BLOOM FORMING AND TOXIC CYANOBACTERIA IN NEW ZEALAND

*Species diversity and distribution, cyanotoxin production and
accumulation of microcystins in selected freshwater
organisms*

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Abstract

The objective of this doctoral study was to identify waterbodies in New Zealand containing cyanobacterial blooms and mats, to assess the species composition and to establish types and concentrations of cyanotoxins in samples obtained.

A total of 271 water and cyanobacterial mat samples from 147 different waterbodies around New Zealand were collected or received between January 2001 and December 2004. Sixty seven percent were found to contain cyanobacteria and 41% of the samples contained cyanobacteria in sufficiently high concentrations to be termed a bloom. A total of 54 different cyanobacteria species were recorded.

Prior to 1987, 33 lakes in New Zealand were recorded as having experienced cyanobacterial blooms. In the present study an additional 18 lakes were recorded as having experienced blooms. Eight bloom forming cyanobacteria species not previously identified in New Zealand (*Anabaena planktonica*, *Cylindrospermopsis raciborskii*, *Microcystis botrys*, *M. panniformis*, *M. ichthyoblabe*, *M. wesenbergii*, *Planktothrix peronata* f. *attenuata* and *Sphaerocavum brasiliense*) were found to be dominant or co-dominant in at least some cyanobacterial blooms. In addition to these species, three non-dominant species were identified that were also new records for New Zealand.

The water and cyanobacterial mat samples were analysed using ELISA's, LC/MS, HPLC and neuroblastoma assays for cyanotoxins and identified; microcystins/nodularins (102 samples from 54 different locations), anatoxin-a (3 samples from 3 different locations), cylindrospermopsins (1 sample) and saxitoxins (48 samples from 41 different locations). Species from the genera *Microcystis*, *Anabaena*, *Cylindrospermopsis*, *Nostoc*, *Planktothrix*, *Oscillatoria* and *Phormidium* produce these cyanotoxins.

An in-depth study in Lakes Rotoehu and Rotoiti investigated the relationship between cyanobacteria species composition and levels of microcystins over a six month period and examined accumulation of microcystins in rainbow trout (*Oncorhynchus mykiss*) and freshwater mussels (*Hydridella menziesi*).

Weekly water samples collected from both lakes showed that cyanobacteria species and levels of microcystins can vary over time during a bloom event. When phytoplankton samples were dominated by very high cell concentration of *Microcystis* spp. they were found to contain microcystins. Generally cell counts were found to be a poor indicator of levels of microcystins in the water samples, suggesting that cell counts alone are not a satisfactory way of identifying human health dangers in waterbodies containing cyanobacteria.

Microcystins were detected in rainbow trout liver and muscle tissue, and in freshwater mussels. The Total Daily Intake of microcystins recommended by the World Health Organisation for humans is $0.04 \mu\text{g kg}^{-1} \text{ day}^{-1}$. A 70 kg human consuming 300 g of muscle tissue would have exceeded this level in 50% (Lake Rotoiti) and 71% (Lake Rotoehu) of the samples. Health problems could result if more than 300 g of trout muscle tissue was consumed on a regular basis over an extended period.

This study has demonstrated that the prevalence of freshwater cyanotoxin-producing cyanobacteria is widespread in New Zealand waterbodies and these cyanotoxins pose a potential health risk to both humans and animals either directly or indirectly.

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1. Thesis Introduction and Objectives

In many parts of the world cyanobacterial blooms (commonly known as blue-green algae) are causing concern. Blooms appear to be occurring more frequently as eutrophication of waterbodies increases. A recent survey showed that 54% of lakes in the Asia Pacific region are eutrophic; the proportion in Europe, Africa, North America and South America are 53%, 28%, 48% and 41% respectively (ILEC/Lake Biwa Research Institute 1988-1993). Blooms lead to deterioration in water quality and can result in human and animal health problems (Chorus & Bartram 1999, Haider et al. 2003). Recent outbreaks of human poisoning attributed to toxic cyanobacteria have been reported in Australia, following the contamination of drinking water (Byth 1980), in England after recreational activity (Turner et al. 1990) and the most severe in Brazil, where contaminated water supplied through renal dialysis led to the death of 50 patients (Azevedo et al. 2002).

Prior to this study, cyanobacterial bloom formation in New Zealand have been investigated in selected lakes in relation to species identification and population dynamics (e.g. Etheredge & Pridmore 1987, Pridmore & Etheredge 1987, Viner 1987). The relationship between nutrient availability and bloom formation had also received some attention (Viner & White 1987). Much of this work was undertaken in the 1980s and since then there has only been limited research on cyanobacteria. Investigation into production of cyanotoxins has been intermittent, sparse and predominantly in response to adverse situations rather than systematic studies.

In New Zealand, there were clearly gaps in information on recent distributions and species composition of bloom-forming cyanobacteria, the types and levels of cyanotoxins present in waterbodies and changes in cyanotoxin levels over time. Little was known about cyanotoxin accumulation in aquatic organisms and the possible human health risks associated with consumption of contaminated tissue. New Zealand has lagged behind many other developed countries in its research into toxic cyanobacteria and the present study has begun to address some of the gaps in our scientific knowledge and provide important data which future research can build on. In addition, it was anticipated that the present study would provide information that would be useful to managers of waterbodies, such as local authorities, drinking water plant

managers and to government agencies that are now developing food safety, recreational water and drinking water guidelines for cyanobacteria in New Zealand.

During the present study water samples and cyanobacterial mats were obtained from a variety of freshwater bodies throughout New Zealand. Cyanobacteria species and the levels and types of cyanotoxins in the samples were identified. An in-depth study of Lakes Rotoiti and Rotoehu (Rotorua) investigated the relationships between levels of microcystins and specific cyanobacteria species, accumulation of microcystins in selected freshwater organisms and potential human health risks.

This thesis is divided into four main sections:

1. Cyanobacteria species diversity and bloom formation in New Zealand, (Chapter 2).
2. New records of planktonic cyanobacteria in New Zealand freshwaters, (Chapter 3).
3. Cyanotoxins in New Zealand (Chapter 4).
4. Microcystins in rainbow trout, freshwater mussels and phytoplankton in lakes Rotoiti and Rotoehu (Chapter 5).

Chapter 2 reports on examination of cyanobacteria species diversity and bloom formation in New Zealand. It sets out to respond to the following objectives:

- To determine which cyanobacteria species are present in waterbodies and cyanobacterial mat samples.
- To establish which cyanobacteria are dominant cyanobacterial bloom and mat samples.
- To compare the present day species diversity and locations of cyanobacterial blooms in lakes with data collated in the 1987 study of Pridmore & Etheredge.

Chapter 3 describes new records of planktonic cyanobacteria identified in this study. Morphological descriptions, drawings, photographs and in some instances molecular techniques are used to identify species. It sets out to respond to the following objective:

- To determine if there are cyanobacteria species present that have not been previously recorded in New Zealand.

Chapter 4 describes the range, location and levels of cyanotoxins identified in the samples examined. It sets out to respond to the following objectives:

- To determine which cyanotoxins are present in waterbodies and cyanobacterial mat samples.
- To identify which cyanobacteria species are responsible for cyanotoxin production.
- To assess if cyanotoxins are a potential risk to animal and human health in New Zealand.

Chapter 5 presents a case study which examines the relationship between temporal changes in cyanobacteria species diversity in Lakes Rotoehu and Rotoiti (Rotorua). It also examines the relationship between variation in cyanobacteria species and microcystins in water samples and the presence of microcystins in rainbow trout (*Oncorhynchus mykiss*) tissues and indigenous freshwater mussels (*Hyridella menziesi*). Potential human health risks are investigated. It sets out to respond to the following objectives:

- To establish how levels of microcystins vary during a six month period in the waters of Lakes Rotoehu and Rotoiti.
- To identify whether or not microcystins accumulate in the liver and muscle tissue of rainbow trout in Lakes Rotoiti and Rotoehu.
- To establish whether or not microcystins could reach levels in trout tissue that are hazardous to health if consumed by humans.
- To establish whether or not significant levels of microcystins accumulate in the native freshwater mussel (*Hydridella menziesi*), and if they accumulate, how the concentrations of microcystins change with exposure time.

The investigations undertaken to meet these objectives have produced some valuable findings adding considerably to current knowledge of cyanobacteria species identification, location of bloom formation, types of cyanotoxins present and confirmation of the health risks that some cyanobacteria pose in New Zealand. Important topics for future research are identified.

Each Chapter includes an abstract, specific methodology, results, discussion, conclusions and references. The Appendixes for all Chapters are given at the end of the thesis.

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2. Cyanobacteria Species Diversity and Bloom Formation in New Zealand

2.1 Abstract

The initial aim of this part of the study was to assess the species diversity and distribution of cyanobacterial blooms/mats in New Zealand. The results obtained also allowed comparisons with historical data on cyanobacterial blooms in lakes in New Zealand. Prior to 1987, 33 lakes were recorded as having experienced cyanobacterial blooms. Between 2001 and 2004 this study identified an additional 18 lakes experiencing blooms. Reasons for this increase are hard to ascertain as most lakes have very little historical phytoplankton and/or limnological data available. However, it appears that at least some of this increase reflects more intensive and extensive sampling. Twelve of the lakes that experienced cyanobacterial blooms during this study were not sampled during or prior to the 1987 study.

A total of 271 water and cyanobacterial mat samples from 147 different waterbodies around New Zealand were collected or received between January 2001 and December 2004. Sixty seven percent were found to contain cyanobacteria and 41% of the samples contained cyanobacteria in sufficiently high concentrations to be termed a "bloom". A total of 36 different cyanobacteria species were identified during this section of the study (17 Nostocales, 9 Oscillatoriales, 10 Chroococcales).

The species composition of cyanobacterial blooms has changed over time. In the 1970's and 1980's common bloom forming species included: *Anabaena flos-aquae*, *A. spiroides* and *Microcystis aeruginosa*. During the summers of 2001 to 2004 six bloom forming species not previously identified in New Zealand (*A. planktonica*, *Cylindrospermopsis raciborskii*, *M. panniformis*, *M. ichthyoblabe*, *M. wesenbergii*, and *Planktothrix peronata* f. *attenuata*) were found to be dominant or co-dominant in at least some blooms.

Anabaena planktonica dominated in 24% of blooms and *C. raciborskii* 8%. The geographic range of *A. planktonica* also seems to be expanding. These highly adaptive species may be a threat to the water quality and usability of an increasing number of

New Zealand's lakes in the future. Further research into the distribution, genetics, physiology and ecology of these two species in New Zealand is recommended.

2.2 Keywords

cyanobacteria; cyanobacterial blooms; cyanobacterial mats; *Anabaena*; *Microcystis*; *Cylindrospermopsis*

2.3 Introduction

Broady (2000) constructed a checklist of cyanobacteria in New Zealand. A total of 336 taxa, including 319 species distributed amongst 65 genera, were recorded from both terrestrial and freshwater habitats. However, at least 32 of these records are dubious due to a lack of preserved specimens, photographs and drawings (Broady 2000). Globally approximately 2 000 species are known from 215 genera (i.e. less than 30% of known genera and 16% of known species have been recorded in New Zealand), although with the advent of molecular studies many of the species and genera descriptions are subject to on-going review and debate.

The objectives of this part of the present study were:

- To determine which cyanobacteria species are present in New Zealand waterbodies and cyanobacterial mat samples.
- To establish which cyanobacteria are dominant in New Zealand cyanobacterial bloom and mat samples.
- To compare the species diversity and locations of cyanobacterial blooms in lakes with data collated in the 1987 study of Pridmore & Etheredge.

Samples from waterbodies across New Zealand (predominately those experiencing cyanobacterial blooms or mats) were collected and received. Dominant cyanobacteria species were identified and quantified. These samples were also tested for the presence of cyanotoxins and these results are given in Chapter 4. Where blooms were conspicuous, the species diversity was compared to data from Pridmore & Etheredge (1987) to assess if any changes in either number of lakes experiencing blooms or species composition of these blooms had occurred. This was not an original objective of the study and samples were not taken from all lakes examined during or prior to 1987, however because the majority were, and marked differences in species composition were noted, it appeared important to make these comparisons. Samples collected or received were not restricted to those waterbodies with obvious cyanobacterial blooms, but included waterbodies with no apparent problems.

A review of cyanobacteria, the causes of cyanobacterial blooms and information published on cyanobacterial blooms in New Zealand prior to 1987 is given below.

2.3.1 What are Cyanobacteria?

Cyanobacteria (blue-green algae) belong to the Kingdom Bacteria, Class Cyanoprokaryote. This class includes about 150 genera and approximately 2 000 species (Hoek et al. 1995). Cyanobacteria are an ancient group of photosynthetic prokaryotic organisms that are found worldwide in diverse environments including oceans, fresh water, bare rock and soil (Whitton & Potts 2000). The oldest known fossils from Archaean rocks in Western Australia and these have been dated to 3.5 billion years old (Schopf 1996). The name 'blue-green algae' was derived from the colour of the first species identified which were blue-green in colour, but the absence of membrane-enclosed chloroplasts distinguishes cyanobacteria from other algae which are all eukaryotic.

The majority of species require only water, carbon dioxide, inorganic substances and light for their life processes with photosynthesis being the principal mode of energy metabolism (Fay 1983). Cyanobacteria are ecologically important as they provide an important source of food for primary consumers which in turn are consumed by secondary and tertiary consumers in the aquatic food web (Whitton & Potts 2000).

Cyanobacteria can exist as solitary, free-living cells or as colonies consisting of a few to thousands of cells enclosed in mucilage. Most colonies and single cells are microscopic but populations of these can be visible, for example, as mats, crusts, gelatinous mats and blooms in water columns.

There are four orders of cyanobacteria:

Chroococcales: Unicellular, or colonial cells, mostly spherical or oblong. Never in true filaments.

Oscillatoriales: Cells arranged in the form of filaments without true-branching, without any heterocytes or akinetes.

Nostocales: Filaments with sheath and heterocytes, without true branching.

Stigonematales: Filaments with sheath, heterocytes and true branching.

In unicellular forms, reproduction occurs by simple, asexual binary fission. Colonies are then formed when the cells aggregate together and are held by the slimy matrix secreted

during the growth of the colony (Chorus & Bartram 1999). Filamentous forms reproduce by trichome fragmentation, or by formation of hormogonia (reproductive segments of the trichomes which exhibit active gliding motion on their liberation). These then develop into new trichomes (Chorus & Bartram 1999).

Some filamentous cyanobacteria produce specialized pale cells called heterocytes (these usually have a well differentiated cell wall). Heterocytes are specialized in nitrogen fixation (Whitton & Potts 2000). The position of the heterocyte, i.e. at the end (terminal) or middle (intercalary), are useful in the identification of species (Baker 1991).

An akinete is a thickened cell distinguished by its larger size. Akinetes are resting spores that store food material. As a population declines the akinetes settle down to the bottom of the waterbody. After a period of dormancy and followed by appropriate environmental triggers they can germinate into filaments (Baker 1999).

2.3.2 Bloom Forming Cyanobacteria

The term “bloom” is poorly defined, but is generally described as an episodic proliferation of algal biomass that is significantly higher than the known average for a given waterbody (Oliver & Ganf 2000). Usually only one or a few species will be responsible for the bloom and often the bloom will result in discoloration of the water caused by high density of pigmented cells.

In potable and recreational waterbodies an “algal bloom” is commonly defined by the concentration of cells per milliliter (e.g. 15 000 cells ml⁻¹ is commonly used in New Zealand recreational waterbodies), and it has been suggested that different definitions of algal blooms are required for non-toxic and toxic algal species because a low concentration of toxic algae may result in a health risk.

For the purpose of this study the first definition was used, although it should be noted that many of the samples were one-off samples, so a comparison to the “average number” of cells ml⁻¹ could not be carried out. It was assumed that a high level of cyanobacteria was not normal for any New Zealand waterbody.

2.3.3 What Causes Cyanobacterial Blooms?

Cyanobacterial blooms are the result of a complex interaction of a multitude of chemical, physical and biological factors including; light and mixing conditions, availability of nutrients, water residence time, waterbody depth, geographical shape of the waterbody and grazing by zooplankton. An in-depth review of how such factors interact is given in Oliver & Ganf (2000).

Some cyanobacteria species possess the ability to regulate buoyancy, to fix nitrogen and to produce akinetes (resting cells). This optimises the available nutrients and light which can give them an advantage over other phytoplankton species. These characteristics are discussed briefly below.

Many species of cyanobacteria contain gas vesicles, which are gas-filled cylindrical structures within the cytoplasm that enable cyanobacteria to regulate their buoyancy (Hayes 1988). This gives cyanobacteria a significant advantage over other phytoplankton (Oliver & Ganf 2000) as it allows them to adjust their vertical position in the water column (Walsby 1987) and exploit nutrients that are unavailable to other species that have to rely on turbulence to resist sinking.

A number of cyanobacterial species can fix atmospheric nitrogen (N_2) by using the enzyme nitrogenase to convert N_2 directly into ammonium (NH_4). Heterocytes are differentiated for this function (Fay 1983). Examples of genera containing heterocytes are *Anabaena*, *Aphanizomenon*, and *Nostoc*. There are also species which fix nitrogen without the presence of heterocytes (Smoker & Barnum 1990). Nitrogen-fixing cyanobacteria have an advantage under predominantly nitrogen-limited conditions and may gain growth and reproductive success over other phytoplankton in these environments (Chorus & Bartram 1999, Oliver & Ganf 2000). Cyanobacteria also have a substantial storage capacity for phosphorus and can store sufficient to perform two to four cell divisions.

Cyanobacteria of the order Nostocales form akinetes (or resting cells). These can survive in the sediment of waterbodies and may provide inoculums for blooms for many years (Baker 1999).

2.3.4 Cyanobacterial Blooms in New Zealand (Prior to 1987)

Pridmore and Etheredge (1987), using data from their own and previously published studies, reported that prior to 1987 conspicuous blooms and scums of planktonic cyanobacteria had been observed in 33 New Zealand lakes (Table 2.1). They were also able to compare this information to the known trophic status of the lakes and found that 29 of the lakes were described as eutrophic, three were mesotrophic and one was oligotrophic.

At least eight species were responsible for the blooms: *Anabaena flos-aquae*, *A. spiroides*, *A. circinalis*, *A. tenericaulis*, *A. minutissima* var. *attenuata* (Pridmore & Reynolds 1989), (originally described by Pridmore & Etheredge as *A. oscillarioides*), *Aphanizomenon flos-aquae*, *Nodularia spumigena* and *Microcystis aeruginosa*. The taxonomy of a further species, *Anacystis incerta* (which bloomed in Tomahawk Lagoon, Dunedin), was uncertain (Pridmore & Etheredge 1987).

The species that were most commonly reported to form blooms were *A. flos-aquae* (27%), *A. spiroides* (21%) and *M. aeruginosa* (21%) (Table 2.2). It is interesting to note that only *A. flos-aquae* and *A. spiroides* formed blooms in both the North and South Island and only blooms of *N. spumigena* were restricted to the South Island. The remaining five species were reported to bloom only in the North Island. The majority of the blooms occurred in summer and autumn although occasionally *A. flos-aquae* formed blooms in winter (Burns & Mitchell 1974, Dryden & Vincent 1986, Vincent 1985).

Pridmore and Etheredge (1987) listed the following species that were frequently found in Northern Hemisphere temperate lakes but had not been reported in New Zealand; *Aphanocapsa pulchra* (Kuetzing) Rabenhorst, *Coelosphaerium naegelianum* Unger, *Gomphosphaerium aponina* Kuetzing, *Anabaena planktonica* Brunnthaler, *Oscillatoria prolifica* (Greville) Gomont and *O. redekei* van Goor.

Table 2.1. New Zealand lakes in which planktonic cyanobacteria have produced blooms or scums prior to 1987 (adapted from Pridmore & Etheredge 1987).

Lake	Species Responsible for Bloom or Scum	Reference
Alexandrina	<i>Anabaena flos-aquae</i>	(Stout 1981)
Butchers Dam	<i>Anabaena flos-aquae</i>	(Burns & Mitchell 1974)
Ellesmere	<i>Nodularia spumigena</i>	(Dodgshun 1981)
Forsyth	<i>Nodularia spumigena</i>	(Flint 1975)
Hakanoa	<i>Anabaena spiroides</i> <i>Microcystis aeruginosa</i>	(Pridmore & Etheredge 1987) (Flint 1975) (Pridmore & Etheredge 1987)
Hayes	<i>Anabaena flos-aquae</i>	(Burns & Mitchell 1974) (Burns 1975)
Horowhenua	<i>Anabaena</i> sp. <i>Microcystis aeruginosa</i>	(Flint 1975) (Flint 1975) (Pridmore & Etheredge 1987)
Horseshoe	<i>Anabaena flos-aquae</i>	(Parsons 1985)
Johnson	<i>Anabaena flos-aquae</i>	(Burns & Mitchell 1974) (Burns 1975)
Kainui	<i>Microcystis aeruginosa</i>	(Etheredge 1987)
Magakaware	<i>Anabaena circinalis</i>	(Etheredge 1987)
Ngahewa	<i>Anabaena flos-aquae</i> <i>Anabaena spiroides</i> <i>Anabaena circinalis</i> <i>Anabaena</i> sp.	(Kellar & Goldman 1979) (Pridmore & Etheredge 1987) (Kellar & Goldman 1979) (Paerl 1979) (Dryden & Vincent 1986) (Paerl 1979) (Fish 1968)
Ngapouri	<i>Anabaena flos-aquae</i> <i>Microcystis</i> sp.	(Dryden & Vincent 1986) (Fish 1968)
Ngaroto	<i>Anabaena circinalis</i> <i>Microcystis aeruginosa</i>	(Etheredge 1987) (Pridmore & Etheredge 1987) (Etheredge 1987)

Table 2.1 continued....

Lake	Species Responsible for Bloom or Scum	Reference
Okaro	<i>Anabaena flos-aquae</i> <i>Anabaena spiroides</i> <i>Anabaena</i> sp. <i>Microcystis aeruginosa</i>	(Dryden & Vincent 1986) (Flint 1975) (Flint 1977) (McColl 1972) (Paerl 1979) (Fish 1968) (Dryden & Vincent 1986)
Omapere	<i>Anabaena flos-aquae</i>	(Pridmore & Etheredge 1987)
Orakai	<i>Anabaena tenericaulis</i>	(Flint 1975)
Pupuke	<i>Microcystis aeruginosa</i>	(Cassie 1979)
Rotoaira	<i>Anabaena flos-aquae</i>	(Pridmore & Etheredge 1987)
Rotoehu	<i>Aphanizomenon flos-aquae</i>	(Cassie 1978)
Rotoiti (Kaikoura)	<i>Anabaena spiroides</i>	(Flint 1975) (Flint 1977)
Rotoiti (NI)	<i>Anabaena flos-aquae</i> <i>Anabaena spiroides</i>	(Dryden & Vincent 1986) (Cassie 1978)
Rotomanuka South	<i>Anabaena tenericaulis</i> <i>Microcystis aeruginosa</i>	(Etheredge 1987) (Etheredge 1987)
Rotongaio	<i>A. minutissima</i> var. <i>attenuate</i> <i>Anabaena circinalis</i> <i>Anabaena</i> sp.	(Paerl 1979) (Vincent 1980) (Forsyth et al. 1983) (Viner & Kemp 1983) (Paerl 1979) (Jolly & Chapman 1977)
Rotorua (NI)	<i>Anabaena circinalis</i> <i>Anabaena spiroides</i> <i>Microcystis aeruginosa</i>	(White et al. 1978) (Cassie 1978) (Pridmore & Etheredge 1987)
Rotorua (Kaikoura)	<i>Anabaena spiroides</i>	(Flint 1975) (Flint 1977)
Roundabout	<i>Anabaena flos-aquae</i>	(Flint 1975)
Tarawera	<i>Anabaena flos-aquae</i>	(Flint 1975) (Pridmore & Etheredge 1987)
Te Koutu	<i>Anabaena tenericaulis</i> <i>Microcystis aeruginosa</i>	(Etheredge 1987) (Etheredge 1987)
Tomahawk Lagoon	<i>Anabaena flos-aquae</i> <i>Anacystis incerta</i>	(Mitchell 1971) (Mitchell 1971)
Tutira	<i>Anabaena</i> sp. <i>Microcystis aeruginosa</i>	(McColl 1972) (McColl 1972)
Waahi	<i>Anabaena spiroides</i>	(Chapman 1980)
Waitawa	<i>Microcystis aeruginosa</i>	(Flint 1975)

Table 1.2. Percentage of planktonic cyanobacteria species recorded in blooms (67 recorded) in New Zealand lakes prior to 1987 (data from Pridmore & Etheredge 1987).

Species	Percentage of Blooms Recorded Where This Species Dominated
<i>Anabaena flos-aquae</i>	27%
<i>Anabaena spiroides</i>	21%
<i>Microcystis aeruginosa</i>	21%
<i>Anabaena circinalis</i>	7%
<i>Anabaena</i> sp.	7%
<i>Anabaena. minutissima</i> var. <i>attenuate</i>	6%
<i>Anabaena tenericaulis</i>	4%
<i>Nodularia spumigena</i>	3%
<i>Aphanizomenon flos-aquae</i>	1%
<i>Microcystis</i> sp.	1%

2.4. Methods

2.4.1 Sample Collection

Initially this study aimed to collect only cyanobacterial bloom or mat samples. Because one of the main aims of the study was to identify dominant species causing blooms and to determine if cyanotoxins were present, it was assumed that these samples would contain the highest concentrations of cyanobacteria and thus provide the greatest chance of detecting cyanotoxins. The patchy nature of blooms and mats both spatially and temporally meant that intermittent field sampling could easily miss bloom formations

and thus it was planned to rely on people working on or in waterbodies or those who lived near or were frequently visiting waterbodies to collect samples. There were no criteria as to the type of waterbody that would be analysed and samples were collected from oxidation ponds, dams and ponds, rivers, streams and lakes. The locations of all waterbodies sampled are given in Tables A1 – A4, Appendix 2.

In the collection of samples the following steps were taken:

Advertising the project was produced (Appendix 3). This flyer described what a cyanobacterial bloom would look like and the potential dangers such blooms posed. A simple sample collection method was given and contact details and how to obtain further information were provided.

An outline of the project and introducing the flyer was written to Health Protection Officers, Environmental Health Officers in New Zealand and other researchers (e.g. NIWA, Cawthron) carrying out freshwater research in New Zealand.

A letter and a flyer were posted to all Health Protection Officers, Environmental Health Officers in New Zealand and other researchers (e.g. NIWA, Cawthron) carrying out freshwater research in New Zealand.

A copy of the letter and flyer was also sent to both the “NZ Algae” email group and the Department of Conservation freshwater email group.

and thus it was planned to rely on people working on or in waterbodies or those who lived near or were frequently visiting waterbodies to collect samples. There were no criteria as to the type of waterbody that would be analysed and samples were collected from oxidation ponds, dams and ponds, rivers, streams and lakes. The locations of all waterbodies sampled are given in Tables A1 – A4, Appendix 2.

To generate interest in the collection of samples the following steps were taken:

- A flyer advertising the project was produced (Appendix 3). This flyer described what a cyanobacterial bloom would look like and the potential dangers such blooms posed. A simple sample collection method was given and contact details and how to obtain further information were provided.
- A letter outlining the project and introducing the flyer was written to Health Protection Officers, Environmental Health Officers in New Zealand and other researchers (e.g. NIWA, Cawthron) carrying out freshwater research in New Zealand.
- The letter and a flyer were posted to all Health Protection Officers, Environmental Health Officers in New Zealand and other researchers (e.g. NIWA, Cawthron) carrying out freshwater research in New Zealand.
- A modified version of the letter and flyer was also sent to both the “NZ Algae” email group and the Department of Conservation freshwater email group.

- In an attempt to obtain samples from private farms a letter and flyer was also sent to each Federated Farmers Association in New Zealand. Articles were written in the “Vetscript” magazine (distributed to all veterinary clinics in New Zealand) in December 2001 and March 2003 and in “Rural News” 2001 (Appendix 5, Tables 5a – 5c).
- At the beginning of this study a presentation titled “*Toxic Cyanobacteria in New Zealand Waterways – our current knowledge and future research*” was given at the joint New Zealand Limnology Society and New Zealand Hydrology Society Conference (Massey University, Palmerston North, November 2001). This presentation detailed knowledge concerning cyanobacterial blooms and cyanotoxins, outlined the project objectives and finished by requesting participants who knew of waterbodies with cyanobacterial blooms to send in samples. A copy of the flyer was also distributed to all attendees at the conference in their registration package (over 300 people).
- A follow up paper was presented at the New Zealand Limnology Society in November 2002 (Greymouth) titled “*Toxic Cyanobacteria in New Zealand Waterways*”. This presented some of the preliminary results and again requested attendees to send samples or to notify the author when cyanobacterial blooms were occurring.

When participants reported or collected a sample they were sent a “Sample Information Sheet” (Appendix 6) and asked to complete as much of this as possible. This sheet asked questions about: sampler’s contact information; time, date, location of sample; method of collection; water use; weather conditions at time of collection and prior to collection; information of the bloom/mat appearance and other available limnological data on the waterbody. Samplers were asked to draw a sketch or preferably to take a photograph of the sampling point.

Instructions for sample collection stated that samples should be sent in well-sealed 500 ml to 1-litre plastic bottles. Cleaned milk or soft drink bottles were suggested. In addition to this information, samplers were also requested to store samples in the dark

and courier them as soon as possible to the Massey University Ecotoxicology Laboratory (Wellington).

The majority of the samples received were collected as “grab” samples and this allowed the cyanotoxins and cyanobacteria cells in the sample to be quantified. Occasionally samples were collected with plankton nets (Appendix 2, Tables A1 - A4). This concentrates the sample and thus increased chances of identifying cyanobacteria and cyanotoxins. When a plankton net sample was collected a grab sample was usually collected as well.

It proved difficult to attract sufficient interest and persuade people to collect samples and field sampling by the author was also carried out. This sampling occurred in the following regions; Blenheim/Nelson, Wellington, Horowhenua/Wanganui, Central North Island, Rotorua and Waikato.

Water, cyanobacterial bloom/scum and mat samples were collected or received between 01-01-2001 and 05-12-2004. The location of samples, date of collection, collection method, main use of water body and species present are given in Appendix 2 (Tables A1 - A4). Each waterbody was assigned to one of the following: lakes (permanent waterbodies with at least one axis greater than 0.5 km long, this group also includes reservoirs), ponds or dams (natural or man-made waterbodies with both axes less than 0.5 km long), oxidation ponds and rivers or streams.

2.4.2 Sample Analysis

Subsamples (50 ml) were preserved immediately with Lugol's solution (Appendix 7), in the field or when the samples were received at the Massey University Ecotoxicology Laboratory. When possible a further subsample (50 ml) of unpreserved material was retained for taxonomic identifications. The remaining sample was frozen immediately and used for cyanotoxin analyses as described in Chapter 4.

Initially, due to the lack of a suitable inverted microscope, cyanobacteria cell concentrations in the samples were quantified using a Olympus light microscope (CHA) and a “quantification index” (Hirooka et al. 1999) at 100x magnification: + (1 colony or filament/ > 10 optical fields); ++ (1 colony or filament/ 1 to 9 optical fields); +++ (1

colony or filament/ optical field); ++++ (≥ 5 colonies or filaments per field). Later in the study, an Olympus inverted microscope became available and the more standard method of using Utermöhl Chambers and sedimentation of samples (Utermöhl 1958) was used (see Chapter 5). The index method of quantification was continued for continuity of results. Cyanobacteria species identifications were initially carried out using an Olympus light microscope (CHA) at 400 – 1000x (under oil) and later using an inverted Olympus microscope (CKX41) at 400 – 800x magnification. Photomicrographs were taken with an Olympus C-5050 digital camera, using both bright field and phase contrast objectives.

Cyanobacteria species identifications were carried out on the preserved material and when the living unpreserved samples were still fresh these were used for further confirmation, e.g. of colour. Identifications were made primarily by reference to; Baker (1991, 1992), Baker & Fabbro (2002), Biggs and Kilroy (2000), Entwistle (1997), Hindák (2003), Komárek & Anagnostidis (1989), Komárek & Anagnostidis (2000), Komárek & Kling (1991), Komárek & Komárková (2003), Komárek et al. (2002), Li et al. (2000b), Ling & Taylor (2000), McGregor & Fabbro (2001) and Sant'Anna et al. (2003).

To enable a comparison of species composition of cyanobacterial blooms prior to 1987 with results for this study, the samples from lakes (the only waterbody type discussed in Pridmore & Etheredge 1987) which had experienced blooms were identified. A combined quantification index score of ++++ or greater across all species in the sample was required before a sample was classified as experiencing a cyanobacterial bloom. If two or more species co-dominated these each were scored as forming a bloom. For lakes sampled more than once, separate blooms were recorded only when the samples were collected more than four weeks apart.

2.5 Results

2.5.1 Occurrence of Cyanobacterial Blooms

A total of 271 samples from 147 different locations were collected personally or received from other sources. Table 2.3 shows the number of samples and different locations for each waterbody type.

Table 2.3. Number of samples and number of different locations collected for each waterbody type.

Type of Water Body	Total Number of Samples	Number of Locations
Lakes	187	75
Oxidation Ponds	12	9
Ponds and Dams	62	54
Rivers and Streams	10	9
TOTALS	271	147

The cyanobacteria present in each sample are given in Tables A1 - A4 (Appendix 2). A summary of the number of times each waterbody was sampled is shown in Appendix 8.

Of the 271 samples, 67% were found to contain some cyanobacteria and 41% of the samples contained cyanobacterial blooms (Table 2.4). In addition to the bloom samples, nine benthic cyanobacterial mat samples were collected or received (Lakes – 2, Ponds/Dams – 1, Rivers/Streams - 6).

Table 2.4. Number of samples for each waterbody type in which cyanobacteria were identified and were experiencing cyanobacterial blooms.

Type of Water Body	No. of Samples Containing Cyanobacteria	No. of Samples with Blooms
Lakes	144 (77%)	94 (50%)
Oxidation Ponds	12 (100%)	9 (75%)
Ponds and Dams	14 (23%)	5 (8%)
Rivers and Streams	10 (100%)	2 (20%)
TOTALS	180 (67%)	110 (41%)

2.5.2 Diversity of Cyanobacteria

A total of 36 different cyanobacteria species were recorded during this section of the study (17 Nostocales, 9 Oscillatoriales, 10 Chroococcales.). A full species list is given in Appendix 9. Further species were identified in an in-depth study of cyanobacteria over six months in Lakes Rotoiti and Rotoehu. These species are discussed in detail in Chapter 5. Eight species: *Anabaena minderi*; *Anabaena planktonica*; *Anabaena smithii*; *Aphanocapsa holsatica*; *Cylindrospermopsis raciborskii*; *Microcystis panniformis*; *Microcystis smithii*; *Microcystis ichthyoblabe* and *Microcystis wesenbergii* were identified for the first time in New Zealand and a further two species (*Sphaerocavum brasiliense* Azevedo & Sant'Anna, *Microcystis botrys* Teiling) were identified for the first time in New Zealand during the in-depth study in Lakes Rotoiti and Rotoehu (Chapter 5). Morphological descriptions, drawings and photographs of these species are presented in Chapter 3. Of these new species records, six were the dominant or co-dominant species in cyanobacterial blooms observed in this part of the study (Table 2.6).

Two other species were recorded that are also likely to be new species records for New Zealand; *Arthrospira maxima* Setchell et Gardner 1917 and *Planktothrix peronata* f. *attenuata* (Skuja) Anagnostidis & Komárek 1988. These matched the descriptions in Baker (1992). Unfortunately images (either drawings or photographs) of these specimens were not taken and for this reason they have not been documented as new records.

In addition to the new species records there are a number of species that are in need of taxonomic revision in New Zealand. Those of particular importance to this study are described below.

The taxonomic status of *Aphanizomenon flos-aquae* as described in Etheredge and Pridmore (1987) is uncertain. It was reported by Cassie (1978) as forming blooms in Lake Rotoehu. A species similar to this was observed in a number of samples collected in samples during this study, including moderate concentration in Lake Rotoehu (sample numbers L86 & L87). Several morphological features of the species observed make it unlikely that this species is *A. flos-aquae* (Figure 2.1). One major difference was that the trichomes were always separate and never in fascicles as is typical of *A.*

flos-aquae (Hindak 2003, Li et al. 2000a). A further characteristic of *A. flos-aquae* not observed is that apical cells are often hyaline and up to 25 μm in length (in the specimens from this study they were only up to 15 μm). This species resembles *A. gracile* Lemmermann 1907 (Baker 1991, Hindak 2003, Li et al. 2000a). However, it differs from the description of *A. gracile* by Baker (1991) who states that the akinetes often have a “collar like” extension over the adjacent vegetative cell. This was not observed in any of the specimens obtained during this study.

Figure 2.1 Light photomicrograph of *Aphanizomenon gracile* (Lake Rotoehu – L86). A, akinete, H, heterocyte. Scale bar = 10 μm .

The species described as *Anabaena flos-aquae* in Etheredge and Pridmore (1987) is likely to be *A. lemmermannii* (Figure 2.2). The most diagnostic feature of *A. lemmermannii* is that akinetes are adjacent to heterocytes on both sides (Komárkova 1988, Li et al. 2000b). This feature is described in Etheredge and Pridmore (1987) and was also seen in many field specimens (e.g.: from Lake Rotoiti, Lake Okaro, Lower Karori Reservoir, Lake Waihola). In most recent publications, spherical vegetative cells and akinetes distant from the heterocytes are considered characteristic for *A. flos-aquae* (Baker 1991), A. Joosten, Stichting Alg, Netherlands, pers. comm.). The samples observed in this study never had akinetes remote from heterocytes and for this reason it would be more taxonomically correct if the name *A. lemmermannii* was used.

Figure 2.1. Light photomicrographs of *Anabaena lemmermannii* (Lower Karori Reservoir – L77). A, akinete, H, heterocyte. Scale bars = 10 μm .

Two *A. spiroides* subspecies should now be reclassified as separate species based on their morphological characteristics. *A. spiroides* var. *crassa* Lemmermann, 1898 as described in Etheredge and Pridmore (1987) should now be named *A. crassa* (Lemm.) Komárková-Legnerová & Cronberg 1992 (Figure 2.3) (Baker & Fabbro 2002, Komárková-Legnerová & Cronberg 1992, Li et al. 2000b). In *A. crassa* akinetes are wider and have a more ellipsoidal shape than those of *A. spiroides*. The filaments of *A. crassa* are also more uniform and the distance between spirals slightly wider than *A. spiroides*. Likewise *A. spiroides* var. *tumida* Nygaard 1945 as described in Etheredge and Pridmore (1987) should now be named *A. perturbata* f. *tumida* (Nyg.) Cronberg & Komárek 1994 (Cronberg & Komárek 1994). The filaments of *A. perturbata* f. *tumida* are not as uniformly spiralled as those from *A. spiroides* and they tend to form an entangled aggregated mass. The akinetes also differ in shape with those from *A. perturbata* f. *tumida* being cylindrical or slightly curved.

Figure 2.3. Light photomicrographs of *Anabaena crassa* (Lake Rotorua – L90). A, akinete, H, heterocyte. Scale bars = 10 μ m.

Since the publication of Etheredge & Pridmore (1987) two *Oscillatoria* species identified in their study have been assigned to different genera. *Oscillatoria limnetica* has been moved to *Pseudanabaena limnetica* and *Oscillatoria mougeotii* moved to *Planktothrix mougeotii* (Anagnostidis & Komárek 1988).

2.5.3 Benthic Mat Samples

The benthic mat samples were commonly dominated by *Oscillatoria* sp. and *Phormidium* sp. (Figure 2.4A). A sample collected from the shoreline of Lake Taupo was identified as *Nostoc commune* (Figure 2.4C). One benthic mat was collected from the saline environment of Lake Grassmere and identified as *Lyngbya majuscula* (Figure 2.4B). This species is a common marine species and can cause problems when large quantities detach and wash up on beaches.

Figure 2.4. Light photomicrographs of A, *Phormidium* sp (Avalon Pond – DP50); B, *Lyngbya majuscula* (Lake Grassmere – L151); C, *Nostoc commune* (Taupo – L160). H, heterocyte. Scale bars = 10 μ m.

2.5.4 Cyanobacterial Blooms

2.5.4.1 Lakes

Cyanobacterial blooms were identified in 31 lakes (Table 2.5, Appendix 1, Table 1d). In addition to these lakes cyanobacterial blooms have been reported recently from Lake Karapiro by Ryan et al. (2003), Waikopiro (B. Stansfield, Hawke's Bay Regional Council, pers. comm), Lake Taupo (Environment Waikato, pers comm.) and Lake Okataina (Environment Bay of Plenty, pers. comm.) taking the total number of lakes reported to have experienced cyanobacterial blooms between 2001 to 2004 to 35. Of these lakes, 18 had not previously been recorded as experiencing cyanobacterial blooms (Pridmore & Etheredge 1987). However, 12 of these were not surveyed by Etheredge & Pridmore (1987) or in prior published studies. Six of the 18 lakes had been studied and blooms not recorded. Twelve lakes which had been recorded as having experienced cyanobacterial blooms by Pridmore & Etheredge (1987) were not surveyed and four lakes that were previously reported as having blooms were not found to contain blooms when sampled in this study.

Ninety two percent of the blooms reported between 2001 to 2004 came from the North Island and only eight percent from the South Island (Appendix 1, Table 1d). However of the samples collected from lakes only 10% of them came from South Island lakes and only 15 (20%) of the lakes sampled were located in the South Island.

Of the bloom samples that were personally collected or received from other sources during this study the majority were collected in January (16%), March (25%) and April (24%) (Figure 2.5).

Figure 2.5. Percentage of bloom samples that were collected or received during this study for each month.

Table 2.2. A comparison of New Zealand lakes in which planktonic cyanobacteria have produced water-blooms and scums prior to 1987 (adapted from Pridmore & Etheredge 1987) and during this study (or from published or available data post 2001). NBR = no bloom/s recorded. NS = lake not studied. * For location refer to map in Appendix 1, N = North Island, S = South Island.

Lake	Lake no. *	Dominant Species in Blooms (prior to 1987)	Dominant Species in Blooms During This Study (or Published Post 2001)
Alexandrina	S1	<i>Anabaena flos-aquae</i>	NBR
Butchers Dam		<i>Anabaena flos-aquae</i>	NS
Dudding	N64	NS	<i>Microcystis aeruginosa</i>
Ellesmere		<i>Nodularia spumigena</i>	NS
Forsyth	S9	<i>Nodularia spumigena</i>	<i>Nodularia spumigena</i>
Hakanoa	N9	<i>Anabaena spiroides</i> <i>Microcystis aeruginosa</i>	<i>Raphidiopsis</i> sp. <i>Microcystis aeruginosa</i> <i>Anabaena planktonica</i> <i>Microcystis flos-aquae</i>
Hayes		<i>Anabaena flos-aquae</i>	NS
Horowhenua	N69	<i>Anabaena</i> sp. <i>Microcystis aeruginosa</i>	<i>Microcystis aeruginosa</i>
Horseshoe		<i>Anabaena flos-aquae</i>	NS
Johnson		<i>Anabaena flos-aquae</i>	NS
Kainui	N12	<i>Microcystis aeruginosa</i>	<i>Microcystis flos-aquae</i>
Kaitoke	N71	NS	<i>Anabaena lemmermannii</i>
Kaiwakatai	N21	NS	<i>Microcystis flos-aquae</i> (<i>Microcystis aeruginosa</i> and <i>Anabaena planktonica</i> – present low levels)
Karapiro	N17	NBR	<i>Cylindrospermopsis raciborskii</i> (Ryan et al. 2003b)
Kimiha	N18	NBR	<i>Anabaena planktonica</i>
Kohangatera	N73	NS	<i>Anabaena lemmermannii</i> <i>Anabaena circinalis</i>
Lower Karori Reservoir	N66	NS	<i>Anabaena lemmermannii</i> <i>Anabaena circinalis</i> <i>Anabaena planktonica</i> (in 2004)
Magakaware		<i>Anabaena circinalis</i>	NS
Maraetai	N23	NBR	<i>Anabaena planktonica</i>

Table 2.5 continued....

Lake	Lake no. *	Dominant Species in Blooms (prior to 1987)	Dominant Species in Blooms During This Study (or Published Post 2001)
Nгахewa	N26	<i>Anabaena flos-aquae</i> <i>Anabaena spiroides</i> <i>Anabaena circinalis</i> <i>Anabaena</i> sp.	<i>Anabaena spiroides</i> <i>Anabaena planktonica</i> (<i>Microcystis aeruginosa</i> – present low levels)
Ngapouri	N27	<i>Anabaena flos-aquae</i> <i>Microcystis</i> sp.	<i>Anabaena planktonica</i> (<i>Microcystis aeruginosa</i> – present low levels)
Ngaroto	N28	<i>Anabaena circinalis</i> <i>Microcystis aeruginosa</i>	<i>Anabaena planktonica</i> <i>Microcystis ichthyoblabe</i> <i>Microcystis flos-aquae</i> <i>Microcystis aeruginosa</i> <i>Cylindrospermopsis raciborskii</i> (Ryan et al. 2003b)
Okaro	N29	<i>Anabaena flos-aquae</i> <i>Anabaena spiroides</i> <i>Anabaena</i> sp. <i>Microcystis aeruginosa</i>	<i>Microcystis protocystis</i> <i>Anabaena lemmermannii</i>
Okataina		NBR	<i>Anabaena</i> sp. (Environment Bay of Plenty, pers. comm.)
Omapere	N30	<i>Anabaena flos-aquae</i>	<i>Microcystis wesenbergii</i> <i>Microcystis aeruginosa</i>
Orakai	N84	<i>Anabaena tenericaulis</i>	<i>Anabaena spiroides</i> <i>Anabaena</i> sp.
Pauri	N87	NS	<i>Planktothrix peronata</i> f. <i>attenuata</i>
Pupuke		<i>Microcystis aeruginosa</i>	NS
Rotoaira	N88	<i>Anabaena flos-aquae</i>	NBR
Rotoehu	N34	<i>Aphanizomenon flos-aquae</i>	<i>Microcystis panniformis</i> <i>Anabaena</i> sp.
Rotoiti (Kaikoura)		<i>Anabaena spiroides</i>	NS
Rotoiti (NI)	N35	<i>Anabaena flos-aquae</i> <i>Anabaena spiroides</i>	<i>Anabaena planktonica</i> <i>Microcystis aeruginosa</i> <i>Anabaena circinalis</i>
Rotomanuka	N38	<i>Anabaena tenericaulis</i> <i>Microcystis aeruginosa</i>	NBR (<i>Microcystis aeruginosa</i> – present low levels)

Table 2.5 continued....

