf, Ma, Ao, Cr | M | | L |
| | Jan 02 | Mf, Ma, Ao, Ab | M | | L |
| Booragoon Lake | Oct 2000 | Ma, Mf | M | | L |
| Lake Goollelal | Dec 2000 | Mf | M | nT | L |
| | Nov 2001 | Mf | L | | L |
| Hyde Park | Nov 2000 | Ma, Mw | M | T | M |
| | June 2001 | Ma, Mw | M | T | H |
| | Nov 2001 | Ma, Mw | M | | M |
| Lake Joondalup | Dec 2000 | Mf, Ma | L | nT | L |
| Lake Claremont | Dec 2000 | Mf | M | nT | L |
| Tomato Lake | Nov 2001 | Ma, Mf, Ac | L | nT | L |
| | Feb 2002 | Ma, Mf, Ac | M | hT | L |
| | Feb 2003 | Ma, Mf, Ac | L | hT | |
| Jackadder Lake | June 2001 | Ma, Mf | M | T | H |
| | Nov 2001 | Ma, Mf | M | nT | L |
| Emu Lake | Jan 2002 | Mf, Ma, Ao | H | T | M |
| | Nov 2002 | Mf, Ao | H | T | |
| Canning River | Feb 2001 | Mf, Ac | M | hT | L |
| Serpentine River | Nov 2000 | Ns, Ae | M | T | |

[a] Refer to Table 9.1 for complete names.
[b] MC-LR equivalent; L, low toxicity (<0.1 μg $L^{-1}$); M, moderate toxicity (0.1-1.6 μg $L^{-1}$); H, high toxicity (>1.6 μg $L^{-1}$).
[c] nT, non-toxic; T, toxic; hT, highly toxic.
[d] Total MC concentration; LOD, Level of Detection; L, low toxicity (<100 μg $L^{-1}$); M, moderate toxicity (100-1000 μg $L^{-1}$); H, high toxicity (> 1000 μg $L^{-1}$).

In contrast, ELISA detected lower MC-LR eq concentrations in samples from Claremont Lake, Lake Goollelal, Tomato Lake and Neil McDougall Park. For Tomato and Claremont, these findings support the low concentrations of MCs detected by the HPLC. The two samples collected from Lake Joondalup during the same summer (December 2001 and January 2001) also showed low toxicity and were non-toxic by BSLT. The Canning River sample, although highly toxic by BSLT, produced low to moderate toxicity by ELISA and HPLC, which are specific to MCs. The Canning River sample (2/2/2001) contained *A. circinalis*, which is known producer of saxitoxin (STX) (Beltran and Neilan 2000), and *M. flos-aquae*, for which MC production is unknown (Steffensen *et al.* 1999). Frequent blooms of *A. circinalis* have been occurring in the Canning River since 1994 and have been proven highly toxic by mouse bioassay with $LD_{50}$ of 50 mg kg i.p. (J Allen, cited in Hosja and Deeley 1994). The production of MCs by *Anabaena* is yet to be reported in Australia, so it is likely this was a fast acting neurotoxin, even though there is no documented evidence of STX production by *Anabaena* in Western Australia.

In spite of the variation in toxicity shown between the two methods for Bibra Lake and Jackadder Lake, ELISA was effective in identifying Yangebup Lake, Herdsman Lake, Hyde Park and Emu Lake as highly toxic sites, and Claremont Lake, Tomato Lake, Lake Goollelal, Neil McDougall Park, Lake Joondalup and Canning River as sites that produce low concentrations of MCs. For the latter sites, cyanotoxins other than MCs may be present in the samples, especially due to the highly toxic result produced by BSLT. For this reason the production of STX by Western Australian populations of *Anabaena* needs further investigation.

### *9.4.2 Discrepancies in the ELISA results*

In the current study, the concentrations determined from ELISA often exceeded those obtained by HPLC. ELISA has been shown to overestimate MC concentrations when compared to HPLC or other chromatographic analysis (Rivasseau *et al.* 1999; Fromme *et al.* 2000, Moreno *et al.* 2005; Conti *et al.* 2005). Rivasseau *et al.* (1999) reported water samples collected from the surface layer where a bloom had occurred produced ELISA MC-LR eq concentrations three to 90 times higher than the values

obtained by liquid chromatography. Samples collected from deeper water, away from the bloom, produced ELISA concentrations that were only one to two times higher. A study by Fromme *et al.* (2000) found samples dominated by *Microcystis*, especially those with high densities, showed greater variability in concentration obtained by HPLC and ELISA. Similar to the current study and the study by Rivasseau *et al.* (1999), ELISA also overestimated the concentration of MC with the HPLC concentration amounting to only 65% of the concentration determined by ELISA (Fromme *et al.* 2000).

The overestimation of MC concentration obtained by ELISA is due to the cross-reactivity of the other MC variants and structurally related compounds. There are over 60 structural variants of MC with the commercially available ELISA kit calibrated to MC-LR. ELISA will not distinguish between the MC variants but measure the cross reactivity with the antibodies for MC-LR and determine the total MC content. Therefore, cross-reactivity will depend on the structural similarity of the MC variant to MC-LR, which can range from good (MC-RR and MC-YR) to poor (MC-LY and MC-LA). Considering this, the overestimation by ELISA in samples 31, 34 and 36 shows MC-RR responding strongly to the antibodies. However, cross-reactivity does not explain the high number of overestimated samples collected from Yangebup Lake, as these samples were dominated by MC-LR. The Yangebup samples, as well as the overestimated samples from Emu Lake, Hyde Park and Herdsman Lake, all were strongly diluted by a factor of 1:2000 to 1:8000, which would significantly lower the MC concentration. It was these samples that showed the greatest overestimation by ELISA.

Conversely, ELISA underestimates the MC concentrations due to poor cross-reactivity among the variants present in the sample. High toxicity but with low corresponding MC content shows the presence of unidentified MCs that do not react with the antibodies for MC-LR. Chromatography only identified the three structural variants MC-LR, MC-RR and MC-YR. Microcystin-LR was identified in all samples analysed by HPLC but varied in relative proportion by 7 to 100 %. Only four samples had concentrations underestimated by ELISA (samples 14, 23, 26 and 38).

Samples 23 and 38 gave values below the detection limit. However, both samples were shown to have low concentrations of MCs by HPLC (5.7 and 10.15 μg $L^{-1}$). For samples 14 and 26, MC-LR only comprised a small percentage of the total MC concentration with the predominant variants being MC-RR. However, this does not explain the underestimation as MC-RR has good cross-reactivity with MC-LR.

ELISA is also more sensitive than other analytical methods and can detect MC in samples where HPLC was unable to do so. This was observed in sample 36, collected from Lake Claremont in December 2000. Only *M. flos-aquae* was identified in the sample, with low MC content (14.5 μg $L^{-1}$ HPLC). ELISA detected a concentration of 76 μg $L^{-1}$, which is still low, compared to other samples. Evidently, low MC-LR eq concentration was also detected in the other Lake Claremont *M. flos-aquae* sample (sample 35), which was collected during the same bloom period. Lake Goolellal was the only other test site to contain only *Microcystis flos-aquae*. Low concentrations were also detected in these samples (0.52 μg $L^{-1}$ and < 0.1 μg $L^{-1}$). It is evident in these results from the two lakes that MCs are associated with *M. flos-aquae* blooms, but are less toxic than blooms that contain *M. aeruginosa*.

It was evident from the results that some samples needed further dilution. As the algal samples used were collected using a phytoplankton net and thus represented concentrated cell material, high concentrations of MCs were anticipated, so the dilution factors were determined considering this. Nevertheless, many of the samples with MC-LR eq concentrations outside the detection range were those that did not have their MC concentration predetermined by HPLC and adequate dilution factors could not be determined by considering the dominant species present and cell numbers (cells $mL^{-1}$). This might be interpreted as supporting the view that species composition and cell numbers may not always reflect the toxin concentration.

### *9.4.3 Sample pre-treatment*

The difference in sample pre-treatment using HPLC and ELISA provides a more feasible explanation for the discrepancies in the results obtained for the two methods. Rapala *et al.* (2002) evaluated the suitability of ELISA and HPLC for detecting MC

variants in pure toxin extracts, cultured material and field samples and found the HPLC analyses of the field samples produced lower values compared to ELISA. This was at least partly due to the need for concentration of the HPLC samples and the extra purification step needed to eliminate impurities, while the samples for ELISA were analysed directly (Rapala *et al.* 2002). Likewise with the current study, the ELISA samples were analysed directly after freeze thawing, sonication and filtration, whereas more extensive clean-up procedures were used for the HPLC samples. The partial decomposition of the toxins due to freezing and length of storage also needs to be considered as an explanation for the variation in the results. Due to lack of funding, many samples had been kept frozen, for up to three years before being used for ELISA and BSLT. Cummings (2002) evaluated the effect of freezing samples before MC analysis and found frozen algal samples tend to produce lower toxin concentration compared to unfrozen samples. Therefore, it is likely that the frozen samples analysed by ELISA may have contained higher concentrations of MC, if they were tested immediately after collection.

The amount of cell material and additional organic compounds present in the algal samples may also explain the variation in results. Any pigment remaining in algal extracts can interfere or mask the amount of MC the assay can read. This interference can be reduced by choosing the appropriate solvent (methanol or water/methanol/butanol mixture) (Carmichael and An 1999). The samples used in this study were aqueous extracts, with no use of a chemical solvent. Furthermore the samples were collected using a phytoplankton net or hand collected from areas showing scum accumulation. Fromme *et al.* (2000) and Moreno *et al.* (2005) found ELISA overestimated toxin content in samples with higher cyanoprokaryota biovolume or cell counts. Similarly, Rivasseau *et al.* (1999) reported greater variation in toxin content and higher ELISA concentrations in algal samples collected from the surface layer where a bloom occurred compared to samples collected from deeper water, away from the bloom. It is possible that ELISA overestimated the concentration of MC in the samples that contained high amounts of cell material so more effective sample preparation is required especially for the samples recording high cell counts.

### *9.4.4 Sample screening using BSLT*

The suitability of *Artemia* larvae for the screening of MCs in both isolated strains and a range of environmental samples has been well established (Kiviranta *et al.* 1991; Lahti *et al*. 1995; Vezie *et al.* 1996; Lee *et al*. 1999), with the larvae being quite sensitive to MCs, which allows moderate to high concentrations to be reliably detected. In the present research, the *Artemia* were responsive to the range of MCs present in the field samples. The $LC_{50}$ values obtained ranged from 0.25 to 5520 μg $L^{-1}$, which clearly shows the array of toxicity, demonstrated by cyanoprokayota blooms in the SCP wetlands

The algal extracts used in this study were deemed toxic, if the highest dose affected over 50% of the *Artemia* population. Of these, the highly toxic trials were those that produced a response in the *Artemia* at the 1:100 and 1:1000 dilutions, with the $LC_{50}$ comprising a small percentage of the total MC concentration. Kiviranta *et al.* (1991), in one of the first studies to evaluate the use of *Artemia* in screening for cyanotoxins, adopted a similar set of criteria. Kiviranta *et al.* (1991) considered bloom material to be toxic if the median concentration (5.0 μg $L^{-1}$) affected over 20% of the larvae and, simultaneously, the highest dose (8.5 μg $L^{-1}$) affected over 50% of the larvae. These criteria provided the best correlation with mouse bioassay. Therefore, using these criteria the toxicity of the algal extracts used in this study was established (Table 9.6).

Toxic to highly toxic samples were collected from Herdsman Lake, Yangebup Lake, Hyde Park and Emu Lake, which related well with the ELISA and HPLC results. Non-toxic samples were collected from Lake Goollelal, Lake Joondalup, Lake Claremont, Tomato Lake and Jackadder Lake, which matched the low and moderate toxicity determined by HPLC and ELISA, respectively (Table 9.6). One sample from Herdsman Lake was also classified as non-toxic, similar to the ELISA and HPLC result.

However, there was an inconsistency between the three methods in the results obtained for Tomato Lake (Feb 2002 and Feb 2003). The BSLT found the samples to

be highly toxic, yet ELISA and HPLC showed MC concentration to be low. As previously discussed, the study only quantified the concentration of MC, so the presence of additional cyanotoxins is unknown. Samples containing other potentially toxic species, such as *A. circinalis, A. ovalisporum, A. bergii* var. *limnetica, C. raciborskii* and *N. spumigena,* are likely to contain STX, CYL and NOD, which may cause mortality to the *Artemia*. Both ELISA and BSLT proved the *N. spumigena* samples from the upper Serpentine River were toxic, which would indicate the presence of NOD. Likewise, the presence of *A. circinalis* in Tomato Lake, and high mortality in *Artemia*, indicates the possible presence of STX. These wetlands are worthy of additional testing by more specific means like HPLC.

The lowest MC concentrations to cause an observed effect in the larvae were 0.26 (Tomato Lake; trial 15), 0.29 μg $L^{-1}$ (Tomato Lake; trial 16) and 0.47 μg $L^{-1}$ (Canning River; trial 19), which supports the view that the samples containing *A. circinalis* also contained STX. The high percentage of mortalities in trials 15 (81% at 26.39 μg $L^{-1}$), 16 (81% at 29 μg $L^{-1}$) and 19 (100% at 4.7μg $L^{-1}$) shows the presence of toxins other than MCs. Trials 15, 16 and 19 were still killing at doses that produced no response in the other trials, suggesting the presence of additional cyanotoxins. Kiviranta *et al* (1991) reported neurotoxic strains to be more toxic to *Artemia* than hepatotoxic strains, with the effect exhibited through the inability of the *Artemia* to swim forward (Lahti *et al.* 1995). Atypically swimming *Artemia* were observed in the Tomato Lake samples. Because STX is Na-channel blocking neurotoxin, which inhibit nerve impulse transmission (Humpage *et al.* 1994), sciatic nerve preparations using the Queensland cane toad (*Bufo marinus*) may be effective in screening for STX in Western Australian samples. Even the desert locust *Schistocera gregaria* has been used for the detection of STX (McElhiney *et al.* 1998).

Trials 6 and 10, from Herdsman Lake and Jackadder Lake, were not lethal to the larvae, even at the higher doses. In studies where there was no response observed in the larvae, or where samples were considered non-toxic (defined by < 10 % mortality), no hepatotoxins had been detected in the algal material (Lahti *et al.* 1995;

Lee *et al.* 1999; Metcalf *et al.* 2002). Microcystins were detected in the extract used for trial 6 (80.9 μg $L^{-1}$), however it may have been too low to produce a response. The other two trials from Herdsman Lake (trials 7 and 8) also showed low mortalities (< 5%) at 84.3 μg $L^{-1}$ and 36 μg $L^{-1}$, respectively. Less than 2% mortality can be related to natural mortality (Campbell *et al.* 1994). Considering this, the Herdsman Lake samples show low to moderate toxicity by *Artemia* bioassay, as high MC concentrations are required to kill over 50% of the larvae.

A similar result was demonstrated in trial 10 from Jackadder Lake. A dose of 13.5 μg $L^{-1}$ failed to produce a response, as did a concentration of 23.8 μg $L^{-1}$ in trial 9. No deaths were recorded at 23.8 μg $L^{-1}$, but three deaths were recorded at 2.38 μg $L^{-1}$. It is more likely the later is related to natural mortality. A similar dosage was given in trial 17 (13.35 μg $L^{-1}$), and like trial 10, low mortality (13%) was observed. These results for Herdman Lake and Jackadder Lake imply that with the dilution of the concentrated samples, the harmful affect of the toxins was weakened. The diluted samples would be indicative of water samples, and as illustrated by BSLT, at concentrations less than 100 μg $L^{-1}$ they were non-toxic.

The low dose used in trial 18 (0.9 μg $L^{-1}$), from Lake Goollelal, can be considered too low to affect the larvae. Nevertheless, the lack of response in this trial supports the view that Lake Goollelal produces blooms (dominated by *M. flos-aquae*) of low toxicity. Lake Claremont (trial 11) was the only other site to have samples containing only *M. flos-aquae* with only 9 % mortality observed at 14.5 μg $L^{-1}$, the highest dose. Based on the results of this research project it can be concluded that MCs are associated with *M. flos-aquae* blooms, but as stated by Orr *et al.* (2004) and Marsálek *et al*. (2001), such blooms exhibit low toxicity.

Tomato Lake had three samples (trials 14, 15 and 16) tested by the BSLT, each from different years. Similar results were obtained for trials 15 (14$^{th}$ February 2002) and 16 (4$^{th}$ February 2003) with 81% mortality at 26.39 μg $L^{-1}$ and 29 μg $L^{-1}$, respectively. The concentrations for the 1:10 and 1:100 concentrations were also similar. This was also evident in the Hyde Park samples. Trials 4 and 5, from

different seasons, were considered toxic, but high percentage mortality was only recorded at the highest doses and no deaths were recorded at the 1:100 and 1:1000 dilutions. This similarity between the years of sampling shows BSLT suitability for long-term sampling.

## 9.5 Conclusions

Most accounts of toxic blooms in the SCP wetlands are reported from the major river systems and the toxicity is assumed using the presence and cell counts of potentially toxic species. In contrast, this study used chemical, immunological and biological techniques to identify toxic blooms in wetlands. These trials indicate the need for using rapid, accurate and cost-effective toxicity tests in Western Australia. Both methods are less precise in quantification and identification than HPLC, but have proved useful for toxin screening. New information is also gained regarding the major toxin-producing species from Western Australian waters.

Overall, the two techniques showed algal samples from lakes Emu, Herdsman, Hyde Park, Jackadder and Yangebup were the most toxic. The presence of *Aphanizomenon* in Emu Lake, *Anabaena* in Herdsman Lake and *Nodularia* in Yangebup Lake indicates the presence of cyanotoxins other than MC. Samples from Claremont Lake, Lake Goollelal and Lake Joondalup were the least toxic. The sole presence of *Microcystis flos-aquae* in Lake Claremont and Lake Goollelal suggests blooms of predominantly this species are less toxic than blooms of *Microcystis aeruginosa*. Two samples from Tomato Lake and one sample from the Canning River were proven highly toxic by BSLT, even though both HPLC and ELISA found low MC content. The presence of *Anabaena* suggests these samples may contain STXs. Artemia bioassays respond to a much broader range of bioactive compounds including STX, CYL and novel as yet uncharacterised compounds. The ELISA was unable to detect MC-LR equivalents in two samples from Bibra Lake, even though the sample contained *Microcystis*. The presence of *A. ovalisporum* and *A. bergii* var. *limnetica* suggests these samples may contain CYL. Moderate MC toxicity was demonstrated in two other Bibra Lake samples. Presence of other cyanotoxins should be considered when undertaking future testing of samples from these wetlands.

# Chapter 10: Conclusions and Recommendations

## 10.1 Synthesis

The premise at the commencement of this thesis was that harmful cyanoprokaryota blooms in the freshwater lakes and sumplands of the Swan Coastal Plain (SCP), Western Australia, had not been thoroughly studied and described. Research defining the temporal frequency, spatial distribution, morphological diversity and toxicity of the common bloom-forming species in this region are crucial for understanding bloom ecology in temperate regions of Australia. Only a few past studies had identified cyanoprokaryota blooms as being problematic in the urban lakes of the SCP (Congdon and McComb 1976; Gordon *et al.* 1981; Bayley *et al.* 1989; Hosja and Deeley 1994). Information on the current situation was absent. By a multidisciplinary approach, this research provided the first study of toxic freshwater cyanoprokaryota blooms on the SCP, presenting baseline data that can be used in future environmental, taxonomic and toxicity studies to broaden the scope of cyanoprokaryota research in Western Australia. The objectives stated at the beginning of this thesis were achieved through studying cyanoprokaryota blooms in a number of urban lakes over four years and including the major river systems in the study. Following are the key findings from this thesis and recommendations for future research.

### *10.1.1 Ecology*

Multivariate analysis of the physico-chemical data and cyanoprokaryota samples collected from the study lakes revealed distinct species assemblages and a distribution pattern that could be related to environmental conditions. Three spatial distribution patterns emerged; sites containing only *Microcystis*, sites containing both *Microcystis* and *Anabaena* and sites containing blooms comprised of multiple genera. This distribution pattern did not correlate with the traditional consanguineous wetland classification defined by Arnold (1990) and Semeniuk (1996) but was more a reflection of the current water quality conditions experienced at each site. For example, the wetlands of the South West Corridor were hyposaline to hypersaline

sumplands that were less likely to experience water blooms, whereas the wetlands the Western Suburbs and East Beeliar Regional Park represented highly eutrophic freshwater lakes, and are most likely to experience extensive *Microcystis* blooms or blooms of multiple genera.

Measuring only three environmental variables (water temperature, pH and salinity) over the four years of sampling provides limited explanation as to why this distribution pattern was observed. Through the use of statistical analysis, salinity/electrical conductivity showed the greatest variation between lakes because of season, location, and depth; and therefore was used to discuss the species distribution pattern and community structure within a bloom. Amalgamating the results of chapters 4 and 6, it can be concluded that other factors such as water temperature, phosphorus concentration and species competition can also influence distribution of bloom-forming species in the SCP lakes. Further research is required to establish the roles of nitrogen and phosphorus in bloom formation.

The main bloom-forming species contributing to this distribution pattern were *M. flos-aquae*, *A. circinalis*, *A. bergii* var. *limnetica*, *Aphanizomenon ovalisporum* and *Anabaenopsis elenkinii*. For *A. bergii* var *limnetica* and *A. ovalisporum* these are the first published reports in the southwest. The distribution of *M. flos-aquae*, *A. ovalisporum* and *A. bergii* var. *limnetica* are not as well defined in Australia as that of *M. aeruginosa*, *A. circinalis* and *A. elenkinii*. The distribution of these three species could not be correlated to environmental conditions because of the limited number of variables that were measured. In addition, their distribution was not isolated to only one wetland suite or set of environmental conditions, but was geographically spread across the study area.

*Microcystis aeruginosa* is the most abundant and widespread species throughout temperate wetlands of Australia, however in the SCP wetlands, *M. flos-aquae* was the more frequently observed cyanoprokaryote. Even though culture studies were not used to confirm *M. flos-aquae* to be more salt tolerant than *M. aeruginosa,* the field data did show *M. flos-aquae* to withstand a wider range of salinities and dominate

sites that recorded higher summer salinity. There is no other field study in Australia to support this result as natural *Microcystis* populations usually contain a combination of morphospecies and genotypes, which can vary in salt tolerance (Orr *et. al.* 2004; Verspagen *et al.* 2006). It is important to establish the salinity tolerance of this SCP strain of *M. flos-aquae* to determine the extent of its occurrence in freshwater lakes that are becoming hyposaline.

The southwest of Western Australia is becoming warmer and drier (IOCI 2002), which will have a noticeable effect on this recognised distribution pattern. Water availability within the study lakes will be affected by the decrease in rainfall, higher temperatures and increase in evaporation rates (Department of Water 2007). Reduced water levels were a barrier to bloom development in some seasonal lakes used in this study, while the evapo-concentration of salts affected community structure and species succession. This may be more pronounced as a result of global warming. In sub-tropical and tropical regions of Queensland, it is predicted that global warming will produce seasonally earlier blooms of *A. circinalis* and *M. aeruginosa,* while *C. raciborskii* will attain higher cell densities for longer periods (Shaw *et al.* 2001). For the SCP wetlands, the proposed cyanoprokaryote succession is harder to define because of the lack of comparable predictive research in Western Australia. The ongoing changes to climate will impact the recognised distribution pattern and occurrence of species that bloom in the SCP wetlands. Based on the observation presented in this thesis, it is predicted that *M. aeruginosa* and *A. circinalis* dominance in spring-early summer is probable, with *M. flos-aquae*, *A. ovalisporum*, *A. bergii* var. *limnetica* and *A. elenkinii* the main bloom forming species in late summer.

The impact of climate change on the distribution of toxic species has been already shown in the recent increase in the worldwide occurrence of *C. raciborskii* in temperate regions (Saker *et al.* 2003; Bouaïcha and Nasri 2004; Manti *et al.* 2005; Mohamed 2007). Global warming and the physiological tolerance of *C. raciborskii* to a wide range of climatic conditions have caused the spread of this species to temperate wetlands (Briand 2004), including the SCP lakes. The presence of

*C. raciborskii* in Bibra Lake during winter 2001 was a significant finding from this thesis. In the future, the distribution of *C. raciborskii* may expand to neighbouring lakes of the East Beeliar Regional Park. It is likely that we may witness more frequent occurrences in Bibra Lake and Emu Lake, which share a similar species assemblage.