Lake	Lake no. *	Dominant Species in Blooms (prior to 1987)	Dominant Species in Blooms During This Study (or Published Post 2001)
Rotongaio		<i>Anabaena oscillarioides</i> <i>Anabaena circinalis</i> <i>Anabaena</i> sp.	NS
Rotorua (Kaikoura)		<i>Anabaena spiroides</i>	NS
Rotorua (NI)	N39	<i>Anabaena circinalis</i> <i>Anabaena spiroides</i> <i>Microcystis aeruginosa</i>	<i>Microcystis aeruginosa</i> <i>Anabaena planktonica</i> <i>Anabaena circinalis</i>
Roundabout		<i>Anabaena flos-aquae</i>	NS
Southern Reservoir (Dunedin)	S8	NS	<i>Anabaena oscillarioides</i>
Spectacle	N46	NS	<i>Microcystis wesenbergii</i> <i>Anabaena planktonica</i> (minor component)
Tarawera	N48	<i>Anabaena flos-aquae</i>	<i>Anabaena lemmermannii</i>
Taupo	N50	NBR	<i>Anabaena</i> sp. (Environment Waikato, pers. comm.)
Te Koutu	N52	<i>Anabaena tenericaulis</i> <i>Microcystis aeruginosa</i>	<i>Anabaena circinalis</i> <i>Anabaena planktonica</i> (minor component)
Tomahawk Lagoon		<i>Anabaena flos-aquae</i> <i>Anacystis incerta</i>	NS
Tutira	N98	<i>Anabaena</i> sp. <i>Microcystis aeruginosa</i>	NBR
Waahi	N55	<i>Anabaena spiroides</i>	<i>Cylindrospermopsis raciborskii</i>
Waihola	S28	NS	<i>Anabaena lemmermannii</i>
Waitawa	N110	<i>Microcystis aeruginosa</i>	<i>Microcystis aeruginosa</i> <i>Anabaena circinalis</i>
Westmere	N111	NS	<i>Microcystis aeruginosa</i>
Waikopiro	N104	NS	<i>Anabaena planktonica</i>
Whangape	N60	NBR	<i>Cylindrospermopsis raciborskii</i>
Wiritoa	N112	NS	<i>Microcystis flos-aquae</i> <i>Microcystis aeruginosa</i>

A comparison of the dominant species in the recent blooms shows some marked differences from the blooms prior to 1987 (Table 2.6). Nine species that were not previously reported as forming blooms in New Zealand were found to be the dominant or co-dominant species and six of these were also species that were recorded in New Zealand for the first time. Of these species, *Anabaena planktonica* dominated 24% of the blooms. This species was also found in low numbers in many other bloom samples (Appendix 2, Table A1 - A4). *Microcystis aeruginosa* was a common bloom forming species prior to 1987 and this appears to be still the case with approximately 20% of the blooms from both study periods being dominated by this species. *Cylindrospermopsis raciborskii* was identified for the first time during this study and was found in 8% of reported blooms. *Microcystis flos-aquae* which was known to occur in New Zealand prior to this study was also found to be dominant in 8% of samples (This species could easily be confused with early stages of *M. aeruginosa* or *M. panniformis*). *Microcystis panniformis* was only found as dominant in one lake (Rotoehu) where it commonly formed dense blooms. Three species; *Aphanizomenon flos-aquae* (which is likely to be *A. gracile* – see Section 2.5.2), *Anabaena tenericaulis* and *A. minutissima* var. *attenuate*, that were recorded as responsible for bloom formation prior to 1987 were either not recorded or not recorded as forming blooms during this study. Cyanobacterial blooms dominated by *A. lemmermannii* and *A. spiroides* had reduced markedly. However, both were commonly present in samples in low numbers (Appendix 2, Tables A1 - A4).

2.5.4.2 Oxidation Ponds

Cyanobacterial blooms were identified in eight different oxidation ponds. *Planktothrix mougeotii* was the dominant or co-dominant species in 75% of these blooms and *Microcystis aeruginosa* in 25%.

2.5.4.3 Dams and Ponds

Cyanobacterial blooms were identified in only five dams and ponds. There was no apparent dominant species with *Anabaena planktonica*, *Microcystis aeruginosa*, *M. flos-aquae*, *Microcystis* sp. and *A. perturbata* f. *tumida* all dominant or co-dominant at one or more locations.

Table 2.6. Comparisons of the percentage of planktonic cyanobacteria species recorded in blooms (67 recorded prior to 1987 & 68 between 2001 to 2004) in New Zealand lakes. *data from Pridmore & Etheredge 1987. Highlighted in yellow indicates that this is also a new species for New Zealand. Highlighted in blue indicates that this species, although known to occur in New Zealand, was not previously recorded as forming blooms. Species are listed in order of decreasing frequency of occurrence in blooms during 2001-4.

Species	Percentage of Blooms Recorded Where Species Dominated Prior to 1987*	Percentage of Blooms Recorded Where Species Dominated 2001 - 2004
<i>Anabaena planktonica</i>	-	24%
<i>Microcystis aeruginosa</i>	21%	19%
<i>Anabaena flos-aquae</i> (<i>A. lemmermannii</i>)	27%	8%
<i>Cylindrospermopsis raciborskii</i>	-	8%
<i>Microcystis flos-aquae</i>	-	8%
<i>Anabaena circinalis</i>	7%	6 %
<i>Anabaena</i> sp.	7%	5%
<i>Microcystis panniformis</i>	-	5%
<i>Anabaena spiroides</i>	21%	3%
<i>Microcystis wesenbergii</i>	-	3%
<i>Raphidiopsis</i> sp.	-	3%
<i>Nodularia spumigena</i>	3%	2%
<i>Microcystis</i> sp.	1%	2%
<i>Planktothrix peronata</i> f. <i>attenuata</i>	-	2%
<i>Microcystis ichthyoblabe</i>	-	2%
<i>Anabaena oscillarioides</i>	-	2%
<i>Anabaena. minutissima</i> var. <i>attenuata</i>	6%	-
<i>Anabaena tenericaulis</i>	4%	-
<i>Aphanizomenon flos-aquae</i> (<i>A. gracile</i>)	1%	-

2.6 Discussion

2.6.1 Study Limitations

It was planned to collect samples from waterbodies experiencing cyanobacterial blooms and/or mats of cyanobacteria, to maximise the efficiency of sample collection and increase the chances of detecting cyanobacteria and cyanotoxins (see Chapter 4 for information on cyanotoxins). However, many of the samples collected/received did not contain cyanobacterial blooms (Appendix 2).

The patchy distribution both spatially and temporally of cyanobacterial blooms raised logistic problems in the collection of field material and initially the study planned to rely on people living in close proximity to waterbodies or working with them to collect and send samples when they observed cyanobacterial blooms. This created several problems: it was difficult to attract sufficient interest and persuade people to collect samples; there was little control over the sample collection methodology and how the sample was handled after collection and it tended to create bias in the number of samples coming from certain locations. If organisations were interested in having samples analysed they tended to send them regularly (e.g. a large proportion of the samples came from the Rotorua area) which made some areas appear to have a higher frequency of cyanobacterial blooms than other areas that were not sampled, or were sampled less frequently. This also made it difficult to draw conclusions about the time of year that blooms were experienced, and to make valid comparisons between the North and South Islands.

It was preferable to collect field material personally, which allowed correct procedures to be followed. However, time available for field collecting was limited and sampling tended to be biased towards easily assessable areas (e.g. lower and central North Island).

Most of the samples collected or received during this study were one-off samples and were collected during field trips or when others supplying the samples were present at a suitable waterbody. The broad geographical scope of this part of the study did not allow for samples to be collected on a weekly, monthly or seasonal basis from any site. It is

therefore likely that the number of waterbodies containing cyanobacteria was underestimated.

The cyanobacterial bloom data was compared to data from a study carried out by Pridmore and Etheredge (1987) (see Section 2.6.2.1). This comparison was not an initial aim and thus not all lakes studied in the 1987 research were sampled. To assist in assessing if cyanobacterial blooms are increasing in New Zealand it would have been desirable to have obtained samples from at least all lakes reported to have experienced blooms prior to 1987. However, because the majority were sampled the comparison of changes in species diversity proved interesting and valuable.

2.6.2 Lakes

The dominant cyanobacteria genera in the lake samples were *Anabaena*, *Microcystis* and *Cylindrospermopsis*. Species from these genera are commonly known to form cyanobacterial blooms in other parts of the world (Chorus & Bartram 1999, Haider et al. 2003). There are species from other genera that commonly form blooms in other parts of the world that have either not been identified or are not commonly forming blooms in New Zealand, e.g. *Aphanizomenon*, *Woronichinia* and *Plankthotrix*. All of the species identified as forming blooms in this study have been reported to have caused blooms in other parts of the world.

The majority of the lakes that were identified as having experienced cyanobacterial blooms came from the North Island (Appendix 1, Table 1d). This may be due to more extensive sampling in there. Pridmore and Etheredge (1987) noted that more cyanobacteria taxa had been reported from the North Island and this may in part be due to the large proportion of glacial lakes in the South Island that are often nutrient limited. Further studies of cyanobacteria in South Island lakes would greatly add to the knowledge of planktonic cyanobacteria distribution.

2.6.2.1 Are Cyanobacterial Blooms Increasing in New Zealand Lakes?

Although the number of lakes known to have experienced cyanobacterial blooms in New Zealand has increased by 18 lakes from 1987 it is hard to ascertain if the incidence of cyanobacterial blooms are actually increasing. Twelve of the lakes recorded as having experienced cyanobacterial blooms in this study were not sampled by Etheredge

and Pridmore (1987) or in previously published studies, thus this increase in lakes experiencing blooms may merely reflect more intensive or extensive sampling. As discussed above, because many of the samples were one-off samples it is likely that some blooms would have been missed. Twelve lakes that were reported to have experienced cyanobacterial blooms in Pridmore & Etheredge (1987) were not sampled, so it is uncertain whether or not these lakes still experience bloom problems. Lowe & Green (1987) report that in New Zealand there are 776 lakes with at least one axis greater than 0.5 km, thus 6.6% of lakes are now known to have experienced blooms. It seems highly likely that this number will rise with increased sampling intensity and sampling of more locations.

While good phytoplankton databases exist for a few lakes in New Zealand (e.g. Lakes Taupo and Rotorua) the majority have been sampled only infrequently or not at all, and often phytoplankton has not been distinguished beyond the level of genus, making it difficult to determine whether the number of blooms have increased. The establishment of routine monitoring programmes by a number of regional councils may allow such comparisons to be made in the future. Anecdotal evidence from residents living near lakes indicates that the incidences of blooms (at least at some locations) has increased and the blooms appear to be more severe and visible for longer periods of time.

Future projects should aim to sample more lakes, and where possible, to sample each lake regularly, particularly over the summer period.

2.6.2.2 Changes in Composition of Cyanobacterial Bloom Forming Species in Lakes

While there is uncertainty about whether or not cyanobacterial blooms are increasing in New Zealand there is little doubt that species composition has changed over the last 15-30 years. This is illustrated by the identification during this study of six new records of species which form blooms species and of three bloom-forming species that were known to occur in New Zealand but at lower levels of abundance.

Three of the newly recorded bloom-forming species are *Microcystis* spp. It is probable that these have been present in New Zealand for some time but have been misidentified as other *Microcystis* species. However, for the third, *M. wesenbergii*, this is unlikely

because its wide gelatinous margin is very distinctive and hard to confuse with other species.

Because cyanobacteria are an ancient group in evolutionary terms they are often thought to have a cosmopolitan distribution. Increasing evidence shows that while some are indeed cosmopolitan others have pantropical or holarctic distributions (Castenholz 1996, Hoffmann 1996, Komárek 1985) and it is also probable that endemic species exist (Broady 2000). Prior to the 1990's *Cylindrospermopsis raciborskii* was considered a tropical species but in the last ten years it has been found in many temperate locations (see discussion below). *Microcystis panniformis* is considered a tropical species (Komárek et al. 2002) and to the best of the author's knowledge this is the first record of this species from a temperate environment.

Reasons for the change in species composition are unclear and were not investigated. Some of these changes will be due to human error in identifying the species (as mentioned above and described for *C. raciborskii* and *A. planktonica* in more detail below). It is also possible that some of these species have only recently arrived in New Zealand and in some cases they may have features that give them a competitive advantage over other cyanobacteria (discussed below for *C. raciborskii* and *A. planktonica*). Changes in nutrient concentration in New Zealand lakes may also have an effect on species composition. It would be interesting to further investigate the trophic status of the lakes sampled in this study and compare this to historic data. A final reason could be climate change. Changes in environmental variables such as temperatures and wind may lead to conditions that favour some cyanobacteria species.

2.6.2.3 The Identification and Distribution of *Cylindrospermopsis raciborskii*

Cylindrospermopsis raciborskii (Woloszynska) Seenayya et Subba Raju is a planktonic freshwater cyanobacterium that was initially reported only from tropical and subtropical areas (Komárek & Kling 1991). However, a later review of its worldwide distribution (Padisák 1997) reported an expansion of its range to include temperate regions of Australia, Europe, and North and South America.

Cylindrospermopsis raciborskii was implicated in one of the most significant cases of human poisoning from exposure to a cyanobacterial toxin. In 1979, 148 people required hospitalisation with symptoms of gastro-enteritis after a local water supply on Palm Island (Australia) was dosed with copper sulphate to control a dense algal bloom (Bourke et al. 1983, Byth 1980). Further information on the toxins of *C. raciborskii* and the New Zealand reports of this toxin are given in Chapter 4.

The identification during this study of *C. raciborskii* in Lake Waahi and subsequently in Lakes Ngaroto and Whangape is the first report of this species in New Zealand. It has also recently been identified in Lake Karapiro (Ryan et al. 2003b). Because *C. raciborskii* is not described in the commonly used New Zealand taxonomic literature (Etheredge & Pridmore 1987) it seems plausible it species has previously been misidentified or not recognised. When the cyanotoxin cylindrospermopsin was detected in Lake Waitawa (Stirling & Quilliam 2001) the causative species was identified as a *Cylindrospermum* sp. (see Chapter 4 for more information on this cyanotoxin in New Zealand). However, *Cylindrospermum* spp. are not known to produce cylindrospermopsin (Chorus & Bartram 1999) and is primarily a benthic species (Baker & Fabbro 2002). Etheredge & Pridmore (1987) describe several species that are very similar morphologically to *C. raciborskii*. The first, *Cylindrospermum minutissimum* Collins 1896 (Figure 2.6A), was described from Lakes Kimihia and Mangahia both of which are in close proximity to the lakes in which *C. raciborskii* was identified in the present study. The identifications were carried out on a few trichomes (Etheredge & Pridmore 1984) with a key feature being the position of the heterocyte adjacent to the akinetes. Another morphologically similar species, *Raphidiopsis mediterranea* Skuja 1937 (Figure 2.6, B), was identified in both Lakes Rotoaira and Taupo by Etheredge and Pridmore (1987). This is very similar to *C. raciborskii* trichomes which lack akinetes and heterocytes. Thus it is possible that *C. raciborskii* has been present in New Zealand as a minor component of New Zealand's phytoplankton for many years and has been misidentified as a *Cylindrospermum* sp. or a *Raphidiopsis* sp. The correct identification of *C. raciborskii* can be difficult demonstrated by several recent taxonomic and molecular investigations (McGregor & Fabbro 2000, Wilson et al. 2000).

Figure 2.6. Line diagrams from Etheredge and Pridmore (1987) of: (A) *Cylindrospermum minutissimum* Collins 1896, and (B) *Raphidiopsis mediterranea* Skuja 1937. Both these species show morphological similarities to *Cylindrospermopsis raciborskii*, which was identified for the first time in New Zealand during this study.

Factors controlling the possible increase in distribution and dominance of *C. raciborskii* in these Waikato lakes are unclear. It is possible that *C. raciborskii* is an introduced exotic species that has been able to gain a unique physiological and ecological advantage over other algae and cyanobacteria. If *C. raciborskii* is recently adventive, then the question as to how it arrived in New Zealand arises. New Zealand has been geographically isolated from the rest of the world for approximately 80 million years. Padisák (1997) suggests two possible ways of dispersal: either as akinetes in the guts of migratory birds, or other “chance events” of primarily human origin. An example of the later would be the occurrence of *C. raciborskii* in Minnesota lakes which has been attributed to shipments of tropical fish. New Zealand has recently seen a dramatic increase in the trade of aquatic plants (Champion 2004), most of which are used in aquariums or ornamental ponds. Importation of these plants is poorly controlled and it is plausible that some new cyanobacteria species may periodically arrive in New Zealand with these plants.

Once present in a waterbody *C. raciborskii* has several features that may give it a competitive advantage over other cyanobacteria. It is highly tolerant of a range of salinity and nutrient concentrations and has a high growth potential at low irradiance. Many other cyanobacteria tend to be well-adapted to high irradiance and this allows *C. raciborskii* to build up large populations before self-shading prevents further growth

(Padisák & Reynolds 1998). *C. raciborskii* also has a high uptake affinity for phosphorus (P) combined with relatively low thresholds and high P storage capacity (Istvanovics et al. 2000). Heterocytes allow for nitrogen (N) fixation when N is limiting, which may provide *C. raciborskii* a competitive advantage over non N-fixing phytoplankton. In addition, *C. raciborskii* has a high uptake affinity for ammonia which allows it to utilize this N-source while other nitrogen fixing species have to fix atmospheric N₂. *Cylindrospermopsis raciborskii* is also considered to be relatively resistant to grazing by zooplankton (Bouvy et al. 2001), which is perhaps another reason for its dominance in some waterbodies.

Padisák (1998) suggested that *C. raciborskii* may be a good indicator of global climate change. In her studies of Lake Balaton (Hungary) she found that optimum rates of germination of akinetes occur over a narrow temperature of 21-22 °C and that such high temperatures only occur in this lake in exceptionally hot years. There was a correlation between hot summers and blooms of *C. raciborskii*, thus indicating that if there is global warming it is predicted that there will be an increased frequency of *C. raciborskii* blooms. A similar situation could occur in New Zealand, with blooms occurring sporadically only in hot summers. It was also interesting to note that the bloom of *C. raciborskii* did not occur until late in the summer (March). Although no water temperature data were collected it is probable that prior to late summer temperatures were not high enough to allow optimum germination of akinetes. Further studies investigating the germination temperature of the New Zealand strain of *C. raciborskii* are required.

Padisák (1997) suggested that *C. raciborskii* has spread from two “evolutionary centers”, Australia and Africa. Molecular studies would help establish the relationship of the New Zealand strain to strains elsewhere.

2.6.2.4 The Identification and Distribution of *Anabaena planktonica*

Pridmore & Etheredge (1987) noted that although *Anabaena planktonica* was frequently found in temperate Northern Hemisphere lakes, it had not been identified in New Zealand. During the present study it was identified in 20 locations and was dominant or co-dominant in blooms from nine. In addition to blooms sampled in the present study a

further bloom was reported from Lake Waikopiro in December 2003 (B. Stansfield, Hawke's Bay Regional Council, pers. comm.)

Anabaena solitaria and *A. affinis* which are morphologically similar to *A. planktonica*, were recorded in New Zealand prior to the present study. It is possible that these could have been misidentifications of *A. planktonica*. Etheredge & Pridmore (1987) recorded *A. solitaria* and noted the presence of akinetes adjacent to heterocytes. This feature is not consistent with taxonomic descriptions of *A. planktonica* and was not observed in any of the specimens examined in the present study. *Anabaena solitaria* in these early New Zealand descriptions was found at only a few locations: Lakes Ngaroto, Omapere, Rotoaira (Etheredge & Pridmore 1987), Okataina, Rotoma, Tikitapu and Middleton (Thomasson 1974). It was not known to form blooms or scums (Pridmore & Etheredge 1987).

The description of *A. affinis* in Etheredge & Pridmore (1987) also shares some similarities to *A. planktonica*. However, again the presence of akinetes on one or both sides of the heterocytes makes it unlikely that these are the same species. *Anabaena affinis* was recorded in samples from a number of lakes: Clearwater, Evelyn, Georgina, Heron, Lyndon, Okataina, Pearson, Pounui, Rotoaira, Rotokakahi, Rotorua (N.I.), Sarah (Etheredge & Pridmore 1987), Okareka, Rotoiti (N.I) and Rotokawau (Thomasson 1974). It was not recorded as forming blooms (Pridmore & Etheredge 1987).

It is likely *A. planktonica* has been present in New Zealand during the last 5 – 10 years but misidentified as one of the above two species. For example, Wilding (2000) reports on a bloom of *A. solitaria* in Lake Rotoiti (May 1999), and it is highly likely that *A. planktonica* was responsible for this bloom.

There are three possibilities with regard to *A. planktonica*: firstly it has been present in New Zealand for sometime but not identified; secondly that it has been present in New Zealand for sometime but has been misidentified (probably as *A. solitaria* or *A. affinis*); or thirdly that it has only recently arrived in New Zealand. Regardless of which of these possibilities is correct, there seems to be little doubt that its geographic range is expanding and that in some waterbodies it has the ability to form very dense blooms and are often almost monospecific. This species dominated in 24% of the blooms recorded

in the present study. Prior to the publication of Pridmore & Etheredge (1987), neither this species nor any morphologically similar species were known to form blooms in New Zealand.

Ryan et al (2003a) reported synchronous blooms of *A. planktonica* in five Waikato lakes and in Lakes Rotoiti and Rotorua. They observed densities of over 120 000 cell ml⁻¹ and active nitrogen fixation in Lake Rotoiti was noted to have likely contributed to its dominance. The ability to form dense blooms suggests that it tolerates low light environments in which self-shading did not appear to affect the bloom intensity.

The Lower Karori Reservoir (Wellington) can be used as an example of how quickly *A. planktonica* may become established and dominate a bloom. As an early part of the present study a preliminary survey of the Lower Karori Reservoir was carried out in 2001 (see Appendix 10 for details of this study). The cyanobacteria species responsible for and possible causes of, the bloom were investigated. The first cyanobacterial bloom was reported in the Lower Reservoir approximately eight years ago and blooming continued to increase in intensity until extremely dense blooms were reported in 1998/99. Samples were first collected from the reservoir in 2001, when the bloom was dominated by two species of *Anabaena* (*A. circinalis* and *A. lemmermannii*) (Appendix 10). This dominance continued until the 2003/4 summer. Late in the summer of 2004 a few filaments of *A. planktonica* were observed in samples collected from the reservoir (K. Smith, Victoria University, pers. comm.). In November 2004 the reservoir experienced a dense monospecific bloom of *A. planktonica*. Thus in the space of approximately 10 months there had been a total species change. It will be interesting to monitor and assess future changes in species composition.

Despite recent dominance of *A. planktonica* there is little information published internationally on its ecology and physiology. This may be due to different taxonomic classifications of this species, i.e. it has probably been mis-identified as *A. solitaria*.

As with *C. raciborskii* the known distribution of *A. planktonica* is restricted to the North Island. There is a need for further investigation into the distribution, rates of nitrogen fixation, light requirements, resistance to zooplankton grazing and genetics of the New

Zealand strains of both species. It would be interesting to monitor their distribution over the next decade.

2.6.3 Oxidation Ponds

The high nutrient concentrations of oxidation ponds make them ideal environments for cyanobacteria. It was therefore not surprising that all samples contained some cyanobacteria and that 75% of samples contained cyanobacterial blooms. Oxidation ponds in New Zealand have previously been reported to have experienced blooms particularly in late summer (Cassie 1983, Cassie-Cooper 1996). Cassie-Cooper (1996) reported a bloom of *Oscillatoria subbrevis* at Mangere Sewerage Treatment Plant (Auckland). In this study the two species responsible for blooms were identified as *Planktothrix mougeotii* and *Microcystis aeruginosa*. Both of these species have been reported from oxidation ponds in other parts of the world (e.g. (Vasconcelos & Pereira 2001). The possible negative downstream effects from the outflows of oxidation ponds are discussed in Section 4.6.1.3.

2.6.4 Ponds and Dams

Given the highly eutrophic nature of many ponds and dams (particularly those situated in agricultural areas) surprisingly few of the samples obtained during the present study contained cyanobacteria (only 23% and 8% with blooms). The dominant species did not differ markedly from those of the lakes with *Microcystis* and *Anabaena* species commonly being identified. The only species recorded in this water body type that was not recorded elsewhere was *A. perturbata* f. *tumida*, however this species is also known to occur in a number of lakes (Etheredge & Pridmore 1987).

2.6.5 Rivers and Streams

Species of *Oscillatoria*, *Phormidium* and *Nostoc* dominated in these samples. *Oscillatoria* and *Phormidium* were not identified to species level. All of these genera are common in New Zealand rivers and streams (Biggs & Kilroy 2000). The number of benthic samples received or collected during this study was too limited to make any comments on their distribution. More detailed surveys of benthic algae have been

carried out in New Zealand (Biggs & Price 1987, Biggs 1990, Biggs & Kilroy 2000). Benthic cyanobacteria in New Zealand are at times known to produce cyanotoxins (see Chapter 4) and have caused a number of animal mortalities (Hamill 2001). Further is strongly recommended, especially as many New Zealand drinking water supplies are dependant on rivers and streams where these benthic species are common.

2.7 Conclusions

The objectives of this part of the present study initially aimed to assess the distribution of cyanobacterial blooms/mats in New Zealand and to identify the cyanobacteria species involved. It subsequently appeared necessary to compare the results obtained during the present study to historical data on cyanobacterial blooms in lakes in New Zealand and to identify whether there have been changes in the number of lakes experiencing blooms or changes in dominant species over time.

While this appears to indicate a growth in the number of lakes experiencing blooms the limited historical phytoplankton data that exist concerning most lakes makes it difficult to establish distribution patterns over time, and to explain changes. It is likely that some of the increase in the number of affected lakes is due to more intensive and extensive sampling. For example, 12 of the lakes that experienced cyanobacterial blooms during the present study were not sampled during or prior to the research of Pridmore and Etheredge (1987). The total number of lakes known to have experienced cyanobacterial blooms in New Zealand is now 51 compared to 33 in 1987.

The species composition of cyanobacteria blooms has changed over time. In the 1970's and 1980's common bloom-forming cyanobacterial species included: *Anabaena flos-aquae*, *A. spiroides* and *Microcystis aeruginosa*. During the summers of 2001 to 2004 six bloom forming cyanobacteria species not previously identified in New Zealand (*A. planktonica*, *Cylindrospermopsis raciborskii*, *M. panniformis*, *M. ichthyoblabe*, *M. wesenbergii*, and *Planktothrix peronata* f. *attenuata*) were found to be dominant or co-dominant in at least some cyanobacterial blooms. A further three species that were already known to occur in New Zealand but not known to be responsible for bloom formation were also found to be dominant or co-dominant in some blooms (*Microcystis flos-aquae*, *Raphidiopsis* sp. and *Anabaena oscillarioides*). Both *A. lemmermannii* and *A. spiroides* which were common bloom forming species in the 1970s and 1980s have decreased markedly in their dominance. Incorrect taxonomic identification of some species, in particular *Microcystis* spp., may have accounted for some proportion of this apparent change.

Anabaena planktonica was dominant in 24% of blooms. The geographic range of *A. planktonica* also seems to be expanding. In 2001/2 this species was only found in waterbodies north of the central North Island. In 2003 this species was found in the Napier region and in 2004 in several locations in Wellington. The identification of *C. raciborskii* in a number of Waikato lakes was also of interest. This species is known to be highly adaptive and may be a future threat to the water quality and usability of an increasing number of our lakes.

Despite factors limiting rigorous comparison over time it appears likely that there has been an increase in the distribution of cyanobacterial blooms in New Zealand lakes during the last two decades. Empirical evidence shows that there has also been a change in species composition over this time. Given the potential hazards associated with cyanobacterial blooms regular and systematic monitoring of the distribution and species composition of blooms (beyond what was possible in this limited study) is strongly recommended.

2.8 References

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3. New Records of Planktonic Cyanobacteria in New Zealand Freshwaters

3.1 Abstract

New records of planktonic cyanobacteria in New Zealand freshwaters are reported. Identified for the first time was one genus, *Sphaerocavum*, represented by one species, and one species of *Aphanocapsa*, five species of *Microcystis*, one of *Cylindrospermopsis* and three of *Anabaena*. *Microcystis panniformis* has previously been recorded only from tropical or subtropical regions. Descriptions, drawings and photographs of species are presented. The identification of a further species remains uncertain. Based on morphological features of both environmental and cultured material it fits the description of a *Raphidiopsis* sp.. However, the results from a *Cylindrospermopsis raciborskii* specific PCR indicate that it maybe *C. raciborskii*.

3.2 Keywords

blue-green algae; cyanobacteria; planktonic; taxonomy; New Zealand; new records

3.3 Introduction

Cyanobacteria were first noted in New Zealand in the 1880s (Lemmermann 1899, Nordstedt 1888). Since then studies have been infrequent and focused mostly on their ecology (e.g. Cassie 1978, Pridmore & Etheredge 1987, Viner & White 1987). In 1987, a major survey studied taxonomy and distribution of planktonic cyanobacteria in 77 lakes (Etheredge & Pridmore 1987). This represents less than 12% of the total number of lakes in New Zealand. There are also many ponds, impoundments and swamps that have been poorly studied. To date, only 339 species distributed amongst 65 genera have been recorded from both terrestrial and aquatic environments in New Zealand, and identification of some of these is dubious (Broady 2000). In the present study (2001 to 2004) a survey of planktonic cyanobacteria in New Zealand lakes and rivers was conducted to determine species responsible for cyanobacterial blooms (Chapter 2) and cyanotoxin production (Chapter 4), and to assess their distribution in New Zealand (Chapters 2 and 4). In this Chapter the 10 species recorded in New Zealand for the first time are described. A further six new species records for New Zealand; *Anabaena*

torulosa (Carm.) Lagerheim 1883, *Arthrospira maxima* Setchell et Gardner 1917, *Coelosphaerium* aff. *natans* Lemmermann 1900, *Planktothrix* aff. *planktonica* (Elenk.) Anagnostidis & Komárek 1988, *Planktothrix peronata* f. *attenuata* (Skuja) Anagnostidis & Komárek 1988 and *Pseudanabaena galeata* Böcher 1949, are not documented due to a lack of drawings or photomicrographs.

3.4 Methods

3.4.1 Identification Based on Morphology

The newly recored planktonic cyanobacteria were collected from 20 North Island lakes, two ponds and two rivers (Table 3.1 and Appendix 2) between 2000 and 2004. Samples were obtained in the months November through to June and analysed as described in Section 2.4.1 and 2.4.2. Photomicrographs were taken with an Olympus C-5050 digital camera, using both bright field and phase contrast objectives on an Olympus CKX41 Inverted microscope. Line drawings were made from living, preserved and photographic material and proportions kept consistent with recorded cell dimensions. For each species fifty microscope measurements of vegetative cell, akinete, and heterocyte lengths and widths were made, except with *Anabaena minderi* and *A. smithii* where fewer than 50 akinetes were observed in samples.

The species identifications of these species were made primarily by reference to Baker (1991,1992), Baker & Fabbro (2002), Cronberg (1973), Hill (1970), Hindák (2000), Komárek & Komárkova (2003), Komárek & Anagnostidis (1989), Komárek & Anagnostidis (2000), Komárek et al. (2002), Li et al. (2000), McGregor & Fabbro (2001), Sant'Anna et al. (2004). Classification follows that of Komárek & Anagnostidis (1989, 2000).

3.4.2 Culture Conditions

Raphidiopsis sp. (strain number MU1) was isolated from Lake Hakanoa (L161, Appendix 2, Table A1) and maintained in the Massey University Ecotoxicology Laboratory (Wellington). It was grown in 250 ml flasks containing 100 ml of BG-11 medium (with and without nitrogen sources) and Jaworski's Medium (see Appendix 11 for recipes). The cultures were maintain at 25°C, 12:12 h light:dark cycle and a light intensity of 2000 lux.

3.4.3 DNA Extraction

Approximately 0.01 g of lyophilised material of *Cylindrospermopsis raciborskii* from the Lake Waahi sample - L114, (Appendix 2, Table A1) was resuspended in 1 ml of autoclaved water. The sample was then pelleted by centrifugation (10 000 rpm, 1 min) and the supernatant removed. Two ml of cultured material of MU1 was pelleted by centrifugation (10 000 rpm, 2 min) and the supernatant removed. The samples were resuspended in 200 µl of solution containing 90% InstaGene matrix (Biorad, Auckland) and 10% Triton X-100 (Biolab, Auckland). The cells were incubated at 55°C for 30 min, vortexed for 1 min, heated to 95°C for 10 min and centrifuged (10 000 rpm, 1 min). 1 - 5 µl of the resulting supernatant was used in the 20 µl PCR reactions.

3.4.4 *Cylindrospermopsis raciborskii* Specific PCR

All PCRs were run in a Px2 thermal cycler PCR system (ThermoHybaid, Medica Pacifica Ltd, Auckland). Primers were purchased from Invitrogen New Zealand Ltd (Auckland). The PCR for the specific identification of *C. raciborskii* using the primers cyl2- 5'-GGC ATT CCT AGT TAT ATT GCC ATA CTA and cyl4- 5'-GCC CGT TTT TGT CCC TTT CGT GC was applied (Wilson et al. 2000).

Each 20 µl reaction mixture contained 1 - 5 µl of the supernatant obtained as described above (this supernatant contains genomic DNA), 1 µM of both PCR primers, 200 µM deoxynucleoside triphosphates (New England Biolabs Inc, Biolab, Auckland), 10 x Proofstart PCR buffer, and 2.5 U of Proofstart DNA Polymerase (Qiagen, Biolab, Auckland).

The thermal-cycling conditions were 95°C for 10 min, 1 cycle; 94°C for 30 s, 45°C for 30 s, and 72°C for 30 s, 35 cycles; 72°C for 15 min, 1 cycle; 4°C, hold.

The amplified PCR products were visualised on 1.5% agarose gels stained with ethidium bromide. Standard DNA fragments (DNA molecular weight marker 100 bp ladder, New England Biolabs Inc, Biolab, Auckland), were used to indicate sizes of the amplification products.

Table 3.1. Location and sample numbers of 20 North Island, New Zealand lakes, two ponds and two rivers in which new records were identified. Some new records came from the in-depth study on Lakes Rotoiti and Rotoehu samples, refer to Chapter 5. * For location refer to map in Appendix 1, N = North Island, S = South Island.

Site	Lake No.*	Sample Number
Arapuni	N1	L116
Hakanoa	N9	L161, L162, L172, L113
Kainui	N12	L173
Lower Karori Reservoir	N76	L186
Lower Whitby Pond	N77	DP64
Maraetai	N23	L85, L99
Ngahewa	N26	L23, L33, L101, L155
Ngaopouri	N27	L100
Ngaroto	N28	L108, L134, L174
Okaro	N29	L75, L102
Omapere	N30	L58, L59, L60
Prawn Farm - Pond 1	N31	DP62, DP 63
Rotoehu	N34	L12, L18, L25, L80, L104, L106, L107, L156, L157, L180, L184 – also refer to Chapter 5
Rotoiti	N35	L26, L36-40, L89, L94-96, L109, L110, L120, L141, L142, L158, L177, L181-183 – also refer to Chapter 5
Rotoroa	N29	L120
Rotorua	N29	L90, L91, L92, L93, L108
Spectacle	N46	L38
Te Ko Utu	N52	L121
Tutira	N88	L187
Utakura River	N54	R9, R10
Waahi	N55	L114, L129, L138, L165, L166, L175
Waikato River	N56	R3
Weavers	N58	L115
Whangape	N60	L176

3.5 Results

3.5.1 Species Descriptions

ORDER CHROOCOCCALES

Family Merismopediaceae

Description (from Komárek & Anagnostidis (2000)): Solitary cells or mucilaginous colonies. Cells spherical, obovate, oval or rod-shaped. Colourless mucilage with the development of a special central stalk system in some spherical colonies. Cell division occurs by binary fission and is always in two perpendicular planes, cells usually grow to original form before division and nanocyte formation is rare. Reproduction is usually by disintegration of colonies.

Aphanocapsa holsatica (Lemmermann) Cronberg et Komárek 1994. Figure 3.1 A - D

Synonyms: *Clathrocystis holsatica* Lemmermann 1903, *Microcystis holsatica* (Lemmermann) Lemmermann 1907.

Description: Young colonies microscopic, spherical or ellipsoidal; later colonies become irregular and often clathrate up to 250 µm diam.; mucilage hyaline and diffuent; cells densely aggregated, spherical, lacking aerotopes and pale green-blue, 0.8 - 1.3 µm diam.

Locations: L. Rotoiti, L. Rotoehu, L. Ngahewa.

Sphaerocavum brasiliense Azevedo et Sant'Anna 2003. Figure 3.2 A - D

Description: Young colonies hollow and spherical, later becoming irregular and at times clathrate; mucilage narrow and indistinct; cells densely aggregated, spherical, lacking aerotopes and pale green-blue, 2.5 - 3.5 µm diam.

Locations: L. Rotoiti, L. Rotoehu.

Family Microcystaceae

Description (from Komárek & Anagnostidis (2000)): Colonial cells arranged in three dimensional perpendicular rows in mucilaginous colonies, with or without individual gelatinous envelopes. Cells with or without aerotopes, spherical becoming hemispherical after cell division. Cell division usually in three planes perpendicular to one another in consecutive generations. Reproduction is usually by disintegration of colonies, facultative nanocyte production.

Microcystis botrys Teiling 1942. Figure 3.3 A - E

Description: Colonies spherical, irregular and at times clathrate; mucilage hyaline, diffuent, wide, forming a broad margin around the colony, gelatinous protuberances (not visible in the Figures 3.3) project radially from colony; cells very densely aggregated, often radially oriented, spherical, blue-green, with aerotopes, 4.5 – 6.0 µm diam.

Locations: L. Rotoiti.

Microcystis panniformis Komárek et al. 2002. Figure 3.4 A - D

Description: Colonies free floating, initially microscopic, later commonly macroscopic; young colonies irregular with densely aggregated cells, later becoming spherical-irregular to lobate and occasionally forming large flat plate-like formations, often with irregular holes; mucilage colourless, homogenous, not extending beyond colony edges; cells spherical and densely distributed, brownish green, with aerotopes, 3.0 - 5.0 µm diam.

Locations: L. Rotoehu, L. Hakanoa.

Microcystis smithii Komárek et Anagnostidis 1995. Figure 3.5 A - C

Synonyms: *Aphanocapsa pulchra* (Kützing) Rabenhorst 1865; *Microcystis grevillei* sensu G.M. Smith 1920.

Description: Colonies free-floating, spherical or sub-spherical, usually 70 – 100 µm diam.; mucilage fine and colourless; cells arranged sparsely throughout colony, blue-green, with aerotopes, 3.0 - 5.0 µm diam.

Location: L. Okaro.

Microcystis ichtyoblabe Kützing 1843. Figure 3.6 A - E

Description: Colonies free floating, irregular-spherical, composed of sub-colonies situated in wide, hyaline mucilage; cells spherical, blue-green, with aerotopes, 2.5 - 3.0 µm diam.

Location: L. Ngaroto.

Microcystis wesenbergii (Komárek) Komárek in Kondrateva 1968. Figure 3.7 A - E

Synonyms: *Coelosphaerium dubium* Gruunow in Rabenhorst sensu auct. post; *Microcystis aeruginosa* Kützing sensu Teiling 1941; *Diplocystis wesenbergii* Komárek 1958.

Description: Colonies spherical when young, later elongated and very lobate, often composed of sub-colonies; mucilage hyaline, firm, smooth, delimited, with refractive outline; gelatinous margin extends 3.0 – 6.0 µm beyond the cell aggregations; cells distributed randomly, spherical, blue-green with distinctive aerotopes, 4.0 – 6.0 µm diam.

Locations: L. Spectacle, L. Omapere.

Order NOSTOCALES

Family Nostacaceae

Trichomes unbranched or falsely branched, iso-polar or heteropolar, heterocytes usually present. All cells are able to divide and cell division always perpendicular to the long axis of the trichome. Reproduction by trichome fragmentation (to form hormogonia) or akinetes.

Anabaena minderi Huber-Pestalozzi, 1938. Figure 3.8 A – C

Synonyms: *Anabaena bergii* var. *limnetica* Couté et Preisig; 1978; *Anabaena bergii* var. *minor* Kiselev sensu Hindák 1992.

Description: Trichomes solitary, straight or somewhat curved, slightly attenuated, up to 350 µm long, with no mucilaginous sheath evident; heterocytes distant from akinetes, spherical to broadly oval, length 6.0 – 9.0 µm, width 6.0 – 8.0 µm; akinetes broadly ovate to longitudinally cylindrical, yellow-brown, solitary or in pairs, length 12.0 – 20.0 µm, width 9.0 – 12.0 µm; vegetative cells quadrate or barrel-shaped, with aerotopes, constricted at the cross walls, length 3.5 – 5.5 µm, width 4.5 – 5.5 µm; apical cells often hyaline and with tapering ends.

Location: L. Weavers.

Anabaena planktonica Brunnthaler 1903. Figure 3.9A - E

Synonym: *Anabaena solitaria* f. *planktonica* (Brunnthaler) Komárek 1958; *Anabaena limnetica*. G.M Smith 1916; *Anabaena solitaria* f. *solitaria* sensu Komárek 1958.

Description: Trichomes solitary, straight, with a broad mucilaginous sheath; heterocytes spherical, length 9.0 – 13.0 µm, width 9.0 – 13.0 µm; akinetes oblong-ovate or oblong with broadly rounded ends, solitary or occasionally in pairs, remote from the heterocytes, length 15.0 – 27.0 µm, width 11.0 – 17.0 µm; vegetative cells spherical or compressed at the ends, with aerotopes, length 6.0 – 12.0 µm, width 8.0 – 14.0 µm; apical cells undifferentiated.

Locations: L. Arapuni, L. Hakanoa, L. Kainui, L. Karapiro, Lower Karori Reservoir, Lower Whitby Pond, L. Maraetai, L. Ngahewa, L. Ngaopouri, L. Ngaroto, L. Okaro, L. Omapere, Prawn Farm - Pond 1, L. Rotoiti, L. Rotoroa, L. Rotorua, L. Spectacle, L. Te Ko Utu, L. Tutira, Utakura River, Waikato River - this study. L. Waikopiro (39° 14' 176° 53', B. Stansfield, Hawke's Bay Regional Council, pes. comm)

Anabaena smithii (Komárek) M.Watanabe 1992. Figure 3.10 A - B

Synonym: *Anabaena solitaria* f. *smithii* Komárek 1958.

Description: Trichomes solitary, straight, with mucilaginous sheath evident; heterocytes spherical, length 9.0 – 12.0 µm, width 9.0 – 12.0 µm; akinetes spherical, yellow-brown, only solitary akinetes seen in our specimens, remote from the heterocytes, length 12.0 – 18.0 µm, width 12.0 – 18.0 µm; vegetative cells spherical or compressed at the ends to shorter than wide, with aerotopes, length 6.0 – 12.0 µm, width 8.0- 14.0 µm; apical cells undifferentiated.

Location: L. Kainui

Cylindrospermopsis raciborskii (Woloszynska) Seenayya et Subba Raju.
Figure 3.11A- E

Synonyms: *Anabaena raciborskii* Woloszynska 1912; *Anabaenopsis raciborskii* (wolosz.) elenkin 1923.

Description: Trichomes solitary and straight with no mucilaginous sheath evident; heterocytes terminal at one or occasionally both ends, elongated and tapered to a rounded point, length 3.0 - 9.0 µm, width 1.5 - 2.5 µm; akinetes oblong-ovate to cylindrical, solitary or in pairs, usually but not exclusively adjacent to heterocyte, length 5.0 - 19.0 µm, width 2.0 – 4.0 µm; vegetative cells quadrate to cylindrical and not constricted at the cross-walls, length 4.0 - 10.0 µm, width 1.5 - 2.5 µm; apical cells often slightly attenuated at apices when heterocytes not present.

Location: L. Waahi, L. Ngaroto, L. Whangape - this study, L. Karapiro (37° 56' 175° 33', Ryan et al., 2003).

Description: Trichomes solitary, straight or slightly bent with no mucilaginous sheath evident; length 75.0 – 460.0 μm ; heterocytes not observed in either environmental or cultured samples; akinetes cylindrical, solitary or in series (up to 7 observed in a Lake Hakanoa environmental sample and in cultured material), length 6.0 – 26.0 μm , width 2.5 - 3.5 μm ; vegetative cells cylindrical and not constricted or only slightly constricted at the cell walls, length 4.0 – 26.0 μm , width 2 - 2.5 μm ; apical cells often narrowed, attenuated, tapered and hair-like length 8 – 40 μm .

Location: L. Hakanoa, L. Ngawera

3.5.2 PCR Results

A 308 bp diagnostic product was amplified from both the lyophilised material of *Cylindrospermopsis raciborskii* and the cultured material of *Raphidiopsis* sp. The results of the PCR as revealed by gel electrophoresis are shown in Figure 3.13 (A – B).

Figure 3.1 (A - D). Light photomicrographs of *Aphanocapsa holsatica*. A, B, Clathrate colonies; C, Young ellipsoidal colony, arrow points to *A.holsatica* colony; D, Young spherical colony. Scale bars = 10 μ m.

Figure 3.2 (A - D). Light photomicrographs of *Sphaerocavum brasiliense*. A, Hollow and lobate colony; B, Old clathrate colony with densely aggregated cells; C, Clathrate colony negatively stained with Indian Ink in order to show the extent of the colonial mucilage; D, Old clathrate colony (indicated by arrow) compared to a colony of *Microcystis aeruginosa* (MA). Scale bars = 10 μ m.

Figure 3.3 (A - D). Light photomicrographs of *Microcystis botrys*. A, C, Clathrate colonies with wide hyaline mucilage and densely aggregated cells; B, D, Negatively stained with Indian Ink to show wide colonial mucilage, arrows in B show examples of radially oriented cells; E, Drawing of *M. botrys* showing wide mucilage and densely aggregated and some radially oriented cells. Scale bars = 10 μ m.

Figure 3.4 (A - D). Light photomicrographs of *Microcystis panniformis*. A, B, C, Large flat plate-like colonies, photomicrographs taken using phase contrast; D, Negatively stained with Indian Ink in to show narrow colonial mucilage. Scale bars: A, B = 50 μ m; C, D = 20 μ m.

Figure 3.5 (A - C). Light photomicrographs of *Microcystis smithii*. A, Spherical colony with cell sparsely arranged cells, fine mucilage visible; B, Spherical colony taken with phase contrast; C, Drawing of slightly irregular *M. smithii* colony with spherical cells. Scale bars = 10 μm .

Figure 3.6 (A - E). Light photomicrographs of *Microcystis ichthyoblabe*. A, B, Irregular sub-colonies in wide, hyaline mucilage, photomicrographs taken with phase contrast; C, Negatively stained with Indian Ink to show the wide mucilage surrounding sub-colonies; D, Spherical cells densely aggregated into sub colonies; E, Drawing of *M. ichthyoblabe* showing sub colonies and wide mucilage. Scale bars = 10 μ m.

Figure 3.7 (A - E). Light photomicrographs of *Microcystis wesenbergii*. A, B, D Spherical, elongated and lobate colonies with refractive outline of mucilage clearly visible, photomicrographs taken with phase contrast; C, Spherical cells; E, Drawing of *M. wesenbergii* showing variations in formations of colonies. Scale bars A, B = 50 μm ; C, D, E = 10 μm .

Figure 3.8 (A - C). Light photomicrographs of *Anabaena minderi*. A, Straight, slightly attenuated trichome with hyaline and pointed apical cell, photomicrograph taken with phase contrast; B, Longitudinally elliptical akinetes and spherical heterocytes; C, Straight trichome with ovate heterocytes, longitudinally elliptical akinete, one bluntly rounded and one tapered apical cell, photomicrographs taken with phase contrast. A, akinete; H, heterocyte. Scale bars = 10 μ m.

Figure 3.9 (A - E). Light photomicrographs of *Anabaena planktonica*. A, B, D Straight trichomes with spherical heterocytes and oblong akinetes with broadly rounded ends; C, Trichome negatively stained with Indian Ink to show broad mucilaginous sheath. E, Drawing of *A. planktonica* showing spherical heterocytes and oblong-ovate akinetes. A, akinete; H, heterocyte. Scale bars = 10 μ m.

Figure 3.10 (A - B). A, Light photomicrographs of *Anabaena smithii* showing spherical akinete and heterocytes; B, Drawing of *A. smithii* with spherical akinete. A, akinete; H, heterocyte. Scale bars = 10 μm .

Figure 3.11 (A - E). Light photomicrographs of *Cylindrospermopsis raciborskii*, A, B, C, D Straight attenuated trichomes some containing terminal pointed heterocytes, B shows cylindrical paired akinetes, C and D single akinetes illuminated using phase contrast; E, Drawing showing akinetes, heterocytes and slightly attenuated apical cells when heterocytes absent. A, akinete; H, heterocyte. Scale bars = 10 μm .

Figure 3.12 (A - H). Light photomicrographs of *Raphidiopsis* sp. A, B, C, E, H Environmental samples. D, F, G cultured material. A, B, C, D taken with phase contrast and show slightly bent trichomes with cylindrical akinetes and long, tapered, hair-like apical cells; E, Akinetes in series and very pointed apical cells in an environmental sample; F, Seven akinetes in series in an cultured sample; G, H, very long tapered hair-like apical cells. Figure F by A. Crowe (Cawthron Institute). A, akinete. Scale bars = 10 μ m.

Figure 3.12 continued....

Figure 3.13 (A - B). A., *Cylindrospermopsis raciborskii*-specific PCR. Genomic DNA from laboratory cultures and environmental samples was amplified with primers cyl2 and cyl4 to produce the diagnostic 305-bp PCR product. Lane 1, blank, lane 2 *Raphidiopsis* sp. MU1 (1 μ l), lane 3 *Raphidiopsis* sp. MU1 (2 μ l), lane 4 *Raphidiopsis* sp. MU1 (3 μ l), lane 5 *C. raciborskii* environmental sample (1 μ l) (Lake Waahi), lane 6 *C. raciborskii* environmental sample (2 μ l) (Lake Waahi), lane 7 *C. raciborskii* environmental sample (3 μ l) (Lake Waahi), lane 8 *C. raciborskii* environmental sample (5 μ l) (Lake Waahi). Lane M molecular sized marker: 100, 200, 300, 400 and 500 bp. B as per A except inverted.

3.6 Discussion

3.6.1. Notes on Species Identifications

The importance and difficulty of distinguishing these from other species is discussed below.

3.6.1.1 *Aphanocapsa holsatica*

The small cell size and distinctly clathrate colonies make identification of mature colonies straightforward. Young colonies could be confused with other *Aphanocapsa* spp. such as *A. delicatissima*. The latter has been recorded in New Zealand (Etheredge & Pridmore 1987).

3.6.1.2 *Sphaerocavum brasiliense*

Azevedo and Sant'Anna (2003) recently described *Sphaerocavum* from Brazil where it forms heavy blooms during spring and summer. At times in Lake Rotoiti this species formed dense blooms of 30 000 cells ml⁻¹ (refer to Chapter 5). *Sphaerocavum brasiliense* could be easily misidentified as a *Microcystis* species. The main difference between these genera is that *Sphaerocavum* has hollow colonies as a consequence of cell division in two planes whereas *Microcystis* cell division is in three planes and results in compact colonies (Sant'Anna et al. 2004). Cell size of *S. brasiliense* also differs from morphologically similar *Microcystis* spp. and *Aphanocapsa* spp.. The cells of *S. brasiliense* observed in the present study were smaller than those of *Microcystis* spp. and larger than *Aphanocapsa* spp.

3.6.1.3 *Microcystis botrys*

Although *M. botrys* has a cosmopolitan distribution it is not commonly mentioned in literature. To date, this species has been identified only in Lake Rotoiti but is probably much more widely distributed in New Zealand. It is likely that this species is commonly mis-identified as *M. aeruginosa* (Kützing) Kützing 1846 (Cronberg & Baalen 2004, Sant'Anna et al. 2004). The radially oriented protuberances and at time radially oriented cells should be used to distinguish between these two species (Sant'Anna et al. 2003, G Cronberg, University of Lund, Sweden, pers. comm.). The range of cell sizes is slightly wider than that described in Komárek & Anagnostidis (2000) and McGregor & Fabbro (2001).

3.6.1.4 *Microcystis panniformis*

This species was recently described from Brazil (Komárek et al. 2002), and also identified in tropical Australia (Baker & Fabbro 2002). *M. panniformis* is considered a tropical species (Komárek et al. 2002), thus, its presence in temperate environments is noteworthy. In New Zealand it is likely that this species has commonly been misidentified as *M. aeruginosa*. The lack of a wide mucilaginous envelope in *M. panniformis* (Komárek et al. 2002) should be used to distinguish between these species (Sant'Anna et al. 2003). Young *M. panniformis* colonies could easily be confused with young *S. brasiliense* colonies. *M. panniformis* often forms heavy blooms and scums in Lake Rotoehu.

3.6.1.5 *Microcystis smithii*

Microcystis smithii is very similar to young *Microcystis protocystis* Crow 1923, in which colonies have sparsely distributed cells of similar size to *Microcystis smithii* (3.5 – 6.5 µm diam.) (Komárek & Anagnostidis 2000). Differences in species are based on the size and form of colonies; spherical or near spherical in *M. smithii* (70 – 100 µm) and irregular and larger in *Microcystis protocystis* (often greater than 200 µm).

3.6.1.6 *Microcystis ichthyoblabe*

The sub-colonies of densely grouped cells, contained within wide mucilage, make *Microcystis ichthyoblabe* distinctive. This species could be confused with old or senescent colonies of *Microcystis* spp., e.g. *M. aeruginosa* or *M. flos-aquae* (Wittrock) Kirchner 1898.

3.6.1.7 *Microcystis wesenbergii*

The mucilage which is smooth, firm, delimited and has a refractive outline makes *M. wesenbergii* very distinctive and it seems unlikely that this species would previously have been confused with other *Microcystis* species. *Microcystis wesenbergii* is reported as having a cosmopolitan distribution (except for sub-polar regions) (Komárek & Anagnostidis 2000). Currently the distribution in New Zealand is limited to waterbodies in the upper North Island.

3.6.1.8 *Anabaena minderi*

Anabaena minderi could easily be confused with *A. bergii* Ostenfeld 1908 (not recorded in New Zealand). However Hindák (2000) noted that the shape and size of akinetes of *A. minderi* and *A. bergii* are different (in *A. bergii* akinetes are oval to spherical) and therefore they warrant being classified as two independent but closely related taxa (Table 3.2). The akinetes observed in the present study most closely represent those of *A. minderi*. The synonym *Anabaena bergii* var. *limnetica* is used in Australian cyanobacteria taxonomic literature (commonly used in New Zealand), however, to avoid confusion between the two species *A. minderi* was chosen for this study as suggested by Hindák (2000). Less than 50 filaments were observed and these were all from Lake Weavers.

3.6.1.9 *Anabaena planktonica*

Anabaena planktonica, *A. solitaria* and *A. smithii* are very similar in appearance except for the shapes of akinetes; spherical in *A. smithii*, cylindrical in *A. solitaria*, and oblong-ovate in *A. planktonica* (Table 3.2). The similarity of these species led Komárek (1958) to place these species into three forms of *A. solitaria* (*A. solitaria* f. *solitaria*, *A. solitaria* f. *planktonica*, *A. solitaria* f. *smithii*). A study of numerous strains of each species in culture (Li et al. 2000) found that shape of akinetes remained consistent and was therefore a key diagnostic characteristic in separating these species. Etheredge & Pridmore (1987) described *A. solitaria* from New Zealand. It is likely that both *A. planktonica* and *A. smithii* have been misidentified as *A. solitaria*. Etheredge & Pridmore (1987) noted akinetes of *A. solitaria* immediately adjacent to the heterocyte on one or both sides; this was not observed in any of the present specimens. *A. planktonica* commonly forms dense blooms (refer to Chapter 2 for more information on bloom formation and possible mis-identification of this species).

3.6.1.10 *Anabaena smithii*

Anabaena smithii could be misidentified as *A. planktonica* or *A. solitaria* discussed above (Table 3.2). In environmental samples, akinetes are commonly absent and it is therefore likely that *A. smithii* occurs in other locations besides Lake Kainui but has not been able to be identified unambiguously.

Table 3.2. Diagnostic morphological features of straight *Anabaena* identified and those with very similar characteristics.* Not observed during this study - description from Pridmore & Etheredge (1987). # Not observed during this study - description from Hindák (2000).

Taxa	Trichomes	Vegetative Cells	Heterocyte	Apical Cells	Akinetes	Akinete Position
<i>A. minder</i>	Straight or slightly flexuous. Gas vacuolate.	Quadrate or barrel-shaped. Width 4.5 - 5.5 μ m, length 3.5 - 5.5 μ m.	Spherical to broadly oval. Width 6.0 - 8.0 μ m, length 6.0 - 9.0 μ m.	Hyaline and with tapering.	Broadly ovate to longitudinally cylindrical. Width 9.0 - 12.0 μ m, length 12.0 - 20.0 μ m.	Remote from the heterocytes. Single.
<i>A. planktonica</i>	Straight. Gas vacuolate	Spherical or compressed. Width 6.0 - 12.0 μ m	Spherical. Width 9.0 - 13.0 μ m	Rounded	Akinetes oblong-ovate or oblong. Width 11.0 - 17.0 μ m, length 15.0 - 27.0 μ m.	Remote from the heterocytes. Single or in pairs.
<i>A. smithii</i>	Straight. Gas vacuolate	Spherical or compressed. Width 6.0 - 12.0 μ m	Spherical. Width 9.0 - 12.0 μ m	Rounded	Spherical. Width 12.0 - 18.0 μ m	Remote from the heterocytes. Single.
<i>A. solitaria</i> *	Straight. Gas vacuolate.	Almost spherical. Width 8 μ m.	Broadly ellipsoidal. Width 8 - 10 μ m.	Rounded	Cylindrical. Width 9 - 10 μ m. Length 20-29 μ m.	On one or both sides of the heterocyte or remote. Single.
<i>A. bergii</i> #	Straight or slightly flexuous. Gas vacuolate.	Quadrate or barrel-shaped. Width 5.0 - 7.0 μ m.	Spherical to broadly oval. Width 7.0 - 8.0 μ m.	Hyaline and with tapering.	Broadly oval to spherical.	Remote from the heterocytes.
<i>A. affinis</i> *	Straight or slightly flexuous. Gas vacuolate.	Almost spherical. Width 5 - 6 μ m.	Spherical. Width 7 - 8 μ m.	Rounded	Broadly ovoid, truncately rounded poles. Width 10 -11 μ m. Length 16-20 μ m.	Adjacent or remote from heterocyte. Single.

3.6.1.11 *Cylindrospermopsis raciborskii*

The Lake Waahi strain of *C. raciborskii* conforms to species descriptions from natural populations described by Baker (1991) and Komárek & Kling (1991). However, the Lake Waahi strain produced only straight trichomes and the vegetative cell and heterocyte dimensions are slightly narrower than those in Baker (1991) but within the

range of those described in Komárek & Kling (1991). Further discussions on the distribution and possible mis-identification of *C. raciborskii* are given in Chapter 2.

3.6.1.12 cf. *Raphidiopsis* sp.

The identity of this species is still unknown. Based on morphological features it would best fit the description of *Raphidiopsis mediterranea* Skuja 1937 as given in Cronberg (1973), Rao (1957) and Hill (1970). The similar vegetative cell and akinete dimensions and extremely tapered hair-like apical cell of this species closely resemble those of *Aphanizomenon issatschenkoi* (Usáček) Proškina-Lavrenko 1968 as described in Baker (1991) and Watanabe (1991). However, heterocytes were not seen in any of the environmental samples or in the cultured material grown in the three different culture media.

The results from the *C. raciborskii* species specific PCR indicate that cf. *Raphidiopsis* sp. could be *C. raciborskii*. Wilson et al. (2000) developed and tested this PCR and used it to distinguish *C. raciborskii* from species of *Anabaena*, *Microcystis*, *Nodularia* and *Anabaenopsis*. Species of *Raphidiopsis* were not tested during their study. In addition, other species of *Cylindrospermopsis* also exist, e.g. *C. Africana* Komárek et Kling 1991, *C. cuspis* Komárek et Kling 1991 and *C. helicoidea* Cronberg et Komárek 2003. Wilson et al. (2000) did not assess the ability of their PCR to distinguish between *Cylindrospermopsis* species and very few of these other *Cylindrospermopsis* species have been cultured. It is therefore also possible that cf. *Raphidiopsis* sp. is a *Cylindrospermopsis* species, other than *C. raciborskii*. Shafik et al. (2003) demonstrated that in culture *C. raciborskii* can display enormous morphological variability including long twisted filaments similar to those observed in the present specimens, further indicating that the latter may be a *Cylindrospermopsis* sp. The genus *Raphidiopsis* needs reassessment using both traditional morphological and modern molecular approaches.

The cf. *Raphidiopsis* sp. formed a very dense bloom of 500 000 cells ml⁻¹ in Lake Hakanoa in April 2003. This was shortly after *C. raciborskii* was identified in nearby Lake Waahi. No *C. raciborskii* was observed in any samples collected from Lake Hakanoa. The cultured material was tested for microcystin production using the ADDA-ELISA as described in Chapter 4 and was found to be producing low levels (8 ng g⁻¹), this result needs confirmation using LC-MS. Both *Raphidiopsis* sp. and

Cylindrospermopsis sp. are known to produce other cyanotoxins (Lagos et al. 1999, Li et al. 2001, Namikoshi et al. 2003, Ohtani et al. 1992) and further cyanotoxin testing of this species is recommended.

3.7 Conclusion

This study has resulted in 11 planktonic freshwater cyanobacteria species being identified in New Zealand for the first time. In addition, a species resembling *Raphidiopsis* sp. requires further molecular work to clarify the identification, and a number of other species still require further documenting. The limited use of molecular methods in this study illustrates the value of combining both traditional morphological and recent molecular methods and future work should aim to do this. Cyanobacteria taxonomy has received little attention in New Zealand and it is likely that further studies of fresh waterbodies will identify additional new records (planktonic and benthic) and the known distribution of species will expand. Of interest was that all new records came from North Island samples, which may reflect the difference between islands of the number of samples received, and/or differences in nutrients or environmental variables in the waterbodies sampled. Future studies on the taxonomy and distribution of freshwater cyanobacteria in the South Island would be beneficial.

3.8 References

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4. Cyanotoxins in New Zealand

4.1 Abstract

In the present study of toxic cyanobacteria in New Zealand a total of 266 water samples collected from 227 different locations between 2001 to 2004 were analysed for cyanotoxins. ELISA's, LC/MS, HPLC and neuroblastoma assays resulted in the identification of: microcystins/nodularins (102 samples from 54 different locations), anatoxin-a (3 samples from 3 different locations), cylindrospermopsins (1 sample) and saxitoxins (48 samples from 41 different locations). Microcystins were the most common. The highest microcystin level was 36 500 $\mu\text{g l}^{-1}$ from a scum sample collected at Lake Horowhenua (Levin). The detection of anatoxin-a in 3 samples is the first report of this toxin from cyanobacterial samples and from planktonic species in New Zealand. Previously only degradation products of anatoxin-a had been detected in benthic species and mouse bioassays used, in which the symptoms of death implied that anatoxin-a was present in the samples. The detection of cylindrospermopsin in a sample from Lake Waahi (Waikato) is only the second recorded incidence of this cyanotoxin in New Zealand. The non-toxic analogue deoxy-cylindrospermopsin was detected from the same sample for the first time in New Zealand. Only low levels of saxitoxins were detected which may be due to the analytical methods used. Nevertheless the results indicate that saxitoxins may be more widespread in New Zealand freshwater waterbodies than previously assumed and this warrants further investigation. Anatoxin-a(S) has not been found in New Zealand.

All of the cyanotoxins were found in planktonic cyanobacteria. Both microcystins and saxitoxins were found in benthic mat samples. The cyanotoxin levels were similar to those reported in studies in other countries. Cyanotoxins are more widespread in New Zealand waterbodies than previously thought, in particular when cyanobacterial blooms or benthic cyanobacterial mats are present. In this chapter the distribution and levels of cyanotoxins identified are discussed and gaps in knowledge highlighted.

4.2 Keywords

cyanotoxins; New Zealand; cyanobacteria; anatoxin-a; cylindrospermopsins; saxitoxins; microcystins; nodularin

4.3 Introduction

Cyanotoxins are a diverse assemblage of natural toxins, which have a very broad range of toxicity mechanisms. Those currently described include hepatotoxic, neurotoxic, dermatotoxic and protein synthesis inhibition (Chorus & Bartram 1999). Based on their chemical structure they can be divided into three broad groups; cyclic peptides (microcystins and nodularins), alkaloids (cylindrospermopsins, anatoxins and saxitoxins) and lipopolysaccharides (LPS). Table 4.1 (adapted from Chorus & Bartram 1999) presents a summary of known cyanotoxins, primary toxicological target in mammals, and known cyanobacterial genera that produce each toxin.

Flint (1966) published the first report of animal poisonings in New Zealand arising from suspected ingestion of toxic cyanobacteria in farm ponds in Waipukurau, Masterton and the Bay of Islands. There is also a long history of unexplained stock deaths at Lake Forsyth and nearby Lake Ellesmere (Canterbury) (Conner 1977). These stock deaths are thought to be caused by ingestion of lake water contaminated with *Nodularia* sp.. Until recently, information on the toxin content of New Zealand blooms of cyanobacteria has been sparse and investigations into cyanotoxins have largely been the result of reactive measures as opposed to being part of a structured monitoring programme or systematic research.

The primary objectives of this part of the study were:

- To determine which cyanotoxins are present in New Zealand waterbodies and cyanobacterial mat samples.
- To identify which cyanobacterial species are responsible for cyanotoxin production.
- To assess if cyanotoxins are a potential risk to animal and human health in New Zealand.

A detailed description of each cyanotoxin is given below along with available data on the presence and distribution of each cyanotoxin in New Zealand prior to the beginning of the present study.

Table 4.1. Summary of the known cyanotoxins, primary toxicological target in mammals, and known cyanobacteria genera that produce each toxin (adapted from Chorus & Bartram 1999). ¹ Not produced by all species of the particular genus.

Toxin Group and Toxin	Primary Target in Mammals	Cyanobacteria Genera ¹
Cyclic Peptides		
Microcystins	Liver	<i>Microcystis</i> , <i>Anabaena</i> , <i>Planktothrix</i> , <i>Nostoc</i> , <i>Hapalosiphon</i> , <i>Anabaenopsis</i> , <i>Aphanocapsa</i> , <i>Aphanizomenon</i> , <i>Arthrospira</i> , <i>Cylindrospermopsis</i> , <i>Oscillatoria</i> , <i>Phormidium</i> , <i>Pseudanabaena</i> , <i>Snowella</i> , <i>Synechocystis</i> , <i>Woronichinia</i> .
Nodularin	Liver	<i>Nodularia</i>
Alkaloids		
Anatoxin-a	Nerve synapse	<i>Anabaena</i> , <i>Planktothrix</i> , <i>Aphanizomenon</i> , <i>Cylindrospermum</i> , <i>Lyngbya</i> , <i>Microcystis</i> , <i>Oscillatoria</i> , <i>Planktothrix</i> , <i>Phormidium</i> , <i>Raphidiopsis</i> .
Homoanatoxin-a		<i>Planktothrix</i> , <i>Raphidiopsis</i> .
Anatoxin-a(S)	Nerve synapse	<i>Anabaena</i> , <i>Oscillatoria</i> .
Cylindrospermopsins	Liver	<i>Cylindrospermopsis</i> , <i>Aphanizomenon</i> , <i>Umezakia</i> , <i>Anabaena</i> , <i>Raphidiopsis</i> .
Saxitoxins	Nerve axons	<i>Anabaena</i> , <i>Aphanizomenon</i> , <i>Lyngbya</i> , <i>Cylindrospermopsis</i> , <i>Planktothrix</i> .
Lipopolysaccharides (LPS)		
LPS	Potential irritant; any exposed tissue	All

4.3.1 Microcystins

Globally, microcystins are the most commonly found cyanobacterial toxins (Chorus & Bartram 1999). Microcystins are cyclic peptides. They were first isolated as a crude extract from *Microcystis aeruginosa* strain NRC-1 by Bishop et al. (1959) and have subsequently been isolated from a variety of other cyanobacteria species (Chorus & Bartram 1999). Their structure was first elucidated by Botes et al. (1982a, 1982b) and to date, more than 60 microcystins have been isolated and characterised (Chorus & Bartram 1999).

Microcystins have the general structure:

cyclo-(D-alanine¹-X²-D-MeAsp³-Y⁴-Adda⁵-D-glutamate⁶-Mdha⁷) – (Figure 4.111)

in which X and Y are variable L amino acids, D-MeAsp³ is *D-erythro*- β -methylaspartic acid, and Mdha is *N*-methyldehydroalanine. The amino acid ADDA, (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, is the most unusual structure in this group of cyanobacterial cyclic peptide toxins and is required for biological activity of this compound (Chorus & Bartram 1999). Separation of the ADDA component from the cyclic peptide causes both compounds to be non-toxic (Carmichael 1992). The molecular weight of microcystins varies between 800-1100 daltons.

Figure 4.1. General structure of microcystins. X and Y are the common variable amino acids.

Microcystins are hepatotoxins which block protein phosphatase 1 and 2a in affected organisms (MacKintosh et al. 1990). Most microcystins are highly toxic with intra-peritoneal (ip) mouse toxicities ranging between 50 - 300 $\mu\text{g kg}^{-1}$ body weight. Only a few non-toxic variants have been identified (Carmichael 2001). Microcystin LR, the most commonly found microcystin, has an LD₅₀ of $\approx 55 \mu\text{g kg}^{-1}$ body weight by ip injection into mice (Watanabe et al. 1988). Oral administration of microcystin-LR to mice has demonstrated a 24 h LD₅₀ of between 5 000 $\mu\text{g kg}^{-1}$ (Fawell et al. 1999) and 10 900 $\mu\text{g kg}^{-1}$ (Yoshida et al. 1997).

Numerous incidents of animal and human poisonings have been attributed to microcystins (Ressom et al. 1994). One of the most severe cases occurred in Caruaru, Brazil, in 1996 when processes at a water treatment plant failed and manual addition of chlorine to tanker loads of water supplying a hospital was insufficient to remove the microcystins. This resulted in 56 fatalities at the dialysis treatment clinic (Azevedo et al. 2002). In a further case from Brazil, 88 people died after drinking water that came from

a newly constructed reservoir where a *Microcystis* sp. bloom occurred (Teixeira et al. 1993).

In addition to these fatalities there are numerous reports of illness associated with recreational contact in or on water contaminated with cyanobacteria (Chorus et al. 2000). In one instance, two British soldiers were hospitalised with pneumonia after kayaking and practising “Eskimo rolls” in a waterbody that contained a bloom of *Microcystis aeruginosa* (Turner et al. 1990).

There are many documented cases of animal fatalities due to cyanobacteria consumption. In earlier studies (1970’s to early 1990’s) the toxins responsible for these deaths were often not identified, but *Microcystis* sp. were commonly found in drinking sources and symptoms were consistent with hepatotoxin poisoning (Ressom et al. 1994).

Microcystins are also considered to be chronic carcinogens and can cause long term liver damage (Falconer et al. 1983). Epidemiological evidence from China (Ueno et al. 1996) has linked the continual consumption of low doses of microcystins in drinking water to primary liver cancer.

Numerous studies overseas have shown that microcystins can accumulate in a variety of freshwater and marine organisms (Kotak et al. 1996, Vasconcelos 1995, Vasconcelos 1999). This is discussed in more detail in Chapter 5 which presents the results of an investigation into the accumulation of microcystins in rainbow trout (*Oncorhynchus mykiss*) and freshwater mussels (*Hydriddella menziesi*) in Lakes Rotoiti and Rotoehu.

4.3.1.1 Known distribution of microcystins in New Zealand (prior to the present study)

Low levels of microcystins were detected in a sample collected from a cyanobacterial bloom in Lake Rotorua (North Island) in 1997 (Wilding 2000). The toxicity of this sample was confirmed using a mouse bioassay and the microcystin content quantified using a protein phosphatase assay. The low levels of microcystins in the sample did not adequately explain the mouse bioassay result which was positive, indicating that there could have been other toxins present, although Wilding (2000) also notes that this could

have been due to sample degradation decreasing the levels of microcystin present in the samples. The dominant species in these samples were *Anabaena circinalis* and *A. flos-aquae*. All other samples analysed for toxicity from the Rotorua lakes between 12-12-1996 and 17-5-1999 showed no toxicity.

Microcystin variants LR ($8.1 \mu\text{g l}^{-1}$), RR ($5.6 \mu\text{g l}^{-1}$), WR, LA and either Fr or M(O)R or HphR were detected in a cyanobacterial bloom in Lake Waitawa, near Wellington by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Stirling & Quilliam 2001). Prior to the present study these were the only microcystin variants that had been shown to occur in New Zealand.

Christoffersen and Burns (2000) used an ELISA to analyse samples from seven South Island lakes for the presence of microcystins. The ELISA used in their study did not distinguish between microcystins and nodularins (refer Section 4.3.2 for description of nodularin), thus it is possible that some of the microcystins detected could have been nodularin.. The levels of microcystins/nodularins that were reported in the seven lakes were low: Lake Johnson ($0.0003 - 0.00128 \mu\text{g l}^{-1}$); Butchers Dam ($0.00211 \mu\text{g l}^{-1}$); Lake Rotorua – South Island ($1.22 \mu\text{g l}^{-1}$); Lake Ellesmere ($5.76 \mu\text{g l}^{-1}$); Lake Forsyth ($0.00523 \mu\text{g l}^{-1}$) and Lake Roundabout ($0.00104 \mu\text{g l}^{-1}$). Nodularin has been identified previously in Lake Ellesmere (Namikoshi et al. 1994) The dominant species in these blooms were from *Anabaena* and *Microcystis*. Using an acute toxicity test with the freshwater daphnid *Daphnia carinata*, algal material from Lakes Rotorua, Forsyth and Ellesmere caused higher mortality than would have been expected with just microcystins/nodularins, thus they concluded the algal material from these lakes must contain toxic substances which were not detected by the ELISA. It is also likely that these unknown substances had a higher toxicity or were in higher concentrations than the measured microcystins/nodularins.

Microcystins ($13.4 \mu\text{g l}^{-1}$) were detected using an ELISA in benthic mats (*Oscillatoria* sp.) from the Mataura river (Hamill 2001).

Recently Mountfort et al. (2004) in an investigation of differences between the microcystin levels obtained using the ADDA-ELISA, protein phosphatase inhibition assay and LC-MS reported on microcystins in Lake Horowhenua and nearby Hokio

Stream. The maximum levels recorded (using the ADDA-ELISA) were 258 $\mu\text{g l}^{-1}$ and 126 $\mu\text{g l}^{-1}$ respectively.

4.3.2 Nodularins

Nodularins have a very similar structure to microcystins (Figure 4.2) and are produced by *Nodularia spumigena* which grows in brackish waters. Following stock deaths around Lakes Ellesmere and Forsyth researchers using samples from these lakes identified nodularin and the ADDA moiety which is required for toxicity of the seven published nodularins and the microcystins (Carmichael et al. 1988, Rinehart et al. 1988). Nodularin has a molecular weight of 824 daltons and an ip LD₅₀ of 60 µg kg⁻¹ body weight (mouse) (Carmichael et al. 1988). Like the microcystins it is a potent inhibitor of protein phosphatases 1 and 2a (Honkanen et al. 1991). The earliest documented stock death from suspected ingestion of cyanobacteria are of *N. spumigena* from Lake Alexandria (Australia) (Francis 1878). Water blooms of *N. spumigena* are a common phenomenon in the Baltic Sea where nodularins accumulate in flounder, mussels and clams (Sipia et al. 2002).

Figure 4.2. Structure of nodularin.

4.3.2.1 Known distribution of nodularins in New Zealand (prior to the present study)

Nodularin has been identified from blooms of *Nodularia spumigena* in Lake Ellesmere (Carmichael et al. 1988). Stock deaths were also reported around the nearby Lake Forsyth when heavy blooms of *N. spumigena* were present (Conner 1977). *N. spumigena* is known to occur in other brackish lakes around New Zealand

(Etheredge & Pridmore 1987) but the occurrence of nodularin has not been investigated.

4.3.3 Cyindrospermopsins

Cyindrospermopsin is a tri-cyclic alkaloid (Figure 4.3) with a molecular weight of 415 daltons (Hawkins et al. 1997, Heintzelman et al. 2001, Ohtani et al. 1992). Two analogues of cyindrospermopsin exist; 7-epicyindrospermopsin, a toxic minor metabolite of the cyanobacterium *Aphanizomenon ovalisporum* (Banker et al. 2000), and the non-toxic deoxy-cyindrospermopsin (Norris et al. 1999).

Globally cyindrospermopsin is known to be produced by *Cyindrospermopsis raciborskii* (Hawkins et al. 1985, Hawkins et al. 1997), *Umezakia natans* (Harada et al. 1994), *Anabaena bergii* (Fergusson & Saint 2003), *Raphidiopsis curvata* (Li et al. 2001) and *Aphanizomenon ovalisporum* (Banker et al. 1997).

Cyindrospermopsin causes extensive damage to the liver and is a potent inhibitor of protein synthesis (Falconer et al. 1999, Griffiths & Saker 2003, Hawkins et al. 1985, Terao, K. S. et al. 1994). In addition, crude extracts of *C. raciborskii* injected or given orally to mice also induce pathological symptoms in the kidneys, spleen, thymus and heart (Seawright et al. 2000).

C. raciborskii was implicated in one of the most significant cases of human poisoning from exposure to a cyanobacterial toxin. In 1979, 148 people required hospitalisation with symptoms of gastro-enteritis after a local water supply on Palm Island (Australia) was dosed with copper sulphate to control a dense algal bloom (Bourke et al. 1983, Byth 1980). The copper sulphate caused the cells to lysis and resulted in the release of cyanotoxins into the water supply. Later studies identified, isolated, and cultured a highly toxic strain of *C. raciborskii* from the water supply (Hawkins et al. 1985) and identified cyindrospermopsin (Ohtani et al. 1992).

Saker et al. (1999) reported on the suspected role of cyindrospermopsin in cattle mortality in North Queensland, Australia. Cyindrospermopsin has also been implicated in the death of large numbers of alligators in Florida (Richey et al. 2001).

Figure 4.3. Structure of cylindrospermopsins.

4.3.3.1 Known distribution of cylindrospermopsins in New Zealand (prior to the present study)

Stirling & Quilliam (2001) reported the first occurrence of cylindrospermopsin in a cyanobacterial bloom sample taken in 1999 taken from Lake Waitawa, near Wellington. The dominant species was preliminarily identified as *Cylindrospermum* sp., however, a rigorous identification of the causative organism was not carried out. Quantification of cylindrospermopsin in the sample was not possible due to the lack of a pure calibration standard. This was the only report of cylindrospermopsin in New Zealand and in addition none of the species known to produce cylindrospermopsin (see Section 4.3.3) had been recorded as occurring in New Zealand (Broady 2000).

4.3.4 Anatoxin-a and Homoanatoxin-a

Anatoxin-a is an alkaloid with a low molecular weight of 165 daltons. It is a secondary amine (Figure 4.4) (Devlin et al. 1977). Anatoxin-a is produced by *Anabaena* spp. (*flos-aquae-lemmermannii* group) (e.g., Devlin et al. 1977, James et al. 1997, Sivonen et al. 1989), *Anabaena planktonica* (Bruno et al. 1994), *Oscillatoria* sp. (Edwards et al. 1992), *Aphanizomenon* sp. (Sivonen et al. 1989), *Planktothrix* sp. (Sivonen et al. 1989), *Planktothrix rubescens* (Viaggiu et al. 2004), *Raphidiopsis mediterranea* (Namikoshi et al. 2003) and *Cylindrospermum* sp. (Sivonen et al. 1989).

Homoanatoxin-a is an anatoxin-a homologue with a molecular weight of 165 daltons. It is produced by *Oscillatoria formosa* (Skulberg et al. 1992) and *Raphidiopsis*

mediterranea (Namikoshi et al. 2003). It has a propionyl group at C-2 instead of the acetyl group in anatoxin-a (Skulberg et al. 1992).

Anatoxin-a and homoanatoxin-a have ip LD₅₀ of 200 - 250 µg kg⁻¹ body weight (mouse) (Devlin et al. 1977, Skulberg et al. 1992).

Anatoxin-a and homoanatoxin-a are neurotoxic poisons. They are powerful depolarising neuromuscular blocking agents (Carmichael et al. 1979). Homotoxin-a is slightly less toxic than anatoxin-a. Because of their small size they are rapidly absorbed when ingested orally. In affected animals these toxins can cause convulsions, coma, rigors, cyanosis, limb twitching, hypersalivation and/or death.

Anatoxin-a has commonly been involved with animal and wildfowl poisonings (Ressom et al. 1994), however, there have being no reported human fatalities from anatoxin-a.

Figure 4.4. Structure of anatoxin-a.

4.3.4.1 Known distribution of anatoxin-a in New Zealand (prior to the present study)

Over a three-day period from 26-12-1998, five dogs died within 24 hours of contact with cyanobacterial mats in the Waikanae River (Edgewood Park, 40°53'S, 175°3'E) just north of Wellington. One dog was observed to lick a cyanobacterial mat and died within 10 minutes (Hamill 2001). An *Oscillatoria* species was identified growing as a mat at the bottom of the river. An algal mat sample collected by a nearby resident at the time of the deaths was subsequently tested by mouse bioassay and found to be very toxic with the average death time being 3 minutes 50 seconds (Dr. D Stirling, ESR, Porirua, pers. comm.). Further samples of this material were sent to Ireland (Chemistry

Department, Cork RTC, Ireland) where they were analysed by high performance liquid chromatography with fluorescence detection (HPLC-FLD) for anatoxin-a and homoanatoxin-a following derivatisation as described in the method of James et al. (1998). The HPLC-FLD analysis of the sample (which by the time it was analysed was quite old) showed the presence of two dihydroanatoxin-a isomers which are natural degradation products of anatoxin-a. No anatoxin-a was detected (Hamill 2001, Dr K James, Chemistry Department, Cork RTC, Ireland, pers. comm.).

Between January and February 1999, six dogs were reported dead along the Mataura River, near Mataura, Southland. As with the Waikanae incidence, dogs were seen to “eat something” from the river or river bed and die suddenly. Samples of river water and benthic cyanobacterial mats were collected. After the possibility of poisoning from cyanide or sodium monofluoroacetate (1080) was eliminated, the samples were analysed for toxicity by mouse bioassays, microcystins (ELISA), and saxitoxins (neuroblastoma assay) (Hamill 2001, Dr. D. Stirling, ESR, Porirua, pers. comm.).

In April 2000 a further dog died in a similar manner adjacent to the Mataura River. This sample was tested for toxicity using a mouse bioassay and a subsample of the mat was examined to identify the cyanobacteria.

Toxicity of Mataura samples was confirmed with the mice dying within five minutes. Low levels of microcystins were detected in one sample (see Section 4.3.1.1), however these levels were not high enough to have caused the death of the dogs. Saxitoxins were not detected. The presence of anatoxin-a in the Mataura samples was not confirmed by any instrumental analysis. Mataura river samples contained *Oscillatoria* sp. (Hamill 2001).

4.3.5 Anatoxin-a(S)

Anatoxin-a(S) is produced only by *Anabaena lemmermannii* (Henriksen et al. 1997) and *A. flos-aquae* (Mahmood & Carmichael 1987). When screening *Anabaena* sp. for toxicity Carmichael and Gorham (1978) encountered a neurotoxin that appeared to be 10 times more toxic than anatoxin-a and produced salivation in the laboratory mice. Because of this salivation it was named anatoxin-a(S). However anatoxin-a(S) is structurally very different from anatoxin-a. It is a unique phosphate ester of a cyclic N-

hydroxyguanine methyl with a molecular weight of 252 daltons (Figure 4.5). It has an LD_{50} of $20 \mu\text{g kg}^{-1}$ body weight (mouse) (Carmichael 1992) and is an irreversible inhibitor of cholinesterase (Mahmood & Carmichael 1986a). There are no known structural variants of anatoxin-a(S).

Figure 4.5. Structure of anatoxin-a(S)

4.3.5.1 Known distribution of anatoxin-a(S) in New Zealand (prior to the present study)

Anatoxin-a(S) had not being detected in New Zealand prior to the present study.

4.3.6 Saxitoxins (Paralytic Shellfish Poison (PSP))

The saxitoxins (or paralytic shellfish poisons (PSPs)) are fast acting neurotoxins that inhibit nerve conduction by blocking sodium channels (Adelman et al. 1982). They are alkaloids (Figure 4.6) with molecular weights between 200 - 500 daltons. There are approximately 24 saxitoxin compounds identified in published documents. They were first isolated from toxic marine shellfish. In the marine environment they are produced by some marine algae and accumulate in shellfish and other marine organisms. In freshwater, saxitoxins are known to be produced by; *Anabaena circinalis* (Humpage et al. 1994), *Aphanizomenon flos-aquae* (Ikawa et al. 1982, Jackim & Gentile 1968, Mahmood & Carmichael 1986b), *Aphanizomenon issatschenkoi* (Li et al. 2003), *Planktothrix* sp (Pomati et al. 2000), *Lyngbya wollei* (Carmichael et al. 1997, Onodera et al. 1997), *Cylindrospermopsis raciborskii* (Lagos et al. 1999) and probably *Anabaena lemmermannii* in Danish lakes (Kaas & Henriksen 2000).

The saxitoxin group can be divided into three sub-groups based on the net charge of the molecule under acidic conditions (Hall et al. 1990, Shimizu 1988): the saxitoxins (saxitoxin (STX), neosaxitoxin (neoSTX) and decarbamoyl derivatives) (charge +2); the

gonyautoxins (GTXs) including decarbamoyl derivatives (charge +1); and the C toxins (charge 0).

Saxitoxin has an ip LD₅₀ of 10 µg kg⁻¹ body weight (mouse) and an oral LD₅₀ of 263 µg kg⁻¹ body weight (mouse). Other analogues are mostly less toxic than saxitoxin. Saxitoxins have caused sheep mortalities in Australia (Negri et al. 1995) and were also identified in a massive bloom of *A. circinalis* on the Murray Darling River (Australia) which resulted in the death of over 1 600 stock (Bowling & Baker 1996). No PSP-like illnesses have been reported in humans from the consumption of drinking water or contact with recreational water containing saxitoxins (Chorus & Bartram 1999).

Figure 4.6. General structure of the saxitoxin (PSP) series of toxins where the various R groups designate variable components.

4.3.6.1 Known distribution of saxitoxins in New Zealand (prior to the present study)

A cyanobacterial bloom (predominately *Anabaena planktonica*) was reported in the Waikato River in 2003. This caused taste and odour problems in the drinking water supplied to the city of Hamilton and other towns along the length of the Waikato River. Saxitoxins were detected (ELISA and a neuroblastoma assay) in water samples taken from the water treatment intake and throughout the water treatment process but these levels were well below the levels allowed under the proposed drinking water guideline (Dr. D Stirling, ESR, Porirua, pers. comm.). The saxitoxin-producing organism was not identified.

4.3.7 Lipopolysaccharides

Lipopolysaccharides (LPS) are an integral component of the cell wall of all gram negative bacteria, including cyanobacteria. They are found in the outer cell membrane where they form complexes with proteins and phospholipids (Chorus & Bartram 1999). They can cause irritant and allergenic responses when humans and animals come in contact with them and numerous types of allergenic responses from contact with cyanobacteria exist (Pilotto et al. 1997, Torokne et al. 2001). Cyanobacterial LPS are considerably less potent than LPSs from pathogenic gram-negative bacteria such as, *Salmonella* (Keleti & Sykora 1982).

Although comparatively poorly studied, LPSs from cyanobacteria have been implicated in human health problems associated with exposure to cyanobacteria (Ressom et al. 1994). A recent study by Pilotto (2004) showed that there was no correlation between dermatological reaction experienced by exposed individuals and the presence/absence of other cyanotoxins in the samples tested.

4.3.7.1 Known distribution of lipopolysaccharides in New Zealand (prior to the present study)

Lipopolysaccharides almost certainly occur in New Zealand cyanobacteria. Although no attempts to detect or quantify them have been made. There have been numerous reports of humans experiencing skin rashes after contact with water containing cyanobacteria, particularly from Waikato and Rotorua lakes (Wilding 2000, M Bloxham, Environment Bay of Plenty, Whakatane, pers. comm., D Hood, Waikato District Health, Hamilton, pers. comm.).

4.4 Methods

4.4.1 Sample collection

Water, cyanobacterial bloom/scum and cyanobacterial mat samples were collected personally or received by individuals and organisations from a variety of waterbodies across New Zealand between 01-01-2001 and 20-01-2004 (samples were collected after this date for species identification (see Chapter 2) but were not tested for cyanotoxins). A full description of sample collection is given in Chapter 2. The location of samples, date of collection, collection method, main use of waterbody, cyanobacteria species present, cyanotoxins tested for and amount of cyanotoxins detected in each sample are given in Appendix 2, Tables A1 - A4 (also refer to Appendix 1 for map of sample locations). To allow comparisons between similar types of waterbodies they were each assigned to one of the following; lakes (permanent waterbodies with at least one axis greater than 0.5 km long, this group also includes reservoirs), ponds or dams (natural or man-made waterbodies with both axes less than 0.5 km long), oxidation ponds and rivers or streams.

For cyanotoxin analysis, all material remaining after subsamples were taken for species identification and quantification (see Chapter 2) was frozen, either when received at the Massey University Ecotoxicology Laboratory (Wellington) or upon returning from field collection. Samples were stored in chilly bins in the dark prior to freezing. Where possible, this occurred within 12 h and no more than 48 h after sampling. Subsequently aliquots were taken from the frozen material and prepared for individual analyses of each cyanotoxin as described in Section 4.4.2. A variety of methods were used to test the samples. In some cases additional methods may have been desirable but were unavailable due to a lack of equipment, time available on existing testing equipment or the expense of testing samples.

4.4.2 Analysis of Selected Cyanotoxins

4.4.2.1 Microcystins and Nodularins

The total content of microcystins (and/or nodularins) in the samples was analysed with a competitive indirect ELISA using the methods of Fischer et al. (2001). This ELISA uses antibodies raised to the ADDA moiety that is present in most (>80%) of the known toxic penta- and heptapeptide toxin congeners.

From each sample an aliquot (10 ml) was freeze-thawed twice to release any intracellular toxins. These were then filtered through a glass fibre filter (Whatman GF/C, 25 mm diam.) or centrifuged at 10 000 rpm for 2 min and the filtrate or supernatant used for the ELISA assay. This method gives a measure of the total microcystins/nodularins present in the samples. It does not distinguish between intra and extra cellular microcystins/nodularins.

An ELISA plate (NUNC) was coated overnight with OVA-ADDA-hemiglutaryl, in 0.05 M Na₂CO₃ buffer at pH 9.6 (100 µl, 20°C). After being washed twice with phosphate buffered saline (PBS), additional binding sites were blocked by incubation with Ovalbumin (98%, Inovatech, BC, Canada) (1% in PBS, 200 µl, 1 h, 20°C). The plate was washed twice with PBS. The samples and Microcystin-LR standard (ALEXIS, 50 µl) were added to the wells together with antiserum (Cat-BSA-ADDA antiserum, AgResearch, Hamilton, New Zealand, 50 µl). After incubation at 20°C for 1.5 h, the wells were washed twice with PBS containing 0.05% Tween 20 (PBST) and twice with PBS. Anti-sheep secondary antibody (Anti-sheep IgG-HRP, Lifescience NZ Ltd, 100 µl) was then added to the wells and the plate incubated for 1.5 h. The wells were then washed twice with PBST and twice with PBS. BioFX (BioFX Laboratories, MD, United States) substrate solution (100 µl) was added and the plate incubated for 15 min. The reaction was stopped by addition of 2 M H₂SO₄ (50 µl) and the absorbance at 450 nm was determined with a microplate spectrophotometer (VERSA_{max}, Molecular Devices, CA, United States).

Four samples (L42, L179, L118 and DP61, see Appendix 2) were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the microcystin variants LR, YR, RR and for nodularin. The high cost of LC-MS/MS analyses meant all samples could not be tested using this method. The four samples analysed were selected as the ELISA results had shown they contained high levels of microcystins. Algal scum material (0.3 ml) was mixed with methanol (0.9 ml), sonicated for 10 min and then centrifuged at 10 000 rpm (10 min). This analysis was carried out by the Cawthron Institute (Nelson).

4.4.2.2 *Cylindrospermopsin*

Two aliquot (300 ml) of the frozen sample (L114) were lyophilised and the resulting powders dissolved in 20 ml of methanol. The solutions were filtered through a bed of Celite (Celite Corporation, CA, United States) followed by a Teflon syringe filter (13 mm diam., 0.45 µm, Phenomenex, Auckland, New Zealand). The resulting solutions were then evaporated under a stream of nitrogen (35°C). One aliquot was reconstituted in methanol and used for LC-MS and LC-MS/MS analysis for cylindrospermopsin, 7 epi-cylindrospermopsin, and deoxy-cylindrospermopsin. The second aliquot was sent to The New Zealand Institute of Crop and Food Research (Palmerston North, New Zealand) for toxicity testing by mouse bioassay.

Liquid chromatography mass spectrometry (LC-MS) was performed using a Shimadzu LC-10ADVP liquid chromatograph system and Gilson 233XL sampling injector (Gilson, Inc. Middleton, WI, United States) coupled to a PE-SCIEX API 2000 mass spectrometer (PE-SCIEX, Thornhill, Ontario, Canada). Separation of analytes was achieved on a TSK-Gel Amide-80 column (5 µm, 250 mm x 4.6 mm i.d., TosoHass) at 40°C with an isocratic solution of 25% eluent A, 75% eluent B. Eluent A was water and eluent B was a 95% acetonitrile/water solution; both eluents contained 2 mM ammonium formate and 3.6 mM formic acid (pH 3.5) (Dell'Aversano et al. 2004, Eaglesham et al. 1999). The liquid chromatographic flow was introduced into the Turbo Ionspray interface with a 9:1 (waste:mass spec.) split.

Single ion monitoring (SIM) and selected reaction monitoring (SRM) experiments for the detection of the cylindrospermopsins were performed in a similar manner to that previously described by Eaglesham et al. (1999).

Due to limited time available on the LC-MS equipment, sample L114 (Lake Waahi) was the only sample in the present study that was analysed for cylindrospermopsin. Assistance in using the LC-MS machine was given by Dr D Stirling (ESR, Porirua, New Zealand).

4.4.2.3 Anatoxin-a

Samples which contained cyanobacteria in high concentration and with genera known to produce anatoxin-a in other parts of the world were selected for anatoxin-a analysis.