### *10.1.2 Morphology*

While research is progressing towards utilising genetic markers for strain identification and toxin production, this project relied on traditional methods. Cyanoprokaryota samples were morphologically examined and screened for toxins using high performance liquid chromatography (HPLC). Species identification based on phenotypic criteria lead to a taxonomic guide of local bloom-forming species. The detailed morphological descriptions, illustrations and record of distribution will benefit the identification of harmful species. The major disadvantage of phenotypic characterisation is that toxic and non-toxic strains cannot be distinguished by sight. Additionally morphological features can vary with changing environmental conditions and therefore differentiating between closely related morphospecies can be difficult. Molecular characterisation aims to resolve these issues and is an area of research that should be further developed in Western Australia.

Although DNA analysis is currently advancing rapidly, there will always be a place in cyanoprokaryota research for traditional morphological identification. Light microscopy continues to provide initial screening for toxic species (Lawton and Edwards 2008) and preliminary characterisation of the community (Wilhelm 2008) based on the diversity of phenotypic traits (Komárek 2005). New morphological data are important in future revisions and new taxonomic descriptions of cyanoprokaryota (Litvatitis 2002). A polyphasic approach is needed for precise species identification (Willame *et al.* 2006; Haande *et al.* 2007; Komárek and Zapomelová 2007). The current study will assist with re-evaluating Australian taxonomy and provide the basis for future molecular work in Western Australia.

Attention was given to the classification of *Anabaena spiroides* f. *spiroides* (Morphotype 2) (Baker 1991) as a new Australian taxon - *Anabaena* sp. nova of Baker and Fabbro (2002). *Anabaena* is a very diverse and variable genus of cyanoprokaryotes (Komárek 2005; Zapomelová *et al.* 2008) and this is particularly true for *A. spiroides* and *A. oumiana,* which can be easily misidentified. Morphological descriptions and illustrations provided by various authors illustrate the close resemblance between *Anabaena* sp. nova, *Anabaena spiroides*, *Anabaena oumiana, A. reniformis* and *Anabaena torques-reginae* (Li *et al.* 2000; Watanabe *et al.* 2004; Komárek 2005; Komárek and Zapomelová 2007). The latter species are yet to be identified in Australia, although Werner *et al.* (2007) and Li *et al.* (2000) suggests *Anabaena* sp. nova may be the same as *A. oumiana* and *A. reniformis.* However there is no Australian literature to support this observation. Until molecular analysis confirms similarities between South American, African and Asian populations of *A. oumiana*, it cannot be justified that *Anabaena* sp. nova is a new taxon. This endorses the need for future molecular work undertaken in Australia to include the temperate strains collected from the SCP wetlands.

The spherical akinetes located adjacent to the heterocytes was the distinguishing characteristic evident in all *A. spiroides* f. *spiroides* (Morphotype 2) samples collected in this study, easily distinguishing this species from *A. circinalis* and *A. flos-aquae*. The reoccurrence of *A. spiroides* in lakes Tomato, North and Joondalup allows samples to be collected to provide a detailed account of morphology. Where the South American populations of *A. oumiana* have been compared (Werner *et al.* 2007), the Australian populations of *A. spiroides* f. *spiroides* (Morphotype 2) need to also be re-evaluated to finalise the identity of this species. Taxonomic relationship between strains of *A. oumiana* and *Anabaena* sp. nova need to be confirmed by 16s rRNA sequencing, as previously done for morphologically similar species of *Anabaena* (Li and Watanabe 2002; Komárek and Zapomelová 2007), Portuguese and Australian strains of *Cylindrospermopsis raciborskii* (Saker *et al.* 2003) and strains of *Phormidium* from different geographical regions (Marquardt and Palinska 2007). Molecular techniques may provide more reliable tools for species recognition (Litvaitis 2002), and will resolve any issues in classifying to species.

### *10.1.3 Toxicology*

Microcystins associated with the *Microcystis* dominated blooms were diverse in structure and concentration. Total concentration varied between study sites, irrespective of cell concentration. Although no attempt was made to isolate the species responsible for the production of microcystins, it can be inferred from the results that *M. aeruginosa* and *M. flos-aquae* are both potentially toxic in Western Australian wetlands.

In recreational water bodies, awareness is being raised on the acute health effects and risks associated when in contact with the bloom material. The relevance of the toxicity analysis presented in this thesis is its usefulness to forewarn which lakes experience toxic spring-summer blooms, and therefore form the basis of an early warning system. Total microcystin concentrations in bloom material from Herdsman Lake, Hyde Park, Yangebup Lake, Jackadder Lake and Bibra Lake were high enough to require a health risk assessment.

Due to financial and time constraints, the project focused primarily on microcystins, so further studies are required investigating the occurrence of saxitoxins in the freshwater lakes of the SCP. This thesis provides evidence that saxitoxins may be present in blooms containing *Anabaena*, exhibited through the abnormally moving *Artemia* observed in the Brine Shrimp Lethality Test. Saxitoxin production by Western Australian populations of *Anabaena* is yet to be confirmed. The enzyme-linked immunoassay (ELISA) and *Artemia* bioassay used in this study proved valid in the preliminary toxin screening of bloom samples collected from the study lakes and Canning and Serpentine rivers. The initial screening of samples, followed by qualitative and quantitative analysis if samples return positive, provides the essential decision making data needed for successful management strategies (Lawton and Edwards 2008). In the present day, ELISA test kits for saxitoxins and cylindrospermopsin have become more widely available and would be suited to sample screening in Western Australia. These immunoassays provide a cost effective method for identifying a collection of toxins in water and bloom material, and allows

a large number of samples to be tested, which will extend on the findings presented in this thesis.

## 10.2 Recommendations for future research

The current study provided valuable insight into the distribution and toxicity of species commonly observed in temperate wetlands and provided a reference to further ecological and morphological research in Western Australia. The following recommendations to further research in this field are made:

- Ongoing collection of phytoplankton samples and physico-chemical data will show long-term changes in the distribution pattern. Additional sampling in late autumn-winter will determine if toxic blooms occur continuously throughout the year.
- Monitoring Bibra Lake and Yangebup Lake for future occurrences of *C. raciborskii* and *N. spumigena* will determine whether these are sporadic events, or if their distribution has extended to the surrounding East Beeliar wetlands.
- The distribution of *M. flos-aquae*, *A. elenkinii*, *A. ovalisporum* and *A. bergii* var. *limnetica* in fresh to hyposaline conditions suggests these species are salt tolerant, therefore their response to varying salt concentrations in culture should be examined.
- Molecular characterisation would complement the morphological work undertaken in this thesis and verify taxonomic accuracy, as well as differentiate between toxin producing and non-toxin producing strains of *Microcystis*.
- As this was a study focusing on microcystins in *Microcystis* blooms, further studies can confirm the production of saxitoxins or cylindrospermopsin in Western Australian strains of *A. circinalis*, *C. raciborskii* and *A. ovalisporum*, respectively. Likewise, microcystin production by *M. flos-aquae* requires further investigation using isolates from natural populations.

- Implement the use of rapid, cost-effective methods in Western Australia for the detection of cyanotoxins in environmental samples.
- This preliminary research forms the foundation to explore the relationship between the physico-chemical environment and total cyanotoxin concentration, diversity of variants and the concentration of the intracellular and extracellular fraction. The spatial and temporal differences in microcystin concentration can be further investigated.

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# Appendix A: Photographs of the Study Sites


**Plate A1.** Wanneroo Linear Lakes (1) Lake Joondalup and (2) Lake Goollelal. Wetlands of the Western Suburbs (3) Lake Gwelup; (4) Lake Jackadder; (5) Herdsman Lake (Floreat Waters) and (6) Lake Monger.


**Plate A2.** Wetlands of the Western Suburbs (7) Perry Lakes Reserve; (8) Lake Claremont; (9) Shenton Park and (10) Hyde Park. Wetlands of the Inner Central Area (11) Emu Lake.


**Plate A3.** Wetlands of the Inner Central Area (12) Lake Bungana; (13) Lake Brearley; (14) Tomato Lake and (15) Neil McDougall Park. Wetlands of the Eastern Coastal Plain (16) Forrestdale Lake and (17) Mary Carroll Park (main water-body of the northern lake).

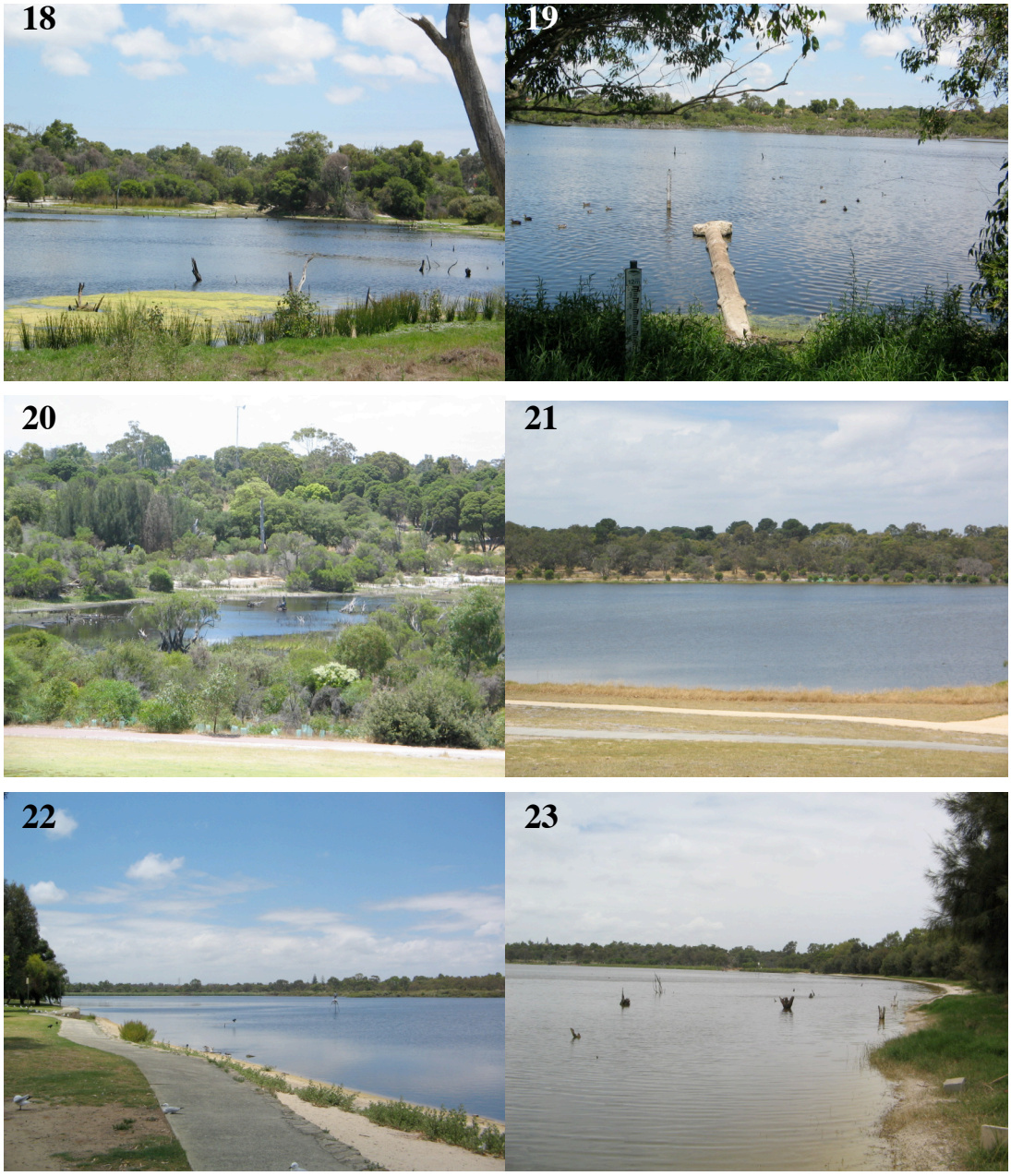


**Plate A4.** Wetlands of the East Beeliar Regional Park (18) Blue Gum Lake. (19) Booragoon Lake; (20) Piney Lakes Reserve; (21) North Lake (22) Bibra Lake and (23) Yangebup Lake.




**Plate A5.** Wetlands of the East Beeliar Regional Park (24) Thomsons Lake. Wetlands of the South West Corridor (25) Manning Lake; (26) Market Garden (Swamp 1) and (27) Lake Coogee.

# Appendix B: Environmental Data

**Table B1.** Environmental data recorded from the Wanneroo Linear lakes.

| | pH | Temp (°C) | Salinity (ppt) | EC (mS cm$^{-1}$) |
|---|---|---|---|---|
| **Lake Joondalup** | | | | |
| 24/2/00 | 9.38 | 27.83 | 2.19 | 4.31 |
| 13/11/00 | 10.12 | 23.82 | 0.69 | 1.51 |
| 22/11/00 | 10.2 | 26.8 | 0.92 | 1.68 |
| 4/12/00 | 9.66 | 26.38 | 0.92 | 1.76 |
| 20/12/00 | 8.91 | 24.3 | 1.21 | 2.14 |
| 9/1/01 | 8.97 | 31.2 | 1.57 | 3.09 |
| 24/1/01 | 8.38 | 23.05 | 2.19 | 3.50 |
| 6/2/01 | 9.15 | 23.9 | 2.57 | 5.33 |
| 4/7/01 | 7.14 | 13.47 | 1.07 | 1.77 |
| 14/9/01 | 7.72 | 18.5 | 0.58 | 1.02 |
| 19/10/01 | 9.55 | 20.03 | 0.69 | 1.21 |
| 30/10/01 | 9.75 | 20.5 | 0.70 | 1.24 |
| 15/11/01 | 8.2 | 26.1 | 0.81 | 1.41 |
| 18/12/01 | 8.87 | 30.57 | 0.90 | 1.81 |
| 9/1/02 | 9.25 | 27.58 | 1.15 | 2.29 |
| 13/2/02 | 8.63 | 30.73 | 2.08 | 4.04 |
| 7/11/02 | 8.62 | 26.3 | 0.75 | 1.60 |
| 13/11/02 | 9.17 | 27 | 0.77 | 1.64 |
| 28/11/02 | 9.35 | 29.13 | 0.86 | 1.83 |
| 2/12/02 | 9.44 | 24 | 0.87 | 1.85 |
| 13/12/02 | 9.7 | 27.93 | 1.02 | 2.14 |
| 20/12/02 | 9.12 | 28.4 | 1.12 | 2.37 |
| 7/1/03 | 9.11 | 26.83 | 1.68 | 3.15 |
| 23/1/03 | 9.35 | 27.7 | 1.12 | 2.25 |
| 30/1/03 | 9.44 | 21.93 | 2.72 | 4.99 |
| 4/2/03 | 9.6 | 24.2 | 2.51 | 4.63 |
| 11/2/03 | 9.06 | 27.27 | 3.26 | 5.92 |
| **Lake Goolelal** | | | | |
| 22/11/00 | 8.52 | 27.73 | 0.61 | 1.12 |
| 4/12/00 | 7.74 | 26.28 | 0.61 | 1.11 |
| 20/12/00 | 8.23 | 24.4 | 0.63 | 1.12 |
| 22/2/01 | 6.99 | 23.4 | 0.76 | 1.32 |
| 4/10/01 | 7.58 | 22.2 | 0.47 | 0.84 |
| 19/10/01 | 9.29 | 20 | 0.50 | 0.89 |
| 30/10/01 | 9.67 | 20.23 | 0.50 | 0.88 |
| 15/11/01 | 7.76 | 27.67 | 0.57 | 1.00 |
| 9/1/02 | 8.88 | 28.9 | 0.58 | 1.20 |
| 13/2/02 | 9.37 | 29.22 | 0.69 | 1.40 |
| 28/2/02 | 9.62 | 24.6 | 0.72 | 1.37 |
| 7/11/02 | 8.47 | 28.2 | 0.48 | 1.05 |

| | | | | |
|---|---|---|---|---|
| Lake Goolelal (*continued*) | | | | |
| 13/11/02 | 8.39 | 27 | 0.50 | 1.08 |
| 28/11/02 | 8.72 | 28.9 | 0.53 | 1.15 |
| 13/12/02 | 8.8 | 29.33 | 0.49 | 1.07 |
| 20/12/02 | 8.7 | 29.3 | 0.86 | 1.27 |
| 7/1/03 | 9.05 | 26.87 | 0.75 | 1.45 |
| 23/1/03 | 9.13 | 28.83 | 0.81 | 1.56 |
| 30/1/03 | 9.31 | 22.5 | 0.86 | 1.65 |
| 4/2/03 | 9.09 | 24.97 | 0.83 | 1.59 |
| 20/2/03 | 8.83 | 28.13 | 0.88 | 1.69 |

**Table B2.** Environmental data recorded from the wetlands of the Western Suburbs.

| | pH | Temp (°C) | Salinity (ppt) | EC (mS $cm^{-1}$) |
|---|---|---|---|---|
| **Lake Gwelup** | | | | |
| 24/2/00 | 9.17 | 25.27 | 0.20 | 0.44 |
| 3/8/00 | 8.19 | 16.25 | 0.14 | 0.28 |
| 7/9/00 | 8.39 | 17.85 | 0.16 | 0.27 |
| 14/9/00 | 7.94 | 18.5 | 0.10 | 0.30 |
| 25/9/00 | 7.78 | 21.58 | 0.11 | 0.32 |
| 4/10/00 | 8.59 | 21.3 | 0.19 | 0.41 |
| 10/10/00 | 8.18 | 19.95 | 0.16 | 0.33 |
| 22/11/00 | 9.39 | 26.1 | 0.21 | 0.41 |
| 4/12/00 | 9.33 | 25.2 | 0.19 | 0.37 |
| 20/12/00 | 9.3 | 23.9 | 0.19 | 0.37 |
| 9/1/01 | 9.43 | 29.8 | 0.19 | 0.42 |
| 24/1/01 | 7.78 | 22.05 | 0.22 | 0.40 |
| 22/2/01 | 8.01 | 26.7 | 0.38 | 0.67 |
| 8/6/01 | 8.18 | 14.43 | 0.44 | 0.77 |
| 22/6/01 | 8.69 | 18.5 | 0.31 | 0.54 |
| 4/10/01 | 8.44 | 20.57 | 0.11 | 0.21 |
| 30/10/01 | 10.15 | 21.87 | 0.10 | 0.20 |
| 18/12/01 | 8.96 | 30.48 | 0.12 | 0.27 |
| 9/1/02 | 8.07 | 28.68 | 163.00 | 0.35 |
| 22/1/02 | 8.04 | 31 | 0.21 | 0.44 |
| **Jackadder Lake** | | | | |
| 13/11/00 | 9.67 | 26.38 | 0.18 | 0.41 |
| 22/11/00 | 9.21 | 24.85 | 0.23 | 0.44 |
| 4/12/00 | 8.29 | 25.3 | 0.25 | 0.46 |
| 20/12/00 | 8.85 | 24.1 | 0.27 | 0.51 |
| 9/1/01 | 9.25 | 31 | 0.30 | 0.62 |
| 24/1/01 | 8.21 | 23.6 | 0.29 | 0.52 |
| 22/2/01 | 9.11 | 24.7 | 0.41 | 0.73 |
| 9/5/01 | 9.37 | 18.78 | 0.46 | 0.83 |
| 8/6/01 | 8.193 | 13.83 | 0.29 | 0.53 |
| 22/6/01 | 8.52 | 15.25 | 0.28 | 0.50 |
| 14/9/01 | 8.39 | 17.7 | 0.18 | 0.34 |
| 4/10/01 | 8.35 | 20 | 0.18 | 0.33 |
| 30/10/01 | 9.29 | 21.67 | 0.20 | 0.36 |
| 15/11/01 | 7.45 | 26.07 | 0.23 | 0.42 |
| 9/1/02 | 8.33 | 27.98 | 0.27 | 0.57 |
| 22/1/02 | 8.06 | 29.8 | 0.29 | 0.61 |
| 13/2/02 | 8.34 | 29.55 | 0.33 | 0.70 |
| 28/2/02 | 8.53 | 25.83 | 0.35 | 0.68 |
| 22/3/02 | 8.51 | 27.2 | 0.37 | 0.76 |

| | | | | |
|---|---|---|---|---|
| **Herdsman Lake** | | | | |
| 10/11/00 | 8.86 | 22.23 | 0.45 | 1.00 |
| 22/11/00 | 8.74 | 24.43 | 0.56 | 1.04 |
| 4/12/00 | 8.24 | 25 | 0.56 | 1.02 |
| 20/12/00 | 8.96 | 25.28 | 0.57 | 1.05 |
| 9/1/01 | 9.43 | 28.9 | 0.52 | 1.07 |
| 24/1/01 | 7.67 | 22.83 | 0.57 | 0.99 |
| 22/2/01 | 9.01 | 25.38 | 0.58 | 1.01 |
| 9/5/01 | 9.14 | 20.1 | 0.58 | 1.05 |
| 8/6/01 | 8.34 | 15 | 0.52 | 0.91 |
| 22/6/01 | 8.84 | 15.9 | 0.52 | 0.90 |
| 14/9/01 | 8.12 | 16.73 | 0.53 | 0.95 |
| 4/10/01 | 8.08 | 19.8 | 0.54 | 0.96 |
| 19/10/01 | 9.18 | 19.76 | 0.58 | 1.03 |
| 30/10/01 | 8.99 | 23.33 | 0.60 | 1.06 |
| 15/11/01 | 7.32 | 24.8 | 0.65 | 1.15 |
| 18/12/01 | 8.12 | 28.93 | 0.64 | 1.30 |
| 9/1/02 | 8.18 | 27.33 | 0.66 | 1.34 |
| 13/2/02 | 8.52 | 28 | 0.71 | 1.43 |
| 28/2/02 | 8.57 | 24.15 | 0.72 | 1.36 |
| 22/3/02 | 8.71 | 26.78 | 0.73 | 1.46 |
| 7/11/02 | 8.16 | 25.9 | 0.71 | 1.51 |
| 13/11/02 | 8.3 | 27.43 | 0.70 | 1.50 |
| 21/11/02 | 8.31 | 27.9 | 0.76 | 1.57 |
| 28/11/02 | 8.32 | 27.63 | 0.78 | 1.67 |
| 13/12/02 | 8.47 | 27.7 | 0.83 | 1.76 |
| 20/12/02 | 8.54 | 30.9 | 0.81 | 1.73 |
| 7/1/03 | 8.64 | 27.23 | 0.79 | 1.51 |
| 23/1/03 | 8.8 | 29.13 | 0.93 | 1.78 |
| 30/1/03 | 8.58 | 25.13 | 0.93 | 1.78 |
| 4/2/03 | 8.65 | 26.3 | 0.91 | 1.25 |
| 11/2/03 | 8.78 | 29.7 | 0.92 | 1.76 |
| 20/2/03 | 8.71 | 28.6 | 0.51 | 0.99 |
| **Lake Monger** | | | | |
| 19/10/01 | 9.3 | 19.33 | 0.69 | 0.38 |
| 30/10/01 | 9.01 | 23.57 | 0.71 | 0.40 |
| 15/11/01 | 7.33 | 24.53 | 0.45 | 0.81 |
| 10/1/02 | 8.38 | 28.08 | 0.54 | 1.11 |
| 14/2/02 | 8.69 | 25.68 | 0.60 | 1.22 |
| 28/2/02 | 8.42 | 23.03 | 0.66 | 1.26 |
| 22/3/02 | 9.33 | 27.38 | 0.72 | 1.45 |
| **Perry Lakes** | | | | |
| 10/11/00 | 8.31 | 24.26 | 0.39 | 0.88 |
| 22/11/00 | 8.68 | 28 | 0.61 | 1.14 |
| 4/12/00 | 7.96 | 26.05 | 0.60 | 1.10 |