An aliquot (10 ml) was freeze-thawed twice and filtered through a glass fibre filter (Whatman GF/C, 25 mm diam.). Benthic samples (approximately 10 g) were lyophilised and the anatoxin-a extracted as described below. The samples were analysed by high performance liquid chromatography with fluorescence detection (HPLC-FLD) for anatoxin-a and homoanatoxin-a following derivatisation as described in the method of James et al. (1998). This method will also detect degradation products of these toxins. The major degradation products are dihydroanatoxin-a, dihydrohomoanatoxin-a, epoxyanatoxin-a and epoxyhomoanatoxin-a.

The aliquot (10 ml) was cleaned up by solid-phase extraction (SPE) cartridges (LC-WCX, 3 ml, Supelco, Sigma, Australia). The SPE cartridges were conditioned with methanol (6 ml, HPLC grade, Biolab, New Zealand) and distilled and deionised water (6 ml). The samples were loaded on to the cartridge and the cartridge was washed with methanol-water (1:1, 3 ml) and air dried. The anatoxin-a was eluted using methanol (10 ml) containing 0.2% trifluoroacetic acid (Aldrich, New Zealand) and the solvent was removed at 35°C, under nitrogen. The samples were reconstituted in methanol (200 µl) and transferred to 2 ml amber vials and evaporated prior to derivatisation.

Anatoxin-a was extracted from lyophilised cyanobacterial material (100 mg) by vortexing (1 min) with methanol (10 ml) containing hydrochloric acid (1 M, 100 µl, Aldrich, New Zealand) and allowing the solution to sit for 10 min. The procedure was repeated four times. The solution was centrifuged at 10 000 rpm for 10 min at 5°C, the supernatant collected and the solvent was removed at 35°C, under nitrogen. The samples were reconstituted in water (5 ml) and loaded onto pre-condition SPE cartridges. The cartridge was then washed with methanol (3 ml), air dried and anatoxin-a eluted as described above.

The dried samples or standards were reconstituted with sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) (1 M, 100 µl, AR grade, Biolab, New Zealand) in a 2 ml amber vial.

4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F, Aldrich, New Zealand) in acetonitrile (Biolab, New Zealand) (1 mg ml^{-1} , $50 \mu\text{l}$) was added to the mixture and allowed to stand (10 min) in the dark at room temperature. Hydrochloric acid (1 M $50 \mu\text{l}$) was added to terminate the reaction and the HPLC-FLD was performed directly on the solution ($3 \mu\text{l}$ injections) using a Luna C_{18} column ($5 \mu\text{m}$, $150 \text{ mm} \times 2 \text{ mm}$, Phenomenex) at 35°C . The mobile phase was acetonitrile–water (45:55), with a flow-rate of $200 \mu\text{l min}^{-1}$, and fluorimetric detection ($\lambda_{\text{excitation}} = 470 \text{ nm}$, $\lambda_{\text{emission}} = 530 \text{ nm}$). A HP1050 quaternary high performance liquid chromatograph and a Perkin Elmer 650-10S fluorescence spectrophotometer were used for the analysis. Chromatography data was transferred to Microsoft Excel for graphical presentation.

Anatoxin-a standard (95% purity) was purchased from Sigma, United States. The standard had been used for a prior study and the exact concentration of the anatoxin-a standard was not known. This meant that a concentration of anatoxin-a in the samples could not be obtained and the analysis was used only to confirm the presence or absence of anatoxin-a, homoanatoxin-a and their degradation products.

To confirm the presence of anatoxin-a in samples, spiking experiments were used. If there was a symmetrical increase in the assigned peak for this toxin in the HPLC–FLD the result was considered positive for anatoxin-a.

4.4.2.4 Saxitoxins

Samples which contained cyanobacteria in high concentration and with genera known to produce saxitoxins in other parts of the world were selected for saxitoxins analysis.

To assist in the detection of saxitoxins, aliquots were concentrated. Subsamples (50 ml) were transferred to polypropylene centrifuge tubes (50 ml , Raylab, New Zealand) and were lyophilised. When only small amounts of lyophilised material were obtained the entire sample was reconstituted in distilled water (1.0 ml). This allowed the amount of saxitoxin per ml to be calculated. When plentiful lyophilised material was obtained, either all the lyophilised material was weighed and reconstituted in distilled water (1.0 ml) (for these samples a saxitoxin per ml of original sample and per kg of lyophilised material was calculated), or a subsample (approx. 0.02 g) was reconstituted in distilled water (1.0 ml). These results are expressed as the amount of saxitoxin (μg)

per kilogram of lyophilised material. The samples were shaken (30 min) and allowed to sit (1 h). The samples were then centrifuged at 10 000 rpm (2 min) and the supernatant used for the ELISA assay.

Initially a sample of lyophilised cell material (approximately 0.1 g) from Australia (A. Humpage, Adelaide University, Australia) known to contain saxitoxins was reconstituted in water (1.0 ml), 1% acetic acid (1.0 ml) and methanol (1.0 ml) to assess which medium extracted the most saxitoxin without affecting the result of the ELISA. This sample was collected from a bloom of *Anabaena circinalis* in Caffey Dam (NSW) in 1993 and contained the saxitoxin variants C1, C2, GTX2, GTX3 and saxitoxins. The toxin content of the sample was analysed by HPLC and was measured at 104 mg kg⁻¹ (saxitoxin equivalents) (A Humpage, Adelaide University, Australia, pers. com.).

An ELISA plate (NUNC) was coated with STX-PSP coater (Jellet Biotek Ltd, Nova Scotia, Canada), in 0.05 M Na₂CO₃ buffer (100 µl, 21°C) sealed with parafilm, wrapped in tinfoil and stored overnight. The antibodies used have been raised to the saxitoxin variant only. After being washed four times with phosphate buffered saline (PBS), additional binding sites were blocked by incubation with Polyvinylpyrrolidone (PVP) (Sigma, 1% in PBS, 200 µl, 1 h, 21°C). The wells were washed twice with PBS containing 0.05% Tween 20 (PBST).

The samples (50 µl) and saxitoxin standard (NRC, Canada, 50 µl) were added to the wells together with antiserum (Anti-STX II, Jellet Biotek Ltd, in PBS containing 5 mg ml⁻¹ albumin bovine serum (BSA) and 0.05% Proclin 300, 50 µl).

After incubation at 21°C for 1 h, the wells were washed four times with PBST. Anti-sheep secondary antibody (Anti-rabbit IgG-HRP, Lifescience NZ Ltd, 100 µl) was then added to the wells and the plate incubated at 21°C for 2 h. The wells were then washed twice with PBST and twice with PBS. BioFX (BioFX Laboratories, MD, United States) substrate solution (100 µl) was added and the plate incubated for 15 min. The reaction was stopped by addition of 2 M H₂SO₄ (50 µl) and the absorbance at 450 nm was determined with a microplate spectrophotometer (VERSA_{max}, Molecular Devices, CA, United States).

Six samples (OP9, L82, L113, L126, L141 and L161) and the Australian sample were selected for further analysis. These samples were selected because they represented a range of saxitoxin levels detected by the ELISA. Lyophilised cell material (0.5 g) of each sample was extracted in 5% acetic acid and the extract tested for saxitoxins by the neuroblastoma assay (Truman et al. 2002). The analysis of these samples was carried out by Dr. P Truman (ESR, Porirua, New Zealand).

4.4.2.5 Other toxins

Occurrence of anatoxin-a(S) and lipopolysaccharides was not investigated in the present study.

4.5 Results

4.5.1 Microcystins and Nodularins

The ELISA used antibodies raised to the ADDA moiety that is present in both nodularins and microcystins; therefore it was not possible to distinguish between these types of cyanotoxins. However, because nodularins are known to be produced only by *Nodularia spumigena* (Chorus & Bartram 1999), it was assumed that unless this species was present in the samples only microcystins were present.

Of the 265 samples tested, 102 tested positive for microcystins/nodularins. One of these samples contained *N. spumigena*. A summary of the number of tested samples for each waterbody type and location is given in Table 4.2 which also shows the number of samples within each category found to contain microcystins.

Table 4.2. Number of New Zealand water/cyanobacteria samples and locations tested for microcystins/nodularins for each waterbody type. The number of positive samples for each waterbody type and location is also given. * One sample contained *N. spumigena* and it is likely that the toxin detected was nodularin.

Waterbody type	No. of samples	Samples containing microcystins/nodularins	No. of different locations	Microcystins/nodularins for each different location
Lakes	183	75*	75	28*
Oxidation Ponds	11	8	8	7
Ponds/Dams	61	12	53	12
Rivers/Streams	10	7	9	6
Totals	265	102	145	54

4.5.1.1 Lakes

Microcystins/nodularins were detected in 28 lakes (Figure 4.7). The highest values measured were 36 500 $\mu\text{g l}^{-1}$ (Lake Horowhenua, L82 – Figure 4.8) and 32 000 $\mu\text{g l}^{-1}$ (Lake Waitawa, L178). These levels were recorded in scum samples collected from the lake shores (refer to Appendix 2, Table A1 for information on whether or not samples were scum material).

The lowest levels recorded were 0.02 $\mu\text{g l}^{-1}$ (Lake Kainui, L82 – not shown on graph as higher values recorded at this lake – refer to Appendix 2, Table A1) and 0.03 $\mu\text{g g}^{-1}$ (lyophilised material, Lake Grassmere, L51).

N. spumigena (Chorus & Bartram 1999) was observed only in samples from Lake Forsyth (L72 - 0 $\mu\text{g l}^{-1}$, L139 - 9 $\mu\text{g l}^{-1}$), thus it is possible that the toxins in sample L139 were nodularins. However *Anabaenopsis* sp. and *Anabaena* sp., both known producers of microcystins (Chorus & Bartram 1999) were also found in these samples, indicating further analysis is required to confirm the presence of nodularins.

Of the 75 positive samples, 73 contained cyanobacteria genera known to produce cyanotoxins. Only two samples, Lake Hatuma (L30, 2.7 $\mu\text{g l}^{-1}$) and Lake Papaitonga (L17, 5 $\mu\text{g l}^{-1}$), contained no detectable cyanobacteria.

Eighty two percent of the 28 lakes found to contain microcystins/nodularin, had recreational activity as a primary use. Some of these lakes, e.g. Lakes Rotoiti and Rotoehu, are also used by a small number of lakeside residents for drinking water, and others, e.g. Lake Dudding, are still accessible to stock on surrounding farms for drinking water. Seven percent of the lakes were used primarily for stock drinking water and a further 11% appeared to have little or no use by humans and were categorised as "scenic".

Figure 4.7. Levels of microcystins/nodularins recorded in samples collected from New Zealand lakes. When microcystins/nodularins were detected in more than one sample from each lake only the highest value was plotted. * Benthic sample and microcystins/nodularins is expressed as $\mu\text{g g}^{-1}$ of lyophilised material.

Figure 4.8 (A - B). Cyanobacterial scum (*Microcystis* sp.), Lake Horowhenua (L82) 29-01-2003. Samples from this scum material had the highest levels of microcystins ($36\,500\,\mu\text{g l}^{-1}$) recorded during the present study.

Figure 4.9 (A - D). A, B, *Nostoc commune* mats along the shores of Lake Taupo (L160) 25-04-2003; C, Low lake levels and high winds caused *Nostoc commune* colonies to be dislodged from rocks in the lake and these accumulated along the shoreline; D, shows *Nostoc commune* viewed under the microscope. Levels of microcystins of $10.9\,\text{mg kg}^{-1}$ were recorded in lyophilised material from these mats.

Ninety percent of samples that contained cyanobacteria and tested positive for microcystins contained species of *Microcystis* (35), *Nostoc* (2) or *Planktothrix* (1), all genera known to produce microcystins. In particular, *Microcystis aeruginosa*, *M. flos-aquae* and *M. panniformis* were commonly associated with presence of microcystins. Seven samples (Table 4.3) contained microcystins but no of *Microcystis*, *Nostoc* or *Planktothrix*. These samples all contained *Anabaena* species and levels of microcystins were quite low.

Table 4.3. Samples from New Zealand lakes in which microcystins were detected but no species from the genera *Microcystis*, *Nostoc* or *Planktothrix* were observed.

Sample no.	Lake	Cyanobacteria species present in samples	Microcystins/ Nodularins ($\mu\text{g l}^{-1}$)
L1	Henly	<i>Anabaena circinalis</i>	0.4
L6	Henly	<i>Anabaena circinalis</i>	2.0
L15	Waiholo	<i>Anabaena lemmermannii</i>	0.7
L27	Orakai	<i>Anabaena spiroides</i> , <i>Anabaena</i> sp.	4.0
L43	Okaro	<i>Anabaena lemmermannii</i>	0.15
L78	Henly	<i>Anabaena</i> sp.	0.5
L116	Arapuni	<i>Anabaena planktonica</i>	0.04

Cyanobacteria were observed in 68 of the 108 samples in which microcystins were not detected, indicating that not all species/strains of cyanobacteria in New Zealand produce these cyanotoxins. The dominant species in the samples without microcystins were *Anabaena* spp. (including *A. planktonica*, *A. oscillarioides*, *A. lemmermannii*, *A. circinalis* and *A. spiroides*, *Cylindrospermopsis raciborskii* and *Planktothrix* sp.). *Microcystis* spp. were present in some of these samples, but only in low concentrations.

Ninety-two of the samples collected from the lakes were from cyanobacterial blooms/scums. Of these samples 60% were found to contain microcystins.

Sample L160 was collected from benthic mats of *Nostoc commune* collected from the eastern shoreline of Lake Taupo (25-04-2003) (Figure 4.9). Microcystins levels of 10.9 mg kg^{-1} were recorded in lyophilised material from these mats. A water sample (L159) was collected at a distance of 1 m from the shoreline to assess if the microcystins contained in these mats were being released back into the waterbodies. A

few filaments of *Nostoc commune* were observed in this sample and levels of microcystins of $1.26 \mu\text{g l}^{-1}$ were recorded.

The four samples (L42, L179, L118 and DP61) tested by LC-MS contained the three microcystin variants RR, YR and LR (Figure 4.10). The microcystin profiles of samples L42 and L118 were similar with approximately 50% of the microcystin in each sample being RR, 40% of LR and only a small amount of YR. Sample L118 had slightly more YR. Sample L179 contained almost entirely YR with only small amounts of RR and LR. No nodularin was detected in the samples.

The total microcystin levels measured by LC-MS were compared to those measured by the ADDA-ELISA (Figure 4.11). There were relatively small differences between the two methods in the samples L179, L118 and DP61. However, there was a large difference between the two methods in the samples from L42. The ELISA detected $36\,500 \mu\text{g l}^{-1}$ and the LC-MS only $3\,777 \mu\text{g l}^{-1}$ (see Section 4.6.1.1 for explanation of this difference).

Figure 4.10. Percentage of microcystin variants (RR, YR and LR) in the samples L118 (Lake Ngaroto), DP61 (Neuman Pond), L179 (Lake Waitawa) and L42 (Lake Horowhenua). The data labels on the bars indicate the amount of each variant found in the samples ($\mu\text{g l}^{-1}$).

Figure 4.11. The different total levels of microcystins recorded in the four samples L179 (Lake Waitawa), L118 (Lake Ngaroto), L42 (Lake Horowhenua) and DP61 (Neuman Pond) by LC-MS and the ADDA-ELISA.

4.5.1.2 Oxidation Ponds

Microcystins were detected in seven of the eight oxidation ponds (Figure 4.13). Only two of the 11 samples tested contained no microcystins (Appendix 2, Table A1). The levels of microcystins ranged from 0.05 $\mu\text{g l}^{-1}$ (OP 2 – Blenheim South) to 100 $\mu\text{g l}^{-1}$ (OP 3 - Kuratau). The dominant cyanobacteria species in all samples tested was *Planktothrix mougeotii*. (*Microcystis aeruginosa* was dominant in OP12 but this sample was not tested for microcystins). Other species that occurred at low levels in some samples were *M. aeruginosa* and an *Arthrospira* sp.. Three samples (OP9 – Kaitaia 2, OP10 - Mt Maunganui, OP11 – Blenheim North) registered no microcystins but there were cyanobacteria present.

4.5.1.3 Dams and Ponds

Microcystins were detected in 12 of the 53 ponds and dams sampled (Figure 4.13). The levels of microcystins ranged from 12 000 $\mu\text{g l}^{-1}$ (DP61 - Neuman Pond) to 0.02 $\mu\text{g l}^{-1}$ (DP3 - Waihopa Cemetery). Of the 12 positive samples, only four contained cyanobacteria. Sample DP61 (Neuman Pond) had a thick bloom/scum of *Microcystis* spp. (Figure 4.12). Avalon Park Pond (DP 50) contained *Phormidium* sp. mats. Richardson Pond (DP14) contained *Microcystis* spp. and the Welt Street Culvet (DP56)

contained a *Planktothrix* sp. Eight of the positive samples contained no visible cyanobacteria, however levels of microcystins were low in these samples (less than $4 \mu\text{g l}^{-1}$). Of the samples that did not contain any microcystins, nine had cyanobacteria present (Appendix 2, Table A3).

4.5.1.4 Rivers/Streams

Ten samples were analysed from rivers and streams. Six were of benthic material and the remainder planktonic. All benthic samples except R2 (Southland Stream) contained microcystins. The levels ranged from $2.1 \mu\text{g kg}^{-1}$ (R8 - Waikanae River) to $200 \mu\text{g kg}^{-1}$ (R5 - Motueka River) (Figure 4.13). Samples with microcystins contained *Phormidium* spp. and *Oscillatoria* spp. The Southland Stream sample (R2) contained *Planktothrix peronata*. Three planktonic samples were collected from rivers; Riverton (R1), Waikato River (R3) and Utaukura River (R9 and R10). Only latter, dominated by *Microcystis wesenbergii*, contained microcystins ($0.35 \mu\text{g l}^{-1}$ in the grab sample and $12.7 \mu\text{g l}^{-1}$ in a concentrated plankton net sample).

Figure 4.12. Cyanobacterial bloom/scum (*Microcystis* spp.) in Neuman Pond (DP61).

Figure 4.13. Levels of microcystins recorded in samples collected from New Zealand ponds, dams, oxidation ponds, rivers and streams. When microcystins were recorded in more than one sample from each location only the highest value was plotted. * Benthic sample – microcystins are expressed as $\mu\text{g g}^{-1}$ of lyophilised material.

4.5.2 Cylindrospermopsins

The dominant cyanobacterial species in sample L114 (Lake Waahi, Waikato, 13-03-2003) was *Cylindrospermopsis raciborskii*. This was the first time this species had been identified in New Zealand (see Chapters 2 and 3 for further information on the identification of *C. raciborskii*). It is known to produce cylindrospermopsin and thus sample L114 was tested for this toxin.

The toxicity of the Lake Waahi sample was confirmed by mouse bioassay, which resulted in a time-to-death of 9.5 h.

The method of analysis for cylindrospermopsin was successfully confirmed using a cylindrospermopsin-containing, Australian *C. raciborskii* sample received from Dr. G Jones (CSIRO, Australia). Deoxy-cylindrospermopsin ($M + H^+$ m/z 400.1) and cylindrospermopsin ($M + H^+$ m/z 416.1) were detected by LC-MS at 5.6 and 6.3 min respectively in the Lake Waahi sample (L114). These retention times corresponded well to those for the Australian sample (Figure 4.14). To provide unequivocal evidence for the presence of cylindrospermopsin, a selected reaction monitoring (SRM) experiment was carried out under identical chromatographic conditions to the single ion monitoring (SIM) experiment. Cylindrospermopsin was positively identified by transitions m/z 416→194 and 416→176 at identical retention times to those obtained in the SIM experiment.

Quantification of cylindrospermopsin in the sample was not possible as no commercial calibration standard was available at the time of testing.

The detection of cylindrospermopsin in the Lake Waahi (L114) sample is the second recorded incidence of this cyanotoxin in New Zealand. The detection of the non-toxic analogue deoxy-cylindrospermopsin is the first New Zealand record.

Lake Waahi is primarily used for recreational activity, including water skiing, kayaking and sailing. Several farms surround Lake Waahi, with stock having access to the water.

Figure 4.14. Single ion monitoring analysis of A, Australian *Cylindrospermopsis raciborskii* and B, comparison to a sample of *C. raciborskii* from Lake Waahi, New Zealand. Chromatogram drawn by Dr. D Stirling (ESR, Porirua).

4.5.3 Anatoxin-a

A total of 57 samples from 40 different locations were analysed for anatoxin-a. The samples were from; lakes (40), oxidation ponds (7), ponds or dams (5) and streams or rivers (5) (Appendix 2, Table A1 - 4).

Anatoxin-a was found in three samples; L12 - Lake Rotoehu, L78 - Lake Henly and L77 - Lower Karori Reservoir. The samples were all analysed twice and on the second analysis each sample was spiked with anatoxin-a standard. In all three samples there was a symmetrical increase in the assigned peak for anatoxin-a (Figure 4.15). NBD-anatoxin-a eluted at 11.4 min.

This is the first report of anatoxin-a from New Zealand cyanobacterial samples. Previously only degradation products of anatoxin-a had been found or a mouse bioassay used, in which symptoms of mice deaths indicated anatoxin-a to be the toxin responsible. It is also the first time that planktonic species have been shown to produce anatoxin-a in New Zealand, the previous cases had all been benthic.

All three samples which tested positive to anatoxin-a contained cyanobacteria. The dominant species in the Lake Rotoehu samples were *Anabaena* cf. *lemmermannii* (no akinetes seen in the sample) and *Microcystis panniformis*. In the Lower Karori Reservoir, *A. lemmermannii* and *A. circinalis* dominated and at the time of sample collection were forming a thin surface scum. The Lake Henly sample contained only fragments of a coiled *Anabaena* sp. and thus identification of this species was not possible. The Lake Rotoehu sample was tested for anatoxin-a many months after it was collected. However, both the Lake Henly and Lower Karori Reservoir samples were analysed within several days of collection. After detecting anatoxin-a in the Lower Karori Reservoir, subsequent samples were collected and analysed (data not shown). Anatoxin-a was not detected in these samples and it was therefore not possible to establish beyond doubt which species was responsible for its production.

The Lower Karori Reservoir is now part of the Karori Wildlife sanctuary in Wellington. Currently its only human use is scenic (see Appendix 10). The main outlet of the reservoir is the Kaiwharawhara stream which flows through urban areas and parks before reaching Wellington Harbour. Lake Henly is used for recreational activities

(Stefanogiannis 2001), in particular dragon boating. Lake Rotoehu is also a popular recreational use lake with activities including fishing, kayaking and water skiing. Thus the human health risk posed by anatoxin-a is quite high in Lakes Henly and Rotoehu due to activities on or in these waterbodies. Because there is little human contact with the Kaiwharawhara stream the health risk is probably much lower.

Figure 4.15. HPLC–FLD of water samples containing cyanobacteria (predominantly *Anabaena* spp.) from A, the Lower Karori Reservoir, Wellington; B, Lake Henly, Masterton; C, Lake Rotorua, Rotorua, after derivatisation with NBD-F. The dashed line represents a chromatogram of the same extracts with a spike of anatoxin-a standard added to them.

4.5.4 Saxitoxins

When the Australian *Anabaena circinalis* sample (see section 4.4.2.4) was reconstituted in distilled water, 1% acetic acid or methanol, the highest levels of saxitoxins were obtained using water, closely followed by acetic acid and methanol. Thus distilled water was used to reconstitute the New Zealand samples. The results obtained with the ELISA ($110 \mu\text{g kg}^{-1}$) were much lower than the original results ($104 \mu\text{g g}^{-1}$) from the HPLC analysis, indicating possibly that the ELISA was not detecting all of the saxitoxin variants present in the sample.

Using the ELISA, 101 samples were tested and 48 from 38 different waterbodies tested positive for saxitoxins. A summary of the number of samples tested for each waterbody type and location is given in Table 4.4 which also shows the number of samples within each category containing saxitoxins.

Table 4.4. Number of New Zealand water/cyanobacterial samples and locations tested for saxitoxins for each waterbody type. The number of positive samples for each waterbody type and location is also given.

Waterbody type	No. of samples tested	Samples containing saxitoxins	No. of different locations	Saxitoxins for each different location
Lakes	97	44	43	36
Oxidation Ponds	2	2	2	2
Ponds/Dams	1	1	1	1
Rivers/Streams	1	1	1	1
Totals	101	48	47	38

Levels of saxitoxins detected in samples in which the whole sample (lyophilised 50 ml) was reconstituted in 1.0 ml of distilled water are shown in Figure 4.16. Using the results obtained for the concentrated samples a value per 1.0 ml of the original water sample was calculated. Although the figures shown on the graph are below the limits of detection for the ELISA they were measured at higher concentration (i.e. level in concentrated 50 ml sample) and this allowed a value to be calculated. The levels of saxitoxins detected in the samples were low. Lake Rotoehu sample (L104) had the highest levels of $0.0005 \mu\text{g l}^{-1}$.

Levels of saxitoxins in the samples where there was sufficient lyophilised material to weigh and thus calculate a quantity (in μg) of saxitoxins per kg are shown in Figure 4.17. The highest levels of saxitoxins were recorded in Lake Kohangatera (L125 – $8.7 \mu\text{g kg}^{-1}$ and

L154 - $2.5 \mu\text{g kg}^{-1}$ – see Appendix 2, Table A1). The highest levels from this location were markedly higher than from other locations, with the next highest value being $5.51 \mu\text{g kg}^{-1}$ (OP5 - Kaitaia Oxidation Pond) and the lowest was from the Prawn Farm - Pond 1 (DP63 - $0.15 \mu\text{g kg}^{-1}$).

Of the seven samples analysed by the neuroblastoma assay all gave positive results. The Australian sample recorded high levels of activity ($\approx 12\,000 \mu\text{g kg}^{-1}$), well above any of the New Zealand samples. The sample from Lake Kohangatera (L126) had the highest level of saxitoxins ($\approx 1\,100 \mu\text{g kg}^{-1}$), followed by Kaitaia - Pond 2 (OP9, $\approx 300 \mu\text{g kg}^{-1}$). These results correlated well with the results of the ELISA as these two samples also recorded the highest levels in that assay. The remaining four samples, Horowhenua (L82), Hakanoa (L113 and L161) and Rotoiti (L141) contained only low ($< 100 \mu\text{g kg}^{-1}$) levels of saxitoxins.

A wide range of cyanobacteria species were found in the samples that tested positive for saxitoxins by the ELISA and neuroblastoma assay (Appendix 2, Table A1 - A4). Additionally, some samples containing no visible cyanobacteria also tested positive with the ELISA. The sample from Lake Kohangatera (L126) which had the highest levels of saxitoxins by both methods contained *Anabaena lemmermannii* and *A. circinalis*. In contrast, the second highest sample from Kaitaia Pond 2 (OP9) contained *Planktothrix mougeotii* and *Microcystis aeruginosa* and the third highest sample from Lake Hakanoa (L113) contained *Anabaena planktonica*, *M. flos-aquae* and *M. aeruginosa*. These results indicate that there could be multiple cyanobacteria species producing saxitoxins in New Zealand.

Seventy six percent of the samples from waterbodies that tested positive for saxitoxins, had recreational activity as their primary use, 11% were used for stock drinking water, 5% were scenic, 5% oxidation ponds and 3% were used for aquaculture.

Figure 4.16. Levels of saxitoxins ($\mu\text{g l}^{-1}$) in samples from New Zealand lakes. These results are from samples in which 50 ml of sample was lyophilised and reconstituted in 1.0 ml distilled water. The values measured were then used to calculate the amount of saxitoxin in 1.0 ml of original sample. When saxitoxins were recorded in more than one sample from each location only the highest value was plotted.

Figure 4.17. Levels of saxitoxins ($\mu\text{g kg}^{-1}$) in samples from New Zealand waterbodies. These results are from samples in which 50 ml of sample was lyophilised and a known weight reconstituted in 1.0 ml distilled water. The measured values were then used to calculate the amount of saxitoxin per kg. When saxitoxins were recorded in more than one sample from each location only the highest value was plotted.

4.6 Discussion

4.6.1 Microcystins and Nodularins

Globally microcystins are the most commonly found cyanotoxin (Chorus & Bartram 1999). The present study indicates that this is also the case in New Zealand. Prior this, microcystins/nodularins were known to occur in nine waterbodies in New Zealand. Microcystins/nodularins were identified in 54 different waterbodies (three of these locations were known to contain microcystins prior to the present study), taking the total number of locations where microcystins/nodularins have been found in New Zealand to 60. Almost all the samples that contained *Microcystis* spp. also contained microcystins. In contrast microcystins were much less frequently found (and were at low levels) in samples containing *Anabaena* spp.

Microcystin levels in the samples collected ranged from $0.02 \mu\text{g l}^{-1}$ (the detection limit of the ELISA used) to a maximum of $36\,500 \mu\text{g l}^{-1}$ in some scum material. Many earlier studies have published their results in terms of dry weight, making comparisons with the values from the present study difficult. Expressing concentrations per litre of water is more useful for human health guideline purposes. The levels of microcystins recorded in the present study do not vary dramatically from other studies where microcystins are expressed in the same format (Cook et al. 2004, Graham et al. 2004, Park et al. 1998).

Microcystins were found in both the North and South Island. The abundance of positive records of microcystins from the North Island reflects the bias towards sample collection from this area. Further studies on South Island waterbodies would be required before a comparison between North and South Islands can be usefully made.

Cyanobacterial blooms occur most commonly during the summer and microcystins were most commonly found between November to April. However, in some lakes, blooms were reported much later in the year. One interesting example was the sample collected from scum material in Lake Waitawa (L178) in June 2003. It recorded the second highest level of microcystins ($32\,000 \mu\text{g l}^{-1}$), demonstrating that high levels of microcystins can be found in blooms outside the period usually considered to be the high risk season for cyanobacterial blooms in New Zealand.

The present study did not include unambiguous identification of microcystin producers (e.g., by culturing and/or testing of individual species/strains). However, by assessing dominant and common species in samples some conclusions about likely microcystin producers in New Zealand can be made. Within the *Microcystis* genera the species *M. aeruginosa* and *M. panniformis* commonly dominated when microcystins were present and both are known to produce microcystins in other countries (Abdel-Rahman et al. 1993, Bittencourt-Oliveira et al. 2004). Other *Microcystis* species that were present when high levels of microcystins occurred (although usually *M. aeruginosa* was also present in the samples) and therefore should be considered as possible microcystin producers include *M. flos-aquae*, *M. wesenbergii* and *M. ichyobabe*. Low levels of microcystins were occasionally found in material containing mostly *Anabaena* spp. indicating that some of these species may be producers of microcystins. *Anabaena flos-aquae* and *A. circinalis* are known microcystin producers (Chorus 1999). Results from oxidation ponds indicate that *Planktothrix mougeotii* is a producer of microcystins. This species and *P. agardhii* are known to produce microcystins (Barco et al. 2004, Keil et al. 2002). During the present study benthic species *Nostoc commune*, *Oscillatoria* spp. and *Phormidium* spp. were found to produce microcystins, these genera are all known to produce microcystins elsewhere (Chorus 1999).

4.6.1.1 Sample Analysis

One disadvantage of the ELISA used in the present study is that it does not give an indication of which microcystin congeners are present in the samples. LC-MS was used to investigate composition of the microcystin variants (RR, YR, LR) and nodularin in four samples. The total microcystin level obtained with LC-MS did not always match with the total microcystin level measured with the ELISA. The results from the LC-MS were slightly lower in two of the samples, slightly higher in one sample and markedly lower in the samples from Lake Horowhenua (L42). There are several probable reasons for this. The most likely is that only three microcystin variants were tested for by LC-MS. Over 60 microcystin variants are now known to occur and it is likely that some of these also occurred in the samples. The ADDA-ELISA used during the present study measures the total amount of ADDA-containing compounds in the sample and should therefore give a measure of total microcystins, even if some variants are only present in very small quantities. The presence of ADDA containing compounds (other than microcystins/nodularins) in cyanobacteria has not been investigated so it is possible that

this may account for the results from the ELISA being higher than the LC-MS. Once the samples were collected they were stored frozen at -20°C and usually analysed with the ELISA within 2 - 4 weeks. As LC-MS analysis did not take place until August 2004 (sample collected in January 2002), it is possible that some sample degradation occurred during the intervening period especially given that the sample had been freeze thawed on numerous occasions. This sample was a scum sample and approximately 2 litres were collected. It is possible that there was considerable heterogeneity in the microcystin concentration within the sample.

Mountford et al. (2004) in a study which used the ADDA-ELISA, protein protein phosphatase inhibition assay and LC-MS to analyse samples also noted a marked difference in levels of microcystins detected via these methods. The ADDA-ELISA usually recorded higher microcystin levels than the other two methods used. They used LC-MS only to screen for microcystin - RR, YR, LR and nodularin. Thus it is possible that not all microcystins were detected via this method.

4.6.1.2 Human Health Risk

The majority of the waterbodies that contained microcystins are used for recreational activity, and a key question for recreational exposure is whether the cyanotoxin levels present in the waterbodies are sufficient to threaten human life or cause serious health problems. Although there have been numerous animal deaths from the ingestion of contaminated water, no human deaths from recreational activity have been reported (Chorus & Fastner 2001). Calculations were used to estimate whether or not the levels of microcystins in New Zealand waterbodies could be dangerous. These were based on a worst-case scenario from toxicological data and the levels in samples containing the highest amounts of microcystins. The following assumptions were made:

- The microcystin in the samples was all microcystin-LR (or of equal toxicity to microcystin-LR). Many of the other microcystins have been found to have similar LD_{50} 's to microcystin-LR (Chorus & Bartram 1999).
- Humans are as sensitive to microcystins as mice on which the toxicological data has been based. (Sensitive individuals are likely to show lethal responses at lower concentrations). Oral administration of microcystin-LR to mice has demonstrated a 24 h LD_{50} of between 5 000 $\mu\text{g kg}^{-1}$ (Fawell et al. 1999) and

10 900 $\mu\text{g kg}^{-1}$ (Yoshida et al. 1997). Thus for a 10 kg toddler a dose of 50 mg could be a lethal dose.

Using these assumptions and the two highest values of microcystin recorded - 36 500 $\mu\text{g l}^{-1}$ (L82 - Lake Horowhenua, Figure 4.8) and 32 000 $\mu\text{g l}^{-1}$ (L178 - Lake Waitawa), the theoretical amount of scum material that would need to be ingested by a 10 kg toddler is given in Table 4.5. The results from the same calculation for each sample location (highest value from each site) are given in Appendix 12.

Table 4.5. Amount of scum material from Lake Horowhenua sample (L82) and Lake Waitawa sample (L178) required to kill a 10 kg toddler – a theoretical worst-case estimate.

Microcystin levels in Lake Horowhenua scum sample = 36.5 mg l^{-1}

Therefore a 10 kg toddler would have to consume 1.37 l of the Lake Horowhenua scum sample to receive a lethal dose.

Microcystin levels in Lake Waitawa scum sample = 32 mg l^{-1}

Therefore a 10 kg toddler would have to consume 1.56 l of the Lake Waitawa scum sample to receive a lethal dose.

Consumption of the amount of scum material given in Table 4.5 during participation in recreational activity is very unlikely, the major risk is more likely to be from repeated or chronic exposure to microcystins at sub-lethal concentrations. It is possible to estimate the amount of this scum material that would need to be consumed to cause health impairment in a toddler by a calculation method adapted from Chorus & Fastner (2001) which uses similar assumptions as above. The results from these calculations for each sample location (highest value from each site) are given in Appendix 12. Results for Lakes Horowhenua and Waitawa are given Table 4.6.

Table 4.6. Amount of scum material from Lake Horowhenua sample (L82) and Lake Waitawa sample (L178) that could cause health impairment in a 10 kg toddler.

The tolerable daily intake, calculated by applying uncertainty factors to the “no observed” or “lowest observed” effect levels in several week exposure experiments (Falconer et al. 1994) is 0.04 $\mu\text{g kg}^{-1}$.

Microcystin levels in Lake Horowhenua scum sample = 36.5 mg l^{-1}

Therefore a 10 kg toddler could experience health impairment if 0.011 ml of the Lake Horowhenua scum sample was consumed.

Microcystin levels in Lake Waitawa scum sample = 32 mg l^{-1}

Therefore a 10 kg toddler could experience health impairment if 0.013 ml of the Lake Horowhenua scum sample was consumed.

Table 4.6 demonstrates that only very small amounts of scum material would need to be ingested to be above the “no observed” or “lowest observed” effect levels. While it is unlikely (but not totally implausible in the case of toddlers) that scum material would be consumed, nearby water of similar toxin level could easily be consumed at this level during activities such as swimming or water skiing. Residents or people using the lake water for drinking, bathing or recreational activity over a period several weeks could be at risk. Lake Waitawa is used extensively for bare foot water skiing and also has a school camp situated on its shoreline, with kayaking, rafting and a water slide (into the lake) being regular activities.

The fact that microcystins were prevalent in so many waterbodies highlights the potential health risk that this toxin might pose in New Zealand. Many of the water bodies experiencing cyanobacterial blooms (and high levels of microcystins) lacked any warning signs to inform water users of the risks involved during contact with the water (discussed in Section 4.6.6).

Only a few waterbodies that supply drinking water were sampled. A survey of cyanobacteria/cyanotoxins in such waterbodies would be beneficial as in New Zealand 73% (in terms of total population) of our drinking water comes from surface water supplies (Water Industry of New Zealand 2004 data, <http://www.drinkingwater.org.nz/>). This includes streams, rivers (discussed further in Section 4.6.1.5) and reservoirs. Many smaller community water supplies do not have adequate treatment plants to remove cyanotoxins (Water Industry of New Zealand 2004 data, <http://www.drinkingwater.org.nz/>).

4.6.1.3 Oxidation Ponds

The identification of microcystins in oxidation ponds appears to be the first record of cyanotoxins in such ponds in New Zealand, although they have been identified elsewhere (Vasconcelos & Pereira 2001). Oxidation ponds are an important form of effluent treatment for domestic sewage in New Zealand. Forty percent of towns (20 000 or more people) in New Zealand have oxidation ponds (Shields 1991), and even larger proportions of smaller communities (1 000 to 20 000 people) have reticulated sewage with oxidation pond treatment (Fitzmaurice 1987, Ministry of Works and Development 1982). Samples were obtained from only 10 oxidation ponds. Toxic cyanobacteria were

found in all samples and microcystins were detected at various times in 80% of the ponds tested. There are many more small oxidation ponds in New Zealand that typically discharge to streams and less often to estuaries (Shields 1991). The outflows of larger ponds tend to be piped and released into the marine environment. The presence of some high levels (during the present study a maximum of $100 \mu\text{g l}^{-1}$ was measured) of microcystins in the oxidation ponds raises questions about potential contamination of downstream waters. Ponds may potentially act as “breeding grounds” for cyanobacteria, since they are rich in nutrients. Once the water is discharged, downstream systems could be contaminated with toxic cyanobacteria and/or cyanotoxins. A larger scale study examining the phytoplankton and cyanotoxins composition and downstream effects from oxidation ponds is recommended. An example of a suitable location for this would be in the Ruamahanga River (Wairarapa) into which several oxidation ponds discharge.

4.6.1.4 Dams and Ponds

Flint (1966) reported on stock deaths in New Zealand resulting from animals consuming farm pond water with heavy blooms of *Microcystis* spp. Since then there have been no published reports of stock deaths from farms related to the consumption of water containing cyanobacteria. Given the large extent of farming land in New Zealand and the multitude of farm ponds/dams and streams/rivers that are used for stock drinking water this seems surprising. Internationally there have been many reports of stock deaths from ingestion of toxic cyanobacteria (Ressom et al. 1994).

During the present study attempts were made to obtain samples from farm dams and ponds. Articles were written in the “Vetscript” magazine (distributed to all veterinary clinics in New Zealand) in December 2001 and March 2003 and in “Rural News” 2001 (Appendix 5, Items 5a – 5c). A promotional flyer and letter asking for samples (Appendices 3 and 4) was also sent to each Federated Farmers association in New Zealand. Each article prompted several responses from concerned farmers. Some had lost stock in circumstances similar to those that could be expected from cyanotoxin poisoning and others reported blooms or mats. However, by the time the stock deaths had been linked to the stock drinking water the blooms had often subsided. Thus most samples contained none, or only low levels, of cyanobacteria and microcystins. Only one sample from Neuman Pond contained high levels ($12\,000 \mu\text{g l}^{-1}$), but stock did not appear to have access to this water. It is likely that many stock deaths that result from

cyanobacteria poisoning in New Zealand go unreported. A more intensive survey of stock drinking water supplies is required; this should begin by focusing on farms that have experienced unexplained stock deaths or are in close proximity to lakes containing cyanobacteria.

4.6.1.5 Benthic Samples

Microcystins have previously been detected in benthic cyanobacteria in New Zealand (Hamill 2001) and thus the detection of them during the present study was not surprising. In Switzerland, cattle deaths have been linked to microcystins and benthic cyanobacteria, including *Oscillatoria*, *Phormidium*, *Tychonema* and *Pseudanabaena* (Mez et al. 1997). They are commonly associated with the production of the neurotoxins anatoxin-a and homoanatoxin-a (Edwards et al. 1992, James et al. 1997). Benthic cyanobacteria are commonly found in flowing waters where they form dark brown-black mats on the river bed. At times, especially following low flow events, these mats will detach from the substrate and form floating mats (Figure 4.18). Benthic mat-forming cyanobacteria are common in New Zealand rivers, including many relatively pristine ones (Biggs & Kilroy 2000). Many of New Zealand drinking water supplies are dependant on rivers and streams (Ministry of Health 2002). Thus these mats may pose a potential human health risk which could be greater than in countries which rely on reservoirs and ground water. Many small or rural communities, e.g. camping grounds, rural schools and holiday homes, use rivers or streams for their supplies and such communities often lack the sophisticated treatment procedures required to remove cyanotoxins. Studies investigating content and release of microcystins in benthic cyanobacteria would be valuable.

A different type of cyanobacterial benthic mat was observed on the eastern shore of Lake Taupo (L160) in March of 2003. The shoreline was lined with thick gelatinous mats of *Nostoc commune* (Figure 4.9). This appeared to result from low lake levels, followed by a storm event in which waves had knocked the *Nostoc commune* off rocks that were usually covered by water and the gelatinous colonies accumulated along the shoreline. *Nostoc* spp. are common in many New Zealand rivers and streams (Biggs & Kilroy 2000), although there is little documentation of their distribution in lakes and reservoirs. These mats contained high levels of microcystins (10.9 mg kg⁻¹ in lyophilised material) and pose a potential human and/or animal health risk. The

gelatinous nature of these colonies could make them particularly appealing to toddlers or young children. One water sample (L159) collected just off the shoreline at Lake Taupo also contained microcystins and demonstrated that some of the microcystins were being released back into the waterbody. This could be a potential health risk if recreational activity was taking place nearby, or if water supply intakes were situated within close proximity.

Figure 4.18. Benthic cyanobacterial mats caught in the trees at the edge of the Hutt River, Wellington.

4.6.2 *Cylindrospermopsins*

The discovery during the present study of the bloom of *Cylindrospermopsis raciborskii* in Lake Waahi (March 2003), was the first reported occurrence of this species in New Zealand. *Cylindrospermopsis raciborskii* is a planktonic freshwater cyanobacterium that is becoming increasingly prevalent in water bodies worldwide. LC-MS and LC-MS/MS were used to confirm the presence of cyanotoxins cylindrospermopsin and deoxycylindrospermopsin.

Because *C. raciborskii* is not described in commonly used New Zealand taxonomic algal literature (Etheredge & Pridmore 1987) it seems plausible that this species has been misidentified previously. When cylindrospermopsin was detected in Lake Waitawa (Stirling & Quilliam 2001) the causative species was identified as *Cylindrospermum* sp.. *Cylindrospermum* sp. is not known to produce cylindrospermopsin (Chorus & Bartram 1999) and is primarily a benthic species (Baker & Fabbro 2002) (see Section 2.6.2.3 for further discussion on the identification of *C. raciborskii*).

The lack of a cylindrospermopsin standard when carrying out the analysis of the Lake Waahi sample meant that the amount of cylindrospermopsin could not be quantified. Future work should attempt to quantify the amounts of cylindrospermopsin in samples and investigate toxicity of the New Zealand strains. Several studies have indicated a substantially increased toxicity for extracts of *C. raciborskii* that cannot be explained solely by the presence of cylindrospermopsin (Metcalf et al. 2002, Norris et al. 1999).

Saker & Eaglesham (1999) have shown in Australia that cylindrospermopsin accumulates in the flesh of a commercially cultured freshwater crayfish and in the freshwater mussel *Anodonta cygnea* (Saker et al. 2004), demonstrating possible risks from the consumption of aquatic organisms during times of *C. raciborskii* blooms. Lake Waahi provides an important habitat for the short-finned eel (*Anguilla australis*), and historically eels from Lake Waahi have provided a significant contribution to the wild eel fishery in the Waikato region (Chisnall 1996, Jellyman & Chisnall 1999). The possibility of bioaccumulation in this species and other aquatic organisms harvested from the lake (or other lakes experiencing *C. raciborskii* blooms) is of concern, especially as this particular toxin is distributed through body tissues and organs (Norris et al. 2001).

Cylindrospermopsin has been shown to degrade rapidly when exposed to sunlight and also in the dark with the presence of plant pigments (Chiswell et al. 1999). However, McGregor & Fabbro (2000) found that cylindrospermopsin could be detected up to six weeks after cells of *C. raciborskii* were below detectable levels. Factors such as turbidity, mixing depth, and depth of the photic zone may affect the rate of

cylindrospermopsin degradation. Cylindrospermopsin degradation kinetics remains unknown for New Zealand waterbodies.

Chiswell et al. (1999) found that at different stages of a bloom of *C. raciborskii* extracellular cylindrospermopsin varied between 19% and 98% of the total toxin content. In New Zealand, cell counts are routinely used as a measure of the risk posed from a cyanobacterial bloom, but these do not take into consideration the extracellular toxin content and therefore may not be a true indicator of the potential risk to human and animal health when *C. raciborskii* is present. Many cyanobacterial blooms (e.g. *Microcystis* spp. and *Anabaena* spp.) are detected by the formation of surface scums, discolouration of water or the production of taste and odour compounds. However *C. raciborskii* does not form scums, will often form subsurface blooms (McGregor & Fabbro 2000) and does not produce taste and odour compounds. Thus blooms of this species may commonly be overlooked in New Zealand. (Figure 4.19) compares blooms/scums formed by different cyanobacterial species).

The identification of *C. raciborskii* from Lake Waahi along with the finding of *C. raciborskii* in other locations (Ryan et al. 2003) demonstrates that *C. raciborskii* is now a species of concern in recreational, stock drinking, and potable waterbodies in New Zealand. Detection of cylindrospermopsin and deoxy-cylindrospermopsin by LC-MS and cylindrospermopsin by LC-MS/MS provide unequivocal evidence of presence of these toxins. Recent research worldwide on the toxicity and adaptive nature of this species highlights the need for further investigation into toxin production, toxicity, genetics, ecology, health risk, and distribution of the New Zealand strain of *C. raciborskii*.

Figure 4.19 (A - C). Comparison of different cyanobacteria species blooms/scums. A, *Cylindrospermopsis raciborskii* bloom (Lake Waahi – L114) of approximately 200 000 cells ml^{-1} . Cells were not visible macroscopically. The water had a slight brown tinge, but was not markedly different from the usual colour of this lake in peat soils; B, *Microcystis panniformis* bloom/scum (Lake Rotoehu – L107), colonies were visible in water and scum had formed along the shoreline; C, *Anabaena circinalis* and *A. lemmermannii* bloom (Lower Karori Reservoir – L50). A thick surface scum covered large areas of the reservoir and filaments were clearly visible in the water.

4.6.3 Anatoxin-a

The identification of anatoxin-a in samples during the present study is the first time this toxin has been identified in New Zealand waterbodies. Quantification was not possible because a quantified standard was unavailable. Previously Hamill (2001) had detected degradation products of anatoxin-a and, using mouse bioassays, symptoms corresponding to anatoxin-a toxicity were demonstrated.

Samples selected for anatoxin-a analysis generally had high concentrations of cyanobacteria and also contained genera known to produce anatoxin-a. Of the 57 samples analysed only 5% contained anatoxin-a. This is much lower than to the detection frequency of microcystins and saxitoxins, but is consistent with studies in other countries where anatoxin-a is not commonly found (Chorus 2001b). Interestingly, anatoxin-a has not been detected in Australia (Baker & Humpage 1994, Velzeboer et al. 2000) despite the presence of species known to produce it.

Although the species responsible for anatoxin-a production was not determined results indicate that it was probably an *Anabaena* species, with *Anabaena circinalis* and *A. lemmermanii* the most likely sources. This is consistent with other studies where the presence of *Anabaena* spp. has been correlated to the presence of anatoxin-a (Bumke-Vogt et al. 1999, Chorus 2001b).

Because the samples were not always analysed immediately after collection, cultures could not be used to determine which species were responsible for anatoxin-a production. However, the Lower Karori Reservoir sample was analysed the day following collection. After determining that anatoxin-a was present in the sample, further samples were collected from the reservoir, however anatoxin-a was not detected in any of the later samples. When the first sample was collected the bloom was in its early stages of proliferation and there was only a thin surface scum visible. The next samples were collected from thick scum material as it was anticipated that the concentration of anatoxin-a would be highest in this material. The inability to detect anatoxin-a in these samples may be due to anatoxin-a being rapidly broken down in sunlight and alkaline conditions (Stevens & Krieger 1991). The scum was exposed to both of these conditions.

The week prior to receiving the sample from Lake Henly there were reports of several dogs becoming sick (although all recovered) after walking near the lake (P Kinaston, Masterton District Health, pers. comm.). Dead ducklings and fish have been reported from the Lower Karori Reservoir during times of dense cyanobacterial blooms (S Fuller, Karori Wildlife Sanctuary, pers. comm.), although these were more likely to be due to a lack of food (ducklings) and low dissolved oxygen (fish) than to cyanotoxins. There were no reports of adverse human or animal health effects at the time of the Lake Rotoehu sample collection.

Since a cluster of dog deaths in the Waikanae and Mataura rivers in 1998-2001 (Hamill 2001) there have been no further published reports of animal fatalities linked to cyanobacteria (and anatoxin-a). It is unclear why there have been no more reports of such fatalities. Further deaths may have occurred but may not have been linked to cyanobacteria. More deaths could be expected considering how common benthic mat-forming cyanobacteria are in New Zealand rivers and streams (Biggs & Kilroy 2000). Only a few benthic samples were analysed during the present study, including mats collected from the same location as the reported dog deaths in Waikanae, but no anatoxin-a was detected in these. A larger scale survey investigating the cyanotoxin content of benthic cyanobacteria and factors affecting this toxin production would be useful.

Future work should aim to quantify the levels of anatoxin-a produced and establish which species are responsible for anatoxin-a production. Studies involving regular sampling of waterbodies experiencing cyanobacterial blooms may also prove useful. Chorus (2001b), in a study of German waterbodies, noted that anatoxin-a showed clear seasonal patterns over three years with most of the anatoxin-a detected towards the end of summer. In some cases this coincided with the development of *Anabaena* spp. and *Aphanizomenon* spp. blooms.

The present study and previous work (Hamill 2001) demonstrate that anatoxin-a does occur in New Zealand and can be produced by both benthic and planktonic cyanobacteria. It does not appear to be a common cyanotoxin, although more studies are required to further define its distribution.

4.6.4 Saxitoxins

Until recently, reports of saxitoxins produced by cyanobacteria were sparse and restricted to a few species and countries (Chorus & Bartram 1999). At least eight species are now known to produce saxitoxins and the number of countries with saxitoxin-producing cyanobacteria is increasing and includes North and South America (Carmichael et al. 1997, Lagos et al. 1999), Australia (Humpage et al. 1994) and a number of European countries (Chorus 2001a, Chorus 2001b, Kaas & Henriksen 2000, Pereira et al. 2000). The results of a survey of water samples for saxitoxins in Denmark suggested that saxitoxins may be more widespread in fresh waters than suggested from previous reports (Kaas & Henriksen 2000).

One possible explanation for the paucity of saxitoxin reports until recently is that many freshwater monitoring programmes have not included analysis for saxitoxins, and neurotoxic responses in mouse bioassays have generally been attributed to anatoxins (Chorus 2001b). The development of simple and reliable techniques (e.g. ELISA, protein phosphatase inhibition assays) which can detect microcystins has meant that many studies have focused on these cyanotoxin. This has certainly been the case in New Zealand where an ELISA test for microcystins is available commercially. Although there are commercial laboratories which are able to test for saxitoxins in marine phytoplankton, these have not been used for freshwater samples. During a Ministry of Health programme, established to check for cyanotoxins in samples taken in response to concerns for human health, mouse bioassays were negative for symptoms indicating the presence of saxitoxins (Dr. D Stirling, ESR, Porirua and A Kouzminov, Ministry of Health, Wellington, pers. comm.).

During the present study using an ELISA the identification of saxitoxins in 38 waterbodies was surprising considering the absence of reported problems potentially related to saxitoxin production in New Zealand. Many of the samples that tested positive contained genera known to produce saxitoxins elsewhere, thus these results are considered reliable. The presence of low levels of saxitoxins in some samples that contained no cyanobacteria raised some concerns about the validity of the results.

The highest levels detected ($8.7\mu\text{g l}^{-1}$) were low considering they were from concentrated cyanobacterial material. The results could indicate that the saxitoxin-

producing species were in low abundance in the samples or only producing saxitoxins at low levels. Some preliminary work by other laboratories has shown that the ELISA does not have high cross reactivity with all other saxitoxin variants (Dr. L Briggs, AgResearch, Ruakura, pers. comm.). This may explain why the concentrations detected in the present study were quite low and were lower than those indicated by the neuroblastoma assay. This is also consistent with the much lower than expected results from the Australian sample tested using the ELISA. Although the samples examined in the present study were concentrated, to maximise the chances of detecting saxitoxins, results may constitute a warning that saxitoxin levels in New Zealand waterbodies are potentially much higher than the results demonstrated. Further research using chemical analysis to confirm the presence and levels of saxitoxins should be a priority.

When analysing samples using the ELISA it was noted that on occasions when a sample was highly pigmented the result was negative. Highly pigmented samples tended to occur in concentrated scum material. These results were not consistent with other results from the same site. For example, Prawn Farm - Pond 1 (DP62) consisted of concentrated scum material and contained no saxitoxins. However DP63 from the same location was not as concentrated (or pigmented) and contained low levels of saxitoxin. This indicates that further sample preparation or clean up may be required before testing samples with the ELISA.

In contrast to the ELISA, the neuroblastoma assay measures the extent to which the voltage-activated sodium channel is blocked. It is an effect-based assay, thus measuring the overall toxicity, and does not rely on knowing which toxin variants are present. Its limit of detection is much higher than that of the ELISA (approx. $100 \mu\text{g l}^{-1}$). The results of the neuroblastoma assay from the sample from Lake Kohangatera and the Kaitaia Oxidation Pond 2, clearly indicated the presence of saxitoxin in these samples. Both types of assays found the same samples to contain most saxitoxins.

Assuming the saxitoxins detected in the New Zealand samples originated from the cyanobacterial population, the results indicate that a number of species may be responsible for the production of these toxins, and quite possibly species additional to those already known as saxitoxin producers. *Anabaena* spp. and *Planktothrix* spp. were abundant in the samples containing significant levels of saxitoxins. Due to the potential

human health hazards constituted by the presence of saxitoxins in fresh waters, further studies on the origin of the toxins in New Zealand are a necessity. Different toxin profiles have been associated with different toxin-producing species (Carmichael et al. 1997, Velzeboer et al. 2001) thus further chemical analysis of samples may also indicate which species are producing the saxitoxins.

Strains of *C. raciborskii* isolated from water supplies in Brazil have been shown to produce the saxitoxins; neosaxitoxin, saxitoxins, the gonyautotoxin - 2/3 isomers, and others yet to be identified (Lagos et al. 1999, Molica et al. 2002). The ELISA results presented in the present study indicated that dense blooms of *C. raciborskii* contained saxitoxins. However testing of a concentrated bloom sample of *C. raciborskii* with the neuroblastoma assay did not show their presence (Wood & Stirling 2003).

Saxitoxin production has been associated also with bacteria (Kodama et al. 1988) and is well documented for dinoflagellates in the marine environment. These two groups of organisms should also be investigated in freshwater bodies. However, because saxitoxin production by cyanobacteria is also well-documented, and many of the New Zealand samples that tested positive were dominated by cyanobacteria, it is likely that cyanobacteria are the primary toxin producers in New Zealand fresh waterbodies.

Cyanobacterial saxitoxins accumulate in freshwater mussels (Negri & Jones 1995, Pereira et al. 2004). Thus the presence of saxitoxin-producing cyanobacteria in New Zealand may affect higher trophic levels and pose a potential health risk if humans are consuming such organisms from contaminated waterbodies. There have been no reports of human illness as a results of saxitoxins produced by freshwater cyanobacteria (Kuiper-Goodman et al. 1999), although in the marine environment the consumption of shellfish containing high levels of saxitoxins has caused acute illness and death (Lehane 2001).

The results of the present study indicate that saxitoxins may be more prolific in New Zealand fresh waterbodies than previously assumed. Further studies on this group of toxins in New Zealand are essential. Such studies should include distribution, levels and variants of the saxitoxins, causative species, toxin production and regulation and investigation of effects on aquatic environments and animal and human health.

4.6.5 Study Limitations

Problems with the general study design are discussed in Section 2.6.1. Samples were collected using both grab sampling and plankton nets. Grab samples allow quantification of samples and the results can be compared to guidelines established by the World Health Organisation for drinking water. Plankton nets concentrate the samples and thus enhance the chances of detecting any cyanobacteria/cyanotoxins that may be present.

In general, samples from waterbodies were collected on a one-off basis during field collecting trips or when other people supplying the samples were present at a suitable waterbody. The scope of this part of the thesis did not allow for samples to be collected on a weekly, monthly or seasonal basis from any site. Given the patchy distribution of blooms and differences in seasonality of some species, it is likely that the present study has underestimated the number of waterbodies experiencing blooms and therefore levels of cyanotoxins. Fastner et al (2001) found that the likelihood of detecting microcystins in a given lake depend to some extent on the frequency of sampling. In lakes sampled only once, 33% contained microcystins compared to 94% for those sampled two to 10 times over three years.

This project did not quantify the absolute concentration of cyanobacterial cells in the samples due to the initial lack of equipment to carry out cell counts. It would have been useful to establish correlations between cell/species numbers and toxin levels and to identify patterns in toxin production.

Analyses performed during the present study measured only the total amounts of toxins present in each sample and thus the proportions of extra-cellular toxins were not established. Recent findings indicate that the amount of extra-cellular toxins in healthy cyanobacteria varies depending on the species, e.g. in *Cylindrospermopsis raciborskii* it can vary between 19% and 98% (Chiswell et al. 1999) compared to approximately 20% in other species (Negri et al. 1997, Sivonen 1990).

4.6.6 Cyanobacterial Bloom Warning Signs

During field sampling a variety of signs warning potential waterbody users of the risk of cyanobacterial blooms were observed. Many waterbodies experiencing blooms lacked any form of signage and others only displayed signs sporadically. At times signs remained in place many months after the blooms had disappeared. Examples of warning signs are given in Figure 4.20 (A – F). Lake Horowhenua (Figure 4.20 A) signs were always displayed clearly and prominently and the Lake Horowhenua Domain Board also took the noteworthy step of fencing off the main public access point to the lake. In contrast, the signs on Lake Waitawa (Figure 4.20 B) did not specify cyanobacteria and when the highest levels of microcystins were recorded at this location there were no signs.

Signage is a continual problem in the Rotorua district with many signs being vandalised shortly after erection. The “algae danger meter” sign shown in Figure 4.20 C was set up as a way of keeping the public informed of the current situation. Figure 4.20 D shows the signs that are put up at all public access points near affected lakes in the Rotorua district. The signs state why the warning is in place and who to contact for further information. Figure 4.20 E and Figure 4.20 F are examples of signs used in the Waikato region. Little information is given on these. They simply read “High blue-green algal levels – avoid contact with the water”. In New Zealand there is a need for a standard sign, containing information allowing the public to make an informed decision. Figure 4.21 is an example of a sign from Queensland, which gives the public information about the danger. It can be updated easily using the arrow to show the current risk level. A similar style of sign seems appropriate for use throughout New Zealand.

Figure 4.20 (A - F). Examples of cyanobacteria warning signs at waterbodies around New Zealand. A, Lake Horowhenua; B, Lake Waitawa; C, Lake Rotoiti (main highway from Rotorua); D, Lake Rotoiti; E, Lake Ngaroto; F, Lake Waahi.

Figure 4.21. Cyanobacteria warning sign in Queensland, Australia.

4.7 Conclusions

The present study showed that cyanobacteria species in New Zealand produce the following cyanotoxins; microcystins, nodularins, cylindrospermopsins, anatoxin-a and saxitoxins. Anatoxin-a(S) has not been found in New Zealand. The cyanotoxins anatoxin-a and saxitoxins were detected for the first time in New Zealand cyanobacterial samples. All of the cyanotoxins were found in planktonic cyanobacteria and both microcystins and saxitoxins were found in benthic mat samples. The cyanotoxin levels were similar to those in waterbodies in other countries.

Cyanotoxins were found in both North and South Islands. Cyanotoxins also occurred in a variety of different waterbodies, including rivers, lakes, ponds, dams and oxidation ponds. Toxic cyanobacteria have been commonly associated with eutrophic waterbodies. In New Zealand, they were identified in waterbodies with a variety of water qualities, ranging from eutrophic e.g. Lake Horowhenua, to oligotrophic e.g. Lake Taupo.

Overseas studies have reported that approximately 60% of recorded cyanobacterial blooms contain cyanotoxins. The present study found that 60% of blooms were positive for microcystins, 10% for anatoxin-a and 77% for saxitoxins (not all blooms were tested for each cyanotoxin). Only one bloom sample was analysed for cylindrospermopsin and this was positive. This indicates that the incidence of toxic blooms in New Zealand is similar to that of other countries.

The results indicate that species from *Microcystis*, *Anabaena*, *Cylindrospermopsis*, *Nostoc*, *Planktothrix*, *Oscillatoria* and *Phormidium* produce cyanotoxins. Further studies to establish which species produce each specific cyanotoxin are essential and would assist in the management of cyanobacteria in New Zealand. Cell counts are regularly used in New Zealand to estimate the health risk posed by cyanobacteria. However, at times large proportions of cyanotoxins can be extracellular and cell counts may not be a good indicator of health risk. Studies investigating extracellular toxin levels maybe warranted. Levels of saxitoxins, anatoxin-a and cylindrospermopsin need to be quantified and additional chemical analyses of samples to establish which variants of each toxin are present in the samples would also be valuable.

The widespread distribution and, in some locations, high levels of cyanotoxins (especially microcystins) indicate that there is potential for a significant threat from cyanotoxins to human and animal health. The possibility of downstream contamination of cyanotoxins should also be investigated. During this research the majority of the samples came from waterbodies that were used for recreational activities. Further studies on waterbodies used for drinking water are recommended, since the direct and long term ingestion of some cyanotoxins poses the greatest health risk.

In New Zealand, guidelines for acceptable levels of cyanobacteria and cyanotoxins in drinking water are being developed by the Ministry of Health. Once these are implemented further monitoring of relevant waterbodies is expected. There are currently no recreational guidelines for cyanobacteria in New Zealand and this has resulted in a lack of national standards for the issuing of health warnings, e.g. warning signs and cell concentration at which health warnings are issued. The development of national protocols is recommended.

The present study has demonstrated that all of the common cyanotoxins are found in New Zealand waterbodies and that these are more widespread than previously assumed. Further research into cyanotoxins in New Zealand is required and this should aim to establish the species responsible for cyanotoxin production, levels of cyanotoxins, structural variants, information on cyanotoxin distribution (especially in the South Island) and the potential human and animal health risks they might pose. As research into cyanotoxins increases it is expected that the number of cyanotoxin records will also rise as a result of this greater research activity.

4.8 References

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5. Microcystins in Rainbow Trout, Freshwater Mussels and Phytoplankton in Lakes Rotoiti and Rotoehu

5.1 Abstract

A range of cyanotoxins have been found in the Rotorua Lakes. At times concentrations of microcystins in Lakes Rotoiti and Rotoehu have reached levels that are potentially dangerous to humans involved in recreational activity on or in these lakes.

Microcystins are known to accumulate in freshwater organisms. In this study the accumulation of microcystins in rainbow trout (*Oncorhynchus mykiss*) and freshwater mussels (*Hydriddella menziesi*) in Lakes Rotoehu and Rotoiti were investigated. The study involved using both rainbow trout that had access to the entire lake and hatchery rainbow trout added to an enclosure in Lake Rotoiti where levels of microcystins in the water and cell counts could be closely monitored. Microcystins in water samples, trout liver, rainbow trout muscle tissue and in the mussels were analysed using the ADDA-ELISA method.

A maximum level of microcystins in the water samples of $760 \mu\text{g l}^{-1}$ was recorded in Te Weta Bay, Lake Rotoiti, in March 2004. ELISA results confirmed the presence of microcystins in rainbow trout liver and muscle tissue, and in freshwater mussels. The Daily Tolerable Intake limit of microcystins for human consumption recommended by the World Health Organisation is $0.04 \mu\text{g kg}^{-1} \text{ day}^{-1}$. A 70 kg human consuming 300 g of rainbow trout muscle tissue from Lakes Rotoiti and Rotoehu would have exceeded this level in 50% and 71% of the samples respectively for the two lakes. Health problems could result if more than 300 g of rainbow trout muscle tissue was consumed on a regular basis over an extended period.

5.2 Keywords

microcystins; cyanobacteria; rainbow trout; freshwater mussels; Lake Rotoiti; Lake Rotoehu

5.3 Introduction

Microcystins are cyclic heptapeptides, which block protein phosphatase 1 and 2a in affected organisms (MacKintosh et al. 1990). To date, more than 60 microcystins have been isolated and characterised (Chu 2000). Microcystins are produced by a variety of cyanobacteria species (Chorus & Bartram 1999), most commonly species from the *Microcystis* genus (Section 4.3.1 for further information on microcystins).

In Chapter 4 the identification of microcystins in water samples from Lakes Rotoiti and Rotoehu is described. At times the levels of microcystins in these lakes reached concentrations that could be hazardous to humans involved in recreational activity on or in the lakes (Wood 2003). In addition to microcystins, both anatoxin-a and saxitoxin have been found in the Rotorua lakes, however, they do not appear to commonly occur or reach high levels (See Chapter 4).

The first objective of the present study was:

- To establish how levels of microcystins vary during a six month period in the waters of Lakes Rotoehu and Rotoiti.

Variation in levels of microcystins over time have not previously been studied in New Zealand water bodies. Lakes Rotoiti and Rotoehu were chosen because they regularly experience cyanobacterial blooms and are known to contain microcystins. Levels of microcystins, and the composition and abundance of cyanobacteria species were monitored weekly in the lakes. Resulting data allowed levels of microcystins and cell concentrations of certain species to be compared, and the results were used to assess periods of greatest human health risk.

Numerous studies overseas have shown that microcystins can accumulate in different organs of freshwater fish (Ernst et al. 2001, Magalhaes et al. 2003), freshwater (Eriksson et al. 1989) and marine mussels (Amorim & Vasconcelos 1999, Vasconcelos 1995), zooplankton and other invertebrates (Kotak et al. 1996, Vasconcelos 1999). Traditionally, microcystins were thought to mostly accumulate in the liver of freshwater fish, thus the risks to humans consuming gutted fish were considered to be low. Current advice given by the Bay of Plenty health protection officers to people eating fish from areas experiencing cyanobacterial blooms is to gut them. However, a recent study in

Egypt detected levels of $102 \mu\text{g kg}^{-1}$ in the muscle of fish (Mohamed et al. 2003) indicating that the consumption even gutted fish might adversely affect human health.

The maximum allowable concentration for microcystins in drinking water was established by Falconer et al. (1994) as $1 \mu\text{g l}^{-1} \text{ day}^{-1}$. A Daily Tolerable Intake (TDI) value of $0.04 \mu\text{g kg}^{-1} \text{ day}^{-1}$ has been proposed as a provisional guideline value by the World Health Organisation (Chorus & Bartram 1999) for total microcystins. TDI is defined as the acceptable amount of a potentially toxic substance that can be consumed daily during a life period.

The rainbow trout (*Oncorhynchus mykiss*) is a species that is of particular importance to recreational fishers and to tourism in the Rotorua lakes. The second objective was to:

- To identify whether or not microcystins accumulate in the liver and muscle tissue of rainbow trout and whether or not microcystins could reach levels in trout tissue that are hazardous to health if consumed by humans.

Because of the variability in levels of microcystins within the Rotorua lakes, trout were sampled over the entire areas of Lakes Rotoiti and Rotoehu, and from a group contained within an enclosure in Lake Rotoiti where microcystins were known to reach high concentrations. In the enclosure, levels of microcystins and cyanobacteria cell counts were closely monitored.

A third objective was to:

- Establish whether significant levels of microcystins accumulate in the native freshwater mussel (*Hydridella menziesi*), and if they accumulate, how the concentration of microcystins changes with exposure time.

Because of the variable distribution of cyanobacteria and cyanotoxins, their concentrations in waterbodies can change very quickly with changing environmental conditions. Routine point sampling may not provide a true indication of the potential risks posed by cyanotoxins in any given area. *Hydridella menziesi* are filter feeders, and it seemed likely that this feeding process would lead to their accumulation of microcystins. If the accumulation builds up and remains in the tissue over a period of time, freshwater mussels may give a useful indication of the toxin levels at a particular

site over a period of time. Additionally, if the mussels accumulate higher levels of microcystins in their tissue than is present in the surrounding water, they could be used as an early warning mechanism in waterbodies with low levels of microcystins, when concentrations in the water might be near the limits of detection of analytical procedures. Proactive measures could then be taken before microcystins reach levels that are deemed a health risk. A British study (Hathaway 2001) found significantly higher levels of microcystins in mussels than in lake water suggesting that this was an area worthy of investigation in the present study.

5.4 Methods

5.4.1 Field Methods

5.4.1.1 *Phytoplankton Sampling*

Surface water samples (400 ml) were collected weekly as part of the Environment Bay of Plenty (ENVBOP) Rotorua Lakes cyanobacteria monitoring program. Collection began on the 14-11-2003 and finished on 02-05-2004. Three samples were collected from both Lake Rotoiti and Lake Rotoehu on each occasion. The sampling sites chosen were areas where cyanobacteria proliferation is known to occur. These were:

Lake Rotoiti - Te Weta Bay (2804295E 6346565N NZ Topographic map Grid Reference, Map U15), Okawa Bay (2803035E 6344690N, Map U15) and Hinehopu (2816215E 6345715N, Map V15) (Figure 5.1).

Lake Rotoehu – Otautu Bay (2821450E 6347065N, NZ Topographic map Grid Reference, Map V15) Kennedy Bay (2821675E 6348380N, Map V15) and Te Pohue Bay (2818190E 6345950N, Map V15) (Figure 5.1).

Figure 5.1. Map showing location of weekly sampling sites on Lakes Rotoiti and Rotoehu.

The water samples were well mixed and a 50 ml sub-sample was taken. This was preserved using Lugol's Iodine, stored in the dark and used later for species identification. The remaining 350 ml was frozen and thawed twice, and then two 2 ml sub-samples were taken and frozen at -20°C for later analysis of microcystins.

Identification, enumeration and analysis of microcystins were carried out on each sample as described in Section 5.4.2.1.

During the enclosure experiment, 400 ml samples were collected near the location of mussel cages at the centre of the enclosure bay. These were collected, preserved and frozen in the same manner as described for the weekly samples. Samples were collected daily at 900 h (surface only) and at 1400 h (surface, 1 m, 2 m and 3 m depths) (New Zealand Standard Time). The dissolved oxygen and water temperature were recorded at each time and depth. Dense blooms were observed at Te Akau Point (Figure 5.2) on several occasions (15-03-2004 and 26-03-2004) and at the jetty area of the nearby Lake Rotoiti campground (26-03-2004) (Figure 5.2). Samples of scums were also collected to obtain an indication of the maximum levels of microcystins reached.

5.4.1.2 Free Roaming Rainbow Trout

Five trout were received from Lake Rotoehu each month from November 2003 to April 2004 except January when four were received (caught by local fishing guides). In Lake Rotoiti five trout were received monthly from December 2003 to April 2004, except February when two were received). The trout were frozen and transported in insulated containers by courier to Massey University, Wellington. Viscera and muscle tissue were removed as described in Section 5.4.2.3 and frozen at -80°C until microcystins analysis was carried out as described in Section 5.4.2.1.

5.4.1.3 Trout Enclosure Experiment

The southern arm of Te Weta Bay, Lake Rotoiti (286460E 6346805N NZ Topographic Map Grid Reference, Map U15 Figure 5.2) was enclosed by a net across its entrance (Figure 5.5 – 5.8). The netted entrance was approximately 100 m wide with a maximum water depth of 4 m. The length of enclosed arm was approximately 350 m. Depth profiles across the entrance and along the length of the arm on the centre-line are shown in Figures 5.3 and 5.4. Resource consent was obtained from Environment Bay of Plenty to establish the enclosure.

The middle 70 m of the net was a length of standard synthetic fishing net with a 20 x 20 mm mesh dimension. The 15 m long end pieces were made from synthetic garden shade cloth with a 4 x 3 mm aperture. The end pieces were sewn to the main section using synthetic cord. This size of mesh allowed water and phytoplankton to move freely into the area, creating an environment similar to other areas of the lake. The main section of fishing net was originally 10 m in depth. To avoid any excess net in the water it was

rolled up so that the depth matched the profile of the bottom of the bay entrance and secured with plastic cable ties. To aid with buoyancy along the top of the net, cork floats were located every 1 m and 5-litre plastic containers were tied to the headline of the net every 3 m.

A rope with a lead core was attached along the middle and on the bottom of the net and, together with lead weights on the bottom, this assisted in weighing down any excess net and held the net to the bottom.

Figure 5.2. Map showing western end of Lake Rotoiti and location of enclosure experiment in Te Weta Bay. The red line shows the location of the net used to form the enclosure.

Figure 5.3. Cross-section profile of net which reached to the bottom across the entrance of southern arm of Te Weta Bay.

Figure 5.4. Longitudinal depth profile of southern arm of Te Weta Bay used for enclosure experiment.

The net was anchored to the bottom sediment of the lake using 1 m long U pins of 12 mm diameter deformed reinforcing rod located about every 2 m. These were pushed into the soft bottom sediment by Scuba divers from ENVBOP and Rotorua District Council. The ends of the net were tied securely to trees at the north end and waratah stakes at the south end. Signs were erected at either end of the net to inform the public about the placement of the net and the study.

The net was installed on 07-03-2004 and removed on 27-03-2004. All other material associated with the project was removed from the site on 27-03-2004.

5.4.1.4 Trout Enclosure Experiment

Eighty, two year old rainbow trout from the Fish and Game New Zealand, Rainbow Valley hatchery were released into the enclosure on 08-03-2004. Each trout was tagged by clipping one pectoral fin before release. Up to five of the released trout were caught every two to three days over a 21 day period. A total of 28 released trout were caught. In addition, three "wild" trout that were in the enclosure area prior to installing the net were captured.

The trout were caught by lure and by using two gill nets with a 50 x 50 mm mesh. Angling took place between 0500 to 0800 h and 1700 to 2000 h. Initially the nets were set and retrieved over these same periods. Careful monitoring was carried out to ensure that water birds did not become entangled in the nets. After the first 14 days, it was apparent that birds were identifying the nets and did not venture into the middle of the enclosed area during the hours of darkness. Following this observation, the nets were sometimes left in place overnight in the middle of the bay. Approximately half of the trout were caught by lure casting and the other half by netting.

Trout were killed immediately after capture by a heavy blow to the top of their heads. The trout were refrigerated shortly after catching and dissected within 12 hours. Approval for the capture of rainbow trout associated with this project was granted by the Victoria University Animal Ethics Committee (Project code 2003R21).

5.5

5.6

Figure 5.5. Enclosure net, showing the main net and the synthetic garden shade cloth end attachments.

Figure 5.6. Enclosure net. 5-litre plastic containers were placed every 3 m along the headline of the net.

5.7

5.8

Figure 5.7. The enclosure net being installed in Te Weta Bay.

Figure 5.8. The enclosure net in place in Te Weta Bay, viewed from the enclosure side.

Viscera and muscle tissue were removed as described in Section 5.4.2.3 and frozen at -80°C until analysis of microcystins was carried out as described in Section 5.4.2.1.

Three trout from the hatchery were used to determine if any baseline microcystins were present. These were killed and dissected at the time the batch was released into the enclosure, thus were not exposed to cyanobacteria in the lake water.

5.4.1.5 Mussel Cage Experiment

Approximately 130 freshwater mussels (*Hydriddella menziesi*) were collected on 06-03-2004 from sediment in Lake Rotoma (free of microcystins) at approximately 10 m depth. A Ministry of Fisheries Special Permit (NN0104) was obtained before collecting these mussels. Mussels were transported in buckets containing Lake Rotoma water to the study site in Lake Rotoiti.

Ten mussels were killed immediately and kept frozen at -80°C until analysis of microcystins was carried out as described in Section 5.4.2.1. These mussels were used to determine if there were any baseline microcystins present. The remaining 120 were placed in plastic mesh cages (Figure 5.9) suspended from buoys 300 mm below the surface in the centre of the enclosure bay about 20 m from the net (Figure 5.10).

Ten mussels were collected every 2-3 days over a period of 21 days, frozen shortly after collection and then the shells were opened and the flesh halved. They were then pooled into two sets each containing ten halves and kept frozen at -80°C until analysis of microcystins was carried out on one of the pools as described in Section 5.4.2.1. The remaining pooled mussels were retained for individual analysis (not carried out in this project) or for use as backup samples.

Figure 5.9. Plastic mesh cages used to hold freshwater mussels during the enclosure experiment.

Figure 5.10. Location of mussel cage mooring buoys inside net buoys in Te Weta Bay.

Figure 5.11. Trout stomach contents (April, Lake Rotoehu). This stomach contained approximately 130 of the gastropod snail *Physa acuta*.

Figure 5.12. Enclosure trout - Lake Rotoiti. Trout stomach contents - Smelt (*Retropinna retropinna*). The large smelt had a smaller fish partly ingested.

5.4.2 Laboratory Methods

5.4.2.1 Microcystins Analysis

The total microcystins content of the samples (phytoplankton and organisms) was analysed with a competitive indirect ELISA using the methods of Fischer et al. (2001). This ELISA uses antibodies raised to the ADDA moiety that is present in most (>80%) of the known toxic penta- and heptapeptide toxin congeners. A full description of this method is given in Section 4.4.2.1.

For phytoplankton analysis, 2 ml subsamples were freeze-thawed twice, centrifuged at 10 000 rpm for 2 min and the supernatant used for the ELISA assay. This gives a measure of the total microcystins present in the lake water. It does not distinguish between intra- and extracellular microcystins.

Trout muscle tissue, trout liver, and freshwater mussels were separately pooled and each pool was homogenised in 75% methanol/water. The homogenate and solvent was centrifuged and the supernatant recovered and washed with hexane. The aqueous phase was retained and processed by solid phase extraction. The fraction containing microcystins was then eluted with 80% methanol/water, diluted in assay buffer (PBS) and analysed by ELISA. For some samples it was necessary to dilute extracts 1:50 to overcome matrix effects.

To assess the effectiveness of the extraction procedure, spiked recovery experiments were carried out on trout muscle tissue, trout liver and mussel samples. The samples were spiked with 100 – 200 ng n⁻¹ of Microcystin-LR (ALEXIS). The samples were then extracted and tested using the ADDA-ELISA as described above.

All trout and mussel samples were analysed by the Toxinology Laboratory, AgResearch, Hamilton, New Zealand.

5.4.2.2 Cyanobacterial Identification and Enumeration

The preserved Lugol's subsamples were used for species identification and enumeration using an inverted Olympus microscope (CKX41) and Utermöhl settling chambers

(Utermöhl 1958). Depending on the density of cyanobacteria, 2-10 ml samples were used for enumeration. When possible, cyanobacteria were identified to species level. The species identifications in this study were made primarily by reference to; Baker 1991, 1992, Baker & Fabbro 2002, Komárek 2000, Komárek et al. 2002, McGregor & Fabbro 2001, Azevedo & Sant'Anna 2003. Cell counts and corresponding levels of microcystins were compared using the Pearson Correlation coefficient with Microsoft Excel Software (2003).

5.4.2.3 Trout Examination

All trout were weighed and the standard length measured. This allowed the Condition Factor (CF) to be calculated for each trout using the following formula (Fish & Game pers. comm.);

$$\text{Condition Factor} = 3612.8 \frac{\text{Weight, g}}{(\text{Length, cm})^3}$$

Necropsy was performed and liver, kidney, gut and muscle tissue removed. Approximately 5 g of muscle tissue was taken from each trout and combined into a pooled sample. A second 5 g of muscle tissue was taken from each trout and kept separately as a back-up sample or for individual muscle tissue sample analysis if required (not carried out in this project). Each liver was halved with one half combined in a pooled sample and the remaining half retained for back-up or individual analysis (not carried out in this project). Kidney samples were also retained for future analysis (not carried out in this project). Samples were stored at -80°C until extracted for ADDA-ELISA analysis as described in Section 5.4.2.1.

An assessment of stomach contents was performed on each trout. The stomachs were opened and the contents were grouped into one of the following groups; plant material, molluscs, insects, fish, pebbles and unidentifiable organic material. The stomach contents were blotted dry, and the wet weight of each category was recorded and a percentage composition of stomach contents then calculated for each trout. The fullness of the stomach contents was not taken into account. An average stomach contents for each pooled sample was then calculated.

5.5. Results

5.5.1 Weekly Phytoplankton Samples

5.5.1.1 Levels of Microcystins

The variations in levels of microcystins at the six weekly sampling locations on Lakes Rotoiti and Rotoehu are shown in Figure 5.13.

Figure 5.13. Levels of microcystins in weekly samples - Lakes Rotoiti and Rotoehu.

Generally no or very low ($<1 \mu\text{g l}^{-1}$) levels of microcystins were detected at the three sampling locations in Lake Rotoehu (Kennedy Bay, Te Pohue Bay and Otatau Bay). This corresponded with low levels of cyanobacteria at these locations (Figure 5.17 – 5.19). When significant levels of microcystins were detected at Te Pohue Bay, 02-05-2004 – $23.5 \mu\text{g l}^{-1}$, these corresponded with an increase in the *Microcystis panniformis* cell counts.

Microcystins were absent from Lake Rotoiti samples until 23-02-2004 when they were detected in both the Te Weta Bay and Okawa Bay sites. Okawa Bay had very high levels of microcystins - $410 \mu\text{g l}^{-1}$. This corresponded with a high cell count of *Microcystis aeruginosa* and *M. botrys* ($940\,000 \text{ cell ml}^{-1}$ – Figure 5.14). Microcystins continued to be recorded at Okawa Bay until the conclusion of sampling. On two other occasions (15-03-2004 and 26-03-2004), the levels were above the proposed Australian recreational guideline ($8 \mu\text{g l}^{-1}$) for microcystins (National Health and Medical Research

Council 2004). The sample from Te Weta Bay on 08-03-2004 recorded the highest levels of the Lakes Rotoiti and Rotoehu study, $760 \mu\text{g l}^{-1}$. This corresponded with a level of $430\,000 \text{ cells ml}^{-1}$ of *Microcystis* spp. (Figure 5.15). Levels in Te Weta Bay were elevated for the remainder of the study with samples regularly above the proposed Australian guideline. Levels of microcystins at Hinehopu remained generally low with only the sample from 22-03-2004 recording a level above the proposed Australian guideline ($9.3 \mu\text{g l}^{-1}$). This also corresponded with an increase in *Microcystis* spp.

5.5.1.2 Cyanobacteria Species Identification and Enumeration

A total of 33 cyanobacteria species were recorded (Table 5. 1). Of these 33 species, six: *Microcystis botrys* Teiling 1942, *Sphaerocavum brasiliensis* Azevedo & Sant'Anna 2003, *Anabaena torulosa* (Carm.) Lagerheim 1883, *Coelosphaerium* aff. *natans* Lemmermann 1900, *Planktothrix* aff. *planktonica* (Elenk.) Anagnostidis & Komarek 1988 and *Pseudanabaena galeata* Böcher 1949 were recorded for the first time in New Zealand. The genus *Sphaerocavum* has not been recorded in New Zealand previously. During the Lakes Rotoiti and Rotoehu study *M. botrys* was identified as *M. aeruginosa*, however, at the 16th Symposium of the International Association for Cyanophyte Research (Luxembourg, 2004) preserved samples from Lake Rotoiti were shown to G. Cronberg (University of Lund, Sweden) who confirmed the presence of *M. botrys* and *M. aeruginosa* in the samples. It was not possible to distinguish these two species in the recorded results and they have been grouped together in the cell count results. Full descriptions of *M. botrys* and *S. brasiliensis* are given in Chapter 3. Unfortunately images (either drawings or photographs) of *A. torulosa*, *Coelosphaerium* aff. *natans*, *Planktothrix* aff. *planktonica* and *P. galeata* were not taken and for this reason they have not been documented as new records in Chapter 3.

Anabaena and *Microcystis* dominate in Lakes Rotoiti and Rotoehu. During the study period, species from these genera usually accounted for over 90% of the cyanobacteria cells in the samples. Figures 5.14 – 5.19 show the total *Anabaena* spp. and *Microcystis* spp. and total cyanobacteria cell counts for each of the six sample sites. Trends at each site are discussed below.

Table 5.1. List of Cyanobacteria Species Observed in Lakes Rotoiti and Rotoehu Samples

Chroococcales
<i>Aphanocapsa</i> cf. <i>delicatissima</i> W. et G. S. West
<i>Aphanocapsa</i> <i>holsatica</i> (Lemmermann) Cronberg et Komárek
<i>Chroococcus</i> cf. <i>minutus</i> (Kütz.) Nägeli
<i>Coelosphaerium</i> aff. cf. <i>natans</i> Lemmermann
<i>Coelosphaerium</i> sp.
<i>Coelosphaerium</i> cf. <i>kutzingianum</i> Nägeli
<i>Microcystis</i> <i>aeruginosa</i> (Kützing) Kützing
<i>Microcystis</i> <i>botrys</i> Teiling
<i>Microcystis</i> <i>paniformis</i> Komárek et al.
<i>Microcystis</i> <i>protocystis</i> Crow
<i>Microcystis</i> <i>flos-aquae</i> (Wittrock) Kirchner
<i>Snowella</i> cf. <i>lacustris</i> (Chodat) Komárek et Hindák
<i>Sphaerocavum</i> <i>brasiliensis</i> Azevedo & Sant'Anna
Oscillatoriales
<i>Geitlerinema</i> <i>splendidum</i> (Gormont) Anagnostidis
<i>Limnothrix</i> aff. <i>planktonica</i> (Van Goor) Meffert
<i>Lyngbya</i> sp.
<i>Oscillatoria</i> sp.
<i>Planktothrix</i> aff. <i>planktonica</i> (Elenk.) Anagnostidis & Komarek
<i>Planktothrix</i> <i>peronata</i> f. <i>attenuate</i> (Skuja) Anagnostidis & Komárek
<i>Planktothrix</i> sp. A
<i>Pseudanabaena</i> <i>galeata</i> Böcher
<i>Pseudanabaena</i> <i>limnetica</i> (Lemm.) Komárek
Nostocales
<i>Anabaena</i> <i>lemmermannii</i> P. Richt
<i>Anabaena</i> sp. A
<i>Anabaena</i> sp. B
<i>Anabaena</i> cf. <i>affinis</i> Bornet et Flahault
<i>Anabaena</i> cf. <i>torulosa</i> * (Carm.) Lagerheim
<i>Anabaena</i> <i>circinalis</i> Rabenhorst
<i>Anabaena</i> <i>planktonica</i> * Brunnthaler
<i>Anabaena</i> <i>spiroides</i> * Klebahn
<i>Aphanizomenon</i> <i>gracile</i> Lemmermann
<i>Gloeotrichia</i> sp.
<i>Raphidiopsis</i> cf. <i>mediterranea</i> Skuja

*no akinetes seen in sample

Anabaena sp. A - straight species - no akinetes/heterocytes seen in sample.

Anabaena sp. B - coiled species - no akinetes/heterocytes seen in sample.

Okawa Bay: Figure 5.14 – Cyanobacteria counts were low in November with the first bloom occurring at this site in December. The bloom comprised of *Anabaena* spp. dominated by *A. circinalis* and *A. planktonica*. Although there were fluctuations in cell numbers, these two species continued to dominate until 16-02-2004 when there was a sudden decline in all cyanobacterial cell numbers. This was followed by a massive bloom of *M. aeruginosa* and *M. botrys* on the 23-02-2004. *Microcystis* species continued to dominate (although in lower numbers) until late March when *Anabaena* spp. once again became dominant.

Te Weta Bay: Figure 5.15 – Cyanobacteria cell numbers remained low in Te Weta Bay until the end of January when there was a bloom (46 000 cells ml⁻¹). The dominant species was *A. planktonica*. As with Okawa Bay, there was a large decrease in cyanobacteria numbers in mid February. This was followed by an increase in *Microcystis* spp. numbers, with a large peak (440 000 cells ml⁻¹) occurring on 08-03-2004. *Microcystis* spp. remained the dominant species in Te Weta Bay until the end of the study period.

Figure 5.14. Cell counts and levels of microcystins in Okawa Bay (Lake Rotoiti) water samples.

Figure 5.15. Cell counts and levels of microcystins in Te Weta Bay (Lake Rotoiti) water samples.

Hinehupu: Figure 5.16 – This site experienced its first bloom (65 000 cells ml⁻¹) on 01-12-2003. The two dominant species in this bloom were *A. lemmermannii* and *A. circinalis*. From 24-01-2004 until 02-02-2004 there was experienced a further bloom with *A. planktonica* being dominant. Cyanobacterial cell numbers remained low from 02-02-2004 to 01-03-2004 when there was an increase in both *Anabaena* spp. and *Microcystis* spp which continued through until the end of April 2004.

Kennedy Bay: Figure 5.17 – *Anabaena* spp. were recorded at very low levels and *Microcystis* spp. were usually dominant but did not exceed 5 000 cells ml⁻¹ until March when *M. panniformis* was dominant.

Te Pohue: Figure 5.18 - Cyanobacteria cell counts remained low (below 3 000 cells ml⁻¹), except on the 02-05-2004 when they peaked at 7 400 cells ml⁻¹. *Microcystis* spp. were most abundant and there were very low numbers of *Anabaena* spp., with *Microcystis panniformis* dominant..

Otauhu Bay: Figure 5.19 – *Microcystis* spp. were dominant. Cell counts fluctuated but were always under 3 500 cells ml⁻¹. *Anabaena* spp. comprised only a small proportion of the total count.

5.5.1.3 Cell Counts Versus Levels of Microcystins

The correlation between cyanobacterial cell counts and levels of microcystins in all samples is shown in Figure 5.20. This is modified version in Figure 5.20 to show these data without the two extremely high levels of microcystins, as including these tended to skew the trend line and obscured trends at lower levels to be observed. Figure 5.21 shows only a very weak correlation ($R^2 = 0.20$) between cell numbers and levels of microcystins. Occasionally there are high cell counts with low or no microcystins detected. On some occasions cyanobacteria cell counts are low and levels of microcystins are elevated, as highlighted by the red circle in Figure 5.21. To assess whether levels of microcystins correlated with cell counts from certain genera, *Microcystis* spp. and *Anabaena* spp. were plotted separately against levels of microcystins for data from the enclosure experiment (Figures 5.22 and 5.23). There was no correlation between cell numbers of these genera and levels of microcystins (*Anabaena* spp. $R^2 = 0.001$, *Microcystis* spp. $R^2 = 0.01$).

Figure 5.16. Cell counts and levels of microcystins in Hinehopu (Lake Rotoiti) water samples.

Figure 5.17. Cell counts and levels of microcystins in Kennedy Bay (Lake Rotoehu) water samples.

Figure 5.18. Cell counts and levels of microcystins in Te Pohue Bay (Lake Rotoehu) water samples.

Figure 5.19. Cell counts and levels of microcystins in Otatau Bay (Lake Rotoehu) water samples.

Figure 5.20. Cyanobacteria cell counts versus levels of microcystins in all water samples.

Figure 5.21. Cyanobacteria cell counts versus levels of microcystins in all water samples. Two extremely high microcystins values removed. Red circle shows data where elevated levels of microcystins corresponded with low cell counts.

Figure 5.22. Total *Anabaena* spp. cell counts versus levels of microcystins in water samples from the Te Weta Bay enclosure, Lake Rotoiti.

Figure 5.23. Total *Microcystis* spp. cell counts versus levels of microcystins in water samples from the Te Weta Bay enclosure, Lake Rotoiti.

5.5.2 Spiked Recovery Experiments

The trout muscle tissue were spiked with microcystin-LR at levels ranging from 100 to 200 $\mu\text{g kg}^{-1}$ and recoveries ranged from 69 - 84% (average 78%).

5.5.3 Monthly Rainbow Trout Samples

5.5.3.1 Trout Muscle and Liver Analysis - Lake Rotoehu

Microcystins were found in liver and muscle tissue samples in all monthly samples from November 2003 to April 2004 (Figure 5.24). Levels of microcystins were always higher in liver than in muscle tissue. Highest levels of 78.92 $\mu\text{g kg}^{-1}$ for liver were recorded in November 2003 and for the trout muscle tissue of 34.56 $\mu\text{g kg}^{-1}$ in April 2004. The amount of microcystins decreased between December 2003 to March 2004 before increasing again in April 2004. These values are the minimum figures and do not consider the possible 20 - 30% not extractable using the method developed in this study.

The average weekly water levels of microcystins from the three sampling sites is plotted for each month in Figure 5.24. Levels were zero or very low. There is no correlation between levels of microcystins in the water and levels of microcystins in the trout samples.

An estimated daily intake (EDI) was calculated using an adult human weight of 70 kg and ingestion of 300 g of trout muscle tissue. The EDI values for monthly samples from Lake Rotoehu are shown in Figure 5.25. The TDI of 0.04 $\mu\text{g kg}^{-1} \text{ day}^{-1}$ suggested by the WHO (Chorus & Bartram 1999) is also shown on the graph. The EDI in the muscle tissue exceeds this guideline in the samples from November and December 2003 and April 2004. The TDI value is not relevant to liver samples as trout liver is not usually consumed.