| | | | | |
|---|---|---|---|---|
| Perry Lakes (*continued*) | | | | |
| 14/9/01 | 8.4 | 18 | 0.18 | 0.34 |
| 4/10/01 | 8.5 | 20.45 | 0.20 | 0.37 |
| 30/10/01 | 9.29 | 24.8 | 0.24 | 0.48 |
| 15/11/01 | 7.25 | 24.4 | 0.33 | 0.59 |
| 9/1/02 | water being pumped in next to the collection site to increase water levels | | | |
| 24/1/02 | 7.91 | 30.68 | 0.65 | 1.32 |
| 13/2/02 | 7.73 | 28.5 | 0.72 | 1.46 |
| **Lake Claremont** | | | | |
| 11/8/00 | 9.21 | 13.3 | 2.04 | 3.01 |
| 7/9/00 | 9.37 | 18.1 | 1.86 | 2.86 |
| 14/9/00 | 9.15 | 19.4 | 1.18 | 3.18 |
| 21/9/00 | 9.3 | 19.55 | 1.22 | 3.26 |
| 4/10/00 | 9.17 | 20.3 | 1.69 | 3.29 |
| 10/10/00 | 9.18 | 19.5 | 1.74 | 3.30 |
| 22/11/00 | 8.94 | 24.2 | 2.92 | 5.08 |
| 4/12/00 | 8.63 | 23.15 | 3.09 | 5.26 |
| 20/12/00 | 8.47 | 21.55 | 3.93 | 6.61 |
| 9/1/01 | 8.5 | 29.2 | 4.61 | 8.92 |
| 24/1/01 | 8.14 | 20.45 | 7.91 | 12.03 |
| 8/6/01 | 8.85 | 13.45 | 4.37 | 7.01 |
| 22/6/01 | 8.75 | 14.6 | 3.75 | 5.90 |
| 4/7/01 | 8.88 | 15.23 | 3.34 | 5.30 |
| 14/9/01 | 7.68 | 17.5 | 2.06 | 3.51 |
| 4/10/01 | 7.92 | 19.33 | 1.98 | 3.34 |
| 30/10/01 | 9.04 | 23.93 | 2.23 | 3.74 |
| 18/12/01 | 9.26 | 28.65 | 2.68 | 5.16 |
| 9/1/02 | 8.3 | 25.95 | 3.40 | 6.45 |
| 22/1/02 | 8.1 | 29.3 | 3.95 | 7.49 |
| 13/2/02 | 8.29 | 27.3 | 5.91 | 10.99 |
| 22/11/02 | 8.96 | 29.93 | 2.83 | 5.67 |
| 13/12/02 | 8.22 | 25.9 | 4.43 | 8.79 |
| 7/1/03 | very low water levels | | | |
| **Shenton Park** | | | | |
| 13/11/00 | 10.13 | 27 | 0.22 | 0.49 |
| 22/11/00 | 10.42 | 24.6 | 0.29 | 0.55 |
| 4/12/00 | 9.61 | 25.53 | 0.27 | 0.50 |
| 20/12/00 | 8.97 | 24.1 | 0.32 | 0.60 |
| 9/1/01 | 9.06 | 30.1 | 0.39 | 0.81 |
| 24/1/01 | 6.96 | 21.4 | 0.47 | 0.80 |
| 6/2/01 | 7.94 | 24 | 0.44 | 0.99 |
| 22/6/01 | 7.25 | 14.3 | 0.13 | 0.23 |
| 14/9/01 | 7.99 | 17.65 | 0.06 | 0.11 |
| 19/10/01 | 8.71 | 21.47 | 0.07 | 0.14 |
| 30/10/01 | 9.29 | 24.53 | 0.10 | 0.19 |
| 15/11/01 | 8.08 | 25.2 | 0.10 | 0.18 |

| | | | | |
|---|---|---|---|---|
| Shenton Park (*continued*) | | | | |
| 9/1/02 | 8.27 | 28.2 | 0.15 | 0.32 |
| 13/2/02 | 7.75 | 28.2 | 0.32 | 0.66 |
| 22/11/02 | 8.23 | 30.33 | 0.18 | 0.40 |
| 13/12/02 | 7.41 | 27.2 | 0.25 | 0.57 |
| 7/1/03 | 7.46 | 25.2 | 0.41 | 0.81 |
| | very low water levels | | | |
| **Hyde Park** | | | | |
| 13/11/00 | 9.35 | 26.35 | 0.32 | 0.72 |
| 22/11/00 | 9.6 | 27.38 | 0.40 | 0.74 |
| 4/12/00 | 7.5 | 24.25 | 0.39 | 0.72 |
| 20/12/00 | 8.03 | 24.15 | 0.37 | 0.66 |
| 9/1/01 | 8.83 | 28.38 | 0.34 | 0.71 |
| 22/2/01 | 8.59 | 24.45 | 0.39 | 0.70 |
| 22/6/01 | 7.31 | 14.8 | 0.30 | 0.52 |
| 4/7/01 | 9.01 | 15.83 | 0.24 | 0.43 |
| 14/9/01 | 7.58 | 17.38 | 0.16 | 0.30 |
| 19/10/01 | 8.67 | 19.6 | 0.21 | 0.39 |
| 30/10/01 | 9.03 | 24.83 | 0.23 | 0.42 |
| 15/11/01 | 7.75 | 25.37 | 0.28 | 0.52 |
| 21/1/02 | 8.04 | 29.8 | 0.39 | 0.80 |
| 14/2/02 | 7.88 | 25.7 | 0.36 | 0.76 |
| 21/3/02 | 8.61 | 27.65 | 0.39 | 0.81 |

**Table B3.** Environmental data recorded from the wetlands of Inner Central Suburban Area.

| | pH | Temp (°C) | Salinity (ppt) | EC (mS $cm^{-1}$) |
|---|---|---|---|---|
| **Emu Lake** | | | | |
| 8/6/01 | 7.89 | 13.8 | 0.12 | 0.22 |
| 4/7/01 | 7.59 | 14.57 | 0.08 | 0.15 |
| 14/9/01 | 7.97 | 17.4 | 0.07 | 0.14 |
| 4/10/01 | 8.45 | 20.6 | 0.07 | 0.14 |
| 19/10/01 | 8.95 | 19.97 | 0.08 | 0.15 |
| 30/10/01 | 9.92 | 20.57 | 0.08 | 0.16 |
| 15/11/01 | 8.12 | 26.43 | 0.09 | 0.17 |
| 9/1/02 | 9.29 | 28.08 | 0.11 | 0.23 |
| 22/1/02 | 9.08 | 31.55 | 0.11 | 0.24 |
| 13/2/02 | 8.63 | 28.45 | 0.13 | 0.28 |
| 28/2/02 | 8.25 | 24.6 | 0.14 | 0.28 |
| 22/3/02 | 7.96 | 27.5 | 0.14 | 0.30 |
| 7/11/02 | 7.81 | 28.2 | 0.11 | 0.25 |
| 13/11/02 | 7.8 | 28.4 | 0.11 | 0.26 |
| 21/11/02 | 8.13 | 27.2 | 0.12 | 0.28 |
| 28/11/02 | 8.13 | 30.9 | 0.13 | 0.29 |
| 13/12/02 | 8.34 | 29.63 | 0.14 | 0.31 |
| 20/12/02 | 7.65 | 29.83 | 0.14 | 0.33 |
| 7/1/03 | 8.14 | 29.16 | 0.19 | 0.39 |
| 23/1/03 | 8.17 | 28.7 | 0.22 | 0.45 |
| 30/1/03 | 8.46 | 24.53 | 0.22 | 0.44 |
| 4/2/03 | 7.99 | 26.4 | 0.20 | 0.41 |
| 11/2/03 | 8.09 | 28.2 | 0.24 | 0.49 |
| 20/2/03 | 7.79 | 28.5 | 0.24 | 0.49 |
| **Lake Bungana** | | | | |
| 8/6/01 | 8.39 | 14.93 | 1.90 | 3.16 |
| 22/6/01 | 8.69 | 16.15 | 2.14 | 3.47 |
| 4/7/01 | 9.29 | 16.13 | 2.06 | 3.34 |
| 4/10/01 | 7.87 | 22.15 | 1.63 | 2.76 |
| 18/10/01 | 9.05 | 21.37 | 1.72 | 2.90 |
| 31/10/01 | 9.21 | 23.96 | 1.79 | 3.03 |
| 14/11/01 | 7.87 | 24.8 | 1.79 | 3.02 |
| 18/12/01 | 8.44 | 29.4 | 1.58 | 3.11 |
| 9/1/02 | 8.49 | 27.98 | 1.64 | 3.19 |
| 14/2/02 | 8.62 | 27.03 | 1.70 | 3.33 |
| 1/3/02 | 8.69 | 25.18 | 1.73 | 3.16 |
| 21/3/02 | 8.73 | 29.38 | 1.78 | 3.45 |
| 7/11/02 | 8.68 | 28.23 | 1.19 | 2.50 |

| | | | | |
|---|---|---|---|---|
| Lake Bungana (*continued*) | | | | |
| 13/11/02 | 8.67 | 28.6 | 1.21 | 2.53 |
| 28/11/02 | 8.67 | 28.03 | 1.25 | 2.59 |
| 13/12/02 | 8.67 | 29.93 | 1.24 | 2.59 |
| 20/12/02 | 8.72 | 30.97 | 1.26 | 2.64 |
| 7/1/02 | 8.88 | 29.9 | 1.42 | 2.68 |
| 23/1/03 | 8.77 | 29.9 | 1.43 | 2.69 |
| 30/1/03 | 8.75 | 27.1 | 1.45 | 2.71 |
| 4/2/03 | 8.72 | 27.67 | 1.43 | 2.71 |
| 11/2/03 | 8.8 | 29 | 1.49 | 2.77 |
| **Lake Brearley** | | | | |
| 4/7/01 | 9.35 | 16.13 | 4.84 | 7.55 |
| 18/10/01 | 8.85 | 21.07 | 4.17 | 6.80 |
| 31/10/01 | 9.41 | 24.4 | 4.14 | 6.73 |
| 14/11/01 | 8.07 | 25.1 | 4.31 | 7.01 |
| 9/1/02 | 8.64 | 28.28 | 4.02 | 7.59 |
| 22/1/02 | 8.83 | 31.8 | 4.14 | 7.79 |
| 14/2/02 | 8.69 | 27.1 | 4.24 | 7.99 |
| 1/3/02 | 8.64 | 26.1 | 4.38 | 7.66 |
| 21/3/02 | 8.79 | 29.1 | 4.56 | 8.50 |
| 7/11/02 | 8.75 | 28.03 | 3.18 | 6.39 |
| 13/11/02 | 8.75 | 27.8 | 3.15 | 6.32 |
| 28/11/02 | 8.87 | 28.83 | 3.27 | 6.56 |
| 13/12/02 | 8.88 | 29.13 | 3.36 | 6.73 |
| 20/12/02 | 9.04 | 31.03 | 3.34 | 6.73 |
| 7/1/03 | 8.97 | 29.7 | 3.84 | 6.94 |
| 23/1/03 | 8.99 | 29.53 | 3.94 | 7.11 |
| 30/1/03 | 8.94 | 27.2 | 3.96 | 7.15 |
| 4/2/03 | 8.99 | 27.43 | 3.96 | 7.15 |
| 11/2/03 | 9.02 | 28.6 | 4.13 | 7.42 |
| **Tomato Lake** | | | | |
| 3/8/04 | 7.6 | 17.18 | 0.29 | 0.56 |
| 7/9/00 | 8.13 | 18.9 | 0.34 | 0.57 |
| 14/9/00 | 7.57 | 19.4 | 0.22 | 0.65 |
| 25/9/00 | 7.32 | 23.65 | 0.24 | 0.70 |
| 4/10/00 | 7.85 | 22.8 | 0.35 | 0.72 |
| 10/10/00 | 8.18 | 22.8 | 0.35 | 0.70 |
| 20/11/00 | 8.16 | 25.4 | 0.43 | 0.96 |
| 7/12/00 | 8.79 | 26.68 | 0.55 | 0.99 |
| 19/12/00 | 8.95 | 28.53 | 0.58 | 1.05 |
| 8/1/01 | 8.65 | 33.2 | 0.57 | 1.17 |
| 23/1/01 | 7.5 | 23.83 | 0.63 | 1.09 |

| | | | | |
|---|---|---|---|---|
| Tomato Lake (*continued*) | | | | |
| 6/2/01 | 8.77 | 24 | 0.58 | 1.29 |
| 22/2/01 | 9.72 | 25.7 | 0.78 | 1.35 |
| 7/6/01 | 7.76 | 15.03 | 0.56 | 0.98 |
| 25/6/01 | 7.82 | 15 | 0.51 | 0.87 |
| 13/9/01 | 8.59 | 19 | 0.33 | 0.60 |
| 18/10/01 | 9.18 | 22.2 | 0.41 | 0.74 |
| 31/10/01 | 9.45 | 25.17 | 0.45 | 0.81 |
| 14/11/01 | 8.96 | 26.8 | 0.48 | 0.86 |
| 18/12/01 | 9.2 | 31.58 | 0.47 | 0.98 |
| 10/1/02 | 9.15 | 28.7 | 0.58 | 1.19 |
| 21/1/02 | 8.38 | 31.68 | 0.65 | 1.28 |
| 14/2/02 | 9.24 | 26.93 | 0.64 | 1.30 |
| 1/3/02 | 9.11 | 25.2 | 0.68 | 1.28 |
| 21/3/02 | 8.42 | 30.8 | 0.86 | 1.71 |
| 7/11/02 | 9.1 | 28.8 | 0.37 | 0.81 |
| 13/11/02 | 9.64 | 30.9 | 0.38 | 0.83 |
| 20/11/02 | 9.63 | 30.5 | 0.40 | 0.87 |
| 27/11/02 | 9.54 | 31.47 | 0.42 | 0.91 |
| 12/12/02 | 9.1 | 31.2 | 0.44 | 0.96 |
| 18/12/02 | 9.57 | 30.9 | 0.46 | 0.99 |
| 10/1/03 | 9.01 | 32 | 0.58 | 1.13 |
| 23/1/03 | 8.56 | 31.43 | 0.68 | 1.31 |
| 30/1/03 | 7.82 | 25.6 | 0.70 | 1.36 |
| 4/2/03 | 7.85 | 26.97 | 0.70 | 1.36 |
| 11/2/03 | 7.9 | 29.43 | 0.73 | 1.40 |
| 20/2/03 | 8.05 | 30.97 | 0.78 | 1.50 |
| **Neil McDougall Park** | | | | |
| 3/8/00 | 7.78 | 16.22 | 0.07 | 0.14 |
| 8/9/00 | 8.47 | 19.43 | 0.07 | 0.13 |
| 14/9/00 | 8.14 | 19.5 | 0.05 | 0.15 |
| 25/9/00 | 8 | 23.67 | 0.05 | 0.17 |
| 4/10/00 | 9.72 | 23.5 | 0.08 | 0.18 |
| 10/10/00 | 9.57 | 23.35 | 0.08 | 0.18 |
| 20/11/00 | 8.61 | 26.05 | 0.15 | 0.35 |
| 4/12/00 | 7.31 | 24.5 | 0.19 | 0.37 |
| 19/12/00 | 9.16 | 27.33 | 0.23 | 0.43 |
| 22/1/01 | 8.46 | 28.93 | 0.27 | 0.46 |
| 23/2/01 | 9.3 | 25.13 | 0.34 | 0.60 |
| 22/6/01 | 7.78 | 12.68 | 0.20 | 0.35 |
| 13/9/01 | 8.98 | 19.33 | 0.07 | 0.14 |
| 4/10/01 | 8.05 | 19.23 | 0.08 | 0.15 |

| | | | | |
|---|---|---|---|---|
| Neil McDougall Park (*continued*) | | | | |
| 31/10/01 | 9.88 | 25.07 | 0.10 | 0.19 |
| 14/11/01 | 7.13 | 26.43 | 0.12 | 0.23 |
| 18/12/01 | 8.5 | 30.3 | 0.15 | 0.32 |
| 9/1/02 | 9.04 | 30.43 | 0.19 | 0.41 |
| 14/1/02 | 8.91 | 30.87 | 0.21 | 0.46 |
| 14/2/02 | 8.77 | 25.3 | 0.24 | 0.51 |
| 28/2/02 | 10.02 | 28.2 | 0.27 | 0.52 |
| 23/3/02 | 9.13 | 26.7 | 0.28 | 0.59 |
| 8/11/02 | 7.86 | 29.53 | 0.12 | 0.28 |
| 13/11/02 | 7.79 | 26.7 | 0.13 | 0.30 |
| 20/11/02 | 8.24 | 27 | 0.15 | 0.34 |
| 27/11/02 | 8.7 | 31.97 | 0.16 | 0.36 |
| 13/12/02 | 7.75 | 28.37 | 0.18 | 0.40 |
| 18/12/02 | 7.85 | 31.2 | 0.20 | 0.44 |
| 10/1/03 | 8.11 | 32.03 | 0.27 | 0.55 |
| 24/1/03 | 8.9 | 26.3 | 0.30 | 0.60 |
| 31/1/03 | 9.11 | 26.76 | 0.29 | 0.59 |
| 5/2/03 | 8.68 | 29.47 | 0.31 | 0.62 |
| 11/2/03 | 8.81 | 31.53 | 0.34 | 0.67 |

**Table B4.** Environmental data recorded from the wetlands of the Eastern Coastal Plain.

| | pH | Temp (°C) | Salinity (ppt) | EC (mS $cm^{-1}$) |
|---|---|---|---|---|
| **Forrestdale Lake** | | | | |
| 21/11/00 | 10.26 | 30.6 | 1.830 | 3.84 |
| 5/12/00 | 8.68 | 26.03 | 2.640 | 4.52 |
| 9/12/00 | 9.14 | 23.75 | 3.990 | 6.78 |
| 8/1/01 | 7.5 | 28.35 | 6.190 | 11.47 |
| **Mary Carroll Park (smaller permanent wetland – 17a)** | | | | |
| 18/10/01 | 9.03 | 22.65 | 2.85 | 4.71 |
| 14/11/01 | 8.56 | 25.67 | 3.25 | 5.35 |
| 10/1/02 | 7.85 | 29.55 | 5.24 | 9.80 |
| 21/1/02 | 8.04 | 35.63 | 6.70 | 12.28 |
| 7/6/01 | 8.91 | 10.18 | 7.35 | 11.49 |
| 21/6/01 | 8.93 | 16.03 | 7.35 | 11.26 |
| 7/11/02 | 8.02 | 30.17 | 2.28 | 4.65 |
| 12/11/02 | 8.04 | 29.7 | 2.49 | 5.19 |
| 20/11/02 | 8.22 | 30.93 | 2.72 | 5.53 |
| 27/11/02 | 8.09 | 28.9 | 2.86 | 5.80 |
| 12/12/02 | 8.13 | 29.43 | 3.50 | 7.03 |
| 18/12/02 | 7.88 | 27.87 | 3.74 | 7.50 |
| 10/1/03 | 8.65 | 29 | 6.31 | 11.21 |
| 23/1/03 | 8.56 | 31.43 | 8.48 | 14.74 |
| 30/1/03 | 8.53 | 30.33 | 10.09 | 17.47 |
| 4/2/03 | 8.46 | 30 | 10.88 | 18.84 |
| 11/2/03 | 8.48 | 30.73 | 13.53 | 22.67 |
| **Mary Carroll Park (larger seasonal wetland – 17b)** | | | | |
| 3/10/01 | 7.76 | 22.15 | 2.12 | 3.55 |
| 18/10/01 | 8.65 | 21.67 | 2.15 | 3.61 |
| 14/11/01 | 7.45 | 25.58 | 3.06 | 5.05 |
| 10/1/02 | 8.06 | 28.28 | 5.09 | 9.53 |
| 21/1/02 | 8.21 | 32.9 | 6.27 | 11.64 |
| 12/2/02 | 8.41 | 33.33 | 9.56 | 17.71 |

**Table B5.** Environmental data recorded from the wetlands of the East Beeliar Regional Park.

| | pH | Temp (°C) | Salinity (ppt) | EC (mS $cm^{-1}$) |
|---|---|---|---|---|
| **Blue Gum Lake** | | | | |
| 3/8/00 | 8.9 | 14 | 0.31 | 0.60 |
| 8/9/00 | 9.52 | 19.5 | 0.36 | 0.60 |
| 14/9/00 | 8.36 | 18.93 | 0.24 | 0.70 |
| 25/9/00 | 9.24 | 22.08 | 0.26 | 0.71 |
| 4/10/00 | 9.42 | 24.3 | 0.36 | 0.75 |
| 20/11/00 | 8.68 | 26.28 | 0.53 | 1.16 |
| 7/12/00 | 9.05 | 30.8 | 0.70 | 1.27 |
| 19/12/00 | 8.69 | 26.65 | 0.77 | 1.39 |
| 8/1/01 | 8.25 | 31.65 | 0.82 | 1.64 |
| 22/1/01 | 8.42 | 27.93 | 0.93 | 1.55 |
| 23/2/01 | 9.78 | 24.8 | 1.18 | 2.00 |
| 7/6/01 | 9.71 | 15.03 | 0.65 | 1.13 |
| 25/6/01 | 9.05 | 16.68 | 0.64 | 1.08 |
| 13/9/01 | 8.67 | 19.75 | 0.36 | 0.65 |
| 3/10/01 | 8.66 | 23.15 | 0.37 | 0.66 |
| 19/10/01 | 8.88 | 23.18 | 0.40 | 0.72 |
| 1/11/01 | 8.75 | 27.6 | 0.45 | 0.81 |
| 14/11/01 | 8.53 | 26.45 | 0.50 | 0.89 |
| 10/1/02 | 9.22 | 29.1 | 0.67 | 1.36 |
| 21/1/02 | 9.17 | 32.63 | 0.75 | 1.51 |
| 12/2/02 | 8.25 | 29.6 | 0.92 | 1.85 |
| 1/3/02 | 8.89 | 26.08 | 1.03 | 1.92 |
| 23/3/02 | 8.48 | 28.5 | 1.09 | 2.15 |
| 8/11/02 | 7.96 | 30.2 | 0.403 | 0.89 |
| 12/11/02 | 8.1 | 29.07 | 0.42 | 0.92 |
| 27/11/02 | 8.39 | 33.07 | 0.47 | 1.03 |
| 12/12/02 | 8.25 | 31.73 | 0.55 | 1.89 |
| 18/12/02 | 8.22 | 31.13 | 0.58 | 1.26 |
| 2/1/03 | 9.43 | 30.9 | 0.74 | 1.42 |
| 10/1/03 | 9.5 | 31.9 | 0.79 | 1.53 |
| 24/1/03 | 8.03 | 27.17 | 0.92 | 1.75 |
| 31/1/03 | 7.82 | 27.76 | 0.97 | 1.86 |
| 5/2/03 | 7.93 | 29.47 | 0.99 | 1.89 |
| **Booragoon Lake** | | | | |
| 21/5/00 | 7.82 | 16.2 | 1.21 | 2.00 |
| 24/5/00 | 8 | 17.17 | 1.15 | 2.06 |
| 29/5/00 | 7.51 | 14.53 | 1.23 | 2.10 |
| 3/8/00 | 7.68 | 13.7 | 0.67 | 1.24 |
| 8/9/00 | 7.5 | 17.93 | 0.72 | 1.16 |
| 14/9/00 | 7.49 | 18.18 | 0.45 | 1.26 |
| 25/9/00 | 7.41 | 20.68 | 0.47 | 1.32 |

| | | | | |
|---|---|---|---|---|
| Booragoon Lake (*continued*) | | | | |
| 20/11/00 | 7.77 | 23.7 | 0.77 | 1.68 |
| 7/12/00 | 8.67 | 27.88 | 0.97 | 1.73 |
| 19/12/00 | 8.92 | 25.18 | 1.14 | 2.03 |
| 8/1/01 | 7.88 | 29.4 | 1.10 | 2.19 |
| 22/1/01 | 7.99 | 26.75 | 1.36 | 2.32 |
| 23/2/01 | 9.25 | 24.08 | 1.64 | 2.74 |
| 13/9/01 | 8.15 | 17.1 | 0.81 | 1.41 |
| 8/5/01 | 9.04 | 18.88 | 2.22 | 3.78 |
| 7/6/01 | 10.06 | 13.93 | 1.54 | 2.57 |
| 21/6/01 | 10.24 | 14.68 | 1.44 | 2.37 |
| 3/10/01 | 7.73 | 19.4 | 0.79 | 1.38 |
| 19/10/01 | 8.38 | 20.27 | 0.82 | 1.43 |
| 1/11/01 | 7.86 | 23.5 | 0.85 | 1.48 |
| 18/12/01 | 7.47 | 28.63 | 1.01 | 2.02 |
| 10/1/02 | 7.68 | 25.7 | 1.21 | 2.24 |
| 12/2/02 | 7.7 | 27.4 | 1.59 | 3.11 |
| 1/3/02 | very low water levels | | | |
| **Piney Lakes Reserve** | | | | |
| 18/10/01 | 8.63 | 21.83 | 0.38 | 0.70 |
| 1/11/01 | 8.11 | 26.47 | 0.30 | 0.55 |
| 21/1/02 | 7.48 | 29.2 | 0.43 | 0.89 |
| 12/2/02 | 7.61 | 29.25 | 0.33 | 0.69 |
| 1/3/02 | 7.65 | 26.53 | 0.37 | 0.71 |
| **North Lake** | | | | |
| 11/11/99 | 7.86 | 16.6 | 0.45 | 0.86 |
| 13/1/00 | 8.04 | 28.1 | 0.74 | 1.41 |
| 18/2/00 | 8.34 | 30.63 | 0.79 | 1.70 |
| 3/8/00 | 7.29 | 14.88 | 0.45 | 0.85 |
| 8/9/00 | 7.35 | 18.1 | 0.46 | 0.75 |
| 10/11/00 | 10.68 | 20.85 | 0.47 | 1.03 |
| 20/11/00 | 10.19 | 21.65 | 0.49 | 1.09 |
| 7/12/00 | 9.27 | 29.85 | 0.67 | 1.22 |
| 19/12/00 | 8.89 | 24.5 | 0.75 | 1.35 |
| 8/1/01 | 8.33 | 30.73 | 0.81 | 1.63 |
| 22/1/01 | 7.36 | 24.7 | 0.97 | 1.61 |
| 6/2/01 | 8.1 | 26.23 | 0.96 | 2.07 |
| 25/6/01 | 9.06 | 18.18 | 0.98 | 1.64 |
| 13/9/01 | 8.48 | 16.9 | 0.49 | 0.86 |
| 3/10/01 | 8.61 | 19.6 | 0.44 | 0.78 |
| 1/11/01 | 8.27 | 22.87 | 0.49 | 0.87 |
| 14/11/01 | 8.58 | 25.37 | 0.52 | 0.92 |
| 10/1/02 | 7.62 | 24.65 | 0.67 | 1.38 |
| 21/1/02 | 7.81 | 25.7 | 0.78 | 1.58 |
| 12/2/02 | 7.79 | 28.13 | 1.03 | 2.05 |
| 1/3/02 | 8.13 | 20.6 | 1.27 | 2.34 |

| | | | | |
|---|---|---|---|---|
| North Lake (*continued*) | | | | |
| 8/11/02 | 8.66 | 25.56 | 0.49 | 1.08 |
| 12/11/02 | 8.9 | 26.9 | 0.51 | 1.10 |
| 20/11/02 | 8.6 | 24.67 | 0.53 | 1.14 |
| 27/11/02 | 8.97 | 27.9 | 0.58 | 1.24 |
| 12/12/02 | 9.61 | 30.7 | 0.67 | 1.43 |
| 18/12/02 | 9.29 | 29.45 | 0.71 | 1.51 |
| 10/1/03 | 8.49 | 31.03 | 1.05 | 2.01 |
| 24/1/03 | 8.32 | 24.63 | 1.28 | 2.42 |
| 31/1/03 | 8.36 | 26.1 | 1.38 | 2.62 |
| 5/2/03 | 8.53 | 28.03 | 1.11 | 2.12 |
| **Bibra Lake** | | | | |
| 31/10/99 | 8.3 | 17 | 0.58 | 1.03 |
| 24/1/00 | 9.02 | 23.2 | 0.74 | 1.41 |
| 3/8/00 | 8.08 | 14.9 | 0.57 | 1.07 |
| 8/9/00 | 7.97 | 18.43 | 0.61 | 0.92 |
| 14/9/00 | 7.96 | 19 | 0.38 | 1.08 |
| 21/9/00 | 8.43 | 18.58 | 0.38 | 1.08 |
| 4/10/00 | 8.33 | 22.8 | 0.53 | 1.09 |
| 10/10/00 | 8.13 | 22.35 | 0.54 | 1.07 |
| 20/11/00 | 8.43 | 22.5 | 0.62 | 1.36 |
| 5/12/00 | 8.42 | 26.45 | 0.78 | 1.40 |
| 19/12/00 | 9.56 | 24.5 | 0.78 | 1.46 |
| 8/1/01 | 9 | 29.53 | 0.79 | 1.59 |
| 22/1/01 | 8.62 | 24.5 | 0.77 | 1.32 |
| 6/2/01 | 9.3 | 24.05 | 0.84 | 1.83 |
| 8/5/01 | 9.14 | 17.68 | 1.56 | 2.69 |
| 7/6/01 | 9.07 | 13.25 | 1.19 | 2.07 |
| 21/6/01 | 9.2 | 14.9 | 1.03 | 1.71 |
| 13/9/01 | 8.57 | 16.83 | 0.64 | 1.13 |
| 3/10/01 | 8.28 | 19 | 0.63 | 0.11 |
| 22/10/01 | 8.56 | 18.13 | 0.65 | 1.14 |
| 1/11/01 | 8.46 | 22.57 | 0.69 | 1.21 |
| 14/11/01 | 7.14 | 25.08 | 0.73 | 1.28 |
| 10/1/02 | 8.56 | 25.2 | 0.81 | 1.63 |
| 21/1/02 | 8.6 | 26.88 | 0.87 | 1.75 |
| 12/2/02 | 8.78 | 30 | 1.01 | 2.03 |
| 1/3/02 | 9.09 | 20.4 | 1.15 | 2.13 |
| 21/3/02 | 9.15 | 26.7 | 1.34 | 2.63 |
| 8/11/02 | 8.54 | 26.4 | 0.65 | 1.40 |
| 12/11/02 | 8.56 | 25.5 | 0.66 | 1.42 |
| 27/11/02 | 8.67 | 28.86 | 0.71 | 1.52 |
| 12/12/02 | 8.83 | 31.53 | 0.77 | 1.66 |
| 18/12/02 | 8.8 | 30.36 | 0.81 | 1.74 |
| 10/1/03 | 9.08 | 30.7 | 1.10 | 2.10 |
| 24/1/03 | 8.89 | 24.37 | 1.26 | 2.38 |

| | | | | |
|---|---|---|---|---|
| Bibra Lake (*continued*) | | | | |
| 31/1/03 | 8.85 | 23.53 | 1.35 | 2.54 |
| 5/2/03 | very low water levels | | | |
| 11/2/03 | 8.99 | 29 | 1.53 | 2.89 |
| 20/2/03 | 9.08 | 33.07 | 1.66 | 3.12 |
| **Yangebup Lake** | | | | |
| 31/10/99 | 8.63 | 20.8 | 1.30 | 2.38 |
| 20/1/00 | 8.81 | 27.9 | 1.60 | 2.97 |
| 18/2/00 | 9.03 | 29.83 | 1.74 | 3.57 |
| 3/8/00 | 8.69 | 15.1 | 1.38 | 2.49 |
| 8/9/00 | 9.06 | 18.9 | 1.43 | 2.24 |
| 14/9/00 | 9.02 | 18.5 | 0.88 | 2.39 |
| 21/9/00 | 9.21 | 19.68 | 0.87 | 2.36 |
| 4/10/00 | 9.21 | 23.7 | 1.17 | 2.30 |
| 10/10/00 | 9.08 | 24.23 | 1.16 | 2.23 |
| 20/11/00 | 8.93 | 22.5 | 1.26 | 2.67 |
| 5/12/00 | 8.49 | 27.8 | 1.56 | 2.71 |
| 19/12/00 | 9.22 | 25.3 | 1.60 | 2.81 |
| 8/1/01 | 8.14 | 29.35 | 1.51 | 2.96 |
| 22/1/01 | 8.27 | 27.67 | 1.65 | 2.62 |
| 23/2/01 | 9.09 | 24 | 1.98 | 3.29 |
| 8/5/01 | 8.8 | 19.1 | 2.18 | 3.71 |
| 7/6/01 | 9.11 | 15.33 | 1.89 | 3.13 |
| 25/6/01 | 9.13 | 17.6 | 1.91 | 3.10 |
| 13/9/01 | 8.32 | 18.85 | 1.41 | 2.41 |
| 3/10/01 | 8.55 | 20.2 | 1.33 | 2.26 |
| 22/10/01 | 8.82 | 19.13 | 1.31 | 2.23 |
| 1/11/01 | 8.53 | 22.9 | 1.34 | 2.29 |
| 14/11/01 | 8.32 | 25 | 1.38 | 2.34 |
| 18/12/01 | 9.06 | 27.8 | 1.25 | 2.49 |
| 10/1/02 | 8.98 | 25.2 | 1.33 | 2.38 |
| 12/2/02 | 9 | 31.2 | 1.50 | 2.95 |
| 1/3/02 | 9.33 | 22.7 | 1.59 | 2.9 |
| 21/3/02 | 9.3 | 27.7 | 1.68 | 3.25 |
| 8/11/02 | 8.87 | 28.6 | 1.07 | 2.25 |
| 12/11/02 | 8.7 | 27.83 | 1.09 | 2.27 |
| 20/11/02 | 8.73 | 25.9 | 1.07 | 2.26 |
| 27/11/02 | 8.75 | 28.93 | 1.06 | 2.29 |
| 12/12/02 | 9.25 | 31.2 | 1.15 | 2.42 |
| 18/12/02 | 8.98 | 29.93 | 1.21 | 2.53 |
| 10/1/03 | 9.7 | 31.43 | 1.42 | 2.69 |
| 24/1/03 | 9.45 | 26.23 | 1.49 | 2.80 |
| 31/1/03 | 9.53 | 25.1 | 1.51 | 2.83 |
| 5/2/03 | 9.75 | 28.97 | 1.32 | 2.50 |
| 11/2/03 | 9.49 | 30.13 | 1.74 | 3.23 |
| 20/2/03 | 9.37 | 31.27 | 1.66 | 3.09 |

| | | | | |
|---|---|---|---|---|
| **Thomsons Lake** | | | | |
| 20/11/00 | 8.35 | 24.2 | 1.67 | 3.52 |
| 5/12/00 | 8.78 | 26.68 | 2.26 | 3.89 |
| | very low water levels | | | |

**Table B6.** Environmental data recorded from the wetlands of the Southern West Corridor.

| | pH | Temp (°C) | Salinity (ppt) | EC (mS $cm^{-1}$) |
|---|---|---|---|---|
| **Manning Lake** | | | | |
| 10/11/00 | 9.09 | 22.77 | 5.35 | 10.71 |
| 20/11/00 | 9.11 | 24.9 | 4.42 | 8.91 |
| 8/12/00 | 8.81 | 25.7 | 5.24 | 8.68 |
| 3/10/01 | 8.43 | 18.7 | 3.82 | 6.25 |
| 1/11/01 | 8.82 | 23.8 | 4.79 | 7.77 |
| 21/6/01 | 9.2 | 15.35 | 5.70 | 8.82 |
| **Market Gardens** | | | | |
| 10/11/00 | 8.71 | 22.2 | 4.74 | 9.55 |
| 20/11/00 | 8.92 | 23.6 | 5.44 | 10.88 |
| 8/12/00 | 8.57 | 24.58 | 8.19 | 13.33 |
| 14/11/01 | 7.95 | 25.08 | 5.85 | 9.38 |
| 18/12/01 | 8.2 | 25.5 | 8.33 | 15.32 |
| 21/6/01 | 8.01 | 14.4 | 7.09 | 10.88 |
| **Lake Coogee** | | | | |
| 10/11/00 | 8.71 | 21.13 | 11.73 | 22.67 |
| 20/11/00 | 8.8 | 22.93 | 12.80 | 24.56 |
| 8/12/00 | 8.47 | 24.65 | 17.13 | 26.83 |
| 19/12/00 | 8.2 | 22.55 | 19.10 | 29.93 |
| 22/1/01 | 7.62 | 25.97 | | |
| 3/10/01 | 7.84 | 20.58 | 11.18 | 17.38 |
| 18/10/01 | 8.59 | 19.2 | 11.97 | 18.65 |
| 1/11/01 | 7.91 | 23.4 | 12.73 | 19.75 |
| 21/1/02 | 8.26 | 29.6 | 21.65 | 37.65 |

**Sampling Period 2000-2001**

| Site | Date | Genera/Species | Cells mL |
|---|---|---|---|
| **Joondalup** | 22/11/2000 | *M. flos-aquae* | 1212255 |
| | | *A. spiroides f. spiroides* | 15063 |
| | 4/12/2000 | *M. flos-aquae* | 70630 |
| | 20/12/2000 | *M. flos-aquae* | 4304575 |
| | | *A. spiroides f. spiroides* | 386640 |
| | | *A. bergii var. limnetica* | 93456 |
| | | *A. elenkiniii* | 8010 |
| | 9/01/2001 | *M. flos-aquae* | 7361649 |
| | | *A. spiroides f. spiroides* | 73964 |
| | | *A. bergii var. limnetica* | 37382 |
| | | *A. elenkiniii* | 590107 |
| | | *A.t anganyikae* | 64885 |
| | 24/01/2001 | *M. flos-aquae* | 9743437 |
| | | *A. spiroides f. spiroides* | 44325 |
| | | *A. bergii var. limnetica* | 1071004 |
| | | *A. elenkiniii* | 775416 |
| | | *A. tanganyikae* | 21895 |
| **Goollelal** | 22/11/2000 | *M. flos-aquae* | 1202890 |
| | 4/12/2000 | *M. flos-aquae* | 12864077 |
| | 20/12/2000 | *M. flos-aquae* | 3877616 |
| **Claremont** | 7/09/2000 | *A. elenkiniii* | 385454 |
| | 14/09/2000 | *A. elenkiniii* | 361687 |
| | 21/09/2000 | *A. elenkiniii* | 391646 |
| | 4/10/2000 | *A. elenkiniii* | 312815 |
| | 10/10/2000 | *A. elenkiniii* | 250721 |
| | 22/11/2000 | *M. flos-aquae* | 551820 |
| | 4/12/2000 | *M. flos-aquae* | 602533 |
| | 20/12/2000 | *M. flos-aquae* | 1310251 |
| | 9/01/2001 | *M. flos-aquae* | 7380340 |
| | 24/01/2001 | *M. flos-aquae* | 5393736 |
| **Gwelup** | 22/11/2000 | *M. aeruginosa* | 278839 |
| | 4/12/2000 | *M. aeruginosa* | 1374612 |
| | 9/01/2001 | *M. aeruginosa* | 16430335 |
| | | *M. flos-aquae* | 4866912 |
| | 24/01/2001 | *M. aeruginosa* | 148728 |
| | | *M. flos-aquae* | 23622962 |
| **Herdsman** | 22/11/2000 | *M. aeruginosa* | 1519220 |
| | 4/12/2000 | *M. aeruginosa* | 387295 |
| | | *M. flos-aquae* | 7224871 |
| | 20/12/2000 | *M.aeruginosa* | 831757 |
| | | *M. flos-aquae* | 11103887 |
| | 9/01/2001 | *M. aeruginosa* | 801050 |
| | | *M. flos-aquae* | 10398429 |
| | 22/02/2001 | *M. aeruginosa* | 585300 |
| | | *M. flos-aquae* | 4658105 |
| **Hyde Park** | 22/11/2000 | *M. aeruginosa* | 5026128 |
| | | *M. wesenbergii* | 411731 |
| | 4/12/2000 | *M. aeruginosa* | 5799881 |

| | | | |
|---|---|---|---|
| | | *M. wesenbergii* | 231473 |
| | 20/12/2000 | *M. aeruginosa* | 2434658 |
| | | *M. wesenbergii* | 498520 |
| | | *M. flos-aquae* | 1156983 |
| | 9/01/2001 | *M. aeruginosa* | 5628711 |
| | | *M. wesenbergii* | 583965 |
| | | *M. flos-aquae* | 745777 |
| **Shenton Park** | 4/12/2000 | *M. flos-aquae* | 532907 |
| | 20/12/2000 | *A. elenkinii* | 17810 |
| | 9/01/2001 | *A. elenkinii* | 25634 |
| | | *M. flos-aquae* | 1805567 |
| | 24/01/2001 | *M. flos-aquae* | 534033 |
| | | *A. elenkinii* | 692908 |
| **Jackadder** | 22/11/2000 | *M. flos-aquae* | 1542484 |
| | | *M. aeruginosa* | 691741 |
| | 4/12/2000 | *M. aeruginosa* | 620274 |
| | 20/12/2000 | *M. aeruginosa* | 962595 |
| | 9/01/2001 | *M. aeruginosa* | 546850 |
| | 24/01/2001 | *M. aeruginosa* | 2359359 |
| **Tomato** | 7/09/2000 | *M. flos-aquae* | 6572462 |
| | | *Anabaena* | 129377 |
| | 25/09/2000 | *Microcystis* | 2910066 |
| | | *Anabaena* | 3035092 |
| | 4/10/2000 | *Microcystis* | 16140014 |
| | | *Anabaena* | 3035092 |
| | 10/10/2000 | *Microcystis* | 9680360 |
| | | *Anabaena* | 176073 |
| | 20/11/2000 | *M. aeruginosa* | 671155 |
| | | *M. flos-aquae* | 3207316 |
| | | *Anabaena* | 111636 |
| | 7/12/2000 | *M. aeruginosa* | 1271512 |
| | 19/12/2000 | *M. flos-aquae* | 2169510 |
| | | *M. aeruginosa* | 952715 |
| | 8/01/2001 | *M. aeruginosa* | 527625 |
| | | *M. flos-aquae* | 5923764 |
| | 23/01/2001 | *M. aeruginosa* | 1003983 |
| | | *M. flos-aquae* | 3294985 |
| | | *T. iwanoffianum* | 6309336 |
| | | *A. spiroides f. spiroides* | 747647 |
| | | *A. circinalis* | 2815156 |
| | 7/02/2001 | *M. aeruginosa* | 6734961 |
| | | *M. flos-aquae* | 1077412 |
| | | *A. circinalis* | 2340668 |
| | | *A. spiroides f. spiroides* | 1622927 |
| **Neil McDougall** | 3/08/2000 | *A. circinalis* | 751542 |
| | | *M. aeruginosa* | 1374274 |
| | | *M. flos-aquae* | 13032620 |
| | 11/08/2000 | *A. circinalis* | 101371 |
| | | *M. aeruginosa* | 751542 |
| | | *M. flos-aquae* | 6782583 |
| | 7/09/2000 | *A. circinalis* | 7567 |
| | | *M. flos-aquae* | 412488 |

| | | | |
|---|---|---|---|
| | 14/09/2000 | *M. aeruginosa* | 346791 |
| | | *M. flos-aquae* | 4740762 |
| | 25/09/2000 | *A. circinalis* | 190300 |
| | | *M. aeruginosa* | 443531 |
| | | *M. flos-aquae* | 30124133 |
| | 4/10/2000 | *A. circinalis* | 294404 |
| | | *M. aeruginosa* | 412902 |
| | | *M. flos-aquae* | 7017667 |
| | 10/10/2000 | *A. circinalis* | 347293 |
| | | *M. aeruginosa* | 391479 |
| | | *M. flos-aquae* | 8277797 |
| | 20/11/2000 | *M. flos-aquae* | 25549569 |
| | | *M. aeruginosa* | 114314 |
| | | *A. elenkinii* | 958529 |
| | 4/12/2000 | *M. flos-aquae* | 51566771 |
| | 19/12/2000 | *M. flos-aquae* | 69200032 |
| | | *M. aeruginosa* | 213613 |
| | | *A. circinalis* | 44325 |
| | 22/01/2001 | *M. aeruginosa* | 77969 |
| | | *M. flos-aquae* | 1304376 |
| | | *A. circinalis* | 4806 |
| **Forrestdale** | 21/11/2000 | *P. planctonica* | 1022632 |
| | 5/12/2000 | *P. planctonica* | 623789 |
| | 19/12/2000 | *P. planctonica* | 609599 |
| **Thomson** | 5/12/2000 | *O. sancta* | 1097112 |
| **North** | 20/11/2000 | *Anabaena* | 2486452 |
| | 7/12/2000 | *Anabaena* | 123854 |
| | | *Microcystis* | 4795157 |
| | 8/01/2001 | *Anabaena* | 28838 |
| | | *Microcystis* | 781558 |
| | 22/01/2001 | *Anabaena* | 41842 |
| **Bibra** | 8/09/2000 | *M. flos-aquae* | 1590854 |
| | | *A. elenkinii* | 58914 |
| | | *A. gracile* | 352314 |
| | 14/09/2000 | *M. flos-aquae* | 1480892 |
| | | *M. aeruginosa* | 352147 |
| | | *A. elenkinii* | 39667 |
| | 21/09/2000 | *M. flos-aquae* | 8756811 |
| | | *M. aeruginosa* | 6556394 |
| | | *A. gracile* | 204694 |
| | | *A. elenkinii* | 51885 |
| | 4/10/2000 | *M. flos-aquae* | 1008740 |
| | | *A. elenkinii* | 18451 |
| | | *A. gracile* | 75317 |
| | 10/10/2000 | *M. flos-aquae* | 1117196 |
| | | *M. aeruginosa* | 2740186 |
| | | *A. elenkinii* | 687 |
| | 20/11/2000 | *M. flos-aquae* | 1048240 |
| | | *A. elenkinii* | 226452 |
| | 5/12/2000 | *M. flos-aquae* | 2352054 |
| | 19/12/2000 | *M. aeruginosa* | 1100108 |

| | | | |
|---|---|---|---|
| | | *M. flos-aquae* | 7783000 |
| | 8/01/2001 | *M. aeruginosa* | 350860 |
| | | *M. flos-aquae* | 267017 |
| | | *A. elenkinii* | 932956 |
| | 22/01/2001 | *M. aeruginosa* | 254467 |
| | | *M. flos-aquae* | 310006 |
| | | *A. elenkinii* | 531897 |
| **Yangebup** | 11/08/2000 | *M. aeruginosa* | 3722649 |
| | 8/09/2000 | *M. aeruginosa* | 2120246 |
| | 14/09/2000 | *M. aeruginosa* | 2704034 |
| | | *M. flos-aquae* | 6634891 |
| | 21/09/2000 | *M. aeruginosa* | 3786417 |
| | | *M. flos-aquae* | 7180183 |
| | 4/10/2000 | *M. aeruginosa* | 2948896 |
| | | *M. flos-aquae* | 4401503 |
| | 10/10/2000 | *M. flos-aquae* | 84053167 |
| | 20/11/2000 | *M. flos-aquae* | 24338143 |
| | | *Anabaena* | 176910 |
| | 5/12/2000 | *M. flos-aquae* | 7093151 |
| | 19/12/2000 | *M. flos-aquae* | 38450390 |
| | 8/01/2001 | *M. flos-aquae* | 54671649 |
| | 22/01/2001 | *M. aeruginosa* | 671013 |
| | | *M. flos-aquae* | 44592 |
| **Booragoon** | 7/12/2000 | *M. aeruginosa* | 2417328 |
| | | *M.flos-aquae* | 9543451 |
| | 19/12/2000 | *M. aeruginosa* | 1949221 |
| | | *M.flos-aquae* | 7003845 |
| | 8/01/2001 | *M. aeruginosa* | 7609973 |
| | | *M.flos-aquae* | 6361670 |
| | 22/01/2001 | *M. aeruginosa* | 282771 |
| | | *M.flos-aquae* | 3111544 |
| **Blue Gum** | 25/09/2000 | *M. flos-aquae* | 5504305 |
| | | *M. aeruginosa* | 167370 |
| | 4/10/2000 | *M. flos-aquae* | 1296785 |
| | | *M. aeruginosa* | 679021 |
| | 10/10/2000 | *M. flos-aquae* | 6025329 |
| | | *M. aeruginosa* | 809402 |
| | 20/11/2000 | *M. flos-aquae* | 497090 |
| | | *P. planctonica* | 748647 |
| | 7/12/2000 | *M. aeruginosa* | 398843 |
| | | *M. flos-aquae* | 6349692 |
| | 19/12/2000 | *M. aeruginosa* | 2551878 |
| | | *M. flos-aquae* | 3146791 |
| | 8/01/2001 | *M. aeruginosa* | 1707838 |
| | | *M. flos-aquae* | 3334503 |
| | 22/01/2001 | *M. aeruginosa* | 526023 |
| | | *M. flos-aquae* | 808259 |
| | | *A. elenkinii* | 155404 |
| **Coogee** | 8/12/2000 | *A. bergii var. limnetica* | 56906 |
| | 19/12/2000 | *A. bergii var. limnetica* | 80105 |
| | 22/01/2001 | *A. bergii var. limnetica* | 59278 |