The CF's for the Lake Rotoehu rainbow trout are shown in Figure 5.26. During the five month study period the CF of trout varied between 33.37 (December 2003) to 41.60 (March 2004).

Figure 5.24. Levels of microcystins in rainbow trout (*Oncorhynchus mykiss*) muscle tissue and liver, and in the monthly water samples from Lake Rotoehu. The water microcystins level is an average of weekly samples at three locations in lake.

Figure 5.25. Estimated human daily intake of microcystins from Lake Rotoehu based on a 70 kg person ingesting a 300 g serving of rainbow trout (*Oncorhynchus mykiss*). The water microcystins level is an average of weekly samples at three locations in Lake Rotoehu.

5.5.3.2 Stomach Contents of Lake Rotoehu Trout

Stomach contents of Lake Rotoehu trout were varied (Figure 5.27). However, in all monthly samples over 40% of the diet was fish. The majority of these fish were common smelt (*Retropinna retropinna*) (Figure 5.12), with bullies (*Gobiomorphus* sp.) identified in some samples. Molluscs, mainly the gastropod snail (*Physa acuta*), made up a large component of trout diet in December 2003, February and April 2004. One stomach in the April 2004 sample contained over 130 *P. acuta* (Figure 5.11). The stomachs of two trout in February 2004, and one in November 2003 were empty.

Figure 5.26. Condition factor of rainbow trout (*Oncorhynchus mykiss*) received monthly from Lakes Rotoehu and Rotoiti. Bars show one standard deviation.

Figure 5.27. Percentage composition by weight of organic material in stomach contents of Lake Rotoehu rainbow trout (*Oncorhynchus mykiss*).

5.5.3.3 Trout Muscle and Liver Analysis - Lake Rotoiti

Microcystins were detected in all trout muscle tissue and liver samples from the Lake Rotoiti monthly samples (Figure 5.28). Levels in muscle tissue did not varied little been between $8.0 \mu\text{g kg}^{-1}$ (March, 2004) to $12.3 \mu\text{g kg}^{-1}$ (February, 2004). Levels of microcystins varied more widely in liver samples with peaks in December 2003 ($44.5 \mu\text{g kg}^{-1}$) and February 2004 ($46.4 \mu\text{g kg}^{-1}$).

No microcystins were recorded in weekly water samples until February 2004 (Figure 5.28). The highest level was recorded in March 2004 ($62.5 \mu\text{g l}^{-1}$). Water microcystins levels do not correlate with trout microcystins levels.

Figure 5.29 shows the EDI for Lake Rotoiti trout. On three occasions (December 2003, February and April 2004) levels of microcystins in muscle tissue were just above the TDI recommended by the WHO.

The Condition Factor (CF) for the Lake Rotoiti rainbow trout is shown in Figure 5.26. During the five month study period the CF of trout gradually decreased from 46.5

(December 2003) to 38.5 (April 2004). The CFs of trout caught during this study were compared to data compiled by Fish and Game for other trout caught in Lake Rotoiti during the equivalent six month study period (R Pitkethley, Fish and Game, unpublished data). Using a student t-test a significant difference was found between the mean CF of the two samples ($n_1 = 58$, $n_2 = 21$, $t = 4.64$, $p < 0.05$). This result may reflect differences in measurement methods and small sample sizes. It could also be due to the diversity of habitats and feeding sources available in Lake Rotoiti.

5.5.3.4 Stomach Contents of Trout from Lake Rotoiti

With the exception of December 2003 the majority of the stomach contents of the Lake Rotoiti trout were fish (Figure 5.30). In January 2004, both smelt (*Retropinna retropinna*) and bully (*Gobiomorphus* spp.) were identified. In all other months only smelt could be identified. In December 2003 insects were present in the stomach contents, but a large portion of the material could not be identified. Two trout also had empty stomachs in this month. In contrast to Lake Rotoehu, molluscs were not identified in any trout stomachs.

Figure 5.28. Levels of microcystins in rainbow trout (*Oncorhynchus mykiss*) muscle tissue and liver, and in the monthly water samples from Lake Rotoiti. The water microcystins level is an average of weekly samples at three locations in lake.

Figure 5.29. Estimated human daily intake of microcystins from Lake Rotoiti based on a 70 kg person ingesting a 300 g serving of rainbow trout (*Oncorhynchus mykiss*). The water microcystins level is an average of weekly samples at three locations in Lake Rotoiti

Figure 5.30. Percentage composition by weight of organic material in stomach contents of Lake Rotoiti rainbow trout (*Oncorhynchus mykiss*).

5.5.4. Enclosure Experiment

5.5.4.1 Microcystins Levels in Water Samples and Cell Counts

Levels of microcystins in the enclosure water varied between $0 \mu\text{g l}^{-1}$ and $38.3 \mu\text{g l}^{-1}$ as shown in Figure 5.31. The depth at which the highest levels of microcystins were recorded varied from day to day - for example, on 13-03-2004 these at the surface, but on 26-03-2004 at 1 m depth.

Average levels of microcystins in upper samples - surface morning and afternoon and 1 m depth (afternoon only), and in the lower samples - 2 and 3 m depths were calculated. A smoothed microcystins level was calculated for the upper and lower samples using the following formula; smoothed value = (microcystins on day)/2 + (microcystins previous day)/4 + (microcystins next day)/4 (Figure 5.32). This formula is applied in engineering to smooth data curves and eliminate the effects of measurement errors or random variations in the data to highlight general trends.

Between 07-03-2004 to 18-03-2004 the microcystins were always higher in upper samples. Between the 19-03-2004 to 28-03-2004 microcystins were higher in lower samples and from the 26-03-2004 to the completion of the study levels were higher in upper samples.

Figure 5.31. Levels of microcystins in water samples - enclosure experiment (Lake Rotoiti).

Cyanobacteria cell count data for each sample taken during the enclosure experiment are listed in Appendix 13. Figure 5.33 shows distribution of cells with depth.. Highest cell counts ($69\,000\text{ cell ml}^{-1}$) were recorded in the surface sample on 08-03-2004 (afternoon). On 18-03-2004 a high cell count was recorded at 2 m depth and on 19-03-2004 at 3 m depth (Figure 5.33). *Microcystis aeruginosa* and *Anabaena planktonica* were present in almost all samples and made up the majority of cells in counts. *Sphaerocavum brasiliensis* was also present in high numbers in many samples.

Average levels of microcystins in the five samples (surface am, pm, and 1, 2, 3 m depths pm) from each day were calculated. Figure 5.34 plots this average together with the average of the total cyanobacteria cell counts per day. The proposed recreational Australian guideline of $8\text{ }\mu\text{g l}^{-1}$ (National Health and Medical Research Council 2004) and the ENVBOP guideline for issuing a health alert ($15\,000\text{ cell ml}^{-1}$) is also shown. On 18-03-2004 and 19-03-2004 the number of cells ml^{-1} exceeded the ENVBOP health alert but on these days microcystins were below the proposed Australian guideline. On six days, the microcystins exceeded the proposed Australian guideline and on four of these occasions cell counts were below the ENVBOP health alert. There was no clear correlation between levels of microcystins and cell counts.

Samples collected from Te Akau Point on 15-03-2004 and 26-03-2004 contained microcystins levels of 414 and $214\text{ }\mu\text{g l}^{-1}$ respectively. The campground sample collected on 26-03-2004 contained $50\text{ }\mu\text{g l}^{-1}$.

Figure 5.32. Smoothed average levels of microcystins in upper (surface and 1 m) and lower (2 and 3 m) levels during enclosure experiment.

Figure 5.33. Total cyanobacteria cell counts for each sample during enclosure experiment (Lake Rotoiti).

Figure 5.34. Average cyanobacteria cell counts per day and average levels of microcystins per day during enclosure experiment. Bars show maximum and minimum values for each day.

5.5.4.2 Levels of Microcystins in Rainbow Trout Samples from within the Enclosure

Of the 80 trout released into the enclosure 28 were recaptured. Three “wild” trout were also caught in the enclosure. The number of trout caught on each date is listed in Table 5.2. Trout caught on the same day were pooled for sample analysis. When only one or two “released” trout were caught these were pooled with the “released” trout caught on the preceding or following day, with the aim of obtaining pooled sample of three to five trout (see Table 5.2 for details of combined catches).

No microcystins were found in the three trout from the hatchery retained to check for background levels of microcystins. Microcystins were found in all muscle tissue and liver samples from trout caught during the enclosure experiment. Levels of microcystins in trout samples are plotted together with the average smoothed levels of microcystins (Section 5.5.4.1) in the water samples (Figure 5.35). Levels of microcystins were also analysed for two of the three “wild” trout caught in the enclosure experiment (Figure 5.35). Levels in the “wild” trout do not differ markedly from those found in enclosure trout caught on similar dates.

Table 5.2. Capture date and number of rainbow trout (*Oncorhynchus mykiss*) caught during enclosure experiment.

Catch Date	Number of Trout Caught
08-03-04	3 (Control – Fish & Game Hatchery)
10-03-04	5
13-03-04	3
16-03-04	4
19-03-04	3
19-03-04	1 "wild" trout
20-03-04	1 "wild" trout
23-03-04	1 (pooled with trout caught on 24-03-04)
24-03-04	4
25-03-04	3
25-03-04	1 "wild" trout
26-03-04	2 (pooled with non "wild" trout caught on 25-03-04)
27-03-04	3

Figure 5.35. Levels of microcystins in rainbow trout (*Oncorhynchus mykiss*) muscle tissue and liver samples and in water samples from enclosure experiment. Water microcystins levels are an average of five samples per day. Only two of the three "wild" trout were analysed for microcystins.

Liver microcystins levels were always higher than those recorded in muscle tissue samples. From day 8-3-2004 to 18-03-2004 the liver microcystins levels increased to a maximum value of $46.6 \mu\text{g kg}^{-1}$. From day 19-3-2004 to 25-3-2004 the levels decreased to $23.7 \mu\text{g kg}^{-1}$, before increasing again to $45.4 \mu\text{g kg}^{-1}$ in the final sample (day 21). Muscle tissue microcystins levels increased from days 1 to 5 to a maximum of $22.7 \mu\text{g kg}^{-1}$. Levels then decreased and stayed between $7.3 \mu\text{g kg}^{-1}$ and $12.2 \mu\text{g kg}^{-1}$ for the remainder of the enclosure experiment.

There was no clear correlation between levels of microcystins in the water and those in the trout muscle tissue and liver samples. However, lower levels of microcystins in the water and the sudden rise between 23-03-2004 to 26-03-2004 was possibly the cause of the fall and rise in liver microcystins values over the period 23-03-2004 to 27-03-2004.

Figure 5.36 shows the EDI for enclosure trout. Five of the seven muscle tissue samples were above the TDI for microcystins.

5.5.4.3 Condition Factors of Rainbow Trout

The CF of released trout increased slightly to 45.6 during the first two days of the enclosure experiment and then gradually decreased to 37.1 on 25-03-2004 (Figure 5.37).

5.5.4.4 Stomach Contents of Rainbow Trout

The percentage composition by weight of stomach contents of the trout from the enclosure experiment is shown in Figure 5.38. For days 1 to 11 of the experiment a large portion of the trouts' diet was made up of insects. The dominant insect was the water boatman (*Corixidae* sp.). Smelt (*Retropinna retropinna*) and bullies (*Gobiomorphus* spp.) were absent or only present as a small proportion of the stomach contents. After 16 days, the trout diet contained greater quantities (30 - 60%) of fish. Both smelt and bully were identified. Stomach contents of 3 "wild" trout are also shown in Figure 5.38. Fish (including goldfish (*Carassius auratus*), smelt and bullies) made up 58% of the stomach content of these trout.

Figure 5.36. Estimated human daily intake (EDI) of microcystins from enclosure experiment based on a 70 kg person ingesting a 300 g serving of rainbow trout (*Oncorhynchus mykiss*). The water microcystins level is an average of 5 samples per day.

Figure 5.37. Condition Factor of rainbow trout (*Oncorhynchus mykiss*) caught during enclosure experiment. Values are averages for each pooled group. The data on the 08-03-2004 is from the trout retained to check background levels of microcystins. Bars show one standard deviation

Figure 5.38. Percentage composition by weight of stomach content of enclosure and “wild” rainbow trout (*Oncorhynchus mykiss*).

5.5.4.5 Levels of Microcystins in Mussels

Levels of microcystins measured in the enclosure mussels are plotted in Figure 5.39 together with a smoothed surface water microcystins level (calculated by taking the average am and pm surface samples for each day and applying the smoothing formulae given in Section 5.5.4.1). Only surface water samples were used as the mussels were positioned just below the surface.

After four days in the enclosure the mussels began to accumulate microcystins. Initially this accumulation was gradual with only $4.5 \mu\text{g kg}^{-1}$ recorded after five days and $4.6 \mu\text{g kg}^{-1}$ after seven days. Over the next two days microcystins in the mussels increased dramatically to reach a peak of $65.1 \mu\text{g kg}^{-1}$. Levels of microcystins in the water were much lower than those in mussels. With the exception of the first six days, the increase and decrease in levels of microcystins in mussels approximately tracked the trend in surface water of microcystins levels with a two day delay.

Figure 5.39. Levels of microcystins in freshwater mussels (*Hydriddella menziesi*) and water during enclosure experiment. The mussels took approximately six days to acclimatise to the new environment.

5.5.4.6 Water Temperature

Afternoon surface water temperatures were always higher than the morning surface temperature (Figure 5.40). In the afternoon, when samples were also taken at depth, the temperatures always decreased with depth. At times differences between surface and 3 m depth reached 3°C. The temperatures increased over the first three days of the enclosure experiment and then generally decreased over the remaining 18 days to the end of the experiment. The surface temperatures on 18-03-2004 were above the general trend and this peak was attributed to a fine calm day following cooler cloudy weather. Surface temperature appeared to be quite sensitive to the cloud cover (or solar radiation) and wind speed. A significant drop in temperatures between the 22-03-04 and 25-03-04 was related to the moderate to fresh south west wind blowing over this period. Water temperature did not generally appear to have a significant effect on cell numbers with the exception of the 18-03-04 when high cell counts did correspond with an increase in water temperature. Wind also had an effect on cell counts with calm days tending to have higher cell counts. This is probably due to the wind causing the buoyant

cyanobacteria cells to accumulate on the leeward side of the bay away from the mid-bay sampling location.

Figure 5.40. Water Temperatures: 0900 h and 1400 h (NZ standard time) during enclosure experiment, Te Weta Bay, Lake Rotoiti.

5.5.4.7 Dissolved Oxygen

Dissolved oxygen (DO) levels in the water column recorded are shown in Figure 5.41. At surface and 1 m and 2 m depths, DO levels did not vary greatly during the duration of the enclosure experiment with the levels always higher in the afternoon than in the morning. Dissolved oxygen levels at the 3 m depth changed more markedly on a daily basis, sometimes changing by 2.3 mg l^{-1} from day to day. Readings at this depth may have been influenced to some extent by benthic weed. Recorded DO levels do not indicate oxygen depletion at this site in Lake Rotoiti.

Figure 5.41. Dissolved Oxygen: 900 h and 1400 h (standard time) in Te Weta Bay enclosure, Lake Rotoiti.

5.6 Discussion

5.6.1 Changes in Cyanobacteria Abundance and Composition

In Lake Rotoiti the cyanobacteria species abundance and composition varied during the six month study period. In the Te Weta Bay and Okawa Bay sites *Anabaena* spp. dominated cyanobacteria blooms until February when *Microcystis* spp. occurred in increasing numbers. A similar trend (although with lower cell numbers) was also seen at the Hinehopu site. Of particular interest was the decline in cyanobacteria cell numbers in mid-February. This was followed by large blooms of *Microcystis* spp in Te Weta Bay and Okawa Bay.

Reasons for the switch in species dominance were not investigated in this study. However, the change is likely to be due to variations in environmental and limnological variables. For example, *Anabaena* species fix nitrogen and *Microcystis* species do not. Therefore variations in nutrient levels may influence species abundances. Other factors such as temperature tolerance, growth rates and light tolerance are also likely to have an effect. The reason for the decline in all cyanobacteria in mid-February is unknown.

In a study in Grangent Reservoir (Loire, France) Latour and Giraudet (2004), noted that colonies of *Microcystis aeruginosa* remained on the sediment as vegetative cells during winter and constituted an inoculum for the following year. Thus benthic *M. aeruginosa*, play an important role in the cycle of this species. It is possible that a similar situation occurs in Lake Rotoiti and that an unknown environmental trigger results in this species again becoming dominant in the water column. It is also interesting to note that Sirenko (1972) found that fewer colonies survive in oxygenated shallow water compared with Brunberg (1995) who noted that anoxic conditions found in deeper water seem to prolong the survival of *Microcystis* in the sediment.

There were also variations in cell counts between locations within Lake Rotoiti. For example, cell counts were often lower at the Hinehopu site. This can be explained by the limnology and geographic and environmental variables. Both Te Weta Bay and Okawa Bay are sheltered, shallow, nearly enclosed bays, whereas Hinehopu is an exposed site at the eastern end of the lake. Factors such as wind direction are more likely to affect cell counts there. Cyanobacteria accumulation can be a result of their

positive buoyancy and wind driven hydrodynamics, with downwind (leeward) shorelines and embayments being likely sites for scum formation (Hutchinson & Webster 1994).

Phytoplankton samples were collected in the enclosure daily at 0900 h and 1400 h. Highest cell counts were usually recorded at 1400 h at the surface, but high cell counts were sometimes recorded at lower depths. These results are not unexpected as cyanobacteria are able to regulate their buoyancy. This is achieved by using mechanisms that collapse gas vacuoles or result in gas vesicle synthesis (Oliver & Ganf 2000). However, the most frequent method of buoyancy regulation is by adjusting cell density. Usually cyanobacteria will accumulate at the surface early in the morning and evening (Wallace et al. 2000). During daylight, through photosynthesis the cyanobacteria accumulate carbohydrates which increase the cell density, thus the cells sink. As this carbohydrate store is used up they will often rise to the surface again. It is possible therefore that the sampling times in this study lay outside the times of highest surface density and this may explain high cell counts recorded at other depths. To obtain a true reflection of the concentration of cyanobacteria in the water column, depth integrated samples should be used in regular sampling programmes.

Cyanobacteria cell numbers remained relatively low in Lake Rotoehu and dominated by *Microcystis*. This is in contrast to previous summers when severe blooms were reported there (Wilding 2000). Between 1995 and 1999 a gradual increase in cell concentrations was recorded. Large numbers of *Microcystis* spp. were first noted in January 1997 and became the autumn dominant algae in 1998. Prior to this *Anabaena* spp. had dominated (Wilding 2000). The reason for the low cell concentrations in 2003/4, might be related to nutrient levels or environmental factors, but this was not investigated.

5.6.2 Microcystins in Phytoplankton

Oral administration of microcystin-LR to mice has demonstrated a 24 h LD₅₀ of between 5 mg kg⁻¹ (Fawell et al. 1999) and 10.9 mg kg⁻¹ (Yoshida et al. 1997). These studies led the World Health Organisation (WHO) to develop drinking water guidelines for total microcystins. The guideline value was set at 1 µg l⁻¹. In Germany, recreational guidelines state that if cyanobacteria are found at a site, users of the water body must be

warned and receive information on cyanobacteria. The German guidelines recommend closure at a total microcystins concentration above $100 \mu\text{g l}^{-1}$ (Frank 2002). Recreational guidelines are currently being developed for New Zealand.

The National Health and Medical Research Council of Australia has recently released the document *Guidelines for Managing Risks in Recreational Water* (National Health and Medical Research Council 2004), which includes a section on cyanobacteria. These guidelines reason that any toxin guidelines should be based primarily upon risk of exposure to known toxins via ingestion. This is because potential adverse health effects from dermal exposure are not as significant as from ingestion of toxic cells. Only guideline limits for microcystins of $8 \mu\text{g l}^{-1}$ are given in the Australian document, as there was insufficient toxicology data for the other cyanotoxins.

The Australian recreational guideline values are based on the lowest observed effect levels for microcystin-LR of $100 \mu\text{g kg}^{-1}$ body weight/day derived from a 44-day study in pigs (Kuiper-Goodman et al. 1999). The Guideline value for microcystins is calculated on the accidental swallowing of approximately 100 ml of lake water per day. The Guideline also acknowledges that because toxin testing is not widely available, cell counts (e.g. 5 000 – 40 000 cells ml^{-1} *Microcystis aeruginosa* triggers further testing) are likely to be used as a first measure, and this would be followed by other measures such as toxin testing.

Environment Bay of Plenty and Pacific Health, which are responsible for the issuing of health warnings with regard to cyanobacteria concentrations in the Rotorua Lakes, base their warnings on cell counts and currently do not use cyanotoxin levels. When concentrations reach $>15,000$ cells ml^{-1} the water is considered unsuitable for general contact recreation, and warnings are issued.

Microcystins reached high levels in both Te Weta Bay and Okawa Bay from 23 February 2004 to the end of the study. Before this date, microcystins were not detected at significant levels. Figure 5.14 shows that Okawa Bay was above the Australian recreational microcystins guideline on three sampling occasions, Te Weta Bay on seven, and Hinehopu on one sampling occasion. However, Okawa Bay was above the ENVBOP cell concentration guideline on 14 sampling occasions, Te Weta

Bay for 12, and Hinehopu on three sampling occasions. This demonstrates that relying on cell counts alone does not necessarily give an indication of levels of microcystins.

Only one sample from Lake Rotoehu (Te Pohue Bay – 2 May 2004) contained levels of microcystins ($23.5 \mu\text{g l}^{-1}$) above the proposed Australian recreational guideline. On no occasion did cell counts exceed the ENVBOP cell concentration guideline.

The high levels of microcystins in the scum samples collected at Te Akau point and in the water near the Lake Rotoiti campground illustrate that potentially dangerous levels of microcystins can occur rapidly due to the formation of wind accumulated scum.

It is not possible to establish unambiguously which species are responsible for toxin production without culturing individual strains or using sensitive detection methods with single cell/filament picking. However, a comparison of species composition and levels of microcystins demonstrated that when very high levels of *Microcystis* spp. (in particular *M. aeruginosa* and *M. botrys* in Rotoiti and *M. panniformis* in Rotoehu) are found, there are higher levels of microcystins. *Anabaena* spp. are not associated with high levels of microcystins. The identification of *Microcystis* species can be very problematic as separate species have similar morphology at varying stages in their lifecycle. A number of *Microcystis* species were observed in the study, including *M. aeruginosa*, *M. botrys*, *M. panniformis*, *M. protocystis* and *M. flos-aquae*. Several other species from different genera with morphological similarities to *Microcystis*, e.g. *Aphanocapsa* spp. and *Sphaerocavum brasiliensis* were also identified. The unequivocal identification of *Microcystis* spp. to species level can be difficult and time consuming. However, identification of potentially toxic genera does not require such a high skill level. Further work is required to establish which species in these lakes produce microcystins. Further assessments correlating cell counts and levels of microcystins could be made.

Comparison of all cell count and microcystins data showed that there is usually no correlation these, even when cell count data are compared at a generic level. This could be due to different species within the genus being responsible for toxin production or to genetic variability within the species. That is, some strains have the ability to produce toxin and others do not. Environmental triggers, such as increase in temperature (Rapala

1998), may also increase the production of microcystins. Additionally, microcystins may be produced only at specific stages during the life-cycles of some species. The lack of correlation could also be explained by continual death of cells, which subsequently lyse and release toxin into the lake, but death will not necessarily be at a constant rate. This may explain why elevated levels of microcystins can coincide with low cell numbers. The data suggest that cell counts alone are not a reliable method for assessing potential health risk from microcystins. Testing for microcystins should be incorporated into routine monitoring programmes where possible.

The presence of cyanobacteria species known to produce cyanotoxins other than microcystins in Lakes Rotoiti and Rotoehu means that tests for other cyanotoxins and/or toxicity should not be ignored until it is known which species are responsible for toxin production in the Rotorua Lakes and generally New Zealand. Cyanobacteria species composition and toxin types/levels can change during a cyanobacterial bloom (Baker et al. 2002) suggesting the need for regular monitoring.

Microcystins occur primarily intracellularly with the majority of microcystins released into the waterbody when cyanobacteria cells are decaying. Once released into the water body the toxins can persist for a few days to a few weeks (Welker et al. 2001). Thus, even after a bloom has subsided, microcystins may still be present. Monitoring should continue for a number of weeks following the subsidence of a bloom.

Given the current levels of microcystins in Lakes Rotoiti and Rotoehu, acute intoxication with microcystins during human recreational activity is unlikely. Chronic liver damage from microcystins is a potential risk, especially from repeated exposure. Of particular concern is the use of Lake Rotoiti waters by residents for bathing/showering and drinking.

5.6.3 Trout Samples and Microcystins

The levels of microcystins in the trout liver samples were higher than the levels detected in the muscle tissue samples on all occasions. This result is consistent with other studies (Mohamed et al. 2003) and is expected, as the liver is the organ most affected by microcystins.

Microcystins were recorded in trout that were ranging through both lakes, at times when no, or very low levels, of microcystins were present in the lake water. This could indicate that the water samples were not indicative of circumstances in the lake as a whole, or more likely that there is a persistence of microcystins in the food sources of trout (See Section 5.6.6).

Detection of microcystins in trout muscle tissue from Lake Rotoehu in November 2003 and from Rotoiti in December 2003 was not expected as cyanobacterial blooms had not been reported in the previous months. It is possible that some *Microcystis* was overwintering in the sediment and because part of the trout diet comes from benthic grazers this caused bioaccumulation of microcystins. Ihle et al. (2004) have detected microcystins in benthic *Microcystis* spp.

In an investigation of the biochemistry of microcystins in rainbow trout, Tencalla & Dietrich (1994) observed that the absorption of microcystin-LR from the gastrointestinal tract into the blood occurred rapidly. However, the intestinal tract represented an important barrier and less than 5% of the applied microcystins reached the blood. Once in the blood, the microcystin-LR was quickly transported to the liver.

In the enclosure study, levels of the microcystins in trout muscle tissue decreased after five days and in the liver after 12. Tencalla (1997) noted that the decrease of microcystins concentrations in the liver occurred more slowly than uptake, and that microcystins could still be detected in the liver three days after exposure. Microcystins are known to be metabolised by Zebra Fish (*Danio rerio*) (Wiegand et al. 1999). Enzymes known as soluble glutathione S-transferase is active in detoxification (Pflugmacher et al. 1998). It is likely that at least some of the decrease in the levels of microcystins in the enclosure trout is due to detoxification process.

Further investigation of variation in microcystins in individual trout, rather than the pooled samples used in this study, could prove useful. This information could be used to assess the sample size required to obtain a true reflection of levels of microcystins in trout populations. The decrease in the levels of microcystins in the muscle tissue and liver might be related to condition factor and stomach contents and investigating this requires the study of individual samples.

5.6.4 Human Health Risk from Trout Consumption

Results confirm that microcystins do accumulate and persist in muscle tissue and liver of rainbow trout in Lakes Rotoiti and Rotoehu. There is a potential human health risk from consumption of trout muscle tissue, as 50% (Rotoehu), 60% (Rotoiti) and 71% (enclosure) of the muscle tissue samples were above the Total Daily Intake (TDI) of $0.04 \mu\text{g kg}^{-1}\text{day}^{-1}$ recommended by the WHO (calculations based on a 70 kg person eating 300 g per day). Using levels found in muscle tissue sampled in this study and ignoring covalently bound microcystins (see Section 5.6.5), the health risk from microcystins consumed while eating muscle tissue from these lakes is very low because trout is not usually consumed every day.

Using the results of this study, a 70 kg person can safely eat a 300 g serving of trout every 3.6 days. This calculation used a muscle tissue concentration of $34.5 \mu\text{g kg}^{-1}$, (the highest value recorded– Rotoehu, April 2004), the TDI of $0.04 \mu\text{g kg}^{-1} \text{day}^{-1}$ (WHO), a 70 kg human and a trout meal size of 300 g. If 70 kg humans eat trout at less than this rate, they will consume levels of microcystins below the WHO standards and there is probably no need for concern. The TDI is a recommended limit for a healthy adult. Children, elderly and sensitive individuals may be at a higher risk. The levels of microcystins are significantly higher in trout liver, but current advice given by Fish and Game New Zealand and local district health boards to gut and thoroughly wash the fish before eating would substantially reduce any potential health risks. Microcystins are heat stable and therefore would not be denatured by cooking (Harada et al. 1996).

5.6.5 Covalently Bound Microcystins

The present study investigated and reported levels of only free ADDA-containing microcystins in trout and mussels. It is likely these will also contain conjugates of microcystins which are produced during detoxification (Pflugmacher et al. 1998), and covalently bound microcystins. Covalent binding of microcystins can occur within hours (Williams 1997). It involves the formation of a covalent linkage between the microcystins and PP-1 and PP-2A enzymes which is the result of a secondary in vitro interaction (Craig et al. 1996, MacKintosh et al. 1990, Runnegar et al. 1995). The presence of conjugates was not tested in this study, although it is likely that they would be detected using the ADDA-ELISA and therefore included in our results. Chemical methods such as LCMS are required to distinguish conjugates from other microcystins.

Few studies have addressed the issue of covalently bound microcystins in freshwater organisms. The levels of covalently bound microcystins (Table 5.3) vary dramatically and depend on the study species, variants of microcystin used, methodology used to test for covalent microcystins, the method of administration to the organism and the length of time following administration.

Table 5.3. Summary of published studies recording levels of covalently bound microcystins in freshwater organisms.

Species (Organ)	Reference	Percentage of Total Microcystins Detected That is Covalently Bound
Atlantic Salmon (Liver)	Williams et al. (1997)	76%
Mussel - <i>Mytilus edulis</i>	Williams et al. (1997)	99.9%
Rainbow trout - (<i>Oncorhynchus mykiss</i>)	Tencalla & Dietrich (1997)	63%
Cypress Island Dungeness Crab Larvae	Williams et al. (1997)	Covalently bound microcystins 10,000 x higher than free microcystins
Zebra mussel - <i>Dreissena polymorpha</i>	Pires et al. (2004)	38%

In Zebra mussels (Pires et al. 2004) only low amounts of covalently bound microcystins could be detected in the first week of exposure. In the second week, covalently bound microcystins increased dramatically and accounted for approximately 62% of total microcystins. Similarly, Williams et al. (1997) found that in the marine mussel (*Mytilus edulis*) almost all the microcystins existed as a covalent complex after three days. These results may explain why levels of microcystins in enclosure trout decreased after five days in muscle tissue and 12 days in the liver. Over time, more microcystins may have become covalently bound and were not able to be extracted and detected. The ADDA-ELISA used in this study should detect covalently bound microcystins as they would still contain the ADDA molecule. However, it is likely that very little of the covalently bound microcystins would have been extracted using the methods applied.

There is very little published information on the toxicity and bioavailability of covalently bound microcystins complexes and also on whether there are enzymes capable of reversing the covalent linkage and “freeing” the toxic microcystins.

It is recommended that future studies assess levels of covalently bound microcystins in Lakes Rotoiti and Rotoehu rainbow trout and attempt to establish whether the total microcystins burden in the trout is a potential health risk to human consumers. If covalently bound microcystins are found in large quantities there is also a need to investigate the toxicity and bioavailability of such microcystins. The setting of guideline quantities of trout that can be safely consumed will not be fully relevant until these data are obtained.

5.6.6 Effect of Diet on Levels of Microcystins in Trout

Laboratory studies (Tencalla et al. 1994) indicated that the main route of uptake for microcystins in rainbow trout is the gastro-intestinal tract and that only very small amounts are taken up through skin or gills. Thus to understand how the microcystins might be bio-accumulating in Lakes Rotoiti and Rotoehu trout an attempt was made to assess dietary composition.

Studies have shown that gastric emptying in rainbow trout is between 6 – 18 h (Grove et al. 1978) thus stomach content analysis gives some indication of diet prior to capture.

Hatchery trout were fed on formulated feed pellets. Prior to the enclosure experiment the length of time taken for them to adapt to feeding in the lake environment was uncertain. Stomach analysis showed that hatchery trout released into the enclosure began feeding within two days. However, for the first 16 days the diet of released trout was quite different to that of free-roaming trout in Lake Rotoiti. A large portion of their diet was made up of insects and mostly water boatmen (*Corixidae* sp.). It is likely that the water boatmen have themselves accumulated microcystins as they feed on algae and minute aquatic organisms. It is possible that different organisms accumulate different quantities of microcystins and the bioavailability of the microcystins may determine how much is transferred to the trout.

The results of the stomach analysis infer that microcystins from phytoplankton accumulate in many different aquatic organisms. The different feeding modes of these organisms may be one reason why microcystins were detected even when cyanobacterial blooms were absent. Benthic grazers might accumulate microcystins from the sediment during winter while cyanobacteria are over-wintering. Further research investigating microcystins and their transfer through the Rotorua lakes food webs would throw further light on this issue. Christoffersen and Burns (2000) found that microcystins were toxic to some indigenous zooplankton, indicating that it is not just the transfer of microcystins through the food web that is important but also the effect of these cyanotoxins on aquatic life.

5.6.7 Mussels and Microcystins

Mussels accumulated microcystins at a slow rate for the first six days of the enclosure experiment. This is in contrast to other studies which have shown that freshwater and marine mussels accumulate microcystins rapidly (Yokoyama & Park 2003, Amorim & Vasconcelos 1999). The mussels used in this experiment were from Lake Rotoma sediment, and transferred to Lake Rotoiti within 12 hours. The initial low accumulation rate suggests mussels required several days to acclimatise to this new environment and to begin feeding normally. After seven days the mussels appeared to commence normal feeding and there was a rapid increase in microcystins in their tissue. This followed the trend of the water microcystins levels which increased two days previously (see Figure 5.39). Laboratory studies on levels of microcystins in marine mussel *Mytilus galloprovincialis* (Vasconcelos 1999) found that when they were placed in an environment free of microcystins there was a 50% decrease in the amount of detectable toxin in the mussels within two days. Yokoyama & Park (2003) investigated the depuration of microcystin-LR in the freshwater bivalve *Unio douglasiae* and demonstrated that microcystins are removed faster at higher temperatures. Because of the change in levels of microcystins in water in the enclosure the degree to which the rapid change in microcystins levels in mussels was due to increase in water microcystins levels or to resumption of normal feeding habits is not clear.

Because of the rapid depuration of microcystins from mussel tissue, freshwater mussels will be poor indicators of the accumulated levels of microcystins at any one location

over an extended period of time. However, the enclosure results show that it may be possible to correlate the toxin levels in mussels with levels in the water approximately two days prior to the collection.

Because mussel microcystins concentrations were higher than in lake water, *Hydriddella menziesi* may be useful as an early warning organism. Their higher levels of microcystins would be easier to detect than in water samples with low levels of microcystins which could be near the limit of quantification of some detection methods. Further studies in controlled laboratory-type situations in which mussels were removed from high microcystins environments and placed in ones free of microcystins would provide valuable information on depuration rates. Controlled experimental work may also assist in developing a method using *H. menziesi* as an early warnings system.

5.7 Recommendations

5.7.1 Research Recommendations

- *Establish unambiguously which species are responsible for the synthesis and release of microcystins.*
- *Investigate the fate of microcystins in water column and sediment.*
- *Investigate levels of microcystins and feeding patterns of organisms ingested by trout.*
- *Develop methods of analysing for covalently bound microcystins and to assess their toxicity and bioavailability. Until this work is completed guidelines for safe levels of trout consumption cannot be stated precisely.*
- *Conduct controlled studies investigating the value of *H. menziesi* in monitoring programmes.*

5.7.2 Management Recommendations

- *Monitor levels of microcystins and species composition of blooms in Lakes Rotoiti and Rotoehu.*
- *Inform the public of potential dangers.*
- *Test for microcystins as part of the regular phytoplankton monitoring programme.*
- *Monitor trout over time to track levels of microcystins in muscle tissue during periods of both cyanobacterial bloom and low cell counts.*
- *Provide clear, unambiguous health information to enable the public to make informed decisions regarding consumption of trout (the quantities that can be safely eaten should be given) based on the best information available at the time the health information is issued.*
- *Issue warnings concerning consumption of *H. menziesi*.*

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6. Thesis Conclusions

Conclusions are detailed at the end of each Chapter of this thesis. This section collates and summarises major conclusions highlighting important issues and making recommendations for further research.

A total of 56 different cyanobacteria species were recorded. These included new records of both dominant and sub-dominant species. Cyanobacterial blooms were recorded in 18 lakes where they had not previously been reported. When the results from this study and a study in 1987 are combined a total of 51 lakes are now known to have experienced cyanobacterial blooms or scums in New Zealand. It is likely that this number will increase with further surveys. It was found that the species composition of cyanobacterial blooms has changed from predominantly *Anabaena flos-aquae*, *A. spiroides* in the 1970s and 1980s to *Anabaena planktonica*. Currently *Microcystis aeruginosa* has remained a constantly dominant species. Eight bloom forming cyanobacteria species not previously identified in New Zealand (*A. planktonica*, *Cylindrospermopsis raciborskii*, *M. botrys*, *M. panniformis*, *M. ichthyoblabe*, *M. wesenbergii*, *Sphaerocavum brasiliense* and *Planktothrix peronata* f. *attenuata*) were found to be dominant or co-dominant in at least some cyanobacterial blooms.

Anabaena planktonica and *C. raciborskii* appear to be highly adaptive, to establish easily and in the case of *C. raciborskii* produce cyanotoxins. Thus they are a threat to water quality and human and animal health in some situations. It is likely that the known distribution of both of these species will increase.

Cyanotoxins produced in New Zealand were found to be microcystins, nodularin, cylindrospermopsin, anatoxin-a and saxitoxins. These have all been found in other parts of the world. In New Zealand species of *Microcystis*, *Anabaena*, *Cylindrospermopsis*, *Nostoc*, *Planktothrix*, *Oscillatoria* and *Phormidium* can produce cyanotoxins. The widespread distribution of these species, and in some locations high levels of microcystins, indicate that there is potential for a significant threat from cyanotoxins to human and animal health. When these genera are present in waterbodies (especially in high concentrations) cyanotoxins are likely to be produced. When waterbodies are used as sources of drinking water by humans or stock, or when recreational activities take

place on or in them, there must be careful monitoring and management programmes in place. Further studies to establish which species produce each specific cyanotoxin are essential and would assist in the management of cyanobacteria in New Zealand.

The in-depth study of Lakes Rotoiti and Rotoehu revealed that cyanobacteria species and microcystin levels can vary over time during a bloom event. Total cyanobacteria cell counts were found to be a poor indicator of microcystin levels in the water samples, indicating that testing for microcystins should be part of any regular phytoplankton monitoring programme.

It was confirmed that microcystins accumulate and persist in both muscle tissue and liver of rainbow trout (*Oncorhynchus mykiss*) in Lakes Rotoiti and Rotoehu. While the levels of microcystin in the muscle tissue were at times found to exceed Total Daily Intakes recommended by the World Health Organisation, the health risk is assessed as very low unless large quantities of trout are consumed regularly. However, because the present study did not measure covalently bound microcystins, the health risk could be greater than currently indicated and further research to establish the significance of covalently bound microcystins in rainbow trout muscle tissue is recommended. Freshwater mussels (*Hyridella menziesi*) were also found to accumulate microcystins.

The research carried out in the present study has successfully answered the objectives outlined in Chapter 1. It has added considerably to knowledge of species diversity and distribution, changes in composition of cyanobacterial blooms, cyanotoxin types and species responsible for their production, and accumulation of cyanotoxin in selected freshwater organisms. It has provided direction for further monitoring and research which will be essential in New Zealand's efforts to address the problems associated with the occurrence and proliferation of toxic cyanobacteria.

Appendix 1. Locations of Sampling Sites 2001 - 2004

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Appendix 1a. Key for Locations of Sampling Sites – Upper North Island

Site Label	Waterbody Name	Waterbody Type
1	Arapuni	Lake
2	Coxe Farm - Big Dam	Pond/Dam
3	Coxe Farm - Boundary Dam	Pond/Dam
4	Coxe Farm - Kahakatra Dam	Pond/Dam
5	Coxe Farm - Middle Dam	Pond/Dam
6	Coxe Farm - Woolshed Pond	Pond/Dam
7	Ellis Farm Pond	Pond/Dam
8	Glen Innis Pond	Pond/Dam
9	Hakanoa	Lake
10	Helensville Dam 1	Pond/Dam
11	Helensville Dam 3	Pond/Dam
12	Kainui	Lake
13	Kaitaia (Ponds 1)	Oxidation Pond
14	Kaitaia (Ponds 2)	Oxidation Pond
15	Kaitaia (Ponds 3)	Oxidation Pond
17	Karapiro	Lake
18	Kimiha	Lake
19	Koromatua	Lake
20	Kuratau	Lake
21	Kuwakatai	Lake
22	Labonte Fish Farm - Pond	Pond/Dam
23	Maraetai	Lake
24	Maratoto	Lake
25	Mt Maunganui	Oxidation Pond
26	Ngahewa	Lake
27	Ngaopouri	Lake
28	Ngaroto	Lake
29	Okaro	Lake
30	Omapere	Lake
31	Prawn Farm - Pond 1	Pond/Dam
32	Rerewhakaaitu	Lake
33	Richardson Farm Pond	Pond/Dam
34	Rotoheu	Lake
35	Rotoiti	Lake

Site Label	Waterbody Name	Waterbody Type
36	Rotokaeo	Lake
37	Rotokawa	Lake
38	Rotomanuku North	Lake
39	Rotorua	Lake
40	Rotorua/Taupo Rd Pond	Pond/Dam
41	Rotowhero	Lake
42	Serpentina East	Lake
43	Serpentina North	Lake
44	Simons Farm - Big Pond	Pond/Dam
45	Simons Farm - Little Pond	Pond/Dam
46	Spectacle	Lake
47	Steedman Farm - Trough	Pond/Dam
48	Tarawhera	Lake
49	Taupo	Lake
50	Taupo	Lake
51	Te Amamutu Pond	Pond/Dam
52	Te Ko Utu	Lake
53	ToRepatutaki Swamp	Pond/Dam
54	Utakura River	Lake
55	Waahi	Lake
56	Waikato River - Wgtn St Beach	River/Stream
57	Waipapa	Lake
58	Weavers	Lake
59	Whakamaru	Lake
60	Whangape	Lake
61	Winchester Farm - Pond	Pond/Dam

Appendix 1a. Locations of Sampling Sites – Upper North Island

Appendix 1b. Locations of Sampling Sites – Lower North Island

Appendix 1b. Key for Locations of Sampling Sites - Lower North Island

Site Label	Waterbody Name	Waterbody Type
62	Avalon Park Pond	Pond/Dam
63	Belmont Park - Pond	Pond/Dam
64	Dudding	Lake
65	Elizabeth Park Pond - Masterton	Pond/Dam
66	Georges Dr Pond	Pond/Dam
67	Hatuma	Lake
68	Henly	Lake
69	Horowhenua	Lake
70	Hutt River - Kennedy Good bridge	River/Stream
71	Kaitoke	Lake
72	Kohangapiripiri	Lake
73	Kohangatera	Lake
74	Lindale - Sth Pond	Pond/Dam
75	Lindale Pond - Nth Pond	Pond/Dam
76	Lower Karori Reservior	Lake
77	Lower Whitby Pond	Pond/Dam
78	Mangamahoe	Lake
79	Masterton (Pond 3)	Oxidation pond
80	Mcintosh Farm - Bottom Dam	Pond/Dam
81	Mcintosh Farm - Culvet	Pond/Dam
82	Neuman Pond	Pond/Dam
83	Ngaumanu Park pond	Pond/Dam
84	Orakai	Lake
85	Otamangakau	Lake
86	Papaitonga	Lake
87	Pauri	Lake
88	Rotoaira	Lake
89	Rotokare	Lake
90	Rotopounamu	Lake
91	Te Whaiiau	Lake
92	Tikotu Creek	River/Stream
93	Torrington Farm - Lower Dam	Pond/Dam
94	Torrington Farm - Sth Pond	Pond/Dam
95	Torrington Farm - Stream	Pond/Dam

Site Label	Waterbody Name	Waterbody Type
96	Torrington Farm - Upper Dam	Pond/Dam
97	Torrington Farm - West Pond	Pond/Dam
98	Tutira	Lake
99	Upper Karori Reservior	Lake
100	Virginia	Lake
101	Waikanae	Pond/Dam
102	Waikanae Estuary	Pond/Dam
103	Waikanae River - Edge Park	River/Stream
104	Waikopiro	Lake
105	Waimanu Lagoon - South	Pond/Dam
106	Waimanu Lagoons - North	Pond/Dam
107	Waimarua - North Pond	Pond/Dam
108	Waimarua - South Pond	Pond/Dam
109	Wairapara	Lake
110	Waitawa	Lake
111	Westmere	Lake
112	Wiritoa	Lake

Appendix 1c. Locations of Sampling Sites – South Island

Appendix 1c. Key for Locations of Sampling Sites – South Island

Site Label	Waterbody Name	Waterbody Type
1	Alexandrina	Lake
2	Bleheim (Nth Pond)	Oxidation Pond
3	Blenheim (Sth Pond)	Oxidation Pond
4	Brunner	Lake
5	Burns Loop Farm Pond	Pond/Dam
6	Chalice	Lake
7	Coleridge	Pond/Dam
8	Dunedin South Reservoir	Lake
9	Forsyth	Lake
10	Golf Course Pond - Blenheim	Pond/Dam
11	Grassmere	Lake
12	Horseshoe	Lake
13	Kaniere	Lake
14	Lyndon	Lake
15	Mahinapua	Lake
16	McGregor	Lake
17	Motueka River - Alexanders Bridge	River/Stream
18	Motupiko River - Quinneys Bush	River/Stream
19	Onamalutu Domain Pond	Pond/Dam
20	Opuha	Lake
21	Pearson	Lake
22	Pond - Blenheim	Pond/Dam
23	Riverton - Creek	River/Stream
24	St Arnaud Pond	Pond/Dam
25	Taylors Dam - Blenheim	Pond/Dam
26	Taylors Dam Pond 1	Pond/Dam
27	Taylors Dam Pond 2	Pond/Dam
28	Waihola	Lake
29	Waihopa Cemetery Pond	Pond/Dam
30	Wairau River Gravel Pit	Pond/Dam
31	Wairua Lagoon	Pond/Dam
32	Welt St Culvet - Blenheim	Pond/Dam
33	Witherhills Park Pond	Pond/Dam
34	Woods Fam - Dam	Pond/Dam

**Appendix 1d. Locations of Lakes in Which Cyanobacteria
Produced Blooms During this Study (or Published
Prior to 2001).**

Appendix 2. Data for Each Sample Collected

Table A1. Lakes.....	237
Table A2. Oxidation Ponds	246
Table A3. Ponds and Dams	247
Table A4. Rivers and Streams	251

Table A1 – Lakes

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty.	Cyano. Sp. 2	Qty.	Cyano. Sp. 3+	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax. (ug kg ⁻¹ FDW)	Sax. (ug l ⁻¹)
L1	Henly	40° 57'	175° 40'	6/01/2001	R	G	N	Anabaena circinalis	+					0.40	NT	NT	NT
L2	Wairapara	41° 10'	175° 18'	6/01/2001	R	G	N							0.00	NT	NT	NT
L3	Coleridge	43° 20'	171° 34'	8/01/2001	R	G	N							0.00	NT	NT	NT
L4	Lyndon	43° 17'	171° 42'	8/01/2001	R	G	N							0.00	NT	NT	NT
L5	Lyndon	43° 17'	171° 42'	8/01/2001	R	G	N							0.00	NT	NT	NT
L6	Henly	40° 57'	175° 40'	19/01/2001	R	G	N	Anabaena circinalis	++					2.00	Negative	NT	NT
L7	Horowhenua	40° 36'	175° 15'	21/10/2001	R	G	N	Microcystis aeruginosa	+					0.00	NT	NT	NT
L8	Mangamahoe	39° 7'	174° 24'	21/10/2001	H	G	N							0.00	NT	NT	NT
L9	Rotokare	39° 27'	174° 24'	21/10/2001	R	G	N							0.00	NT	NT	NT
L10	Virginia	39° 55'	175° 1'	21/10/2001	S	G	N							0.00	NT	NT	NT
L11	Wirirua	39° 58'	175° 5'	21/10/2001	R	G	N							0.00	NT	NT	NT
L12	Rotoehu	38° 2'	176° 30'	6/11/2001	R	G	Y	Anabaena spp.	+++	Microcystis panniformis	+			0.00	Positive	NT	NT
L13	Horowhenua	40° 36'	175° 15'	7/11/2001	R	G	Y	Microcystis aeruginosa	+++					76.60	NT	NT	NT
L15	Waiholia	46° 1'	170° 0'	17/11/2001	R	G	N	Anabaena lemmermannii	+					1.70	NT	NT	NT
L16	Horowhenua	40° 36'	175° 15'	25/11/2001	R	G/S	Y	Microcystis aeruginosa	++++					1700.00	NT	NT	NT
L17	Papaitonga	40° 38'	175° 13'	25/11/2001	S	G	N							5.00	NT	NT	NT
L18	Rotoehu	38° 2'	176° 30'	26/11/2001	R	G	Y	Microcystis panniformis	++++					5.00	NT	NT	NT
L19	Ngaopouri	38° 20'	176° 20'	10/12/2001	R	G	Y	Anabaena sp. *	+++					0.00	Negative	NT	NT
L20	Brunner	43° 38'	171° 24'	18/12/2001	R	G	N							0.00	NT	NT	NT
L21	Kaniere	42° 48'	171° 8'	18/12/2001	R	G	N							0.00	NT	NT	NT
L22	Pearson	43° 6'	171° 46'	18/12/2001	R	G	N							0.00	NT	NT	NT

Table A1. Location, date of collection, water use, sampling method, presence/absence of bloom, cyanobacteria species present and cyanotoxins and levels detected in samples for lakes. G = grab sample, G/S = Grab sample of scum material, P = Plankton net (mesh size), B = Benthic sample, R = Recreational, S = Scenic, DW = Drinking water, SDW = Stock drinking water, H = Hydro electricity, OP = Oxidation Pond, I = Irrigation, A = Aquaculture, * = Coiled Species, # = Straight Species, + = Microcystins measured in ug kg⁻¹ of freeze dried weight. Quantification Index: + (1 colony or filament/ > 10 optical fields); ++ (1 colony or filament/ 1 to 9 optical fields); +++ (1 colony or filament/ optical field); ++++ (≥ 5 colonies or filaments per field) at 100x amplification.