**Sampling Period 2001-2002**

| Site | Date | Genera/Species | Cells mL |
|---|---|---|---|
| **Yangebup** | 7/06/2001 | *M. aeruginosa* | 7017731 |
| | | *M. flos-aquae* | 26381778 |
| | 25/06/2001 | *M.aeruginosa* | 3019157 |
| | | *M. flos-aquae* | 13207444 |
| | 13/09/2001 | *M. aeruginosa* | 33647034 |
| | | *M. flos-aquae* | 81612301 |
| | 3/10/2001 | *M. aeruginosa* | 29010377 |
| | | *M. flos-aquae* | 111194544 |
| | 22/10/2001 | *M. aeruginosa* | 15383000 |
| | | *M. flos-aquae* | 61311682 |
| | | *N. spumigena* | 27225 |
| | 1/11/2001 | *M. aeruginosa* | 5281106 |
| | | *M. flos-aquae* | 35507414 |
| | 10/01/2002 | *M. aeruginosa* | 334454 |
| | | *M. flos-aquae* | 8093913 |
| | | *A. elenkinii* | 25131 |
| | 19/01/2002 | *M. flos-aquae* | 593305 |
| | 12/02/2002 | *M. aeruginosa* | 307438 |
| | | *M. flos-aquae* | 8054959 |
| | 21/03/2002 | *M. aeruginosa* | 749956 |
| | | *M. flos-aquae* | 21988519 |
| **Bibra** | 8/05/2001 | *C. raciborskii* | 6439640 |
| | | *A. ovalisporum* | 792772 |
| | | *M. aeruginosa* | 9205666 |
| | | *M. flos-aquae* | 40288272 |
| | 21/06/2001 | *C. raciborskii* | 386640 |
| | | *A. ovalisporum* | 499855 |
| | | *M. aeruginosa* | 442447 |
| | | *M. flos-aquae* | 15290441 |
| | 13/09/2001 | *M. aeruginosa* | 4869315 |
| | | *M. flos-aquae* | 12525217 |
| | 22/10/2001 | *M. aeruginosa* | 375502 |
| | | *M. flos-aquae* | 414455 |
| | 1/11/2001 | *M. aeruginosa* | 5769279 |
| | | *M. flos-aquae* | 907235 |
| | 10/01/2002 | *M. aeruginosa* | 1976357 |
| | | *M. flos-aquae* | 701369 |
| | | *A. ovalisporum* | 64503 |
| | 21/01/2002 | *M. aeruginosa* | 2209449 |
| | | *M. flos-aquae* | 11518 |
| | | *A. ovalisporum* | 10681 |
| | | *A. bergii var. limnetica* | 642311 |
| | 12/02/2002 | *M. aeruginosa* | 2867886 |
| | | *M. flos-aquae* | 713725 |
| | 21/03/2002 | *M. aeruginosa* | 20083995 |
| | | *M. flos-aquae* | 31309251 |

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|---|---|---|---|
| | | *A. ovalisporum* | 445241 |
| | | *A. bergii var. limnetica* | 80210 |
| **Blue Gum** | 7/06/2001 | *M. aeruginosa* | 10069465 |
| | 25/06/2001 | *M. aeruginosa* | 1950557 |
| | 10/01/2002 | *A. circinalis* | 119582 |
| | | *P. amoenum* | 624928 |
| | | *M. flos-aquae* | 9758225 |
| | | *P. planktonica* | 134452 |
| | 21/01/2002 | *M. flos-aquae* | 9568065 |
| | | *M. aeruginosa* | 929225 |
| | | *P. amoenum* | 1846722 |
| | | *P. planktonica* | 220945 |
| | | *A. circinalis* | 6492 |
| | 12/02/2002 | *M. aeruginosa* | 452152 |
| | | *M. flos-aquae* | 9841576 |
| | 21/03/2002 | *M. aeruginosa* | 730898 |
| | | *M. flos-aquae* | 3936379 |
| **Piney** | 21/01/2002 | *M. aeruginosa* | 293616 |
| | 12/02/2002 | *M. aeruginosa* | 18100726 |
| **North** | 1/11/2001 | *A. circinalis* | 155671 |
| | 21/01/2002 | *M. flos-aquae* | 2472907 |
| **Booragoon** | 7/06/2001 | *M. aeruginosa* | 5479715 |
| | | *M. flos-aquae* | 11047813 |
| | 21/06/2001 | *M. aeruginosa* | 249127 |
| | | *M. flos-aquae* | 5752339 |
| | 3/10/2001 | *M. aeruginosa* | 1371130 |
| | | *M. flos-aquae* | 3614070 |
| | 12/02/2002 | *M. aeruginosa* | 3073961 |
| **Herdsman** | 9/05/2001 | *M. aeruginosa* | 3959323 |
| | | *M. flos-aquae* | 41014023 |
| | 8/06/2001 | *M. aeruginosa* | 16328068 |
| | | *M. flos-aquae* | 69385075 |
| | 4/10/2001 | *M. aeruginosa* | 5701873 |
| | | *M. flos-aquae* | 4492288 |
| | | *A. circinalis* | 1870719 |
| | 19/10/2001 | *M. aeruginosa* | 3230367 |
| | | *M. flos-aquae* | 220022 |
| | 30/10/2001 | *M. aeruginosa* | 3167156 |
| | | *M. flos-aquae* | 2934064 |
| | | *A. circinalis* | 117487 |
| | 24/01/2002 | *M. aeruginosa* | 66807 |
| | | *M. flos-aquae* | 5931375 |
| | 10/01/2002 | *M. aeruginosa* | 919173 |
| | | *M. flos-aquae* | 13082031 |
| | 13/02/2002 | *M. flos-aquae* | 1708082 |
| | 22/03/2002 | *M. aeruginosa* | 259689 |
| | | *M. flos-aquae* | 77069 |
| **Monger** | 19/10/2001 | *Microcystis* | 9723144 |
| | 22/03/2002 | *Microcystis* | 4707487 |
| **Hyde Park** | 22/06/2001 | *M. aeruginosa* | 8117306 |

| | | | |
|---|---|---|---|
| | | *M. wesenbergii* | 6613735 |
| | 4/07/2001 | *M. aeruginosa* | 4291758 |
| | | *M. wesenbergii* | 5290401 |
| | 14/09/2001 | *M.aeruginosa* | 5939543 |
| | | *M. wesenbergii* | 2520657 |
| | 19/10/2001 | *M. aeruginosa* | 8186730 |
| | | *M. wesenbergii* | 9075913 |
| | 30/10/2001 | *M. aeruginosa* | 5749803 |
| | | *M. wesenbergii* | 5355662 |
| | 21/01/2002 | *M. aeruginosa* | 14474705 |
| | | *M. wesenbergii* | 1941211 |
| | | *M. flos-aquae* | 186111 |
| | | *P. mougeotii* | 3122493 |
| | 14/02/2002 | *M. aeruginosa* | 8423759 |
| | | *M. wesenbergii* | 758752 |
| | | *M. flos-aquae* | 12524329 |
| | | *P. mougeotii* | 118745 |
| | 21/03/2002 | *M. aeruginosa* | 4375546 |
| | | *M. wesenbergii* | 433513 |
| | | *M. flos-aquae* | 405659 |
| | | *P. mougeotii* | 613619 |
| **Shenton Park** | 13/02/2002 | *A. spiroides f. spiroides* | 1236872 |
| | | *A. elenkinni* | 282307 |
| | 20/02/2002 | *A. spiroides f. spiroides* | 607965 |
| | | *A. elenkinni* | 362936 |
| | | *M. flos-aquae* | 2361074 |
| **Perry** | 22/06/2001 | *M. wesenbergii* | 676086 |
| | | *M. aeruginosa* | 469415 |
| | Jan 2001 | no cyanoprokaryota cells in net sample | |
| | Feb 2001 | no cyanoprokaryota cells in net sample | |
| **Claremont** | 8/06/2001 | *M. aeruginosa* | 379698 |
| | 20/02/2002 | *A. arnoldii* | 2077510 |
| **Joondalup** | 9/01/2002 | *M. flos-aquae* | 2989981 |
| | 13/02/2002 | *M. flos-aquae* | 437073 |
| **Goollelal** | 30/10/2001 | *M. flos-aquae* | 1720857 |
| | 9/01/2002 | *M. flos-aquae* | 4831468 |
| | 13/02/2002 | *M. flos-aquae* | 27541042 |
| **Emu** | 8/06/2001 | *M. aeruginosa* | 1094234 |
| | | *M. flos-aquae* | 8886314 |
| | 4/07/2001 | *M. aeruginosa* | 49398 |
| | | *M. flos-aquae* | 6164880 |
| | 30/10/2001 | *M. aeruginosa* | 143038 |
| | | *M. flos-aquae* | 2975950 |
| | 9/01/2002 | *M. aeruginosa* | 418015 |
| | | *M. flos-aquae* | 2526730 |
| | 22/01/2002 | *M. aeruginosa* | 480843 |
| | | *M. flos-aquae* | 19785981 |
| | | *A. ovalisporum* | 87959 |
| | | *A. bergii var. limnetica* | 43980 |
| | 13/02/2002 | *M. aeruginosa* | 756448 |

| | | | |
|---|---|---|---|
| | | *M. flos-aquae* | 3556480 |
| | 22/03/2002 | *M. aeruginosa* | 1500331 |
| | | *M. flos-aquae* | 3227471 |
| **Tomato** | 7/06/2001 | *M. aeruginosa* | 2100620 |
| | 25/06/2001 | *M. aeruginosa* | 860328 |
| | 10/01/2002 | *M. aeruginosa* | 449848 |
| | | *M. flos-aquae* | 3338886 |
| | 31/10/2001 | *M. aeruginosa* | 1811539 |
| | | *Anabaena* | 516236 |
| | 21/01/2002 | *M. aeruginosa* | 136546 |
| | | *M. flos-aquae* | 863256 |
| | | *Anabaena* | 215290 |
| | 23/01/2002 | *M.aeruginosa* | 473932 |
| | | *Anabaena* | 1961697 |
| | | *Planktothrix* | 2784324 |
| | 14/02/2002 | *M. aeruginosa* | 402727 |
| | | *M. flos-aquae* | 1522530 |
| | | *Anabaena* | 1140746 |
| | 21/03/2002 | *M. aeruginosa* | 2122746 |
| | | *M. flos-aquae* | 5464983 |
| **Neil McDougall** | 24/01/2002 | *M. flos-aquae* | 186390 |
| | | *A. circinalis* | 13822 |
| | 14/02/2002 | *M. flos-aquae* | 3199408 |
| | 22/03/2002 | *M. aeruginosa* | 121258 |
| | | *M. flos-aquae* | 6937879 |
| | | *A. elenkinii* | 335082 |
| **Jackadder** | 9/05/2001 | *M. aeruginosa* | 7845750 |
| | | *M. flos-aquae* | 27922464 |
| | 8/06/2001 | *M. aeruginosa* | 2171379 |
| | | *M. flos-aquae* | 11579978 |
| | 22/06/2001 | *M. aeruginosa* | 1320071 |
| | | *M. flos-aquae* | 11448500 |
| | 9/01/2002 | *M. aeruginosa* | 159164 |
| | | *M. flos-aquae* | 4296802 |
| | 13/02/2002 | *M. aeruginosa* | 1241061 |
| | | *M. flos-aquae* | 578436 |
| | 22/03/2002 | *M. aeruginosa* | 533619 |
| | | *M. flos-aquae* | 7719458 |
| **Gwelup** | 22/01/2002 | *M. flos-aquae* | 10767995 |
| | Oct 2001 | low amounts of Microcystis in net samples | |
| | Jan 2002 | low amounts of Microcystis in net samples | |
| **Bungana** | 22/06/2001 | *M. aeruginosa* | 2821031 |
| | | *M. flos-aquae* | 1151643 |
| | 16/10/2001 | *M. aeruginosa* | 2230390 |
| | 31/10/2001 | *M. aeruginosa* | 1451535 |
| | 14/02/2002 | *M. aeruginosa* | 1291114 |
| | 21/03/2002 | *M. aeruginosa* | 2994170 |
| **Brearley** | 22/01/2002 | *A. bergii var. limnetica* | 101781 |
| | 14/02/2002 | M. flos-aquae | 589117 |
| | | M. aeruginosa | 97802 |

| | | | |
|---|---|---|---|
| | | *A. bergii var. limnetica* | 18011 |
| | 21/03/2002 | *M. aeruginosa* | 1136976 |
| | | *M. flos-aquae* | 5520481 |
| **Mary Carroll Park** | 21/01/2002 | *Anabaenopsis* | 79582 |
| | | *Anabaena* | 67016 |
| | 12/02/2002 | *Anabaenopsis* | 234558 |
| | | *Anabaena* | 858648 |
| | 21/02/2002 | *Anabaenopsis* | 242516 |
| | | *Anabaena* | 395607 |

**Sampling Period 2002-2003**

| Site | Date | Genera/Species | Cells mL |
|---|---|---|---|
| **Tomato** | 7/11/2002 | *Anabaena* | 1798973 |
| | 13/11/2002 | *Anabaena* | 1322199 |
| | 20/11/2002 | *Anabaena* | 1526719 |
| | 27/11/2002 | *Anabaena* | 1976199 |
| | 12/12/2002 | *M. aeruginosa* | 21571 |
| | | *P. mougeotii* | 167541 |
| | 18/12/2002 | *Anabaena* | 2004420 |
| | | *P. mougeotii* | 2513117 |
| | 10/01/2003 | *P. mougeotii* | 3230822 |
| | | *Anabaena* | 423041 |
| | 23/01/2003 | *M. flos-aquae* | 15523315 |
| | 30/01/2003 | *M. aeruginosa* | 1682323 |
| | | *M. flos-aquae* | 258432 |
| | 11/02/2003 | *M. aeruginosa* | 1907875 |
| | 20/02/2003 | *M. aeruginosa* | 6784387 |
| **Neil McDougall** | 13/11/2002 | *Anabaena* | 270788 |
| | 20/11/2002 | *Anabaena* | 1614502 |
| | 13/12/2002 | *M. flos-aquae* | 467021 |
| | | *Anabaena* | 87959 |
| | 24/01/2003 | *M. flos-aquae* | 1368875 |
| | 31/01/2003 | *M. flos-aquae* | 156179 |
| | | *A.elenkinii* | 135414 |
| | 11/02/2003 | *M. flos-aquae* | 1110379 |
| **Emu** | 7/11/2002 | *M. flos-aquae* | 7294323 |
| | | *M. aeruginosa* | 589117 |
| | | *A. ovalisporum* | 115185 |
| | 13/11/2002 | *M. flos-aquae* | 3445065 |
| | 21/11/2002 | *A. ovalisporum* | 108273 |
| | | *A. affinis* | 25759 |
| | 28/11/2002 | *M. flos-aquae* | 67334512 |
| | | *A. ovalisporum* | 15440 |
| | 13/12/2002 | *M. aeruginosa* | 245982 |
| | | *M. flos-aquae* | 2839621 |
| | 20/12/2002 | *A. ovalisporum* | 464927 |
| | | *A. affinis* | 13822 |
| | | *M. flos-aquae* | 3648837 |
| | 7/01/2003 | *A. ovalisporum* | 109503 |
| | | *M. aeruginosa* | 855968 |
| | | *M. flos-aquae* | 602887 |
| | 23/01/2003 | *M. flos-aquae* | 3281005 |
| | 30/01/2003 | *A. ovalisporum* | 57173 |
| | | *M. flos-aquae* | 341365 |
| | 20/02/2003 | *M. flos-aquae* | 896788 |
| | | *A. ovalisporum* | 281300 |
| **Brearley** | 13/11/2002 | *M. flos-aquae* | 8138531 |
| | 30/01/2003 | *A. bergii var. limnetica* | 55917 |
| **Bungana** | 20/12/2002 | *M. aeruginosa* | 1274779 |

| | | | |
|---|---|---|---|
| | 28/12/2002 | *M. flos-aquae* | 1848593 |
| | 7/01/2003 | *M. aeruginosa* | 899905 |
| | 30/01/2003 | *M. aeruginosa* | 591529 |
| | 11/02/2003 | *M. aeruginosa* | 1174361 |
| **Goollelal** | 13/11/2002 | *M. flos-aquae* | 4095976 |
| | 13/12/2002 | *M. flos-aquae* | 5600691 |
| | 20/12/2002 | *M. flos-aquae* | 1332847 |
| | 7/01/2003 | *M. flos-aquae* | 8554441 |
| | | *T. iwanoffianum* | 418853 |
| | 23/01/2002 | *M. flos-aquae* | 2666937 |
| | 20/02/2003 | *M. flos-aquae* | 6649860 |
| **Herdsman** | 7/11/2002 | *M. aeruginosa* | 1822682 |
| | | *M. flos-aquae* | 17481417 |
| | | *Anabaena* | 945949 |
| | 13/11/2002 | *M. flos-aquae* | 31683603 |
| | | *Anabaena* | 189367 |
| | | *M. aeruginosa* | 1089705 |
| | 21/11/2002 | *M. flos-aquae* | 16260380 |
| | 28/11/2002 | *M. flos-aquae* | 14890219 |
| | | *M. aeruginosa* | 8733082 |
| | 13/12/2002 | *M. flos-aquae* | 2127941 |
| | 20/12/2002 | *M. aeruginosa* | 1380233 |
| | 23/01/2003 | *M. aeruginosa* | 877975 |
| | | *M. flos-aquae* | 822248 |
| | 30/01/2003 | *M. flos-aquae* | 32047898 |
| | | *M. aeruginosa* | 201049 |
| | 11/02/2003 | *M. flos-aquae* | 23817321 |
| | 20/02/2003 | *M. flos-aquae* | 11582748 |
| **Joondalup** | 7/11/2002 | *M. flos-aquae* | 19665770 |
| | 13/11/2002 | *M. flos-aquae* | 47869646 |
| | 28/11/2002 | *M. flos-aquae* | 4561674 |
| | 13/12/2002 | *A. spiroides f. spiroides* | 663254 |
| | | *M. flos-aquae* | 18372352 |
| | 20/12/2002 | *M. flos-aquae* | 13666 |
| | 7/01/2003 | *M. flos-aquae* | 11606413 |
| | | *A. spiroides f. spiroides* | 309323 |
| | | *A. ovalisporum* | 677704 |
| | 23/01/2003 | *M. flos-aquae* | 3496283 |
| | | *A. bergii var. limnetica* | 480961 |
| | 30/01/2003 | *A. bergii var. limnetica* | 614068 |
| | | *Anabaenopsis* | 149080 |
| | 11/02/2003 | *A. bergii var. limnetica* | 851737 |
| | | *Anabaenopsis* | 98430 |
| **Yangebup** | 8/11/2002 | *M. flos-aquae* | 8010038 |
| | | *M. aeruginosa* | 5802943 |
| | 12/11/2002 | *M. aeruginosa* | 2136778 |
| | | *M. flos-aquae* | 2407776 |
| | 20/11/2002 | *M. flos-aquae* | 1690700 |
| | | *M. aeruginosa* | 2128401 |
| | 12/12/2002 | *Anabaena* | 2467043 |
| | | *M. flos-aquae* | 81959033 |

| | | | |
|---|---|---|---|
| | | *M. aeruginosa* | 5656608 |
| | 18/12/2002 | *Anabaena* | 546095 |
| | | *M. flos-aquae* | 33826985 |
| | | *M. aeruginosa* | 1993591 |
| | 10/01/2003 | *M. flos-aquae* | 5465738 |
| | | *M. aeruginosa* | 1189269 |
| | | *Anabaena* | 685059 |
| | | *A. elenkinii* | 200016 |
| | | *N. spumigena* | 26089 |
| | 24/01/2003 | *M. aeruginosa* | 2702229 |
| | | *M. flos-aquae* | 464927 |
| | 31/01/2003 | *M. aeruginosa* | 1745125 |
| | | *M. flos-aquae* | 455582 |
| | | *Anabaena* | 88738 |
| | | *A. elenkinii* | 14198 |
| | 11/02/2003 | *M. aeruginosa* | 473862 |
| | | *A. elenkinii* | 61584 |
| | | *M. flos-aquae* | 143223 |
| | 20/02/2003 | *M. flos-aquae* | 271626 |
| | | *M. aeruginosa* | 413198 |
| | | *N. spumigena* | 73299 |
| | | *A. elenkinii* | 236024 |
| **Bibra** | 8/11/2002 | *M. aeruginosa* | 402339 |
| | 27/11/2002 | *M. flos-aquae* | 1325216 |
| | 10/01/2003 | *Microcystis* | low amounts |
| | 31/01/2003 | *Microcystis* | low amounts |
| | 11/02/2003 | *A.ovalisporum* | 159196 |
| | | *M. aeruginosa* | 863777 |
| | 20/02/2003 | *M. aeruginosa* | 134350 |
| | | *A.ovalisporum* | 720554 |
| **North** | 20/11/2002 | *Anabaena* | 78622 |
| | 12/12/2002 | *Anabaena* | 157070 |
| | 31/01/2003 | *M. flos-aquae* | 20288559 |
| | 24/01/2003 | *Anabaena* | 650806 |
| **Blue Gum** | 12/11/2002 | *M. aeruginosa* | 2044002 |
| | 27/11/2002 | *M. aeruginosa* | 295291 |
| | | *M. flos-aquae* | 5195241 |
| | | *P. mougeotii* | 69739 |
| | 12/12/2002 | *P. mougeotii* present in net sample | |
| | 18/12/2002 | *M. flos-aquae* | 4146015 |
| | | *A. circinalis* | 14032 |
| | 2/01/2003 | *A. circinalis* | 1283686 |
| | 10/01/2003 | *A. circinalis* | 83414 |
| **Mary Carroll Park** | 13/11/2002 | *Anabaenopsis* | 9459 |
| | | *A. bergii var. limnetica* | 5324 |
| | 27/11/2002 | *A. bergii var. limnetica* present in net samples | |
| | | *Anabaenopsis* | 82881 |
| | 18/12/2002 | *Anabaenopsis* | 59268 |
| | 23/01/2003 | *Anabaenopsis* | 91310 |
| | | *A. bergii var. limnetica* | 421575 |
| | | *A. aphanizomenoides* | 139687 |

| | | |
|---|---|---|
| 30/01/2003 | *A. bergii var. limnetica* | 716238 |
| | *A. aphanizomenoides* | 490058 |
| | *Anabaenopsis* | 228903 |
| 11/02/2002 | *Anabaenopsis* | 307566 |
| | *A. bergii var. limnetica* | 590464 |

# Appendix D: BG-11 Medium

**Table D1.** BG-11 medium adapted from Stanier *et al.* (1971).

| **Compound** | **Amount** |
|---|---|
| $NaNO_3$ | 1.5 g |
| $K_2HPO_4$ | 0.04 g |
| $MgSO_4.7H_2O$ | 0.075 g |
| $CaCl_2.2H_2O$ | 0.036 g |
| Citric Acid | 0.006 g |
| Ferric ammonium citrate | 0.006 g |
| EDTA(disodium salt) | 0.001g |
| $NaCO_3$ | 0.02 g |
| Trace metal mix A5 | 1.0 mL |
| **Trace metal mix A5:** | |
| $H_3BO_3$ | 2.86 g |
| $MnCl_2.4H_2O$ | 1.81 g |
| $ZnSO_4.7H_2O$ | 0.222 g |
| $NaMoO_4.2H_2O$ | 0.39 g |
| $CuSO_4.5H_2O$ | 0.079 g |
| Co $(NO_3)_2.6H_2O$ | 49.4 mg |
| Distilled $H_2O$ | 1.0 L |