Table A1 continued....

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty.	Cyano. Sp. 2	Qty.	Cyano. Sp. 3+	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax (ug kg ⁻¹ FDW)	Sax (ug l ⁻¹)
L23	Ngaehewa	38° 19'	176° 22'	5/01/2002	R	G	N	Anabaena planktonica	+	Microcystis aeruginosa	+			0.00	Negative	NT	NT
L24	Okaro	38° 17'	176° 23'	5/01/2002	R	G	N	Microcystis protoeensis	+	Anabaena lemmermannii	+			0.00	Negative	0.0000	0.0000
L25	Rotohu	38° 0'	176° 32'	5/01/2002	R	G	N	Microcystis panniformis	+					0.00	Negative	0.0002	0.0002
L26	Rotiti	38° 3'	176° 20'	5/01/2002	R, DW	G	N	Anabaena planktonica	+	Anabaena circinalis	+	Anabaena lemmermannii	+	0.00	Negative	0.7686	0.0154
L27	Orakai	39° 14'	176° 33'	18/01/2002	SDW	G	Y	Anabaena spiroides	+++	Anabaena sp?	+++			4.00	Negative	NT	NT
L28	Tutira	39° 14'	176° 33'	18/01/2002	R	G	N							0.00	NT	NT	NT
L29	Waikopiro	39° 14'	176° 33'	18/01/2002	R	G	N							0.00	NT	NT	NT
L30	Hatuma	40° 1'	176° 31'	19/01/2002	R	G	N							2.70	Negative	NT	NT
L31	Ngaehewa	38° 19'	176° 22'	28/01/2002	R	G	Y	Anabaena planktonica	+++	Microcystis aeruginosa	+			0.15	Negative	NT	NT
L32	Rotohu	38° 2'	176° 30'	28/01/2002	R	G	Y	Microcystis panniformis	+++		+			25.00	NT	NT	NT
L33	Rotokawa	38° 6'	176° 19'	28/01/2002	S	G	N							0.00	NT	NT	NT
L34	Rotorua	38° 7'	176° 15'	28/01/2002	R	G	N	Planktothrix sp.	+					0.00	Negative	NT	NT
L35	Rotiti	38° 2'	176° 26'	28/01/2002	R, DW	G	N							0.00	NT	NT	NT
L36	Rotiti	38° 1'	176° 20'	28/01/2002	R, DW	G	Y	Anabaena planktonica	+++	Microcystis aeruginosa	+			0.60	NT	NT	NT
L37	Rotiti	38° 3'	176° 20'	28/01/2002	R, DW	G	Y	Anabaena planktonica	+++	Microcystis aeruginosa	+			0.25	Negative	NT	NT
L38	Rotiti	38° 3'	176° 20'	28/01/2002	R, DW	G	Y	Anabaena planktonica	+++	Microcystis aeruginosa	+	Anabaena circinalis	+	0.25	NT	NT	NT
L39	Rotiti	38° 3'	176° 20'	28/01/2002	R, DW	G	Y	Anabaena planktonica	+++	Microcystis aeruginosa	+			0.27	NT	NT	NT
L40	Rotiti	38° 1'	176° 21'	28/01/2002	R, DW	G	Y	Anabaena planktonica	+++	Microcystis aeruginosa	+			0.40	NT	NT	NT
L41	Rotowhero	38° 19'	176° 22'	28/01/2002	S	G	N							0.00	Negative	NT	NT
L42	Horowhenua	40° 36'	175° 15'	29/01/2002	R	G/S	Y	Microcystis aeruginosa	+++					4500.00	NT	NT	NT
L43	Okaro	38° 17'	176° 23'	29/01/2002	R	G	N	Anabaena lemmermannii	++					0.15	Negative	NT	NT
L44	Otamangakau	39° 0'	175° 37'	29/01/2002	R, H	G	N							0.00	NT	NT	NT
L45	Rotaira	39° 4'	175° 41'	29/01/2002	R	G	N							0.00	NT	NT	NT
L46	Rotopounamu	39° 1'	175° 44'	29/01/2002	S	G	N							0.00	NT	NT	NT

Table A1 continued....

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty	Cyano. Sp. 2	Qty.	Cyano. Sp. 3+	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax (ug kg ⁻¹ FDW)	Sax (ug l ⁻¹)
L47	Te Whaitau	33° 1'	175° 44'	29/01/2002	R, H	G	N	Anabaena circinalis		Microcystis aeruginosa	+			0.00	NT	NT	NT
L48	Rotiti	38° 3'	176° 20'	30/01/2002	R, DW	G	Y	Anabaena circinalis	++					0.35	NT	NT	NT
L49	Rotiti	38° 3'	176° 20'	30/01/2002	R, DW	G	Y	Anabaena circinalis	++	Microcystis aeruginosa	+			0.35	NT	NT	NT
L50	Lower Karori Reservoir	41° 17'	174° 45'	6/02/2002	S	G	Y	Anabaena circinalis	+++	Anabaena lemmermannii	+			0.00	NT	NT	NT
L51	Lower Karori Reservoir	41° 17'	174° 45'	7/02/2002	S	G	Y	Anabaena circinalis	+++	Anabaena lemmermannii	+			0.00	NT	NT	NT
L52	Lower Karori Reservoir	41° 17'	174° 45'	8/02/2002	S	G	Y	Anabaena circinalis	+++	Anabaena lemmermannii	+			0.00	NT	NT	NT
L53	Upper Karori Reservoir	41° 17'	174° 44'	9/02/2002	S	G	N							0.00	NT	NT	NT
L54	Horowhenua	40° 36'	175° 15'	17/02/2002	R	G/S	Y	Microcystis aeruginosa	+++					9000.00	NT	NT	NT
L55	Papaitonga	40° 38'	175° 13'	17/02/2002	S	G	N	Planktothrix peronata	++					0.90	Negative	NT	NT
L56	Pauri	39° 58'	175° 6'	22/02/2002	R	G	Y	Planktothrix peronata	+++					0.00	NT	NT	NT
L57	Wiriota	39° 58'	175° 5'	22/02/2002	R	G	N							0.00	NT	NT	NT
L58	Omapere	35° 20'	173° 46'	2/03/2002	R	G	Y	Microcystis aeruginosa	++	Microcystis wesenbergii	++			8.30	NT	NT	NT
L59	Omapere	35° 20'	173° 46'	2/03/2002	R	G	Y	Microcystis aeruginosa	++	Microcystis wesenbergii	++			0.18	NT	NT	NT
L60	Omapere	35° 20'	173° 46'	2/03/2002	R	G	Y	Microcystis aeruginosa	+	Microcystis wesenbergii	+			1.15	NT	NT	NT
L61	Rotoroa	37° 47'	175° 16'	10/03/2002	R	G	N	Microcystis aeruginosa	+					0.00	NT	NT	NT
L62	Waiholia	46° 1'	170° 0'	12/03/2002	R	G	Y	Anabaena lemmermannii	+++					0.00	Negative	NT	NT
L63	Kanieri	42° 48'	171° 8'	13/03/2002	R	G	Y	Anabaena lemmermannii	+					0.00	NT	NT	NT
L64	Mahinapua	42° 47'	170° 54'	14/03/2002	S	G	N							0.00	NT	NT	NT
L65	Waiholia	46° 1'	170° 0'	14/03/2002	R	G	Y	Anabaena lemmermannii	+++					0.00	NT	NT	NT
L66	Horseshoe	43° 35'	172° 31'	16/03/2002	S	G	N	Planktothrix peronata	+					0.00	NT	NT	NT
L67	Rotiti	41° 48'	172° 50'	16/03/2002	R, DW	G	N							0.00	NT	NT	NT
L68	Rotoroa	41° 47'	172° 36'	16/03/2002	R	G	N							0.00	NT	NT	NT
L69	Chalice	41° 34'	173° 18'	17/03/2002	S	G	N							0.00	NT	NT	NT
L70	Henly	40° 57'	175° 40'	18/03/2002	R	G	N	Anabaena circinalis	++					0.00	NT	NT	NT

Table A1 continued....

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty	Cyano. Sp. 2	Qty.	Cyano. Sp. 3+	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax (ug kg ⁻¹ FDW)	Sax (ug l ⁻¹)
L71	Omapere	35°20'	173°47'	26/03/2002	R	G	Y	Microcystis aeruginosa	++	Anabaena planktonica	++		++	5.06	NT	NT	NT
L72	Forsyth	43°47'	175°45'	27/03/2002	R	G	N	Nodularia spumigena	++					0.00	NT	NT	NT
L73	Taupo	38°43'	175°45'	27/03/2002	R, DW	G	N	Microcystis aeruginosa	+	Anabaena circinalis	+		+	0.00	NT	NT	NT
L74	Waitawa	43°10'	175°10'	28/11/2002	R	P (40 um)	Y	Microcystis aeruginosa	++	Anabaena circinalis	++		++	11.30	Negative	NT	NT
L75	Okaro	38°17'	176°23'	1/12/2002	R	G	N	Microcystis sp.	+	Anabaena lemmermannii	+	Microcystis smithii	+	0.00	Negative	NT	NT
L76	Okaro	38°17'	176°23'	1/12/2002	R	G	Y	Microcystis protocystis	++	Anabaena lemmermannii	+	Microcystis smithii	+	0.00	Negative	NT	NT
L77	Lower Karori Reservoir	41°17'	174°45'	8/01/2003	S	G	Y	Anabaena circinalis	+++	Anabaena lemmermannii	+++		+++	0.00	Positive	NT	NT
L78	Henly	40°57'	175°40'	15/01/2003	R	G	N	Anabaena sp. *	+		+		+	0.50	Positive	0.0000	0.0000
L79	Waitawa	43°10'	175°10'	29/01/2003	R	G	N	Microcystis aeruginosa	+		+		+	4.45	NT		0.0004
L80	Waitawa	43°10'	175°10'	29/01/2003	R	G	Y	Microcystis aeruginosa	+++	Anabaena cf. circinalis	+		+	5.20	NT	1.7219	
L81	Waitawa	43°10'	175°10'	29/01/2003	R	G	Y	Microcystis aeruginosa	++	Anabaena cf. circinalis	+		+	8.61	NT	0.9294	
L82	Horowhenua	40°36'	175°15'	29/01/2003	R	G/S	Y	Microcystis aeruginosa	+++					36500.00	NT	0.0000	0.0000
L83	Lower Karori Reservoir	41°17'	174°45'	29/01/2003	S	G	Y	Anabaena circinalis	+++	Anabaena lemmermannii	+++		+++	0.00	NT	0.0000	0.0000
L84	Kimiha	37°31'	175°11'	15/02/2003	R	G	Y	Anabaena planktonica	++					0.00	Negative	1.2214	0.0244
L85	Maraetai	38°22'	175°47'	16/02/2003	R, DW, H	P (23 um)	N	Anabaena planktonica	+					0.00	NT		0.0002
L86	Rotoehu	38°2'	176°30'	24/02/2003	R	G	N	Microcystis panniformis	+	Aphanizomenon gracile	+		+	0.20	Negative	0.9265	0.0185
L87	Rotoehu	38°1'	176°32'	24/02/2003	R	G	N	Aphanizomenon gracile	++					0.00	Negative	0.8564	0.0171
L88	Rotoehu	38°0'	176°32'	24/02/2003	R	G	N	Aphanizomenon gracile	++					0.00	NT	0.5698	0.0118
L89	Rotoiti	38°1'	176°20'	24/02/2003	R, DW	G	Y	Microcystis aeruginosa	+	Anabaena planktonica	+	Anabaena crassa	+	0.00	NT		0.0001
L90	Rotorua	38°2'	176°17'	24/02/2003	R	G	Y	Microcystis aeruginosa	+	Anabaena planktonica	+	Anabaena crassa	+	0.00	Negative		0.0002
L91	Rotorua	38°2'	176°17'	24/02/2003	R	G	Y	Microcystis aeruginosa	+	Anabaena planktonica	+	Anabaena crassa	++	0.30	NT		0.0001
L92	Rotorua	38°7'	176°17'	24/02/2003	R	G	Y	Microcystis aeruginosa	++	Anabaena planktonica	+	Anabaena crassa	+	0.00	NT		0.0000
L93	Rotorua	38°7'	176°17'	24/02/2003	R	G	Y	Microcystis aeruginosa	+	Anabaena planktonica	+	Anabaena crassa	+	0.20	NT		0.0000

Table A1 continued.....

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty	Cyano.Sp. 2	Qty.	Cyano. Sp. 3+	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax (ug kg ⁻¹ FDW)	Sax (ug l ⁻¹)
L94	Rotiti	38° 3'	176° 26'	24/02/2003	R, DW	G	Y	Microcystis aeruginosa	+	Anabaena planktonica	+			0.00	NT		0.0001
L95	Rotiti	38° 2'	176° 29'	24/02/2003	R, DW	G	Y	Microcystis aeruginosa	+	Anabaena planktonica	+			0.00	NT		0.0001
L96	Rotiti	38° 3'	176° 20'	24/02/2003	R, DW	G	Y	Microcystis aeruginosa	+	Anabaena planktonica	++	Anabaena crassa		0.00	NT		0.0001
L97	Kuwakatai	36° 17'	174° 14'	6/03/2003	R	G	Y	Microcystis flos-aquae	++	Microcystis aeruginosa	+	Anabaena cf. planktonica	+	1.06	NT	1.0218	0.0204
L98	Spectacle	36° 10'	174° 37'	6/03/2003	S	G	Y	Microcystis wesenbergii	++	Anabaena planktonica	+	Anabaena cf. circinalis*	+	0.00	Negative	NT	NT
L99	Maraetai	38° 22'	175° 47'	11/03/2003	R, DW, H	G	Y	Anabaena planktonica	++					0.00	NT		0.0002
L100	Ngaopouri	38° 20'	176° 20'	12/03/2003	R	G	Y	Anabaena planktonica	+++	Microcystis aeruginosa	+			0.38	NT	1.4170	0.0002
L101	Ngahewa	38° 19'	176° 22'	12/03/2003	R	G	Y	Anabaena cf. spiroides	++	Anabaena planktonica	+	Aphanizomenon gracile, Pseudanabaena limnetica	+	0.00	NT		0.0001
L102	Okaro	38° 17'	176° 23'	12/03/2003	R	G	N	Anabaena planktonica	+	Microcystis smithii	+			0.00	NT		0.0001
L103	Rerewhakaaitu	38° 18'	176° 30'	12/03/2003	R	G	N							0.00	Negative		0.0002
L104	Rotoehu	38° 1'	176° 32'	12/03/2003	R	G	N	Microcystis panniformis	+					0.11	NT		0.0005
L105	Rotoehu	38° 0'	176° 32'	12/03/2003	R	G	N	Aphanizomenon gracile	+					0.00	NT		0.0005
L106	Rotoehu	38° 2'	176° 30'	12/03/2003	R	G	Y	Microcystis panniformis	+	Aphanizomenon gracile	+	Pseudanabaena sp., Oscillatoria sp.	+	0.00	NT	0.0000	0.0000
L107	Rotoehu	38° 2'	176° 30'	12/03/2003	R	G/S	Y	Microcystis panniformis	+++					0.40	NT	0.2566	0.0001
L108	Rotorua	38° 7'	176° 17'	12/03/2003	R	G	N	Anabaena planktonica	+	Anabaena sp.*	+			0.00	NT		0.0000
L109	Rotiti	38° 2'	176° 29'	12/03/2003	R, DW	G	N	Anabaena planktonica	+					0.00	Negative		0.0001
L110	Rotiti	38° 3'	176° 20'	12/03/2003	R, DW	G	Y	Anabaena planktonica	++	Microcystis aeruginosa	+	Anabaena sp. 1*	+	0.00	NT		0.0000
L111	Tarawhera	38° 12'	176° 22'	12/03/2003	R, DW	G	N	Anabaena lemmermannii var. minor	+					0.00	NT		0.0003
L112	Tarawhera	38° 10'	176° 32'	12/03/2003	R, DW	G	N	Anabaena lemmermannii var. minor	++					0.00	Negative	0.7622	0.0003
L113	Hakanoa	37° 33'	175° 10'	13/03/2003	R	P (23 um)	Y	Anabaena planktonica	++	Microcystis flos-aquae	+	Microcystis aeruginosa	+	90.00	NT	2.3187	0.0060
L114	Waahi	37° 34'	175° 7'	13/03/2003	R	P (23 um)	Y	Cylindrospermopsis raciborskii	+++					0.00	Negative		0.0002
L115	Weavers	37° 34'	175° 8'	13/03/2003	S	G	N	Anabaena milderi	+					0.00	NT	1.3014	0.0004

Table A1 continued....

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty	Cyano. Sp. 2	Qty.	Cyano. Sp. 3+	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax (ug kg ⁻¹ FDW)	Sax (ug l ⁻¹)
L116	Arapuni	38° 8'	175° 8'	14/03/2003	H, R	P (23 um)	N	Anabaena planktonica	+					0.04	NT	0.4412	0.0001
L117	Karapiro	37° 56'	175° 33'	14/03/2003	R	P (23 um)	N	Anabaena planktonica	+					0.00	NT	0.7962	0.0002
L118	Ngaroto	37° 57'	175° 17'	14/03/2003	R	G/S	Y	Anabaena planktonica	+++	Microcystis flos-aquae	++	Microcystis aeruginosa	+	423.00	NT	0.0000	0.0000
L119	Rotokaeo	37° 46'	175° 15'	14/03/2003	R	G	N							0.00	NT	0.8247	0.0002
L120	Rotorua	37° 47'	175° 16'	14/03/2003	R	P (23 um)	N	Anabaena planktonica	+					0.00	NT		0.0000
L121	Te Ko Ulu	37° 53'	175° 28'	14/03/2003	R	G	Y	Anabaena circinalis	+++	Anabaena planktonica	+	Microcystis flos-aquae	+	2.14	Negative		0.0001
L122	Waipapa	38° 17'	175° 40'	14/03/2003	H, R	P (23 um)	N							0.00	NT	0.8854	0.0001
L123	Whakamaru	38° 25'	175° 48'	14/03/2003	H, R	P (23 um)	N							0.00	NT	0.9141	0.0001
L124	Horowhenua	40° 36'	175° 15'	15/03/2003	R	P (23 um)	N	Microcystis aeruginosa	+	Anabaena cf. circinalis	+			0.00	NT	1.1848	0.0004
L125	Kohangapiri	41° 21'	174° 51'	16/03/2003	SDW	P (23 um)	N							0.00	NT	NT	NT
L126	Kohangaterua	41° 22'	174° 52'	16/03/2003	SDW	P (23 um)	N	Anabaena lemmermannii	+	Anabaena circinalis	+			0.00	Negative	8.6988	NT
L127	Dunedin South Reservoir	45° 53'	170° 26'	19/03/2003	DW	G	Y	Anabaena oscillarioides	+++					0.00	NT	0.0000	0.0000
L128	Rotiti	38° 3'	176° 20'	24/03/2003	R, DW	G	Y	Anabaena planktonica	+++	Anabaena circinalis	+	Microcystis flos-aquae	+	0.30	NT	0.0000	0.0000
L129	Waahi	37° 34'	175° 7'	25/03/2003	R	G	Y	Cylindrospermopsis raciborskii	+++					0.00	NT	0.4458	0.0002
L130	Hakanoa	37° 33'	175° 10'	28/03/2003	R	G	N	Microcystis flos-aquae	+	Microcystis aeruginosa	+			10.60	NT	0.4165	0.0001
L131	Kainui	37° 40'	175° 13'	28/03/2003	R	G	N	Microcystis aeruginosa	+					0.54	NT	1.7833	0.0003
L132	Koromatua	37° 50'	175° 13'	28/03/2003	R	G	N	Merismopedia sp.	+	Anabaena sp. #	+			0.00	NT	1.0101	0.0003
L133	Maratoto	37° 53'	175° 18'	28/03/2003	R	G	N							0.00	NT	1.1341	0.0002
L134	Ngaroto	37° 57'	175° 17'	28/03/2003	R	G	Y	Anabaena planktonica	++	Microcystis litchyblake	++	Cylindrospermopsis raciborskii, Microcystis aeruginosa, Microcystis flos-aquae	+	0.89	NT	0.0000	0.0000
L135	Rotomanuku North	37° 55'	175° 18'	28/03/2003	R	G	N	Microcystis aeruginosa	+					0.00	NT		0.0000
L136	Serentina East	37° 57'	175° 19'	28/03/2003	R	G	N							0.00	NT		0.0001
L137	Serentina North	37° 57'	175° 19'	28/03/2003	R	G	N							0.00	NT		0.0003

Table A1 continued....

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty.	Cyano. Sp. 2	Qty.	Cyano. Sp. 3+	Qty.	MC (µg l ⁻¹) except where noted	Anatoxin	Sax (µg kg ⁻¹ FDW)	Saxi (µg l ⁻¹)
L138	Waahi	37° 34'	175° 7'	28/03/2003	R	G	Y	<i>Cylindrospermopsis raciborskii</i>	+++					0.00	NT	0.7320	0.0003
L139	Forsyth	43° 47'	175° 45'	2/04/2003	R	G	Y	<i>Nodularia spumigena</i>	++	<i>Anabaena</i> sp. *	+	<i>Anabaenopsis</i> sp.	+	9.00	NT	0.2643	0.0004
L140	Rotiti	38° 3'	176° 20'	7/04/2003	R, DW	P (23 µm)	Y	<i>Anabaena planktonica</i>	++++	<i>Microcystis flos-aquae</i>	+++	<i>Microcystis aeruginosa</i> , <i>Anabaena</i> spp. *	++	44.00	Negative	1.9031	NT
L141	Rotiti	38° 1'	176° 21'	7/04/2003	R, DW	P (23 µm)	Y	<i>Microcystis flos-aquae</i>	+++	<i>Microcystis aeruginosa</i>	+++	<i>Anabaena planktonica</i> , <i>Aphanocapsa holstica</i> , <i>Anabaena</i> sp. *	++	350.00	Negative	0.4270	NT
L142	Rotiti	38° 2'	176° 21'	7/04/2003	R, DW	P (23 µm)	Y	<i>Microcystis flos-aquae</i>	++	<i>Microcystis aeruginosa</i>	++	<i>Anabaena planktonica</i> , <i>Aphanocapsa holstica</i> , <i>Anabaena</i> sp. *, <i>Pseudanabaena</i> sp., <i>Aphanocapsa holstica</i> , <i>Anabaena</i> spp. *	++	315.00	Negative	1.0601	NT
L143	Dudding	40° 6'	175° 16'	16/04/2003	R	P (23 µm)	Y	<i>Microcystis aeruginosa</i>	+++	<i>Anabaena</i> sp. *	+			20.000	NT	NT	NT
L144	Horowhenua	40° 36'	175° 15'	16/04/2003	R	P (23 µm)	Y	<i>Microcystis aeruginosa</i>	++					10.00	NT	0.4128	NT
L145	Kaitake	37° 40'	175° 4'	16/04/2003	SDW, S	P (23 µm)	N	<i>Anabaena lemmermannii</i>	++					0.62	Negative	0.2862	0.0002
L146	Pauri	39° 58'	175° 6'	16/04/2003	SDW, S	P (23 µm)	N							0.00	NT	0.4993	0.0002
L147	Virginia	39° 55'	175° 1'	16/04/2003	R	P (23 µm)	N	<i>Microcystis aeruginosa</i>	++	<i>Anabaena lemmermannii</i>	+			2.10	Negative	1.4682	0.0005
L148	Waitawa	40° 43'	175° 10'	16/04/2003	R	P (23 µm)	Y	<i>Microcystis aeruginosa</i>	++	<i>Anabaena cf. circinalis</i> *	+			3700.00	NT	0.3591	NT
L149	Westmere	39° 53'	173° 59'	16/04/2003	S	P (23 µm)	Y	<i>Microcystis aeruginosa</i>	++	<i>Anabaena</i> sp. *	+			13.10	NT	0.2006	NT
L150	Wiriota	39° 58'	175° 5'	16/04/2003	R	P (23 µm)	Y	<i>Microcystis flos-aquae</i>	++	<i>Microcystis aeruginosa</i>	++			20.000	NT	0.0000	0.0000
L151	Grassmere	41° 44'	174° 10'	18/04/2003	SDW	B	Y	<i>Lyngbya majuscula</i>	MAT					0.03	NT	NT	0.0004
L152	Grassmere	41° 44'	174° 10'	18/04/2003	S	G	N							0.00	NT	NT	NT
L153	Kohangatera	41° 22'	174° 52'	20/04/2003	SDW	P (23 µm)	N	<i>Anabaena circinalis</i>	+	<i>Anabaena lemmermannii</i>	+			0.00	NT	0.8185	0.0005
L154	Kohangatera	41° 22'	174° 52'	20/04/2003	SDW	P (23 µm)	Y	<i>Anabaena circinalis</i>	++	<i>Anabaena lemmermannii</i>	+			0.00	Negative	2.4499	0.0016

Table A1 continued....

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/ Scum?	Cyanobacteria Sp. 1	Qty	Cyano. Sp. 2	Qty.	Cyano. Sp. 3+	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sex (ug kg ⁻¹ FDW)	Sex (ug l ⁻¹)
L155	Ngahewa	38° 19'	176° 22'	25/04/2003	R	P (23 um)	Y	Anabaena cf. spiroides	++	Microcystis flos-aquae	+	Aphanocapsa holsatica	+	1.48	Negative		0.0001
L156	Rotoehu	38° 2'	176° 30'	25/04/2003	R	G/S	Y	Microcystis panniformis	+++					43.00	NT		0.0000
L157	Rotoehu	38° 2'	176° 30'	25/04/2003	R	P (23 um)	Y	Microcystis panniformis	+++					16.60	NT	0.0000	0.0000
L158	Rotiti	38° 3'	176° 20'	25/04/2003	R, DW	P (23 um)	Y	Microcystis flos-aquae	+	Microcystis aeruginosa	+	Anabaena planktonica*	+	0.00	NT	0.3037	0.0002
L159	Taupo	38° 47'	176° 4'	25/04/2003	R, DW	G	N	Nostoc commune	+					1.26	NT	NT	NT
L160	Taupo	38° 47'	176° 4'	25/04/2003	R, DW	B	Y	Nostoc commune	MA T					10870*	Negative	NT	NT
L161	Hakanoa	37° 33'	175° 10'	26/04/2003	R	P (23 um)	Y	Raphidiopsis sp.	+++	Microcystis flos-aquae	+	Microcystis aeruginosa, Anabaena planktonica*	+	241.00	NT	1.1805	
L162	Hakanoa	37° 33'	175° 10'	26/04/2003	R	G	Y	Raphidiopsis sp.	++	Microcystis flos-aquae	+	Microcystis aeruginosa, Anabaena planktonica*	+	455.00	NT	0.2229	
L163	Karapiro	37° 56'	175° 33'	26/04/2003	R, DW	P (23 um)	N	Anabaena planktonica	++					0.00	NT	0.0000	0.0000
L164	Kimiha	37° 31'	175° 11'	26/04/2003	R	G	Y	Anabaena planktonica	++	Microcystis flos-aquae	+	Anabaena sp.*	+	0.00	NT	0.0000	0.0000
L165	Waahi	37° 34'	175° 7'	26/04/2003	R	P (23 um)	Y	Cylindrospermopsis raciborskii	+++					0.00	NT	0.6485	
L166	Waahi	37° 34'	175° 7'	26/04/2003	R	G	Y	Cylindrospermopsis raciborskii	+++					0.00	NT	0.3923	0.0002
L167	Weavers	37° 34'	175° 8'	26/04/2003	R	P (23 um)	N	Anabaena cf. affinis	+	Anabaena sp.*	+	Oscillatoria sp.	+	0.00	NT	0.8270	0.0003
L168	Weavers	37° 34'	175° 8'	27/04/2003	R	G	N	Anabaena cf. affinis	+	Anabaena sp.*	+			0.00	NT	NT	NT
L169	Alexandrina	43° 56'	170° 27'	30/04/2003	R	P (23 um)	N							0.00	NT	0.0000	0.0000
L170	McGregor	43° 56'	170° 28'	30/04/2003	R	P (23 um)	N							0.00	NT	0.0000	0.0000
L171	Opuha	43° 59'	170° 53'	30/04/2003	R	P (23 um)	N							0.00	NT		0.0000
L172	Hakanoa	37° 33'	175° 10'	8/05/2003	R	G	Y	Raphidiopsis sp.	+++	Microcystis aeruginosa	+	Anabaena planktonica	+	11.30	NT	0.7094	0.0001
L173	Kainui	39° 57'	175° 13'	8/05/2003	R	G	Y	Microcystis flos-aquae	++	Microcystis aeruginosa	+	Anabaena smithii	+	0.02	NT	NT	NT
L174	Ngaroto	37° 57'	175° 17'	8/05/2003	R	G	Y	Anabaena planktonica	++	Microcystis flos-aquae	+	Microcystis aeruginosa	+	0.00	NT	0.5686	0.0001
L175	Waahi	37° 34'	175° 7'	8/05/2003	R	G	Y	Cylindrospermopsis raciborskii	+++					0.00	NT	0.8206	0.0003
L176	Whangape	37° 28'	175° 3'	8/05/2003	R	G	Y	Cylindrospermopsis raciborskii	++	Pseudanabaena linnelica	+	Anabaena cf. affinis	+	0.00	NT	0.7059	0.0001

Table A1 continued....

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty.	Cyanobacteria Sp. 2	Qty.	Cyanobacteria Sp. 3+	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax (ug kg ⁻¹ FDW)	Sax (ug l ⁻¹)
L177	Rotiti	38° 3'	176° 20'	13/05/2003	R, DW	G	Y	Anabaena planktonica	+++	Anabaena circinalis	+	Microcystis flos-aquae	+	2.51	NT	NT	NT
L178	Wallawa	40° 43'	175° 10'	23/05/2003	R	G/S	Y	Microcystis aeruginosa	++++	Microcystis flos-aquae	+			32000.00	NT	0.7042	0.0001
L179	Wallawa	40° 43'	175° 10'	23/05/2003	R	P (23 um)	Y	Microcystis aeruginosa	++++	Microcystis flos-aquae	+			2180.00	NT	0.0000	0.0000
L180	Rotihu	38° 1'	176° 32'	26/05/2003	R	G	Y	Anabaena lemmermannii	++	Microcystis panniformis	+			5.00	NT	0.0000	0.0000
L181	Rotiti	38° 3'	176° 20'	26/05/2003	R, DW	G	Y	Anabaena planktonica	+++	Anabaena circinalis	+	Microcystis aeruginosa	+	0.00	NT	NT	NT
L182	Rotiti	38° 3'	176° 20'	11/06/2003	R, DW	P (23 um)	Y	Anabaena planktonica	+++	Anabaena circinalis	+	Microcystis aeruginosa	+	26.40	NT	NT	NT
L183	Rotiti	38° 1'	176° 21'	11/06/2003	R, DW	P (23 um)	Y	Anabaena planktonica	+++	Anabaena circinalis	+	Microcystis aeruginosa	+	6.29	NT	NT	NT
L184	Rotihu	38° 2'	176° 30'	12/06/2003	R	G	Y	Anabaena lemmermannii	++	Microcystis panniformis	+	Microcystis protocystis	+	4.50	NT	NT	NT
L185	Tarawera	38° 12'	176° 24'	1/10/2003	R, DW	G	Y	Anabaena lemmermannii	++++					0.00	NT	NT	NT
L186	Lower Karori Reservoir	41° 17'	174° 45'	28/11/2004	S	G	Y	Anabaena planktonica	++++					NT	NT	NT	NT
L187	Tutira	39° 14'	176° 1'	18/01/2002	R	G	N	Anabaena planktonica	+++					NT	NT	NT	NT

Table A2 – Oxidation Ponds

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty.	Cyanobacteria Sp. 2	Qty.	Cyano. Sp. 3	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax (ug kg ⁻¹ FDW)	Sax (ug l ⁻¹)
OP1	Blenheim (Nth Pond)	41°31'	174°2'	1/01/2001	O	G	N	Planktothrix mougeotii	++					6.60	NT	NT	NT
OP2	Blenheim (Sth Pond)	41°31'	174°2'	1/01/2001	O	G	Y	Planktothrix mougeotii	++++					0.50	Negative	NT	NT
OP3	Kuratau	38°54'	175°45'	10/01/2002	O	G	Y	Planktothrix mougeotii	++++	Microcystis aeruginosa	++++			100.00	Negative	NT	NT
OP4	Kaitiaki (Pond 2)	35°6'	173°13'	3/03/2002	O	G	Y	Planktothrix mougeotii	++++					2.00	NT	NT	NT
OP5	Masteron (Pond 3)	40°59'	175°40'	13/03/2002	O	G	Y	Planktothrix mougeotii	++++					17.00	Negative	NT	NT
OP6	Kaitiaki (Pond 1)	35°6'	173°13'	13/03/2002	O	G	Y	Planktothrix mougeotii	++++					0.90	NT	NT	NT
OP7	Kaitiaki (Pond 3)	35°6'	173°13'	13/03/2002	O	G	Y	Planktothrix mougeotii	++++	Arthrospira cf maxima	+			1.50	Negative	NT	NT
OP8	Kaitiaki (Ponds 1)	35°6'	173°13'	25/03/2002	O	G	Y	Planktothrix mougeotii	++++	Microcystis aeruginosa	+++			20.00	Negative	NT	NT
OP9	Kaitiaki (Pond 2)	35°6'	173°13'	22/01/2003	O	G	Y	Planktothrix mougeotii	+++	Microcystis aeruginosa	+			0.00	Negative	5.51	
OP10	Mt Maunganui	37°41'	176°13'	20/03/2003	O	G	N	Planktothrix mougeotii	++					0.00	Negative	NT	NT
OP11	Blenheim (Nth Pond)	41°31'	174°2'	19/04/2003	O	G	N	Planktothrix mougeotii	++					0.00	NT	0.3635	0.0002
OP12	Waikanae	40°51'	175°2'	16/02/2004	O	G	Y	Microcystis aeruginosa	++++					NT	NT	NT	NT

Table A2. Location, date of collection, water use, sampling method, presence/absence of bloom, cyanobacteria species present and cyanotoxins and levels detected in samples for oxidation ponds. G = grab sample, G/S = Grab sample of scum material, P = Plankton net (mesh size), B = Benthic sample, R = Recreational, S = Scenic, DW = Drinking water, SDW = Stock drinking water, H = Hydro electricity, OP = Oxidation Pond, I = Irrigation, A = Aquaculture, * = Coiled Species, # = Straight Species, + = Microcystins measured in ug kg⁻¹ of freeze dried weight. Quantification Index: + (1 colony or filament/ > 10 optical fields); ++ (1 colony or filament/ 1 to 9 optical fields); +++ (1 colony or filament/ optical field); ++++ (≥ 5 colonies or filaments per field) at 100x amplification.

Table A3 – Ponds and Dams

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty.	Cyano. Sp. 2	Qty.	Cyano. Sp. 3	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax (ug kg FDW)	Sax (ug l ⁻¹)
DP1	Pond - Blenheim	41°31'	174°2'	1/01/2001	S	G	N							0.40	NT	NT	NT
DP2	Golf Course Pond - Blenheim	41°30'	174°3'	1/01/2001	S	G	N							0.00	NT	NT	NT
DP3	Waihopa Cemetery Pond	41°31'	173°47'	1/01/2001	S	G	N							0.20	NT	NT	NT
DP4	Taylor's Dam - Blenheim	41°34'	173°47'	1/01/2001	S	G	N							0.00	NT	NT	NT
DP5	Taylor's Dam - Blenheim	41°34'	173°47'	1/01/2001	S	G	N							0.00	NT	NT	NT
DP6	Taylor's Dam Pond 1	41°34'	173°47'	1/01/2001	SDW	G	N							0.00	NT	NT	NT
DP7	Taylor's Dam Pond 2	41°34'	173°47'	1/01/2001	S	G	N							0.00	NT	NT	NT
DP8	Elizabeth Park Pond - Masterton	40°57'	175°40'	6/01/2001	S	G	N							0.45	NT	NT	NT
DP9	Torrington Farm - Stream	41°22'	174°53'	11/01/2001	SDW	G	N							0.00	NT	NT	NT
DP10	Torrington Farm - Sh Pond	41°22'	174°53'	11/01/2001	SDW	G	N							0.00	NT	NT	NT
DP11	Torrington Farm - West Pond	41°22'	174°53'	11/01/2001	SDW	G	N							0.00	NT	NT	NT
DP12	Torrington Farm - Upper Dam	41°22'	174°53'	11/01/2001	SDW	G	N	<i>Coelosphaerium</i> sp.	++					0.00	NT	NT	NT
DP13	Torrington Farm - Lower Dam	41°22'	174°53'	11/01/2001	SDW	G	N							4.00	NT	NT	NT

Table A3. Location, date of collection, water use, sampling method, presence/absence of bloom, cyanobacteria species present and cyanotoxins and levels detected in samples for farm ponds and dams. G = grab sample, G/S = Grab sample of scum material, P = Plankton net (mesh size), B = Benthic sample, R = Recreational, S = Scenic, DW = Drinking water, SDW = Stock drinking water, H = Hydro electricity, OP = Oxidation Pond, I = Irrigation, A = Aquaculture, * = Coiled Species, # = Straight Species, + = Microcystins measured in ug kg⁻¹ of freeze dried weight. Quantification Index: + (1 colony or filament/ > 10 optical fields); ++ (1 colony or filament/ 1 to 9 optical fields); +++ (1 colony or filament/ optical field); ++++ (≥ 5 colonies or filaments per field) at 100x amplification.

Table A3 continued....

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty.	Cyano. Sp. 2	Qty.	Cyano. Sp. 3	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax (ug kg ⁻¹ FDW)	Saxitoxin (ug l ⁻¹)
DP14	Richardson Farm Pond	35° 19'	173° 46'	29/11/2001	SDW	G	Y	<i>Microcystis</i> sp.	+++ +					3.50	NT	NT	NT
DP15	Coxe Farm - Woolshed Pond	35° 56'	174° 12'	29/11/2001	SDW, I	G	N							0.00	NT	NT	NT
DP16	Coxe Farm - Big Dam	35° 56'	174° 12'	29/11/2001	SDW	G	N							0.00	NT	NT	NT
DP17	Coxe Farm - Middle Dam	35° 56'	174° 12'	29/11/2001	SDW	G	N							0.00	NT	NT	NT
DP18	Coxe Farm - Kahakara Dam	35° 56'	174° 12'	29/11/2001	SDW	G	N							0.00	NT	NT	NT
DP19	Coxe Farm - Boundary Dam	35° 56'	174° 12'	29/11/2001	SDW	G	N							0.00	NT	NT	NT
DP20	Labonte Fish Farm - Pond	35° 59'	174° 26'	29/11/2001	A	G	N							0.00	NT	NT	NT
DP21	Richardson Farm Pond	35° 19'	173° 46'	30/11/2001	DW, SDW	G	N	<i>Microcystis</i> sp.	+					0.00	NT	NT	NT
DP22	Simons Farm - Little Pond	37° 11'	174° 54'	10/12/2001	SDW	G	N							0.00	NT	NT	NT
DP23	Simons Farm - Big Pond	37° 11'	174° 54'	10/12/2001	SDW	G	N							0.00	NT	NT	NT
DP24	Mcintosh Farm - Bottom Dam	40° 42'	175° 13'	16/12/2001	S	G	N							0.00	NT	NT	NT
DP25	Mcintosh Farm - Bottom Dam	40° 42'	175° 13'	16/12/2001	S	G	N							0.00	NT	NT	NT
DP26	Mcintosh Farm - Culvet	40° 42'	175° 13'	16/12/2001	S	G	N							0.00	NT	NT	NT
DP27	Georges Dr Pond	39° 30'	176° 54'	27/01/2002	S	G	N							0.00	NT	NT	NT
DP28	To Repatua ki Swamp	38° 29'	176° 20'	28/01/2002	SDW	G	N							0.00	NT	NT	NT
DP29	Rotorua/Tau po Rd Pond	38° 24'	176° 19'	29/01/2002	S	G	N							0.00	NT	NT	NT
DP30	Belmont Park - Pond	41° 9'	174° 54'	9/02/2002	SDW	G	N							0.00	NT	NT	NT
DP31	Ngaumanu Park pond	40° 51'	175° 3'	17/02/2002	S	G	N							0.00	NT	NT	NT
DP32	Waikanae Estuary	40° 52'	175° 0'	17/02/2002	R	G	N	<i>Planktothrix peronata</i>	++					0.00	NT	NT	NT

Table A3 continued....

No.	Sample Location	Lat. ('S)	Long. ('E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty	Cyano. Sp. 2	Qty	Cyano. Sp. 3	Qty	Microcystins (ug l-1) except where noted	Anatoxin	Sax. (ug kg-1 FDW)	Sax (ug l-1)
DP33	Waimanu Lagoon - South	40°52'	175°0'	17/02/2002	R	G	N							0.00	Negative	NT	NT
DP34	Waimanu Lagoons - North	40°52'	175°0'	17/02/2002	R	G	N	<i>Planktothrix peronata</i>	+					0.00	NT	NT	NT
DP35	Lindale Pond	40°54'	175°1'	17/02/2002	S	G	N	<i>Anabaena circinalis</i>	++	<i>Anabaena sp.</i>	+			0.00	Negative	NT	NT
DP36	Ellis Farm Pond	36°6'	174°4'	20/02/2002	SDW	G	N							0.00	NT	NT	NT
DP37	Ellis Farm Pond	36°6'	174°4'	21/02/2002	SDW	G	N	<i>Anabaena sp. *</i>	++					0.00	NT	NT	NT
DP38	Helensville Dam 1	36°40'	174°24'	13/03/2002	DW	G	N							0.00	NT	NT	NT
DP39	Helensville Dam 3	36°40'	174°24'	13/03/2002	DW	G	N							0.00	NT	NT	NT
DP40	St Arnaud pond	41°48'	172°50'	17/03/2002	SDW	G	N							0.00	NT	NT	NT
DP41	Wairau River Gravel Pit	41°29'	173°44'	17/03/2002	S	G	N							0.00	NT	NT	NT
DP42	Taylor's Dam - Blenheim	41°34'	173°47'	17/03/2002	S	G	N							3.20	NT	NT	NT
DP43	Taylor's Dam - Blenheim	41°34'	173°47'	17/03/2002	S	G	N							0.00	NT	NT	NT
DP44	Onamalutu Domain Pond	41°27'	173°42'	17/03/2002	R	G	N							0.00	NT	NT	NT
DP45	Elizabeth Park Pond - Masterton	40°57'	175°40'	18/03/2002	S	G	N							0.00	NT	NT	NT
DP46	Glen Imis Pond	36°52'	174°52'	1/04/2002	S	G	N							0.00	NT	NT	NT
DP47	Wincester Farm - Pond	38°8'	176°14'	4/12/2002	SDW	G	N							0.80	NT	NT	NT
DP48	Wincester Farm - Pond	38°8'	176°14'	4/12/2002	SDW	G	N							0.00	NT	NT	NT
DP49	Woods Farm - Dam	41°48'	173°44'	12/12/2002	I	G	Y	<i>Anabaena perturbata f. tumida</i>	+++ +					0.00	Negative	NT	NT
DP50	Avalon Park Pond	41°14'	174°55'	20/01/2003	S	B	-	<i>Phormidium sp.</i>						26*	Negative	NT	NT
DP51	Lindale - Sth Pond	40°54'	175°1'	29/01/2003	SDW	G	N							0.00	NT	NT	NT
DP52	Waimarua - South Pond	40°52'	175°1'	29/01/2003	S	G	N							0.00	NT	NT	NT

Table A3 continued....