# Appendix E: Morphological Measurements

**Table E1.** Cell diameters of *Microcystis aeruginosa* Kützing.

| | Site | n | Mean (μm) | SD | Range (μm) |
|---|---|---|---|---|---|
| 2000-01 Collection | Bibra Lake | 60 | 5.29 | 0.43 | 5-6.75 |
| | Blue Gum Lake | 82 | 4.87 | 0.45 | 4-6 |
| | Booragoon Lake | 60 | 4.63 | 0.85 | 3.25-6 |
| | Lake Goollelal | 64 | 3.84 | 0.5 | 3 -5 |
| | Lake Gwelup | 80 | 4.66 | 0.74 | 3 -6 |
| | Herdsman Lake | 90 | 4.93 | 0.44 | 4-6 |
| | Hyde Park | 110 | 5.16 | 0.49 | 4-6 |
| | Jackadder Lake | 75 | 4.99 | 0.48 | 3.89-6 |
| | Neil McDougall Park | 80 | 5.35 | 0.68 | 4-7 |
| | North Lake | 50 | 5.43 | 0.66 | 4-7 |
| | Tomato Lake | 60 | 5.36 | 0.46 | 4.5-6 |
| | Yangebup Lake | 65 | 4.99 | 0.43 | 4.44-6 |
| | Kent St. (Canning River) | 80 | 4.91 | 0.42 | 4-6 |
| | Hester Park (Canning River) | 50 | 5.19 | 0.79 | 4-6 |
| | Liege St.(Canning River) | 76 | 4.84 | 0.63 | 3.75-6 |
| 2001-02 Collection | Booragoon Lake | 50 | 5.28 | 0.70 | 3.33-7 |
| | Blue Gum Lake | 50 | 5.10 | 0.55 | 3.76-6 |
| | Lake Goollelal | 50 | 5.11 | 0.54 | 3.89-6 |
| | Lake Monger | 50 | 4.79 | 0.63 | 3.33-6 |
| | Piney Lake Reserve | 50 | 5.52 | 0.48 | 4-6 |
| | Neil McDougall Park | 50 | 5.35 | 0.59 | 4.44-6.5 |
| | Lake Bungana | 50 | 5.30 | 0.49 | 4.16-6 |
| | Bibra Lake | 50 | 5.49 | 0.48 | 5-6.67 |
| | Perry Lakes | 50 | 5.08 | 0.45 | 4-6 |
| | Emu Lake | 50 | 5.35 | 0.63 | 4-6.66 |
| | Tomato Lake | 50 | 5.67 | 0.61 | 5-6.67 |
| | Jackadder Lake | 50 | 4.97 | 0.57 | 3.75-6.5 |
| | Hyde Park | 50 | 5.13 | 0.60 | 3.5-6 |
| | Yangebup Lake | 50 | 5.36 | 0.56 | 4.54-7 |
| | Herdsman Lake | 50 | 5 | 0.36 | 4.17-5.75 |
| | Lake Joondalup | 50 | 4.91 | 0.30 | 4-5.5 |
| | Canning River | 50 | 5.08 | 0.49 | 4-7 |
| 2002-03 Collection | Yangebup Lake | 50 | 5.15 | 0.58 | 4-6.5 |
| | Herdsman Lake | 50 | 5.02 | 0.34 | 4-6 |
| | Lake Bungana | 50 | 5.00 | 0.47 | 4-6 |
| | Tomato Lake | 50 | 5.36 | 0.42 | 5-6 |
| | Lake Brearley | 20 | 5.4 | 0.42 | 5-6 |
| | Blue Gum Lake | 50 | 5.29 | 0.53 | 4-6 |
| | Bibra Lake | 50 | 5.11 | 0.31 | 4.5-6 |
| | Emu Lake | 50 | 5.31 | 0.40 | 5-6 |
| | Neil McDougall Park | 50 | 5.40 | 0.55 | 4-7 |

**Table E2.** Cell diameters of *Microcystis flos-aquae* (Wittrock) Kirchner.

| | Site | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|
| 2000-01 Collection | Bibra Lake | 60 | 3.56 | 0.58 | 2.78-4.78 |
| | Blue Gum Lake | 84 | 3.50 | 0.44 | 2.78-4.44 |
| | Booragoon Lake | 65 | 3.46 | 0.53 | 2.56-4.5 |
| | Lake Claremont | 85 | 3.67 | 0.44 | 3-4.5 |
| | Lake Goollelal | 50 | 3.28 | 0.53 | 2-4.4 |
| | Lake Gwelup | 74 | 3.22 | 0.44 | 2.2-4 |
| | Herdsman Lake | 120 | 3.22 | 0.41 | 2-4 |
| | Hyde Park | 52 | 3.51 | 0.59 | 2.22-4.5 |
| | Jackadder Lake | 66 | 3.13 | 0.42 | 2.22-4 |
| | Lake Joondalup | 100 | 3.54 | 0.42 | 2.78-4.44 |
| | Neil McDougall Park | 80 | 2.95 | 0.39 | 2-4 |
| | North Lake | 65 | 3.65 | 0.53 | 2.78-5 |
| | Tomato Lake | 86 | 3.53 | 0.41 | 3-4 |
| | Yangebup Lake | 85 | 3.15 | 0.45 | 2-4 |
| | Kent St. (Canning River) | 60 | 3.51 | 0.44 | 2.78-4.44 |
| | Hester Park (Canning River) | 54 | 3.43 | 0.43 | 2.75-4 |
| | Liege St. (Canning River) | 90 | 3.48 | 0.43 | 2.75-4.44 |
| | Swan River | 200 | 3.35 | 0.54 | 1.85-4 |
| 2001-02 Collection | Booragoon Lake | 50 | 3.34 | 0.34 | 3-4.16 |
| | Blue Gum Lake | 50 | 3.37 | 0.42 | 2.86-4.28 |
| | Lake Goolellal | 50 | 3.43 | 0.41 | 2.85-4 |
| | Lake Monger | 50 | 4.13 | 0.5 | 2.77-5 |
| | Lake Gwelup | 50 | 2.90 | 0.45 | 1.67-4 |
| | Piney Lake Reserve | 50 | 3.24 | 0.33 | 3-4 |
| | Neil McDougall Park | 50 | 3.00 | 0.42 | 2.08-4 |
| | Lake Bungana | 50 | 3.50 | 0.33 | 3-4 |
| | Bibra Lake | 50 | 3.49 | 0.44 | 3-4 |
| | North Lake | 50 | 3.66 | 0.37 | 3-4 |
| | Emu Lake | 50 | 3.68 | 0.34 | 3-4.16 |
| | Tomato Lake | 50 | 3.61 | 0.46 | 2.86-4.28 |
| | Jackadder Lake | 50 | 3.36 | 0.39 | 2.75-4 |
| | Shenton Park Lake | 50 | 3.62 | 0.50 | 2.22-4.44 |
| | Hyde Park | 50 | 3.23 | 0.36 | 3-4 |
| | Yangebup Lake | 50 | 3.18 | 0.50 | 2.27-4 |
| | Lake Brearley | 50 | 3.88 | 0.36 | 3-4.5 |
| | Lake Bungana | 50 | 3.03 | 0.2 | 2.5-3.5 |
| | Mary Carroll Park | 50 | 3.31 | 0.38 | 3-4 |
| | Herdsman Lake | 50 | 3.14 | 0.39 | 2.5-4.29 |
| | Lake Joondalup | 50 | 3.39 | 0.37 | 3-4 |
| | Canning River | 50 | 3.75 | 0.32 | 3-4 |
| 2002-03 Collection | Yangebup Lake | 50 | 3.41 | 0.42 | 3-4 |
| | Herdsman Lake | 50 | 3.10 | 0.37 | 2.5-4 |
| | Neil McDougall Park | 50 | 3.39 | 0.41 | 3-4 |
| | Mary Carroll Park | 50 | 3.04 | 0.45 | 2-4 |
| | Tomato Lake | 50 | 3.41 | 0.41 | 3-4 |
| | Blue Gum Lake | 50 | 3.41 | 0.42 | 3-4 |
| | Lake Goolellal | 50 | 3.39 | 0.47 | 3-4 |
| | Bibra Lake | 50 | 3.15 | 0.32 | 2.75-4 |
| | Lake Joondalup | 50 | 3.43 | 0.44 | 3-4 |
| | Emu Lake | 50 | 3.40 | 0.44 | 3-4 |
| | Lake Brearley | 50 | 3.37 | 0.41 | 3-4 |
| | Canning River | 50 | 3.18 | 0.29 | 3-4 |

**Table E3.** Cell diameters of *Microcystis wesenbergii* Komárek.

| | Site | n | Mean (μm) | SD | Range (μm) |
|---|---|---|---|---|---|
| 2000-01 Collection | Hyde Park | 130 | 6.17 | 0.70 | 4.44-8 |
| | Tomato Lake | 60 | 6.19 | 0.52 | 4.44-7 |
| 2001-02 Collection | Shenton Park Lake | 50 | 5.83 | 0.59 | 4.44-7 |
| | Hyde Park | 50 | 6.09 | 0.41 | 5-7 |
| | Neil McDougall Park | 50 | 5.57 | 0.59 | 4.54-6.5 |
| Autumn-winter 2001 | Hyde Park | 50 | 5.34 | 0.46 | 5.56-7 |
| | Lake Claremont | 50 | 6.20 | 0.35 | 6-7 |
| | Perry Lakes | 50 | 6.14 | 0.36 | 5.5-7 |

**Table E4.** Mean cell measurements for *Anabaena affinis* Lemmermann collected from Emu Lake.

| | Cell Type | | n | Mean (μm) | SD | Range (μm) |
|---|---|---|---|---|---|---|
| 2001-02 Collection | Vegetative | L | 50 | 5.67 | 0.59 | 4-7 |
| | | B | 50 | 5.83 | 0.45 | 5-7 |
| | Heterocyte | L | 50 | 7.31 | 0.60 | 6-8 |
| | | B | 50 | 7.22 | 0.69 | 6-8 |
| | Akinete | L | 3 | 17.63 | 0.58 | 17-18 |
| | | B | 3 | 8.67 | 1.53 | 9-10 |
| 2002-03 Collection | Vegetative | L | 30 | 5.28 | 0.52 | 4-6 |
| | | B | 30 | 5.90 | 0.31 | 5-6.5 |
| | Heterocyte | L | 30 | 7.12 | 0.69 | 6-9 |
| | | B | 30 | 6.93 | 0.72 | 6-8 |

**Table E5.** Mean cell measurements for *Anabaena aphanizomenoides* Forti.

| | Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 3.48 | 0.77 | 2-5 |
| | | B | 50 | 3.57 | 0.85 | 2.5-5 |
| Mary Carroll | Heterocyte | L | 50 | 4.67 | 1.04 | 3-7 |
| Park | | B | 50 | 3.92 | 1.17 | 3-8 |
| 2001-02 | Akinete | L | 25 | 7.98 | 0.94 | 6-10 |
| | | B | 25 | 6.56 | 0.68 | 5-8 |
| | Apical | L | 21 | 4.79 | 1.19 | 3-6.5 |
| | | B | 21 | 2.69 | 0.92 | 2-4 |
| | Vegetative | L | 30 | 3.80 | 0.71 | 3-5 |
| Mary Carroll | | B | 30 | 2.93 | 0.17 | 2.5-3 |
| Park | Heterocyte | L | 16 | 4.69 | 0.48 | 4-5.5 |
| 2002-03 | | B | 16 | 3.34 | 0.47 | 3-4 |
| | Akinete | L | 3 | 8.67 | 1.15 | 8-10 |
| | | B | 3 | 7.17 | 0.29 | 7-7.5 |
| | Vegetative | L | 30 | 4.18 | 0.87 | 2.5-6.5 |
| Mary Carroll | | B | 30 | 2.92 | 0.32 | 2-3.5 |
| Park | Heterocyte | L | 30 | 4.87 | 0.76 | 3-6 |
| Cultured | | B | 30 | 3.65 | 0.56 | 3-5 |
| | Akinete | L | 21 | 8.14 | 0.96 | 7-10 |
| | | B | 21 | 7.29 | 0.94 | 6-9 |
| | Vegetative | L | 30 | 4.43 | 0.63 | 3-6 |
| Lake | | B | 30 | 2.80 | 0.34 | 2-3 |
| Joondalup | Heterocyte | L | 30 | 4.97 | 0.81 | 4-7 |
| 2002-03 | | B | 30 | 3.23 | 0.63 | 2-5 |
| | Akinete | L | 8 | 7.75 | 1.39 | 7-11 |
| | | B | 8 | 6.63 | 1.60 | 5-10 |

**Table E6.** Mean cell measurements for *Anabaena bergii var. limnetica* Coute & Preisig 1978.

| | Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 2.99 | 0.63 | 2-4.5 |
| | | B | 50 | 5.50 | 0.48 | 5-6 |
| Lake Coogee | Heterocyte | L | 50 | 6.00 | 0.67 | 4.5-8 |
| 2000-01 | | B | 50 | 6.94 | 0.65 | 5.5-8 |
| | Apical | L | 40 | 3.93 | 1.17 | 2-7 |
| | | B | 40 | 2.84 | 0.84 | 1.5-5 |
| | Vegetative | L | 50 | 4.25 | 0.96 | 3-7 |
| | | B | 50 | 3.76 | 1.10 | 3-6 |
| Lake Joondalup | Heterocyte | L | 50 | 5.11 | 0.82 | 3.33-7 |
| 2000-01 | | B | 50 | 4.55 | 0.93 | 2.78-6 |
| | Akinete | L | 7 | 8 | 2.08 | 6-11 |
| | | B | 7 | 7.43 | 2.57 | 5-11 |
| | Apical | L | 30 | 5.58 | 0.94 | 4-8 |
| | | B | 30 | 3.40 | 0.98 | 1.5-5 |
| | Vegetative | L | 40 | 3.28 | 0.88 | 2-5 |
| Lake Joondalup | | B | 40 | 4.75 | 0.61 | 4-5.5 |
| 2002-03 | Heterocyte | L | 30 | 5.60 | 0.72 | 5-7 |
| | | B | 30 | 5.63 | 0.52 | 5-7 |

**Table E6.** (*Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 3.62 | 0.87 | 2-5.5 |
| | | B | 50 | 5.29 | 0.53 | 4-6 |
| Lake Brearley | Heterocyte | L | 50 | 6.26 | 1.27 | 5-10 |
| 2001-02 | | B | 50 | 6.14 | 1.09 | 5-9 |
| | Akinete | L | 30 | 16.35 | 1.75 | 13-19 |
| | | B | 30 | 11.6 | 2.02 | 8-17 |
| | Apical | L | 30 | 5.53 | 1.52 | 3-8 |
| | | B | 30 | 2.90 | 0.53 | 2-4 |
| | Vegetative | L | 37 | 4 | 0.85 | 3-6 |
| Lake Brearley | | B | 47 | 5.65 | 0.82 | 4-8 |
| 2002-03 | Heterocyte | L | 40 | 7.03 | 1.66 | 4-10 |
| | | B | 40 | 7.18 | 1.72 | 4-10 |
| | Akinete | L | 8 | 19.25 | 3.06 | 14-22 |
| | | B | 8 | 13.13 | 0.64 | 12-14 |
| | Apical | L | 5 | 4.2 | 1.10 | 3-5 |
| | | B | 5 | 3.4 | 1.52 | 2-5 |
| | Vegetative | L | 50 | 3.28 | 0.63 | 2-4.5 |
| Mary Carroll | | B | 50 | 6.33 | 0.52 | 5-7 |
| Park | Heterocyte | L | 50 | 7.77 | 1.47 | 5-10 |
| 2001-02 | | B | 50 | 7.89 | 1.48 | 6-11 |
| | Akinete | L | 50 | 17.76 | 2.92 | 11-23 |
| | | B | 50 | 12.74 | 2.22 | 9-19 |
| | Vegetative | L | 50 | 4.09 | 0.96 | 3-7 |
| | | B | 50 | 5.5 | 0.72 | 4-7 |
| Mary Carroll | Heterocyte | L | 50 | 6.27 | 0.69 | 5-8 |
| Park | | B | 50 | 6.16 | 1 | 4-10 |
| 2002-03 | Akinete | L | 50 | 13.06 | 2.15 | 10-20 |
| | | B | 50 | 11.12 | 0.9 | 10-15 |
| | Apical | L | 14 | 6.07 | 1.38 | 3-8 |
| | | B | 14 | 3.25 | 0.58 | 2-4 |
| | Vegetative | L | 50 | 3.51 | 0.77 | 2.5-5 |
| Bibra Lake | | B | 50 | 5.50 | 0.66 | 4-7 |
| 2001-02 | Heterocyte | L | 50 | 8.15 | 1.29 | 5-10 |
| | | B | 50 | 7.86 | 1.34 | 5-9.5 |
| | Akinete | L | 1 | 19 | | |
| | | B | 1 | 6.5 | | |
| | Vegetative | L | 50 | 3.33 | 0.84 | 2-6 |
| | | B | 50 | 4.76 | 0.70 | 4- 6 |
| Bibra Lake | Heterocyte | L | 37 | 6.62 | 1.52 | 5-10 |
| 2002-03 | | B | 37 | 5.76 | 1.35 | 4-10 |
| | Akinete | L | 25 | 14.56 | 5.42 | 11-20 |
| | | B | 25 | 10.32 | 1.07 | 7-12 |
| | Vegetative | L | 60 | 4.95 | 0.72 | 3-6 |
| | | B | 60 | 5.14 | 0.78 | 4-6.5 |
| Bibra Lake | Heterocyte | L | 60 | 5.90 | 0.68 | 5-7 |
| Cultured | | B | 60 | 5.68 | 0.88 | 4-8 |
| | Akinete | L | 35 | 11.27 | 1.16 | 10-14 |
| | | B | 35 | 9.54 | 0.61 | 8-11 |
| | Apical | L | 30 | 8.13 | 2.26 | 4-14 |
| | | B | 30 | 3.12 | 0.34 | 2.5-4 |

**Table E6.** (*Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| | Vegetative | L | 60 | 3.92 | 0.78 | 2-5 |
| | | B | 60 | 4.95 | 1.00 | 3-7 |
| Emu Lake | Heterocyte | L | 60 | 6.01 | 1.24 | 3-9 |
| 2001-02 | | B | 60 | 5.57 | 1.17 | 3-9.5 |
| | Akinete | L | 17 | 13.59 | 3.79 | 9-20 |
| | | B | 17 | 9.29 | 2.54 | 5-17 |
| | Apical | L | 40 | 6.31 | 1.57 | 3-10 |
| | | B | 40 | 2.56 | 0.57 | 2-4 |
| | Vegetative | L | 30 | 3.52 | 0.65 | 2-4.5 |
| Emu Lake | | B | 30 | 3.80 | 0.52 | 3-5 |
| 2002-03 | Heterocyte | L | 7 | 6.14 | 0.69 | 5-7 |
| | | B | 7 | 5.14 | 0.38 | 5-6 |
| | Akinete | L | 2 | 19 | 1.14 | 18-20 |
| | | B | 2 | 10 | | 10 |

**Table E7.** Mean cell measurements for *Anabaena circinalis* Rabenhorst ex Bornet & Flahault 1888 collected from the freshwater lakes.

| **Location** | **Cell Type** | | **n** | **Mean (µm)** | **SD** | **Range (µm)** |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 4.57 | 0.71 | 3.5-6 |
| Yangebup Lake | | B | 50 | 5.38 | 0.72 | 4.44-8 |
| 2000-01 | Heterocyte | L | 50 | 7.46 | 1 | 5.55-9 |
| | | B | 50 | 7.35 | 0.87 | 5.55-9 |
| | Vegetative | L | 50 | 5.38 | 0.59 | 4.5-7 |
| Yangebup Lake | | B | 50 | 7.50 | 0.52 | 6-8 |
| 2001-02 | Heterocyte | L | 50 | 7.54 | 0.79 | 6-9 |
| | | B | 50 | 7.88 | 0.74 | 6-9.5 |
| | Akinete | L | 31 | 20.16 | 1.77 | 15-23 |
| | | B | 31 | 13.18 | 1.84 | 10-16 |
| | Vegetative | L | 30 | 5.30 | 0.79 | 4-7 |
| Yangebup Lake | | B | 30 | 6.97 | 0.93 | 6-9 |
| 2002-03 | Heterocyte | L | 30 | 7.40 | 0.67 | 6-9 |
| | | B | 30 | 7.20 | 0.71 | 6-9 |
| | Akinete | L | 13 | 22.31 | 1.60 | 20-25 |
| | | B | 13 | 12.15 | 1.21 | 10-14 |
| | Spiral | H | 21 | 23 | 4.43 | 15-30 |
| | | W | 23 | 32.31 | 2.17 | 30-35 |
| | Vegetative | L | 50 | 4.94 | 0.54 | 4-6.25 |
| North Lake | | B | 50 | 7 | 0.90 | 5-9 |
| 2000-01 | Heterocyte | L | 50 | 7.97 | 1.01 | 6-10 |
| | | B | 50 | 8.58 | 0.91 | 7-11 |
| | Spirals | H | 30 | 31.33 | 6.82 | 20-45 |
| | | W | 30 | 36.6 | 5.24 | 30-50 |
| | Vegetative | L | 50 | 4.94 | 0.56 | 4-6 |
| North Lake | | B | 50 | 7.99 | 0.56 | 7-9 |
| 2001-02 | Heterocyte | L | 50 | 8.97 | 1.13 | 7-11 |
| | | B | 50 | 9.29 | 1.25 | 7-12 |
| | Spiral | H | 30 | 36.58 | 5.19 | 30-47.5 |
| | | W | 30 | 64.75 | 7.69 | 52.5-85 |

**Table E7.** (*Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 5.30 | 0.61 | 4-7 |
| North Lake | | B | 50 | 7.85 | 0.44 | 7-9 |
| 2002-03 | Heterocyte | L | 30 | 7.80 | 0.92 | 6-9 |
| | | B | 30 | 7.98 | 1 | 6-10 |
| | Akinete | L | 11 | 19.82 | 2.36 | 16-23 |
| | | B | 11 | 13.09 | 1.64 | 10-15 |
| | Vegetative | L | 50 | 4.97 | 0.72 | 4-7 |
| | | B | 50 | 6.32 | 0.69 | 5-7.5 |
| Tomato Lake | Heterocyte | L | 50 | 6.96 | 1.07 | 5-9 |
| 2000-01 | | B | 50 | 7.79 | 1.04 | 6-10 |
| | Akinete | L | 8 | 14.13 | 2.64 | 10-22 |
| | | B | 8 | 9.38 | 1.30 | 8-11 |
| | Vegetative | L | 50 | 4.53 | 0.61 | 3-6 |
| | | B | 50 | 6.03 | 0.57 | 4-7 |
| Tomato Lake | Heterocyte | L | 50 | 6.25 | 0.56 | 5-8 |
| 2001-02 | | B | 50 | 6.66 | 0.70 | 5-8 |
| | Spiral | H | 30 | 19.71 | 3.53 | 15-32.5 |
| | | W | 30 | 44.92 | 5.01 | 37.5-57.5 |
| | Vegetative | L | 50 | 4.80 | 0.39 | 5-5 |
| | | B | 50 | 5.68 | 0.46 | 5-6.5 |
| Tomato Lake | Heterocyte | L | 45 | 5.47 | 0.57 | 4.5-7 |
| 2002-03 | | B | 45 | 5.83 | 0.63 | 5-7 |
| | Akinete | L | 38 | 18.13 | 1.57 | 16-21 |
| | | B | 38 | 9.65 | 0.94 | 7-11 |
| | Spiral | H | 27 | 10.94 | 1.82 | 7.5-15 |
| | | W | 27 | 30.80 | 5.05 | 20-40 |
| | Vegetative | L | 50 | 4.68 | 0.54 | 3.5-5.5 |
| | | B | 50 | 5.81 | 0.39 | 5-6.5 |
| Blue Gum Lake | Heterocyte | L | 50 | 5.82 | 0.569 | 4-7 |
| 2001-02 | | B | 50 | 6.14 | 0.589 | 5-7 |
| | Akinete | L | 20 | 14.6 | 3.033 | 9-19 |
| | | B | 20 | 9.425 | 1.27 | 7-11 |
| | Vegetative | L | 30 | 4.98 | 0.56 | 4-6 |
| Blue Gum Lake | | B | 30 | 5.75 | 0.47 | 5-6.5 |
| 2002-03 | Heterocyte | L | 30 | 5.68 | 0.70 | 5-7 |
| | | B | 30 | 5.72 | 0.60 | 5-7 |
| | Akinete | L | 30 | 18.10 | 2.78 | 15-27 |
| | | B | 30 | 10.90 | 0.99 | 10-13 |
| | Vegetative | L | 84 | 5.86 | 1.04 | 4-9 |
| Neil McDougall | | B | 84 | 7.69 | 0.93 | 6-10 |
| Park | Heterocyte | L | 84 | 8.76 | 1.14 | 6-12 |
| 2000-01 | | B | 84 | 9.98 | 1.17 | 6-12 |
| | Akinete | L | 41 | 22.02 | 5.50 | 10-35 |
| | | B | 41 | 11.44 | 2.12 | 8-19 |
| | Vegetative | L | 50 | 5.42 | 0.75 | 4-7 |
| Neil McDougall | | B | 50 | 6.63 | 0.45 | 6-7 |
| Park | Heterocyte | L | 50 | 7.04 | 0.79 | 5-9 |
| 2001-02 | | B | 50 | 7.31 | 0.83 | 6-9 |
| | Akinete | L | 30 | 21.27 | 3.32 | 15-26 |
| | | B | 30 | 11.45 | 2.08 | 8-15 |

**Table E7.** (*Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| | Vegetative | L | 30 | 5.45 | 0.69 | 4-7 |
| | | B | 30 | 6.65 | 0.51 | 6-7.5 |
| Neil McDougall | Heterocyte | L | 30 | 7.28 | 0.54 | 6.5-8.5 |
| Park | | B | 30 | 7.15 | 0.63 | 6-9 |
| 2002-03 | Akinete | L | 24 | 24.29 | 3.13 | 18-30 |
| | | B | 24 | 11.42 | 2.04 | 8-16 |
| | Spiral | H | 25 | 25.7 | 4.36 | 15-32.5 |
| | | W | 25 | 56.2 | 8.69 | 45-75 |
| | Vegetative | L | 50 | 4.81 | 0.75 | 3-6 |
| | | B | 50 | 8.18 | 0.95 | 6-10 |
| Herdsman Lake | Heterocyte | L | 50 | 7.86 | 1.56 | 5-11 |
| 2001-02 | | B | 50 | 7.74 | 1.35 | 5.5-11 |
| | Akinete | L | 50 | 24.98 | 4.30 | 15-35 |
| | | B | 50 | 13.20 | 2.46 | 9-18 |
| | Spiral | H | 30 | 22.33 | 4.20 | 15-30 |
| | | W | 30 | 54.17 | 12.41 | 25-75 |
| | Vegetative | L | 30 | 5.15 | 0.76 | 4-7 |
| Herdsman Lake | | B | 30 | 5.92 | 0.82 | 5-8 |
| 2002-03 | Heterocyte | L | 30 | 7.07 | 0.94 | 5-9 |
| | | B | 30 | 6.87 | 1.03 | 5-9 |
| | Vegetative | L | 50 | 4.72 | 0.53 | 4-6 |
| Lake Bungana | | B | 50 | 6.21 | 0.50 | 5-7 |
| 2001-02 | Heterocyte | L | 50 | 5.28 | 0.89 | 3.5-7 |
| | | B | 50 | 5.44 | 0.81 | 4-7 |
| | Akinete | L | 3 | 15.67 | 3.79 | 13-20 |
| | | B | 3 | 8.5 | 2.18 | 7-11 |
| Mary | Vegetative | L | 30 | 4.82 | 0.61 | 4-6 |
| Carroll Park | | B | 30 | 6.10 | 0.48 | 5-7 |
| 2002-03 | Heterocyte | L | 30 | 6.50 | 0.74 | 5-8 |
| | | B | 30 | 6.58 | 0.6 | 6-8 |

**Table E8.** Mean cell measurements for *A. circinalis* collected from the Canning River.

| | **Cell Type** | | **n** | **Mean (µm)** | **SD** | **Range (µm)** |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 5.06 | 0.70 | 4-7 |
| Kent St. Weir | | B | 50 | 6.64 | 0.61 | 6-8 |
| 2000-01 | Heterocyte | L | 50 | 7.38 | 1.01 | 6-10 |
| | | B | 50 | 8.14 | 0.95 | 6.5-10 |
| | Vegetative | L | 50 | 5.73 | 0.61 | 4.5-7 |
| | | B | 50 | 7.50 | 0.60 | 6-9 |
| Liege St. | Heterocyte | L | 50 | 8.17 | 1.15 | 6-10 |
| 2000-01 | | B | 50 | 8.57 | 1.18 | 6-10 |
| | Akinete | L | 30 | 19.82 | 2.46 | 15-25 |
| | | B | 30 | 12.15 | 1.45 | 10-15 |
| | Vegetative | L | 50 | 5.18 | 0.53 | 4-6.5 |
| Kent St Weir | | B | 50 | 7.06 | 0.71 | 6-8 |
| 2001-02 | Heterocyte | L | 50 | 7.11 | 0.63 | 6-8 |
| | | B | 50 | 7.45 | 0.78 | 6-9 |
| | Akinete | L | 50 | 18.14 | 3.80 | 13-31 |
| | | B | 50 | 10.6 | 1.29 | 9-14 |

**Table E8.** (*Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| | Vegetative | L | 30 | 5.43 | 0.68 | 5-7 |
| Kent St Weir | | B | 30 | 7.53 | 0.63 | 6-8 |
| (Balbiri Close) | Heterocyte | L | 30 | 7.83 | 0.79 | 6-10 |
| 2002-03 | | B | 30 | 8.10 | 0.80 | 7-10 |
| | Akinete | L | 21 | 21 | 2.17 | 16-25 |
| | | B | 21 | 13.48 | 1.50 | 11-17 |
| | Spiral | H | 20 | 18 | 2.51 | 12.5-22.5 |
| | | W | 20 | 38.38 | 3.83 | 27.5-45 |

**Table E9.** Mean cell measurements for *Anabaena flos-aquae* f. *flos-aquae* (Lyngb.) Komárek.

| **Location** | **Cell Type** | | **n** | **Mean (µm)** | **SD** | **Range (µm)** |
|---|---|---|---|---|---|---|
| Herdsman | Vegetative | L | 50 | 4.58 | 0.53 | 4-6 |
| Lake | | B | 50 | 5.88 | 0.31 | 5-6.5 |
| 2001-02 | Heterocyte | L | 30 | 6.36 | 0.75 | 5-9 |
| | | B | 30 | 6.64 | 0.71 | 5-9 |
| Herdsman | Vegetative | L | 30 | 4.75 | 0.50 | 4-6 |
| Lake | | B | 30 | 5.17 | 0.33 | 5-6 |
| 2002-03 | Heterocyte | L | 20 | 5.25 | 0.85 | 4-7 |
| | | B | 20 | 5.23 | 0.90 | 4-7 |
| | Vegetative | L | 50 | 4.52 | 0.61 | 3-5 |
| North Lake | | B | 50 | 5.64 | 0.53 | 5-6.5 |
| 2002-03 | Heterocyte | L | 30 | 5.70 | 0.74 | 4-7 |
| | | B | 30 | 5.95 | 0.62 | 5-7 |
| | Akinete | L | 7 | 18.29 | 1.60 | 16-20 |
| | | B | 7 | 10.14 | 0.38 | 10-11 |
| | Vegetative | L | 30 | 4.53 | 0.51 | 4-5 |
| Kent St Weir | | B | 30 | 5.27 | 0.61 | 4-7 |
| (Balbiri Close) | Heterocyte | L | 30 | 5.42 | 0.49 | 5-6 |
| 2002-03 | | B | 30 | 5.73 | 0.58 | 5-7 |
| | Akinete | L | 22 | 16.95 | 1.68 | 14-20 |
| | | B | 22 | 10.14 | 1.42 | 8-12 |

**Table E10.** Mean cell measurements for *Anabaena spiroides* f. *spiroides* (Elenkin) Komárek.

| | Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 4.33 | 0.70 | 2.77-5 |
| North Lake | | B | 50 | 5.05 | 0.46 | 4-6 |
| 2000-01 | Heterocyte | L | 50 | 6.53 | 1.14 | 4.44-9 |
| | | B | 50 | 6.50 | 1.11 | 4.44-9 |
| | Akinete | L | 30 | 8.25 | 0.98 | 7-10 |
| | | B | 30 | 8.17 | 1.15 | 5-10 |
| | Spiral | L | 30 | 15.17 | 3.56 | 10-26 |
| | | B | 30 | 24.49 | 4.33 | 15-33.33 |
| | Vegetative | L | 50 | 4.17 | 0.65 | 3-5 |
| North Lake | | B | 50 | 5.01 | 0.54 | 4-6 |
| 2002-03 | Heterocyte | L | 41 | 6.33 | 0.74 | 5-8 |
| | | B | 41 | 6.27 | 0.83 | 4-8 |
| | Akinete | L | 10 | 10.90 | 1.37 | 9-13 |
| | | B | 10 | 10.70 | 1.34 | 9-13 |
| | Spiral | H | 20 | 10.60 | 1.93 | 7-15 |
| | | W | 20 | 21.90 | 2.63 | 18-30 |
| | Vegetative | L | 50 | 4.68 | 0.58 | 3.5-6 |
| Tomato Lake | | B | 50 | 4.98 | 0.43 | 4-6 |
| 2000-01 | Heterocyte | L | 50 | 5.56 | 0.83 | 4-7.5 |
| | | B | 50 | 5.56 | 0.83 | 4- 7.5 |
| | Akinete | L | 50 | 10.20 | 1.52 | 6-15 |
| | | B | 50 | 9.93 | 1.41 | 6-13.33 |
| | Spiral | L | 15 | 14.07 | 3.37 | 7-20 |
| | | B | 15 | 25.87 | 2.88 | 22-31 |
| | Vegetative | L | 50 | 4.7 | 0.65 | 3-6 |
| Tomato Lake | | B | 50 | 5.94 | 0.48 | 5-7 |
| 2001-02 | Heterocyte | L | 50 | 5.64 | 0.78 | 4-8 |
| | | B | 50 | 5.98 | 0.72 | 5-8 |
| | Spiral | H | 30 | 11.5 | 1.59 | 8.75-15 |
| | | W | 30 | 28.92 | 2.22 | 25-32.5 |
| | Vegetative | L | 50 | 4.65 | 0.52 | 4-6 |
| Tomato Lake | | B | 50 | 5.33 | 0.44 | 5-6 |
| 2002-03 | Heterocyte | L | 40 | 5.40 | 0.63 | 4-7 |
| Morphotype 1 | | B | 40 | 6.05 | 0.49 | 5-7 |
| | Akinete | L | 8 | 16.50 | 2.83 | 11-20 |
| | | B | 8 | 9.38 | 1.19 | 8-11 |
| | Spiral | H | 10 | 11.80 | 1.55 | 10-15 |
| | | W | 10 | 24.10 | 2.64 | 20-28 |
| | Vegetative | L | 50 | 4.34 | 0.84 | 2-6 |
| Lake Joondalup | | B | 50 | 4.81 | 1.07 | 3-8 |
| 2000-01 | Heterocyte | L | 50 | 6.19 | 1.13 | 4-9 |
| | | B | 50 | 6.21 | 1.32 | 4-10 |
| | Akinete | L | 50 | 9.38 | 2.89 | 6-17 |
| | | B | 50 | 8.18 | 1.46 | 6-11 |
| | Spiral | L | 30 | 12.70 | 2.58 | 8.5-19 |
| | | B | 30 | 20.83 | 3.44 | 11-30 |

**Table E10.** (*Continued*)

| | | | | | | |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 4.29 | 0.50 | 3-5 |
| Lake Joondalup | | B | 50 | 5.07 | 0.29 | 4.5-6 |
| 2001-02 | Heterocyte | L | 50 | 5.96 | 0.65 | 5-7 |
| | | B | 50 | 5.97 | 0.56 | 5-7 |
| | Akinete | L | 50 | 8.68 | 1.49 | 7-12 |
| | | B | 50 | 8.23 | 1.40 | 7-12 |
| | Spiral | H | 30 | 12.33 | 1.30 | 10-15 |
| | | W | 30 | 26.42 | 2.99 | 22.5-32.5 |
| | Vegetative | L | 30 | 4.60 | 0.50 | 4-5 |
| Lake Joondalup | | B | 30 | 4.15 | 0.35 | 4-5 |
| 2002-03 | Heterocyte | L | 30 | 4.98 | 0.53 | 4-6 |
| | | B | 30 | 4.58 | 0.59 | 4-6 |
| | Akinete | L | 23 | 7.46 | 1.14 | 6-10 |
| | | B | 23 | 7.48 | 1.16 | 6-10 |
| | Vegetative | L | 50 | 4.10 | 0.72 | 3-5 |
| Shenton Park | | B | 50 | 5.01 | 0.07 | 5-5.5 |
| 2001-02 | Heterocyte | L | 50 | 6.14 | 0.82 | 5-8 |
| | | B | 50 | 6.17 | 0.81 | 5-8 |
| | Akinete | L | 50 | 9.53 | 1.31 | 7-13 |
| | | B | 50 | 9.49 | 1.26 | 7-12 |
| | Spiral | H | 30 | 13.83 | 2.51 | 10-20 |
| | | W | 30 | 27.13 | 3.05 | 19-33 |
| | Vegetative | L | 50 | 4.63 | 1.11 | 3-7 |
| Lake Claremont | | B | 50 | 5.76 | 0.99 | 4.5-8 |
| 2001-02 | Heterocyte | L | 50 | 5.96 | 0.90 | 5-8 |
| | | B | 50 | 6.09 | 1.29 | 5-9 |
| | Akinete | L | 15 | 9.40 | 2.41 | 7-17 |
| | | B | 15 | 8.73 | 1.10 | 7- 11 |

**Table E11.** Mean cell measurements for *Anabaena torulosa* var. *tenuis* (Lemmermann) Geitler collected from Mary Carroll Park.

| | **Cell Type** | | **n** | **Mean (µm)** | **SD** | **Range (µm)** |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 3.74 | 0.79 | 2-6 |
| | | B | 50 | 3.20 | 0.40 | 2.5-4 |
| | Heterocyte | L | 30 | 4.27 | 0.92 | 3-6 |
| 2001-02 | | B | 30 | 3.35 | 0.46 | 3-4 |
| Collection | Akinete | L | 13 | 7.88 | 1.50 | 6-10 |
| | | B | 13 | 6.62 | 1.04 | 5-8 |
| | Apical | L | 10 | 4.15 | 0.75 | 3-5 |
| | | B | 10 | 2.30 | 0.42 | 2-3 |

**Table E12.** Mean cell measurements for *Anabaenopsis arnoldii* Aptekarj collected from Lake Claremont.

| | **Cell Type** | | **n** | **Mean (µm)** | **SD** | **Range (µm)** |
|---|---|---|---|---|---|---|
| 2001-02 Collection | Vegetative | L | 50 | 5.47 | 1.5 | 3-9 |
| | | B | 50 | 8.55 | 0.88 | 7-11 |
| | Terminal heterocyte | L | 50 | 9.11 | 1.06 | 8-12 |
| | | B | 50 | 8.74 | 0.99 | 7-12 |
| | Intercalary heterocyte | L | 50 | 9.31 | 1.13 | 7-12 |
| | | B | 50 | 9.27 | 1.47 | 7-12 |
| | Akinete | L | 50 | 13.54 | 1.53 | 11-17 |
| | | B | 50 | 11.72 | 1.16 | 9-15 |
| | Spiral | H | 35 | 16.71 | 3.87 | 10-27.5 |
| | | W | 35 | 40.57 | 3.93 | 32.5-50 |

**Table E13.** Mean cell measurements for *Anabaenopsis elenkinii.* Miller 1923.

| | **Cell Type** | | **n** | **Mean (µm)** | **SD** | **Range (µm)** |
|---|---|---|---|---|---|---|
| Bibra Lake 2000-01 | Vegetative | L | 50 | 4.84 | 1.36 | 2- 9 |
| | | B | 50 | 5.13 | 1.05 | 3-7 |
| | Terminal heterocyte | L | 50 | 7.04 | 1.15 | 5-10 |
| | | B | 50 | 6.06 | 0.93 | 3.5-8 |
| | Intercalary heterocyte | L | 32 | 5.33 | 1.53 | 3-10 |
| | | B | 32 | 5.58 | 0.86 | 5-8 |
| | Akinete | L | 10 | 12.02 | 1.75 | 10-15 |
| | | B | 10 | 7.85 | 1.92 | 5-12 |
| Neil McDougall Park 2000-01 | Vegetative | L | 50 | 7.35 | 1.28 | 5- 10 |
| | | B | 50 | 5.52 | 0.51 | 5- 7 |
| | Terminal heterocyte | L | 50 | 6.35 | 0.92 | 4- 8 |
| | | B | 50 | 5.37 | 0.65 | 4- 6 |
| | Intercalary heterocyte | L | 30 | 6.27 | 1.29 | 4- 9 |
| | | B | 30 | 5.30 | 0.79 | 4- 6.5 |
| | Akinete | L | 50 | 13.19 | 1.44 | 10- 17 |
| | | B | 50 | 8.88 | 1.05 | 6- 11 |
| Neil McDougall Park 2002-03 | Vegetative | L | 30 | 6.23 | 1.50 | 5- 10 |
| | | B | 30 | 5.20 | 0.60 | 4- 6 |
| | Terminal heterocyte | L | 30 | 5.73 | 0.74 | 5- 8 |
| | | B | 30 | 4.87 | 0.39 | 4- 6 |
| | Akinete | L | 30 | 12.30 | 1.49 | 8- 15 |
| | | B | 30 | 7.87 | 1.07 | 6-10 |
| Lake Joondalup 2000-01 | Vegetative | L | 50 | 8.18 | 1.49 | 5-10 |
| | | B | 50 | 4.92 | 0.57 | 4- 6 |
| | Terminal heterocyte | L | 43 | 5.52 | 1.14 | 4- 8 |
| | | B | 43 | 4.20 | 0.41 | 3.5- 5 |
| | Intercalary heterocyte | L | 13 | 4.15 | 0.90 | 3- 6 |
| | | B | 13 | 4.19 | 0.38 | 4- 5 |
| | Akinete | L | 15 | 13.20 | 2.68 | 10-18 |
| | | B | 15 | 8.40 | 0.91 | 7-10 |

**Table E13.** (*Continued*)

| | | | | | | |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 6.13 | 0.84 | 4-8 |
| Lake | | B | 50 | 5.70 | 0.40 | 5- 6 |
| Joondalup | Terminal heterocyte | L | 50 | 5.08 | 0.51 | 4-6 |
| 2001-02 | | B | 50 | 5.12 | 0.47 | 4-6 |
| | Intercalary heterocyte | L | 30 | 5.38 | 0.50 | 4 .5- 6 |
| | | B | 30 | 5.24 | 0.41 | 4-6 |
| | Vegetative | L | 50 | 5.86 | 1.01 | 4-8.89 |
| | | B | 50 | 5.93 | 0.71 | 4.44- 7 |
| Shenton Park | Terminal heterocyte | L | 50 | 7.65 | 1.11 | 5-11 |
| 2000-01 | | B | 50 | 6.69 | 1.04 | 4.44-9 |
| | Intercalary heterocyte | L | 35 | 6.49 | 1.27 | 4.44-9 |
| | | B | 35 | 6.01 | 1.02 | 4.44- 8 |
| | Akinete | L | 50 | 13.03 | 1.17 | 10-19.11 |
| | | B | 50 | 9.15 | 1.38 | 6.67-13 |
| | Vegetative | L | 50 | 6.30 | 1.31 | 4-10 |
| | | B | 50 | 6.39 | 0.56 | 5-7 |
| Shenton Park | Terminal heterocyte | L | 50 | 7.26 | 1.05 | 6-10 |
| 2001-02 | | B | 50 | 6.34 | 0.87 | 5-9 |
| | Intercalary heterocyte | L | 50 | 7.52 | 0.91 | 6-10 |
| | | B | 50 | 6.37 | 0.86 | 5-9 |
| | Akinete | L | 50 | 13.32 | 1.45 | 10-16 |
| | | B | 50 | 9.66 | 1.29 | 7-13 |
| | Vegetative | L | 50 | 7.01 | 2.03 | 3-12 |
| Mary Carroll | | B | 50 | 6.33 | 0.84 | 5-8 |
| Park | Terminal heterocyte | L | 50 | 4.20 | 0.80 | 3-6 |
| 2001-02 | | B | 50 | 4.28 | 0.72 | 3-6 |
| | Akinete | L | 50 | 11.10 | 1.75 | 8-17 |
| | | B | 50 | 9.48 | 1.52 | 7-14 |
| Yangebup | Vegetative | L | 30 | 8.53 | 1.93 | 5-12 |
| Lake | | B | 30 | 3.75 | 0.39 | 3-4 |
| 2002-03 | Terminal heterocyte | L | 30 | 4.65 | 1.04 | 3-8 |
| | | B | 30 | 3.75 | 0.43 | 3-4 |
| | Vegetative | L | 30 | 5.65 | 1.11 | 4-9 |
| Yangebup | | B | 30 | 5.45 | 0.55 | 5-7 |
| Lake | Terminal heterocyte | L | 30 | 5.75 | 0.84 | 4.5-9 |
| 2002-03 | | B | 30 | 5.60 | 0.64 | 4.5-7 |
| | Akinete | L | 30 | 11.33 | 1.32 | 9-14 |
| | | B | 30 | 8.62 | 1.03 | 7-11 |

**Table E14.** Mean cell measurements for *Anabaena elenkinii* Miller 1923 collected from the Serpentine River.

| | Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|---|
| 2000-01 | Vegetative | L | 35 | 7.39 | 1.76 | 4-10 |
| | | B | 35 | 7.56 | 1.01 | 6-9 |
| | Terminal heterocyte | L | 32 | 8.56 | 1.01 | 7-10 |
| | | B | 32 | 7.95 | 0.99 | 6-10 |
| | Akinete | L | 2 | 16.50 | 0.71 | 16-17 |
| | | B | 2 | 10.50 | 0.71 | 10-11 |
| 2001-02 | Vegetative | L | 30 | 6.32 | 1.10 | 4-9 |
| | | B | 30 | 6.37 | 0.89 | 4-8 |
| | Terminal heterocyte | L | 30 | 7.85 | 1.17 | 5-10 |
| | | B | 30 | 6.53 | 0.73 | 5-8 |
| | Akinete | L | 5 | 14.20 | 1.79 | 11-15 |
| | | B | 5 | 10.60 | 0.89 | 9-11 |

**Table E15.** Mean cell measurements for *Anabaenopsis tanganyikae* (G.S. West) Woloszynska & Miller.

| | Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|---|
| Mary Carroll Park 2001-02 | Vegetative | L | 50 | 8.51 | 2.45 | 6-18 |
| | | B | 50 | 4.60 | 0.61 | 3.5-6 |
| | Terminal heterocyte | L | 50 | 4.79 | 0.87 | 4-7 |
| | | B | 50 | 4.13 | 0.56 | 3-5.5 |
| | Intercalary heterocyte (juvenile) | L | 12 | 4.125 | 0.61 | 3-5 |
| | | B | 12 | 4.25 | 0.38 | 4-5 |
| | Akinete | L | 50 | 13.07 | 1.93 | 9-18 |
| | | B | 50 | 7.99 | 1.48 | 5-11 |
| Mary Carroll Park 2002-03 | Vegetative | L | 30 | 7.80 | 1.65 | 6-13 |
| | | B | 30 | 4.88 | 0.34 | 4-5.5 |
| | Terminal heterocyte | L | 30 | 5.43 | 0.62 | 4-6.5 |
| | | B | 30 | 4.93 | 0.55 | 4-6 |
| | Akinete | L | 30 | 12.30 | 1.49 | 8-15 |
| | | B | 30 | 7.87 | 1.07 | 6-10 |
| Mary Carroll Park 2002-03 | Vegetative | L | 30 | 8.07 | 1.23 | 6-10 |
| | | B | 30 | 4.33 | 0.65 | 4-6 |
| | Terminal heterocyte | L | 11 | 4 | 0.77 | 3-6 |
| | | B | 11 | 3.77 | 0.61 | 3-5 |
| | Akinete | L | 6 | 9.33 | 1.37 | 7-11 |
| | | B | 6 | 7.5 | 1.05 | 6-9 |
| Lake Joondalup 2001-02 | Vegetative | L | 33 | 9.45 | 1.03 | 8-13 |
| | | B | 33 | 4.86 | 0.88 | 3-6 |
| | Terminal heterocyte | L | 13 | 6.35 | 1.41 | 5-9 |
| | | B | 13 | 4.85 | 0.66 | 4-6 |
| Lake Joondalup 2002-03 | Vegetative | L | 30 | 9.53 | 1.57 | 7-15 |
| | | B | 30 | 5.30 | 0.48 | 4.5-6 |
| | Terminal heterocyte | L | 30 | 6.57 | 0.97 | 5-9 |
| | | B | 30 | 5.72 | 0.52 | 5-7 |
| | Akinete | L | 30 | 13.47 | 1.57 | 10-16 |
| | | B | 30 | 8.97 | 0.76 | 7-10 |

**Table E16.** Mean cell measurements for *Aphanizomenon gracile* Lemmerman collected from Bibra Lake in spring 2000.

| Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|
| Vegetative | L | 50 | 4.42 | 0.95 | 3-6 |
| | B | 50 | 2.83 | 0.41 | 2-4 |
| Heterocyte | L | 50 | 6.30 | 0.85 | 5-9 |
| | B | 50 | 4.36 | 0.74 | 3-6 |
| Akinete | L | 50 | 19.08 | 4.86 | 10-30 |
| | B | 50 | 4.80 | 1.09 | 3-8 |
| Apical | L | 20 | 7.28 | 2.22 | 4-12 |
| | B | 20 | 2.90 | 0.62 | 2-4.5 |

**Table E17.** Mean cell measurements for *Aphanizomenon ovalisporum* Forti.

| | Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|---|
| | Vegetative | L | 50 | 8.54 | 2.91 | 5-15.56 |
| Bibra Lake | | B | 50 | 4.04 | 0.77 | 3-5.56 |
| 2000-01 | Heterocyte | L | 50 | 6.46 | 0.96 | 4.44-9 |
| | | B | 50 | 4.32 | 0.71 | 2.78-5.5 |
| | Akinete | L | 50 | 12.72 | 1.99 | 8-17 |
| | | B | 50 | 9.83 | 1.87 | 6-16 |
| | Apical | L | 30 | 9.72 | 2.29 | 6-15 |
| | | B | 30 | 3.40 | 0.69 | 2-5 |
| | Vegetative | L | 30 | 7.52 | 1.5 | 5-10 |
| Bibra Lake | | B | 30 | 3.82 | 0.69 | 3-5 |
| May-June 2001 | Heterocyte | L | 30 | 6.37 | 0.99 | 4-9 |
| | | B | 30 | 4.85 | 1.07 | 4-9 |
| | Akinete | L | 25 | 13.42 | 2.57 | 10-18 |
| | | B | 25 | 10.32 | 1.02 | 8-12 |
| | Apical | L | 30 | 8.68 | 1.8 | 5-13 |
| | | B | 30 | 3.42 | 0.54 | 2-4 |
| | Vegetative | L | 50 | 4.94 | 1.10 | 3-8 |
| Bibra Lake | | B | 50 | 3.66 | 0.49 | 3-5 |
| 2001-02 | Heterocyte | L | 12 | 6.29 | 1.10 | 5-8 |
| | | B | 12 | 4.33 | 0.62 | 3-5 |
| | Vegetative | L | 37 | 5.51 | 0.73 | 5-8 |
| Bibra Lake | | B | 50 | 3.69 | 0.44 | 3-4.5 |
| 2002-03 | Heterocyte | L | 50 | 5.77 | 0.73 | 4-7 |
| | | B | 50 | 4.45 | 0.81 | 3-6 |
| | Akinete | L | 3 | 16.33 | 2.31 | 15-19 |
| | | B | 3 | 9.67 | 3.21 | 6-12 |
| | Apical | L | 32 | 8 | 2.21 | 5-13 |
| | | B | 32 | 2.78 | 0.42 | 2-3.5 |
| | Vegetative | L | 50 | 6.4 | 1.39 | 4-10 |
| Emu Lake | | B | 50 | 2.85 | 0.34 | 2-3 |
| 2001-02 | Heterocyte | L | 50 | 6.99 | 1.26 | 5-10 |
| | | B | 50 | 3.65 | 0.49 | 3-5 |
| | Akinete | L | 7 | 12.57 | 2.15 | 10-17 |
| | | B | 7 | 9.14 | 1.21 | 7-10 |
| | Apical | L | 50 | 7.15 | 1.49 | 5-11 |
| | | B | 50 | 2.41 | 0.49 | 2-4 |

**Table E17.** *(Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| | Vegetative | L | 35 | 5.51 | 0.82 | 4- 7 |
| Emu Lake | | B | 35 | 2.66 | 0.44 | 2-3 |
| 2002-03 | Heterocyte | L | 31 | 6.71 | 1.30 | 5-10 |
| | | B | 31 | 2.9 | 0.55 | 2-4 |
| | Vegetative | L | 50 | 5.38 | 0.7 | 4-7 |
| Lake Joondalup | | B | 50 | 4.48 | 0.48 | 4-5 |
| 2002-03 | Heterocyte | L | 50 | 5.45 | 0.59 | 5-7 |
| | | B | 50 | 5.09 | 0.73 | 4-7 |
| | Akinete | L | 50 | 11.58 | 1.11 | 10-15 |
| | | B | 50 | 10.9 | 0.79 | 10-13 |

**Table E18.** Measured attributes of *Cylindrospermopsis raciborskii* collected from Bibra Lake in May and June 2001.

| Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|
| Vegetative | L | 30 | 7.32 | 1.58 | 5-11 |
| | B | 30 | 2.11 | 0.37 | 1.5-3 |
| Heterocyte | L | 50 | 5.58 | 1.15 | 4-9 |
| | B | 50 | 2.23 | 0.44 | 1.5-3 |

**Table E19.** Mean cell measurements for *Nodularia spumigena* Mertens ex Bornet & Flahault.

| | Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|---|
| | Vegetative | L | 92 | 2.92 | 0.69 | 2-5 |
| | | B | 92 | 10.94 | 1.31 | 9-13 |
| Serpentine River | Heterocyte | L | 90 | 6.12 | 1.25 | 4-10 |
| 2000-01 | | B | 90 | 12.56 | 1.37 | 10-17 |
| | Akinete | L | 52 | 6.31 | 1.34 | 4-10 |
| | | B | 52 | 11.32 | 1.50 | 9-18 |
| | Vegetative | L | 30 | 2.85 | 0.60 | 2-4 |
| | | B | 50 | 7.45 | 0.63 | 6-9 |
| Serpentine River | Heterocyte | L | 50 | 4.56 | 0.76 | 3-7 |
| 2001-02 | | B | 50 | 9.25 | 1.10 | 7-11 |
| | Akinete | L | 50 | 7.34 | 0.98 | 5-10 |
| | | B | 50 | 10.75 | 0.69 | 9-12 |
| | Vegetative | L | 50 | 2.545 | 0.69 | 2-4 |
| | | B | 50 | 10.7 | 1.05 | 8-13 |
| Yangebup Lake | Heterocyte | L | 50 | 5.53 | 0.96 | 4-8 |
| 2001-02 | | B | 50 | 11.85 | 1.24 | 9-15 |
| | Akinete | L | 50 | 8.06 | 1.00 | 6-10 |
| | | B | 50 | 12.27 | 0.59 | 11-14 |
| | Vegetative | L | | | | 2-3 |
| Yangebup Lake | | B | 30 | 10.23 | 0.68 | 9-12 |
| 2002-03 | Heterocyte | L | 30 | 4.93 | 0.5 | 4-6 |
| | | B | 30 | 10.28 | 0.74 | 8-12 |

**Table E19.** *(Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| | Vegetative | L | | | | 2-3 |
| Yangebup Lake | | B | 30 | 9.67 | 0.76 | 8-11 |
| Cultured | Heterocyte | L | 30 | 5.15 | 0.76 | 4-7 |
| | | B | 30 | 9.83 | 0.56 | 9-11 |

**Table E20.** Mean cell measurements for *Nostoc linckia* (Roth) Bornet and Flahault collected from North Lake (2002-03).

| Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|
| Vegetative | L | 30 | 2.32 | 0.4 | 2-3 |
| | B | 30 | 3.13 | 0.22 | 3-3.5 |
| Heterocyst | L | 30 | 4.72 | 0.65 | 3-6 |
| | B | 30 | 4.08 | 0.77 | 3-6 |
| Vegetative | L | 30 | 3.87 | 0.78 | 3-5 |
| | B | 30 | 3.67 | 0.87 | 2.5-5 |
| Heterocyst | L | 30 | 4.07 | 0.58 | 3-5 |
| | B | 30 | 3.80 | 0.39 | 3-4 |
| Akinete | L | 14 | 6.29 | 1.27 | 5-9 |
| | B | 14 | 5.21 | 0.87 | 4.5-8 |

**Table E21.** Mean cell measurements for *Oscillatoria sancta (*Kütz) Gomont collected from Thomsons Lake in December 2000.

| Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|
| Trichome | B | 30 | 13 | 0.91 | 10-15 |
| Sheath | B | 30 | 3.13 | 0.98 | 2-5 |
| Total width | B | 30 | 19.92 | 1.78 | 17-24 |
| Apical | L | 30 | 6.43 | 1.14 | 4.75-9 |
| | B | 30 | 12.12 | 1.45 | 5-13.5 |

**Table E22.** Mean cell measurements for *Phormidium* aff. *amoenum (*Kütz) Anagnostidis & Komárek.

| | Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|---|
| Blue Gum Lake 2001-02 | Vegetative | B | 50 | 5.51 | 0.521 | 5 – 7 |
| | Apical | L | 17 | 3.44 | 0.864 | 2 – 5 |
| | | B | 17 | 4.15 | 0.931 | 2 – 6 |
| Neil McDougall Park 2002-03 | Vegetative | B | 50 | 4.99 | 0.49 | 4-6 |

**Table E23.** Mean trichome width for *Planktothrix mougeotii* (Küetz. ex Forti) Anagnostidis & Komárek.

| | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|
| Tomato Lake | 2001-02 | 40 | 5.93 | 0.41 | 5-6.5 |
| Tomato Lake | 2002-03 | 30 | 5.47 | 0.54 | 5-6.5 |
| Hyde Park | 2001-02 | 50 | 5.97 | 0.31 | 5-6.5 |
| Neil McDougall Park | 2002-03 | 30 | 5.08 | 0.53 | 4-6 |
| Blue Gum Lake | 2002-03 | 30 | 5.31 | 0.14 | 5-6 |

**Table E24.** Mean cell measurements for *Planktothrix perornata* f. *attenuata* (Skuja) Anagnostidis & Komárek collected from Lake Coogee in spring 2001.

| Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|
| Vegetative | L | 25 | 3.26 | 0.78 | 2-5 |
| | B | 50 | 9.51 | 0.78 | 8-11 |
| Apical | L | 50 | 5.51 | 1.52 | 3-13 |
| | B | 50 | 7.66 | 0.87 | 6-10 |

**Table E25.** Mean cell measurements for *Planktothrix planctonica* (Elenk.) Anagnostidis & Komárek.

| | Cell Type | | n | Mean (µm) | SD | Range (µm) |
|---|---|---|---|---|---|---|
| Canning River 2000-01 | Vegetative | B | 44 | 15.68 | 0.95 | 14-19 |
| | | L | 20 | 2.35 | 0.80 | 1-4 |
| | Apical | L | 40 | 4.46 | 0.94 | 2-6 |
| | | B | 40 | 14.56 | 1.02 | 13-17 |
| Canning River 2001-02 | Vegetative | B | 31 | 16.35 | 1.34 | 14-18 |
| Blue Gum Lake 2000-01 | Vegetative | B | 30 | 14.95 | 0.85 | 13-16.5 |
| | Apical | L | 30 | 4.50 | 1.13 | 3-7 |
| | | B | 30 | 13.98 | 0.96 | 12.5-17 |
| Blue Gum Lake 2001-02 | Vegetative | B | 50 | 13.90 | 0.83 | 12-16 |
| Forrestdale Lake 2000-01 | Vegetative | B | 30 | 14.85 | 1.06 | 13-17 |
| | Apical | L | 30 | 4.98 | 1 | 3-7 |
| | | B | 30 | 13.37 | 1.10 | 11-15 |
| Tomato Lake 2001-02 | Vegetative | B | 30 | 13.30 | 0.92 | 11-15 |

**Table E26.** Mean cell measurements for *Trichodesmium iwanoffianum* Nygaard.

| | **Cell Type** | | **n** | **Mean (µm)** | **SD** | **Range (µm)** |
|---|---|---|---|---|---|---|
| | Vegetative | L | 37 | 2.45 | 0.42 | 2-3 |
| Tomato Lake | | B | 37 | 5.55 | 0.42 | 5-6 |
| 2000-01 | Apical | L | 30 | 4.25 | 0.64 | 3-5.5 |
| | | B | 30 | 5.19 | 0.38 | 5-6 |
| | Vegetative | L | 11 | 2.09 | 0.44 | 1.5-3 |
| Blue Gum Lake | | B | 30 | 5.72 | 0.41 | 5-6.25 |
| 2000-01 | Apical | L | 21 | 2.73 | 0.93 | 1.5-5 |
| | | B | 21 | 4.67 | 0.81 | 2-6 |
| | Vegetative | L | 50 | 2.96 | 0.59 | 2-4.5 |
| Neil McDougall | | B | 50 | 6.11 | 0.58 | 4.5-7 |
| Park 2000-01 | Apical | L | 30 | 5.54 | 2.48 | 3-15 |
| | | B | 30 | 5.22 | 0.88 | 4-7 |
| | Vegetative | L | 30 | 2.41 | 0.44 | 2-4 |
| Neil McDougall | | B | 50 | 5.40 | 0.43 | 5-6 |
| Park 2001-02 | Apical | L | 30 | 3.55 | 2.03 | 2-9 |
| | | B | 30 | 4.14 | 0.55 | 3-5 |
| Neil McDougall | Vegetative | L | | | | 2-3 |
| Park 2002-03 | | B | 30 | 5.26 | 0.50 | 4-6.5 |
| | Vegetative | L | 30 | 3.22 | 0.84 | 2-5.5 |
| Lake Goollelal | | B | 35 | 5.17 | 0.42 | 4-6 |
| 2001-02 | Apical | L | 4 | 6.5 | 0.58 | 6-7 |
| | | B | 4 | 4.25 | 0.5 | 4-5 |
| Lake Bungana | Vegetative | L | 50 | 3.39 | 0.57 | 2-4 |
| 2001-02 | | B | 50 | 7.52 | 0.78 | 6-9 |

# Appendix F: Summary of Morphological Features

**Table F1.** Characteristic features for Nostocales species with circinate or spiralled trichomes. Akinete location (1) remote from heterocyte, (2) adjacent to one side of heterocyte, (3) adjacent to both sides of heterocyte.

| Site<br>Collection period | Trichome shape | Vegetative cell shape | Heterocyte shape | Akinete position | Akinete location | Akinete shape |
|---|---|---|---|---|---|---|
| ***Anabaena circinalis* Rabenhorst ex Bornet & Flahault 1888** | | | | | | |
| Yangebup Lake | circinate, flexuous, regularly spiralled | spherical, subspherical | spherical | solitary, pairs | (1) | oblong, oval |
| North Lake | circinate, regularly spiralled | spherical, subspherical | spherical, oval | solitary | (1) | oblong |
| Tomato Lake | circinate, regularly spiralled, irregularly spiralled | spherical, subspherical | spherical | solitary | (1) | oblong, pyriform |
| Blue Gum Lake | circinate, flexuous, regularly spiralled | spherical, subspherical | spherical, oval | solitary | (1) | oblong, oval |
| Neil McDougall Park | circinate, flexuous | spherical, subspherical | spherical subspherical | solitary, pairs | (1) | oblong |
| Herdsman Lake | circinate, irregularly spiralled regularly spiralled | subspherical | spherical | solitary, pairs | (1) | oblong |
| Lake Bungana | curled fragments | spherical | spherical | only 3 were observed | | |
| Mary Carroll Park | circinate | spherical, subspherical | oval | NP | | |
| Canning River | circinate regularly spiralled | spherical, subspherical | spherical, oval | solitary, pairs, in series | (1) | oblong, pyriform |

**Table F1.** *(Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Anabaena flos-aquae* (Lyngb.) Komárek** | | | | | | |
| Herdsman Lake | circinate, irregularly spiralled | spherical | spherical | NP | | |
| North Lake | circinate | spherical | spherical | solitary | (1) | oblong |
| Canning River | irregularly spiralled | subspherical | oval | solitary, pairs | (1) | oblong |
| ***Anabaena spiroides* f. *spiroides* (Elenkin) Komárek** | | | | | | |
| Tomato Lake | regularly spiralled, irregularly spiralled | spherical | spherical | solitary | (1) (2)(3) | spherical, oval |
| North Lake | regularly spiralled, irregularly spiralled | spherical ,oval | spherical, oval | solitary | (1) (2)(3) | spherical, oval |
| Lake Joondalup | regularly spiralled, irregularly spiralled | spherical, oval | spherical | solitary | (1) (2)(3) | spherical, oval |
| Shenton Park | regularly spiralled | spherical | spherical | solitary | (2)(3) | spherical, oval |
| Lake Claremont | irregularly spiralled | spherical | spherical | solitary | (2)(3) | oval |
| ***Anabaenopsis arnoldii* Aptekarj** | | | | | | |
| Lake Claremont | regularly spiralled | barrel-shaped | oval | solitary, pairs, in series | (1)(2) | oval |
| ***Anabaenopsis elenkinii* Miller 1923** | | | | | | |
| Bibra Lake | circinate | quadrate, barrel-shaped | spherical, oval | solitary, pairs, in series | (1) | oblong |
| Neil McDougall Park | circinate | barrel-shaped, cylindrical | oval | solitary | (1) | oblong |
| Lake Joondalup | circinate | barrel-shaped | oval, oval-oblong | solitary | (1) | oblong |

**Table F1**. *(Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Anabaenopsis elenkinii* Miller 1923** | | | | | | |
| Shenton Park | circinate | barrel-shaped | oval, oval-oblong | solitary, pairs | (1) | oblong, oval-oblong |
| Mary Carroll Park | circinate | quadrate, barrel-shaped | spherical | solitary, pairs | (1) | sausage shaped |
| Yangebup Lake | circinate | barrel-shaped | oval | NP | | |
| Serpentine River | circinate | quadrate, barrel-shaped | oval | solitary, pairs | (1) | oblong |
| ***Anabaenopsis tanganyikae* (G. S. West) Woloszynska & Miller** | | | | | | |
| Lake Joondalup | zig-zag shaped, regularly spiralled | cylindrical | oval, ovate-oblong | solitary, pairs | (1) | oblong, kidney |
| Mary Carroll Park | zig-zag shaped | cylindrical (curved) | spherical, oval | solitary, pairs | (1) | oval, kidney |

**Table F2.** Characteristic features for Nostocales species with straight trichomes. Akinete location (1) remote from heterocyte, (2) adjacent to one side of heterocyte, (3) adjacent to both sides of heterocyte. Metameric refers to the trichome consisting of several identical sections (Baker 1991).

| Site | Trichome | Vegetative Cell shape | Heterocyst shape | Akinete position | Akinete location | Akinete shape | Apical cell shape |
|---|---|---|---|---|---|---|---|
| ***Anabaena affinis*** Lemmerman | | | | | | | |
| Emu Lake | uniformly broad | spherical | spherical | solitary | (2) | oblong | undifferentiated |
| ***Anabaena aphanizomenoides*** Forti | | | | | | | |
| Mary Carroll Park | attenuated, constricted | quadrate (bead) | spherical, oval | solitary | (2)(3) | oval | rounded conical |
| Lake Joondalup | attenuated, constricted | quadrate (bead) | spherical | solitary | (2) | oval | |
| ***Anabaena bergii* var. *limnetica*** Coute & Preisig 1978 | | | | | | | |
| Lake Coogee | attenuated, metameric | discoid | oval | NP | | | |
| Lake Joondalup | attenuated, metameric | discoid, quadrate | oval, spherical | solitary | (1) | oval | |
| Bibra Lake | attenuated, metameric | discoid, quadrate | spherical | solitary pairs | (1) | oblong-ovate | rounded conical |
| Lake Brearley | attenuated, metameric | discoid | spherical, oval | solitary | (1) | oval | bulbous |
| Mary Carroll Park | attenuated, metameric | discoid | spherical | solitary pairs | (1) | oblong-ovate | rounded conical |
| Emu Lake | attenuated metameric | quadrate | spherical | solitary pairs | (1) | oblong-ovate | rounded conical |

**Table F2.** *(Continued)*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Anabaena torlusa* var. *tenuis*** (Lemmerman) Geitler | | | | | | | |
| Mary Carroll Park | uniformly broad | quadrate | oval | solitary | (2) | oblong-ovate | rounded-conical |
| ***Aphanizomenon gracile*** Lemmerman | | | | | | | |
| Bibra Lake | slightly constricted | quadrate, cylindrical | oval | solitary, pairs | (1) | cylindrical (sheath) | rounded |
| ***Aphanizomenon ovalisporum*** Forti | | | | | | | |
| Bibra Lake | constricted | cylindrical | oval | solitary, pairs | (1) | oval | rounded |
| Emu Lake | constricted | quadrate, cylindrical | oval | solitary, pairs | (1) | oval | rounded |
| Lake Joondalup | constricted | cylindrical | oval | solitary, pairs | (1) | oval | rounded |
| ***Nodularia spumigena*** Mertens ex Bornet & Flahault | | | | | | | |
| Yangebup | metameric | discoid | oval | paired | (1) | oblong | |

# Appendix G: Publications

The following publications have resulted from this research project.

## G1 Conference Proceedings

### *G1.1 Poster Presentations*

John, J. and Kemp, A. (2006) Harmful cyanobacterial blooms in the wetlands of the Swan Coastal Plain, Western Australia. INTERACT Conference, Perth, Western Australia, 24th-28th September 2006.

John, J., Kemp, A. and Pennacchio, M. (2001) Distribution Pattern, Taxonomy and Toxicity of Cyanobacterial Blooms in the Swan Coastal Plain, Western Australia. 5th International Conference on Toxic Cyanobacteria, Noosa, Queensland, 16th-20th July 2001.

Kemp, A., John, J. and Pennacchio, M. (2004) Cyanobacterial blooms and microcystins in freshwater urban wetlands of Perth, Western Australia. 11th International Conference on Harmful Algae, Cape Town, South Africa, 14th-19th November 2004.

Kemp, A., Pennacchio, M. and John, J. (2000) Toxic blue-green algal blooms in the metropolitan wetlands and Swan River Estuary, Western Australia. Western Australia Marine Science Conference, Perth, Western Australia, 27th-28th April 2000.

### *G1.2 Oral Presentations*

Kemp, A. (2005) Toxic cyanobacterial blooms in the freshwater urban wetlands on the Swan Coastal Plain. Royal Society of Western Australia 7th Annual Postgraduate Symposium, Perth, Western Australia, 3rd April 2005.

Kemp, A. and John, J. (2004) Cyanobacterial blooms in the wetlands of the Swan Coastal Plain, Western Australia: systematics and ecology. 43rd Annual Australian Society for Limnology Congress, Adelaide, South Australia, 29th November-3rd December 2004.

Kemp, A., Pennacchio, M. and John, J. (2002) Cyanobacterial blooms in Perth metropolitan wetlands: distribution and composition. 17th Australasian Society for Phycology and Aquatic Botany Conference, Perth, Western Australia, 1st-4th October 2002.

## G2 Journal Publications

John, J. & Kemp, A. (2006) Cyanobacterial blooms in the wetlands of the Perth region, taxonomy and distribution: an overview. *Journal of the Royal Society of Western Australia,* 89: 51-56.

Kemp, A. & John, J. (2006) Microcystins associated with *Microcystis* dominated blooms in the southwest wetlands, Western Australia. *Environmental Toxicology,* 21: 125-130.