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/Scum?	Cyanobacteria Sp. 1	Qty	Cyano. Sp. 2	Qty.	Cyano. Sp. 3	Qty.	Microcystins (ug l-1) except where noted	Anatoxin	Sax (ug kg-1 FDW)	Sax (ug l-1)
DP53	Waimarua - North Pond	40° 52'	175° 1'	29/01/2003	S	G	N							0.00	NT	NT	NT
DP54	Steedman Farm - Trough	36° 17'	174° 14'	9/02/2003	SDW	G	N							0.00	NT	NT	NT
DP55	Te Anamutu Pond	38° 0'	175° 19'	14/03/2003	S	G	N							0.00	NT	NT	NT
DP56	Wet St Culvet - Blenheim	41° 44'	174° 9'	18/03/2003	SDW	G	N	Planktothrix sp.	+					0.47	NT	NT	NT
DP57	Burns Loop Farm Pond	41° 41'	174° 8'	18/03/2003	SDW	G	N							0.00	NT	NT	NT
DP58	Witherhills Park Pond	41° 33'	173° 56'	20/03/2003	SDW	G	N							3.40	NT	NT	NT
DP59	Wairua Lagoon	41° 30'	174° 3'	20/03/2003	S	G	N							0.00	NT	NT	NT
DP60	Lindale Pond - Nth Pond	40° 54'	175° 1'	29/03/2003	SDW	G	N							0.79	NT	NT	NT
DP61	Neuman Pond	40° 7'	175° 20'	16/04/2003	SDW	G	Y	Microcystis aeruginosa	+++ +	Microcystis flos-aquae	+++			12000.00	NT	NT	NT
DP62	Prawn Farm - Pond 1	38° 37'	176° 6'	28/04/2003	A	P (23 um)	Y	Anabaena planktonica	+++ +	Anabaena cf torulosa	+	Anabaena cf spiroides	+	0.00	Negative	0.0000	0.0000
DP63	Prawn Farm - Pond 1	38° 37'	176° 6'	28/04/2003	A	G	Y	Anabaena planktonica	++	Anabaena cf torulosa	+			0.00	NT	0.1548	0.0001
DP64	Lower Whitby Pond	41° 07'	174° 53'	5/12/2004	s	G	N	Anabaena planktonica	+					NT	NT	NT	NT

Table A4. Rivers and streams

No.	Sample Location	Lat. (°S)	Long. (°E)	Date	Water Use	Sampling method	Bloom/ Scum?	Cyanobacteria Sp. 1	Qty.	Cyano. Sp. 2	Qty.	Cyano. Sp. 3	Qty.	Microcystins (ug l ⁻¹) except where noted	Anatoxin	Sax (ug kg ⁻¹ FDW)	Sax (ug l ⁻¹)
R1	Riverton - Creek	43°42'	172°24'	5/02/2001	SDW	G		<i>Planktothrix</i> sp.	+					0.00	NT	NT	NT
R2	Southland Stream (unknown location)	-	-	1/03/2002	S	B		<i>Planktothrix peronata</i>	+++					0.00	NT	NT	NT
R3	Waikato River - Wgtn St Beach	37°46'	175°16'	8/01/2003	R	G		<i>Anabaena planktonica</i>	++					0.00	Negative	NT	NT
R4	Hutt River - Kennedy Good bridge	41°11'	174°55'	12/02/2003	R	B		<i>Phormidium</i> sp.						4.8*	Negative	NT	NT
R5	Motueka River - Alexanders Bridge	41°27'	174°50'	18/02/2003	R	B		<i>Oscillatoria</i> sp.						200*	Negative	NT	NT
R6	Motupiko River - Quineys Bush	41°47'	174°48'	19/02/2003	R	B		<i>Oscillatoria</i> sp.						44*	Negative	NT	NT
R7	Tikotu Creek	40°53'	174°58'	20/03/2003	S	B		<i>Oscillatoria</i> sp.	++					26*	Negative	NT	NT
R8	Waikanae River - Edge Park	40°53'	175°3'	16/04/2003	S	B		<i>Oscillatoria</i> sp.						2.1*	NT	0.2885	
R9	Utakura River	35°21'	173°44'	19/01/2004	SDW	G		<i>Microcystis wesenbergii</i>	+++	<i>Anabaena planktonica</i>	+	<i>Anabaena cf. circinalis</i>	+	0.35	NT	NT	NT
R10	Utakura River	35°21'	173°44'	20/01/2004	SDW	G/S		<i>Microcystis wesenbergii</i>	++++	<i>Anabaena planktonica</i>	+	<i>Anabaena cf. circinalis</i>	+	12.70	NT	NT	NT

Table A4. Location, date of collection, water use, sampling method, presence/absence of bloom, cyanobacteria species present and cyanotoxins and levels detected in samples for rivers and streams. G = grab sample, G/S = Grab sample of scum material, P = Plankton net (mesh size), B = Benthic sample, R = Recreational, S = Scenic, DW = Drinking water, SDW = Stock drinking water, H = Hydro electricity, OP = Oxidation Pond, I = Irrigation, A = Aquaculture, * = Coiled Species, # = Straight Species, + = Microcystins measured in ug kg⁻¹ of freeze dried weight. Quantification Index: + (1 colony or filament/ > 10 optical fields); ++ (1 colony or filament/ 1 to 9 optical fields); +++ (1 colony or filament/ optical field); ++++ (≥ 5 colonies or filaments per field) at 100x amplification.

Survey of Toxic Cyanobacteria (Blue-Green Algae)

SUSIE WOOD (PHD STUDENT)

Cyanobacteria (blue-green algae) are found naturally in many inland waters, estuaries and the sea. In slow moving water-bodies such as lakes, ponds and some rivers, these cyanobacteria may multiply sufficiently in summer months to discolour the water so that it appears green, blue-green or greenish brown. During calm conditions the cyanobacteria can rise to the surface to form a scum, which may resemble a blue-green paint or jelly. These scums can be blown around the water surface and thus may disappear and reappear in different places and at different times.

Some cyanobacteria species can produce toxins known as cyanotoxins. These toxins represent a health risk to both animals and humans. When ingested cyanotoxins have been known to kill cattle, sheep, waterfowl and dogs. Humans may develop skin irritations or rashes from contact with affected water. Ingestion of affected water can lead to vomiting, diarrhoea, fever, and pains in muscles and joints.

Although not all algal scums are harmful, it is a sensible precaution to avoid contact with the scum and water close to it.

As part of my PhD study at Victoria and Massey Universities, Wgtn, algal bloom/scum samples from around New Zealand are being screened for cyanotoxins. If there is an algal bloom affecting a water body in your area, I would greatly appreciate being sent a sample for testing. In return, I would inform you if the bloom contains any cyanotoxins and recommend appropriate actions. Samples should be sent in well sealed 500ml-1L plastic bottles. Cleaned milk or soft drink bottles are suitable.

For further Information contact:

Susie Wood. Ph: (04) 801 2794 extn. 6918 or A/H (04) 589 9782

Fax (04) 568 6276

Email: susie.wood@vuw.ac.nz

Address for delivery of samples: Atten. Susie Wood, School of Biological Sciences, Level 5, Room 508, New Kirk Bldg, Victoria University, Kelburn Parade, Wellington.

Appendix 4. Letter Introducing the Study and Flyer

School of Biological Sciences
Victoria University
PO Box 600
Wellington
Phone (04) 801 2794 ext 6918
A/H (04) 589 9782
Fax (04) 568 6276
email susie.wood@vuw.ac.nz

10 February 2001

Survey of Toxic Cyanobacteria (Blue-Green Algae)

As part of my PhD study I need cyanobacterial bloom samples from around New Zealand. The samples will be screened to assess the frequency, toxicity, toxin content and species composition of cyanobacterial blooms throughout New Zealand. If you are aware of any cyanobacterial blooms in your area I would appreciate being sent a sample.

I have enclosed an information flyer on toxic cyanobacteria. Some cyanobacteria (blue-green algae) can produce toxins that can be a health risk to animals and humans.

Please take time to read the enclosed flyer. It contains information about cyanobacterial blooms and details of how to send a sample. Please share the flyer with other people by passing it on to your friends or displaying it in an appropriate public place.

Yours sincerely

Susie Wood

Appendix 5. Articles to Advertise the Project to Farmers and Veterinarians

- 5a. Wood, S. A. 2001: Cyanobacteria – an underestimated risk to animal health in New Zealand? Vetscript December 2001: 4-5..... 255
- 5b. Wood, S. A. 2001: Livestock could be at risk. Rural News November 26 2001: 55..... 257
- 5c. Wood, S. A.; Parton, K. 2003: Livestock poisoning by blue-green algae Vetscript March 2003:12-14..... 258

TOXIC ALGAE

Cyanobacteria – an underestimated risk to animal health in New Zealand?

by Susie Wood, Wellington

As the summer months approach, warmer temperatures and calm conditions may trigger the onset of a potentially toxic hazard in many of New Zealand's slow moving fresh or brackish water bodies.

Cyanobacteria (also known as blue-green algae) are microscopic organisms with some characteristics of algae, such as the ability to photosynthesise, and some characteristics of bacteria, such as the absence of a nucleus. They occur naturally in many inland waters, estuaries and the sea.

In slow moving bodies of water, such as lakes, ponds and some rivers, cyanobacteria may multiply sufficiently in summer to discolour the water green, blue-green or greenish brown. During calm conditions they can rise to the surface to form a scum, which may resemble a blue-green paint or jelly. Although they do occur naturally, pollution with nutrients from agriculture and urbanisation has led to eutrophication of many water bodies and proliferation of cyanobacteria.

Some species can produce toxins (cyanotoxins). Within potentially toxic species there are toxic and non-toxic strains, thus just because cyanobacteria are found in a water body it does not necessarily mean that cyanotoxins will also be found. What triggers a strain to produce toxins is not fully understood but is believed to be linked to the genetics of individual species. Overseas research has shown that when a potentially toxic species does occur there is a 50-75% likelihood that it will produce cyanotoxins.

Cyanotoxins

Cyanotoxins are a diverse group, both chemically and toxicologically, and

appear to be more hazardous to terrestrial mammals than to aquatic biota. Mechanisms of toxicity are diverse and range from hepatotoxic, neurotoxic and dermatotoxic effects to general inhibition of protein synthesis. Cyanotoxins fall into three broad groups of chemical structure: cyclic peptides, alkaloids and lipopolysaccharides (LPS).

The hepatotoxic cyclic peptides (such as microcystins and nodularin) are found most frequently. Ingested in large doses, microcystins are highly toxic causing severe liver damage. The alkaloids (such as anatoxin-a, saxitoxins), a diverse group, generally have strong neurotoxic effects. LPSs are believed to occur in many cyanobacteria species but there has been little research

Risks to health

Cyanotoxins have been known to kill cattle, sheep, waterfowl and dogs when ingested. The first recorded animal poisonings were in Lake Alexandrina in South Australia in

Cyanobacterial bloom, Lake Rotoehu, March 2001. Inset: Photomicrograph of *Microcystis aeruginosa* from toxic bloom in Lake Horowhenua, 2001.

1878. Since then, toxic cyanobacteria have been reported from all continents, with numerous instances of animal poisonings.

Humans may develop skin irritations or rashes from contact with affected water. Ingestion can lead to vomiting, diarrhoea, fever, and pain in muscles and joints, and several fatalities have occurred. In Brazil, 50 dialysis patients developed fatal intoxication by microcystin, and in Australia 140 children were hospitalised after receiving contaminated drinking water.

Toxic cyanobacteria in New Zealand

Cyanotoxins have been identified from at least 11 lakes and two rivers here, and there is evidence of animal deaths caused by ingestion of affected farm dam water. Around Lake Forsyth and Ellesmere (Canterbury) there is a long history of nodularin poisoning of stock. Cyanobacteria in the Maitai River are suspected to have killed six dogs, some sheep and possibly rabbits and seagulls in early 1999. In a similar incident on the Kapiti Coast, five dogs died in December 1998.

Clinical signs

The speed of death or illness is determined primarily by the dose of cells ingested and the type of cyanotoxin. Signs may include rapid death, massive haemorrhage and acute hepatic necrosis, trembling, shaking, vomiting, muscle tremors, frothing at the mouth, diarrhoea, anorexia, mental derangement, dehydration, recumbency and ruminal atony, paralysis, enlarged liver, rapid respiration, respiratory failure, convulsions.

If such signs are observed in animals, check water bodies for green or green/brown discoloration, scums on the surface or shoreline, or any distinctive smell. Cyanotoxins can occur even when no scum or bloom is visible.

Analysis of water samples for cyanotoxin and cyanobacteria species should help establish if cyanotoxin poisoning was the cause of death or illness. If cyanotoxins are present, a first simple preventive measure is to restrict stock access and find alternative water supplies. Although not all algal scums are harmful, it is a sensible precaution to avoid contact with the scum and water close to it.

Algal bloom and scum samples are being screened for cyanotoxins as part of a PhD study at Victoria and Massey Universities, Wellington. If there is an algal bloom affecting a water body in your area, samples can be sent in for free analysis. You will be informed if the water body contains cyanotoxins, and appropriate actions will be recommended. Samples should be sent in well sealed 500 ml or 1 litre plastic bottles. Cleaned milk or soft drink bottles are suitable.

Anabaena sp bloom, Lower Karori Reservoir, Wellington, February 2001.
Inset: Photomicrograph of *Anabaena* sp.

For further information on sample collection and analysis, contact Susie Wood (s.wood@massey.ac.nz, phone 04 801 2794 extn 6918, or A/H 04 589 9782), School of Biological Sciences, New Kirk Building, Victoria University, Wellington. ■

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Within many farm dams, ponds and drinking troughs lurks a potentially hazardous organism that may be endangering the lives of farm stock and posing potential human health risks — PhD student Susie Woods explains:

Livestock could be at risk

CYANOBACTERIA — what is it?

Cyanobacteria — also known as blue-green algae — are microscopic organisms found naturally in many inland waters, estuaries, and the sea.

In slow-moving water bodies such as lakes, ponds, and some rivers, cyanobacteria may multiply sufficiently in summer months to discolour the water so that it appears green, blue-green or greenish brown. During calm conditions, the cyanobacteria can rise to the surface to form a scum, which may resemble a blue-green paint or jelly. Although cyanobacteria occur naturally, increasing runoff of nutrients from agriculture and urbanisation has caused the proliferation of some cyanobacteria species.

About 50 different cyanobacteria species can produce toxins known as cyanotoxins. Not all strains within a species will produce toxins, thus just because cyanobacteria occur in a water body, it does not necessarily mean that cyanotoxins will also be found. However, overseas research has shown that when a potentially toxic species does occur there is 50-75 per cent likelihood that it will be producing cyanotoxins.

Cyanotoxins are a diverse group of natural toxins, both from a chemical and toxicological point of view. In spite of their aquatic origin they appear to be more hazardous to terrestrial mammals than aquatic biota.

Risks to animal and human health.

When ingested, cyanotoxins have been known to kill cattle, sheep, waterfowl and dogs.

Humans may develop skin irritations or rashes from contact with affected water. Ingestion of affected water can lead to vomiting, diarrhoea, fever, and pains in muscles and joints.

Toxic cyanobacteria in New Zealand

Cyanotoxins have now been identified in at least 11 lakes and two rivers in New Zealand. There is evidence of suspected animal deaths caused by ingestion of water containing cyanobacteria from farm dams and drinking troughs. There is a long history of stock deaths from ingestion of cyanotoxins from around Lake Forsyth and Ellesmere (Canterbury). More recently, cyanobacteria in the Maitai River is suspected to have killed dogs, sheep and possibly rabbits and seagulls in January and February 1999. In a similar incident on the Kapiti Coast, five dogs died in December 1998.

Symptoms of cyanobacterial poisoning.

The speed of death or illness is determined primarily by the dose of cells ingested and the type of cyanotoxin.

Symptoms may include: trembling; shaking; vomiting; muscle tremors; frothing at the mouth; diarrhoea; anorexia; dehydration; paralysis; enlarged liver; hurried respiration; respiration failure; convulsions. Death may be rapid.

If any of the above symptoms are observed, check water bodies for green or green/brown discolouration, the presence of any scum on surface/shoreline or any unusual smell. Note that cyanotoxins can occur even when no scum or bloom is visible.

Although not all algal scums are harmful, it is a sensible precaution to avoid contact with the scum and water close to it. Many algal blooms are caused by harmless species of algae.

As part of a PhD study at Victoria and Massey Universities, Wellington, algal bloom/scum samples from around New Zealand are being screened for cyanotoxins. If there is an algal bloom affecting a water body in your area samples can be sent in for free analysis.

In return you will be informed if the water body contains any cyanotoxins and appropriate actions will be recommended. Samples should be sent in well-sealed 500ml-litre plastic bottles. Cleaned milk or soft drink bottles are suitable.

For further information on sample collection/analysis, please contact: Susie Wood, S.Wood@mas-vic.ac.nz; Ph: (041) 801 2794 ext. 6918 or A: (041) 589 9762; Fax: (041) 568 6276.

Address for courier delivery of samples: Attn: Susie Wood, School of Biological Sciences, c.c. 5 Room 308 New Kirk Bldg, Victoria University, Kallikoro Parade, Wellington.

Do you know what's lurking in your animal's drinking water?

Image: AgResearch/PhotoDisc 7704

Livestock poisoning by blue-green algae

by Susie Wood, Massey/Victoria University, and Kathy Parton, Pathobiology Group, IVABS, Massey University

Toxic cyanobacteria (blue-green algae) in waterbodies have been implicated in many stock poisoning incidents throughout the world. Research in New Zealand is increasing our knowledge of them, but their role in stock poisoning in this country is still unknown.

Cyanobacteria

Cyanobacteria are a group of photosynthetic bacteria also known as blue-green algae. There are approximately 2000 species worldwide and more than 40 are known to have toxin-producing strains.

The cyanobacteria of concern to stock health are generally freshwater or brackish water species and are commonly found as 'blooms' in slow-flowing, nutrient-rich waters, usually in the warmer months. Highly toxic 'scum' material can form on the water surface, creating a potential danger for livestock and humans. Some species can also grow on the bottom sediments sometimes forming coherent mats. These benthic species can be a problem especially during low flow periods when the mats become available to livestock.

Cyanotoxins

Cyanotoxins, the toxins sometimes produced by cyanobacteria, are a diverse group of natural toxins. They can be divided into three broad groups by chemical structure: cyclic peptides, alkaloids and lipopolysaccharides (LPS).

- The hepatotoxic cyclic peptides (eg microcystins and nodularin) are the largest group and most commonly found. They are protein phosphatase inhibitors.
- The alkaloids (eg anatoxin-a, saxitoxins, cylindrospermopsin) are a diverse group, which generally have strong neurotoxic effects.
- LPSs are believed to occur in many species but there has been little research on these.

Stock poisonings

The 2003 summer has already seen health warnings issued at four of New Zealand's recreational lakes and along 400 km of the Waikato River, which is widely used for recreation and as a water supply. Such warnings advise against drinking untreated water, and against recreational contact such as swimming and boating. However, despite the fact that bloom forming cyanobacteria have become increasingly prevalent, records of actual stock poisoning by cyanobacteria in New Zealand are scarce.

A duck swims in a bloom caused by *Anabaena lemmermanii* and *Anabaena circinalis* on the lower Karori reservoir, 2003

The first reports of suspected animal poisonings from ingestion of toxic cyanobacteria in freshwater ponds in New Zealand were published in 1966¹. Stock deaths from nodularin have also been recorded around Lakes Ellesmere and Forsyth. There are few other records of stock deaths and although there have almost certainly been other cases in New Zealand they have not been reported.

Recent research is increasing our knowledge and the toxic cyanobacteria species known to cause toxic blooms in New Zealand waterways are *Microcystis* sp, *Anabaena* sp, *Oscillatoria/Phormidium* sp and *Cylindrospermum* sp.

- Work by Susie Wood at Massey/Victoria University, Wellington, has identified microcystin toxins from more than 80 waterbodies with levels of up to 9000 ng/ml in one sample. The toxin nodularin was isolated from Lake Forysth in 1988 and toxic blooms continue to form here.
- In 1999, Institute of Environmental Science and Research Limited (ESR) scientist David Stirling identified the toxin cylindrospermopsin, which has caused numerous stock deaths in tropical Australia, in Lake Waitawa². To date this toxin has not been found elsewhere in New Zealand.
- The lethal neurotoxin anatoxin-a is believed to be responsible for a number of dog deaths in the Waikanae and Maitara Rivers. Current work at ESR and Massey/Victoria University is investigating further suspected occurrences of anatoxin.
- There are no reports of cyanobacteria producing saxitoxins (which are responsible for numerous stock deaths in Australia).

Clinical findings and pathology

Clinical signs depend on the quantity and type of toxin ingested. The range of clinical signs can be wide especially if more than one type of cyanotoxin is involved.

- **Anatoxin-a** mimics the effect of acetylcholine. The onset of clinical signs is abrupt (within 60 minutes). Signs include lethargy, muscle tremors, hyperpnoea.

Appendix 5c continued....

A bloom of *Microcystis aeruginosa* on Lake Horowhenua, 2003

continued from page 12

cyanosis, paralysis, hyperaesthesia, convulsions and death within 30 minutes after signs appear as a result of respiratory arrest.

- **Anatoxin-a (S)** is an anticholinesterase, and has not been recorded in New Zealand. Exposure causes an increase in salivation, lacrimation, ataxia, diarrhoea, dyspnoea and death within an hour. Signs similar to anatoxin-a may also occur.
- **Saxitoxins** block nerve cell sodium channels. They cause paralysis, respiratory depression and respiratory failure. In people incoordination, confused speech, nausea, vomiting, eye irritation, and respiratory distress may occur. In severe cases respiratory paralysis requires artificial respiration.

There are no specific lesions in animals that die of cyanobacterial neurotoxicoses.

- **Hepatotoxins (eg microcystins, nodularin)** cause intrahepatic haemorrhage and liver damage. Sometimes convulsions, paralysis or muscle tremors precede death. Depending on the amount of toxin ingested, signs can be acute or chronic. The liver may appear normal, but is usually enlarged and dark red from congestion and intrahepatic haemorrhage. Histologically, a widespread centrilobular to midzonal necrosis is observed.
- **Cylindrospermopsin** blocks protein synthesis with a major effect on liver cells. Effects include extended fibrosis and bile duct proliferation with isolated groups of hepatocytes remaining. Extensive subserosal haemorrhages of heart, and extended epicardial haemorrhages of the small intestine and omentum.

Milk found safe from toxic algae

Australian research has shown that even if dairy cattle drink water polluted by toxic cyanobacteria there is little risk of the toxins getting into their milk. In a study in which dairy cows were dosed with water contaminated

with a typical bloom concentration of the cyanobacteria *Microcystis* sp (which produces microcystin), the cows showed no ill effects. Two highly sensitive tests detected no microcystin in their milk. These results are reassuring because the Waikato River provides stock drinking water to many of the farms along its bank.

Diagnosis

Diagnosis of cyanobacterial poisoning should be based on the identification of cyanobacteria and cyanotoxins in the water source as well as the clinical signs and pathology. Gastrointestinal contents can be examined for the presence of cyanobacteria or tested for cyanotoxins. ESR and AgResearch have a range of commercially available assays for detecting cyanotoxins.

Treatment

If asymptomatic, small animals should be decontaminated with an emetic followed by activated charcoal. Treat large animals with activated charcoal. Animals with clinical signs require aggressive symptomatic treatment.

Prevention and control

Stock should be prevented from access to water that is visibly contaminated with cyanobacteria. All blooms should be regarded as potentially toxic.

Various physical, chemical and biological techniques have been used to prevent or control cyanobacterial blooms, including algicides, water filtration, water destratification and biomanipulation. The appropriate management technique will depend on the type of waterbody.

Care should be taken when applying management options such as algicides, as some techniques can result in the death of the cyanobacteria, causing a massive release of the cyanotoxins from the cells, thus escalating any potential risks.

For further information or sample analysis contact Susie Wood, PhD Student, Massey/Victoria University, Wellington, tel 04 801 2794, extn 6918, email S.Wood@massey.ac.nz

Animals suspected of cyanotoxin poisoning would be of interest to veterinary pathology at Massey University. Contact Mark Collett, tel 06 350 5799, extn 7884, email M.G.Collett@massey.ac.nz

References

- 1 Flint E. Toxic Algae in some New Zealand freshwater ponds. *New Zealand Veterinary Journal* 14. 181-184. 1966.
- 2 Stirling DJ, Quilliam MA. First report of the cyanobacterial toxin cylindrospermopsin in New Zealand. *Toxicon* 39. 1219-1222. 2001. ■

Appendix 6. Sampling Information Sheet

Cyanobacteria Sampling Sheet (please complete as much as possible)

SAMPLE NO.

Contact Information:

Name of sampler: ..... Ph: ..... Fax: .....
 Email: ..... Address: .....
 .....

Sample Information:

Date: ..... / ..... / ..... Time: .....

Sample Location (please be as detailed as possible e.g.: North end of Green Bay, Lake Karori):

.....
 .....
 .....

Sketch of Sample
Location or attach

Sampling method (i.e. filled plastic bottle): .....

Depth of sample(m): ..... Distance from shore (m): .....

Water Use (Circle more one than choice if necessary):

Human Drinking Water   Stock Drinking Water   Irrigation
                                          Oxidation pond

Recreation (please state activities e.g. swimming/sailing/fishing): .....

Other (please state): .....

Weather Conditions:

Weather at time of collection (circle): Clear Drizzling Light Rain Moderate Rain Heavy Rain

Cloud cover (1-8/1=clear): ..... **Wind strength** (1-8/1= calm):   **Wind direction:** .....

Weather conditions for 24hrs prior to sampling: .....

Bloom/mat Information:

What percentage of waterbody does bloom/mat cover: .....

What colour is the bloom/mat? ..... Is there any distinctive smell? .....

Are there any signs of animal/human poisonings? e.g. dead birds, fish, stock, rashes on swimmers: .....

Other Limnological Data:

Max. Water Depth: ..... Ave. Water Depth: .....

Area of Waterbody: ..... Water Temperature: .....

Predominant catchment cover (e.g.: farmland): .....

What wildlife is present on or in waterbody (e.g. trout/ducks): .....

Appendix 7. Lugol's Solution Recipe

Mix 20 g of KI (potassium iodide, AR grade) with 200 ml distilled water and dissolve 10 g pure iodine in this solution. Add 20 g of glacial acetic acid a few days before use. The solution must be stored in the dark.

Appendix 8. Number of Times Each Waterbody was Sampled

Lake	No. of Samples
Alexandrina	1
Arapuni	1
Brunner	1
Chalice	1
Coleridge	1
Dudding	1
Dunedin South Reservoir	1
Forsyth	2
Grassmere	2
Hakanoa	5
Hatuma	1
Henly	4
Horowhenua	8
Horseshoe	1
Kainui	2
Kaitoke	1
Kaniere	2
Karapiro	2
Kimiha	2
Kohangapiripiri	1
Kohangatera	3
Koromatua	1
Kuwakatai	1
Lower Karori Reservoir	5
Lyndon	2
Mahinapua	1
Mangamahoe	1
Maraetai	2
Maratoto	1
McGregor	1
Ngahewa	4
Ngaopouri	2
Ngaroto	3
Okaro	5
Omapere	4
Opuha	1
Orakai	1
Otamangakau	1
Papaitonga	2
Pauri	2
Pearson	1
Rerewhakaaitu	1
Rotoaira	1
Rotoehu	15

Rotoiti	25
Rotokaeo	1
Rotokare	1
Rotokawa	1
Rotomanuku North	1
Rotopounamu	1
Rotoroa (NI)	8
Rotoroa (SI)	1
Rotowhero	1
Serpentina East	1
Serpentina North	1
Spectacle	1
Tarawera	3
Taupo	3
Te Ko Utu	1
Te Whaiau	1
Tutira	1
Upper Karori Reservoir	1
Virginia	2
Waahi	6
Waihola	3
Waikopiro	1
Waipapa	1
Wairapara	1
Waitawa	7
Weavers	2
Westmere	1
Whakamaru	1
Whangape	1
Wiritoa	3
TOTAL SAMPLES	183
TOTAL LAKES	75

Oxidation Pond	No. of Samples
Bleheim (Nth Pond)	2
Bleheim (Sth Pond)	1
Kaitaia (Pond 1)	2
Kaitaia (Pond 2)	2
Kaitaia (Pond 3)	1
Kuratau	1
Masterton (Pond 3)	1
Mt Maunganui	1
Waikanae	1
TOTAL SAMPLES	12
TOTAL OXIDATION PONDS	9

Pond/Dam	No. of Samples
Avalon Park Pond	1
Belmont Park - Pond	1
Burns Loop Farm Pond	1
Coxe Farm - Big Dam	1
Coxe Farm - Boundary Dam	1
Coxe Farm - Kahakatra Dam	1
Coxe Farm - Middle Dam	1
Coxe Farm - Woolshed Pond	1
Georges Dr Pond	1
Glen Innis Pond	1
Golf Course Pond - Bleheim	1
Helensville Dam 1	1
Helensville Dam 3	1
Labonte Fish Farm - Pond	1
Lindale - Sth Pond	1
Lindale Pond	1
Mcintosh Farm - Culvet	1
Neuman Pond	1
Ngaumanu Park pond	1
Onamalutu Domain Pond	1
Pond - Bleheim	1
Richardson Farm Pond	1
Rotorua/Taupo Rd Pond	1
Simons Farm - Big Pond	1
Simons Farm - Little Pond	1
St Arnaud pond	1
Steedman Farm - Trough	1
Taylors Dam Pond 1	1
Taylors Dam Pond 2	1
Te Amamutu Pond	1
ToRepatutaki Swamp	1
Torrington Farm - Lower Dam	1
Torrington Farm - Sth Pond	1
Torrington Farm - Stream	1
Torrington Farm - Upper Dam	1

Torrington Farm - West Pond	1
Waihopa Cemetry Pond	1
Waikanae Estuary	1
Waimanu Lagoon - South	1
Waimanu Lagoons - North	1
Waimarua - North Pond	1
Waimarua - South Pond	1
Wairau River Gravel Pit	1
Wairua Lagoon	1
Welt St Culvet - Blenheim	1
Witherhills Park Pond	1
Woods Fam - Dam	1
Ellis Farm Pond	2
Elizabeth Park Pond - Masterton	2
Mcintosh Farm - Bottom Dam	2
Prawn Farm - Pond 1	2
Winchester Farm - Pond	2
Taylors Dam - Blenheim	4
TOTAL SAMPLES	61
TOTAL PONDS	53

Rivers/Streams	No. of Samples
Riverton - Creek	1
Southland Stream (unknown location)	1
Waikato River - Wgtn St Beach	1
Hutt River - Kenndy Good bridge	1
Motueka River - Alexanders Bridge	1
Motupiko River - Quinneys Bush	1
Tikotu Creek	1
Waikanae River - Edge Park	1
Utakura River	2
TOTAL SAMPLES	10
TOTAL RIVERS	9

Appendix 9. Species List for Chapters 2 – 4

<u>Chroococcales</u>
<i>Aphancapsa holsatica</i> (Lemmermann) Cronberg et Komárek
<i>Coelosphaerium</i> sp.
<i>Merismopedia</i> sp.
<i>Microcystis aeruginosa</i> (Kützing) Kützing
<i>Microcystis flos-aquae</i> (Wittrock) Kirchner
<i>Microcystis ichyobabe</i> Kützing
<i>Microcystis panniformis</i> Komárek et al.
<i>Microcystis smithii</i> Komárek et Anagnostidis
<i>Microcystis</i> sp.
<i>Microcystis wesenbergii</i> (Komárek) Komárek in Kondratieva
<u>Oscillatoriales</u>
<i>Arthrospira</i> cf. <i>maxima</i> Setchell & Gardner
<i>Lyngbya majuscula</i> Harvey ex Gormont
<i>Oscillatoria</i> sp.
<i>Phormidium</i> sp.
<i>Planktothrix mougeotii</i> (Kütz. ex Forti) Anagnostidis & Komárek
<i>Planktothrix peronata</i> f. <i>attenuate</i> (Skuja) Anagnostidis & Komárek 1988
<i>Planktothrix</i> sp.
<i>Pseudanabaena limnetica</i> (Lemm.) Komárek
<i>Pseudanabaena</i> sp.
<u>Nostocales</u>
<i>Anabaena affinis</i> Lemmermann
<i>Anabaena circinalis</i> Rabenhorst
<i>Anabaena crassa</i> (Lemm.) Komárková-Legnerová & Cronberg
<i>Anabaena lemmermannii</i> P. Richt
<i>Anabaena lemmermannii</i> var. <i>minor</i> (Uterm.) comb. nova
<i>Anabaena minderi</i> Huber-Pestalozzi
<i>Anabaena oscillarioides</i> Bory ex Bornet et Flahault
<i>Anabaena perturbata</i> var. <i>tumida</i> (Nyg.) Cronberg & Komárek
<i>Anabaena</i> sp.
<i>Anabaena planktonica</i> Brunnthaler
<i>Anabaena spiroides</i> Klebahn
<i>Anabaenopsis</i> sp.
<i>Aphanizomenon gracile</i> Lemmermann
<i>Cylindrospermopsis raciborskii</i> (Woloszyńska) Seenayya et Subba Raju
<i>Nodularia spumigena</i> (Mert.) Nordin et Stein
<i>Nostoc commune</i> Vaucher ex Bornet et Flahault
cf. <i>Raphidiopsis</i> sp.

Appendix 10. Cyanobacterial Blooms in the Karori Reservoirs

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1. Introduction

The study of the Karori reservoirs was carried out as a preliminary investigation for the main doctoral project. It provided good background knowledge into issues involved with formation and management of cyanobacterial blooms and was used to develop skills in the identification of cyanobacteria species. The data collected on the cyanobacteria species in the bloom later proved useful as this has allowed species composition changes in the Lower Reservoir to be assessed (see Section 2.6.2.4).

The Karori Reservoirs are located in the Wellington suburb of Karori - (Upper Reservoir $41^{\circ} 18'S$, $174^{\circ} 44'E$; Lower Reservoir $41^{\circ} 17'S$, $174^{\circ} 45'E$ (Figure 1).

Figure 1. Location of the Upper and Lower Karori Reservoirs (Wellington).

The Lower Karori Reservoir (Figure 2) was a major part of the first comprehensive water supply scheme in Wellington City. The construction of the large earth dam was completed on 24-02-1874. Early photos (Figure 3) show that the land was cleared on the eastern side of the valley and sparsely planted with pine trees while the western slope remained mostly in native scrub and bush.

The land surrounding the reservoirs was privately owned and farmed until purchased by the Wellington City Council in 1908. The Upper Dam was constructed

in 1908 (Figure 4) to supplement the Wellington water supply, which could not keep up with demand. The dam is a concrete gravity structure with a 90 m radius curve. It was considered a major engineering feat of the time (Figure 5). The maximum depth of the resulting reservoir was approximately 20 m and it had an impounding capacity of 62.5 million litres.

The Upper Dam was partially emptied in 1992 (Figure 6) following an engineers report which found that it did not have “sufficient factors of safety” to cover the risk of an earthquake on the Wellington faultline which runs through both dams. The Lower Dam was considered safe provided it did not have to withstand additional water pressure or over topping if the Upper Dam collapsed.

Figure 2. The Lower Reservoir. Source: www.sanctuary.org.nz.

Figure 3. The Lower Reservoir Dam, valve tower and boat shed (1873). Source: Alexander Turnbull Library.

Figure 4. Construction of Upper Dam in 1908. Source: Alexander Turnbull Library.

Figure 5. Upper Reservoir Dam when filled (1908). Source: Alexander Turnbull Library.

Figure 6. Upper Reservoir 2004. The tree line shows the former water level.
Source: www.sanctuary.org.nz.

The completion of a new enclosed water supply reservoir for Wellington at Johnsonville resulted in the Lower Dam being withdrawn from Wellington's water supply system in 1998. It is still retained as an emergency water supply.

The Karori reservoirs were used to store water from other locations prior to it being distributed to Wellington water supply and it is likely that this had an affect on the current day biota in the reservoirs. For example, Vidal & Maris-McArthur (1973) note that after 1966, excess fluoridated water from other water supplies (e.g. Morton Dam – Wainuiomata) was pumped into the Upper Karori Reservoir to raise the fluoride content of the stored water. The crustacean *Boeckella minuta*, was only recorded in the Upper Reservoir after this pumping commenced.

The Morton Dam (Wainuiomata) is recorded as having experienced blooms of *Anabaena circinalis* and a further unidentified *Anabaena* sp. between 1947 - 66

(Vidal & Maris-McArthur, 1973). Water from this dam was pumped to the Karori reservoirs and it is possible that this could have been the original source of *Anabaena* spp..

Both reservoirs are now part of a unique conservation project - "The Karori Wildlife Sanctuary." An 8.6 km predator-proof fence, specifically designed to exclude non-native mammals, encircles an area of 252 ha containing both reservoirs (see www.sanctuary.org.nz for further information on this project). All non-native mammals have been removed from the area and native flora and fauna is being reintroduced. The 500 year goal of the project is to return the valley to a pristine state.

Limnology data for both reservoirs is given in Table 1 including some historical data for the Upper Reservoir prior to draining.

Table 1. Limnological data for the Upper, Lower Reservoirs and the Upper Reservoir prior to draining. * Data from Vidal & Maris-McArthur (1973).

	Lower Karori Reservoir	Upper Karori Reservoir	Upper Karori Reservoir 1947 - 66*
Catchment Area (km ²)		2.6	2.6
Reservoir Area (km ²)	0.025	0.005	0.034
Storage Capacity (10 ⁶ m ³)	0.18	0.012	0.266
Average Depth (m)	8.2	4.2	7.8
Max. Depth (m)	20+	8+	20

Both Karori reservoirs are classified as warm monomictic lakes and mix only once during the year. During the study of the Upper Karori Reservoir carried out by Vidal & Maris-McArthur (1973) the water in this reservoir did not stratify. This is probably because large amounts of water were removed from the lower layers of the reservoir for water supply.

In the summer of 1996 - 97, whilst being used as the municipal water supply, the Lower Reservoir had to be closed due to a bloom of *Anabaena* spp. In the summer

of 1997-98 there was a further dense bloom but no species identification was carried out, as the reservoir was not being used as a water supply at the time (S Fuller, Karori Wildlife Sanctuary Trust, pers. comm). During this bloom a number of fish deaths were reported. After 1998, the reservoir had a number of small blooms but the species causing these were not identified (S Fuller, Karori Wildlife Sanctuary Trust, pers. comm). In the summer of 2000 - 01 there was a dense bloom that covered the entire reservoir (Figures 7 – 9) and lasted from November to April. The Upper Reservoir contains cyanobacteria species but no blooms have been reported.

Figure 7. Overview of the cyanobacterial bloom in the Lower Karori Reservoir. December 2000. Source: Evening Post.

Figure 8. Cyanobacterial scum at the South End of the Lower Reservoir. March 2001.

Figure 9. Cyanobacterial bloom at the North End of the Lower Reservoir. January 2001.

2. Objectives

The objectives of this preliminary study were:

- To establish which species were responsible for the cyanobacterial blooms in the Lower Reservoir.
- To establish if the cyanobacterial bloom contained any cyanotoxins.
- To identify physical and chemical parameters that could be causing the formation of cyanobacterial blooms in the Lower Reservoir and to establish why similar blooms do not occur in the Upper Reservoir.
- To identify possible management options that with further research could be applied to help reduce the cyanobacterial bloom problems in the Lower Reservoir.

3. Methods

Longitudinal depth profiles were constructed for both reservoirs by measuring the depth every 10 m along longitudinal transects. In addition, a longitudinal depth profile for the Lower Reservoir was constructed using depths given on an old drawing of this reservoir.

Grab samples (1-litre, in glass bottles) or depth integrated samples were collected fortnightly or monthly from February 2001 to September 2001. The samples were collected at three locations in the Lower Reservoir. The first site was a surface sample from the north end (close to dam). The second site was a mid-lake site – at this site three depth integrated samples were collected 1 – 5 m, 6 – 11 m and 12 – 15 m. These samples were collected using a Van Dorn sampler (1-litre) at 1 m intervals, the samples were then pooled and a 1-litre subsample was taken. The third site was a surface site at the south end of the Lower Reservoir. At the Upper Reservoir there was one surface sampling point, this was located next to the dam, just off the shoreline on the southern side.

Temperatures in the Lower Reservoir were measured (YSI, John Morris Scientific Ltd) at 0.5 m depth increments at the mid-lake site fortnightly or monthly from February 2001 to September 2001 and then intermittently between November 2001 to June 2002. For the Upper Reservoir they were measured fortnightly or monthly from March 2001 to September 2001 and then intermittently from November 2001 to June 2002. In the Upper Reservoir the temperature was measured close to the dam where this reservoir is deepest. Dissolved oxygen was measured intermittently in both reservoirs from March 2001 to September 2001. The dissolved oxygen meter could only measure to a depth of 7 m.

The water samples were well mixed and a 50 ml subsample was taken. It was preserved using Lugol's Iodine and stored in the dark and used later for species identification. Enumeration and identification was carried out using an inverted Olympus microscope and Utermöhl settling chambers (Utermöhl 1958).

At each site the Secchi disk depth was recorded. The following parameters were measured in each sample; turbidity, nitrate, nitrite, total phosphorus, dissolved phosphorous, ammonia and pH. In addition, on each sampling day the air temperature, wind direction and strength were recorded.

In November 2000 toxin analysis of the cyanobacterial bloom was carried out in conjunction with ESR (Porirua). A sample was sent to AgResearch (Ruakura) for microcystins analysis using the ADDA-ELISA (Fischer et al. 2001). The toxicity of the sample was assessed using a mouse bioassay (as described in Section 4.4.2.2).

Sediment cores were collected from both reservoirs on the 03-07-2001. The sediment core samples were divided into two subsamples, the first was sediment between 0 – 100 mm and the second 100 – 200 mm. The samples were then analysed for a range of elements and for carbon content by Spectra Chem Analytical (Lower Hutt).

4. Results

4.1 Longitudinal Depth Profiles of Reservoirs

The Lower Reservoir is an old flooded stream bed which is shown by the deep channel and large variation in the depths on the depth profile. The water height at the time of measuring the profile was 0.97 m below the top edge of the dam. The longitudinal profile from the old drawings did not differ noticeably from the measured profile (Figure 10). The Upper Reservoir is only a fraction of the size it once was and now has a maximum depth of only 8 m (Figure 11).

Figure 10. Longitudinal depth profile of the Lower Karori Reservoir. The dotted red line is a depth profile taken from measurements on an old drawing of the reservoir.

Figure 11. Longitudinal depth profile of the Upper Karori Reservoir.

4.2 Cyanobacteria Species Identification

The cyanobacteria species responsible for the 2000 – 2001 bloom in the Lower Karori Reservoir were identified as *Anabaena lemmermannii* (Figure 12) and *A. circinalis* (Figure 13). Both of these species are known to produce a range of cyanotoxins in other countries.

Figure 12. *Anabaena lemmermannii* from the Lower Karori Reservoir.

Figure 13. *Anabaena circinalis* from the Lower Karori Reservoir.

4.3 Cyanotoxins

The results of the ADDA-ELISA showed no evidence of microcystins. The mouse bioassay was also negative.

4.4 Cyanobacteria Species Enumeration

Concentrations of over 200 000 cells ml^{-1} of *Anabaena* spp. were recorded at Site 3 (surface, south end) in March 2001 (Figure 14). As the bloom subsided at the surface sites in April the cell concentrations of *Anabaena* spp. increased at the lower depths of the reservoir. *Anabaena* spp. were also recorded at in the Upper Reservoir and these reached a peak of 5 000 cells ml^{-1} in March 2001. Cyanobacteria were not observed in samples between March 2001 to September 2001.

Figure 12. Cyanobacteria cell concentration (cells ml^{-1}) of *Anabaena* spp. at five sampling sites in the Lower Karori Reservoir and one site at the Upper Karori Reservoir.

4.5 Temperature Depth Profiles

The Lower Reservoir was stratified when sampling commenced in February 2001 (Figure 15). It remained stratified until April 2001. Stratification was also recorded in November and February 2002. A maximum temperature of 22.1°C was recorded in February 2001 and a minimum of 7.5°C in August 2001. Some stratification occurred in the Upper Reservoir (Figure 16). In March 2001, April 2001, September 2001 and November 2001 there was approximately 2°C difference between the surface and bottom water temperatures. The maximum temperature of 19.5 °C was recorded in February 2001 and a minimum of 6.7°C in July 2001.

Figure 13. Temperature depth profile for the Lower Karori Reservoir at Site 2 (mid-lake) February 2001 to June 2002.

Figure 14. Temperature depth profile for the Upper Karori Reservoir, next to dam in deepest section, February 2001 to June 2002.

4.6 Dissolved Oxygen/Depth Profiles

The levels of dissolved oxygen in the Lower Reservoir did not vary with depth except in February 2001 where at 7 m depth there was 7 mg l^{-1} less dissolved oxygen than at the surface (Figure 17). In the Upper Reservoir in March, April and September 2001 the dissolved oxygen was $1 - 3 \text{ mg l}^{-1}$ less at 7 m depth than at the surface (Figure 18).

Figure 15. Dissolved oxygen depth profile for the Lower Karori Reservoir February 2001 to September 2001.

Figure 16. Dissolved oxygen depth profile for the Upper Karori Reservoir March 2001 to September 2001.

4.7 Chemical, Physical and Biological Data for Karori Reservoirs

The wind strength and wind direction for each sampling day is given in Table 2. The predominate wind direction on the sampling days was from the north. Tables 2 – 7 give chemical, physical and biological data for the six sample locations. In general Secchi disk depths increased and turbidity and *Anabaena* spp. concentration decreased over the February to September 2001 recording period. Ammonia was markedly higher at Site 2 (11 – 15 m) than the other sites in February and March. In April to September the levels of ammonia were similar at all sites. Total phosphorus was very high at Site 3 on the 26-03-2001. Nitrate was elevated at all sites in the Lower Reservoir on the 25-06-2001 otherwise it did not vary greatly. Nitrite levels were similar at all sites in the Lower Reservoir and were generally higher than those recorded in the Upper reservoir. Dissolved phosphorus was noticeably higher at Site 2 (11 – 15m) than other sites on the 07-03-2001.

Date	22/2/01	07/03/01	26/03/01	09/04/01	26/04/01	08/05/01	21/05/01	06/06/01	25/06/01	03/08/01	13/9/01
Wind Strength (knots)	15	0	15	15	5	0	5	10	5	5	10
Wind Direction	N	-	N	N	N	-	N	N	N	N	N
Water Temp (°C)	19.6	19.5	18.1	15.9	14.1	15.2	13.5	9.8	10.5	NS	NS
Air Temp (°C)	21	22	19.5	16	15	18	15	10	12	NS	NS
Secchi Disk (m)	0.8	0.4	0.6	2.5	2.1	3	3.2	3.2	3.4	NS	NS
Turbidity (NTU's)	14	35	26.5	2	1.6	1.4	1.2	2.2	0.8	NS	NS
Nitrate (mg l ⁻¹)	0.30	0.10	0.20	0.00	0.30	0.30	0.20	0.60	0.90	NS	NS
Nitrite (mg l ⁻¹)	0.006	0.002	0.000	0.000	0.008	0.019	0.010	0.013	0.005	NS	NS
Tot. Phosphorus (mg l ⁻¹)	1.13	2.44	1.04	0.90	0.73	1.04	2.18	1.05	1.89	NS	NS
Diss Phosphorous (mg l ⁻¹)	NS	0.68	NS	0.65	1.15	0.31	0.82	1.82	0.99	NS	NS
Diss. Oxygen (mg l ⁻¹)	5.40	6.65	4.00	2.26	4.25	5.10	5.20	5.29	5.95	NS	NS
Ammonia (mg l ⁻¹)	0.17	0.06	0.07	1.14	0.88	0.75	0.76	0.42	0.10	NS	NS
pH	9.11	8.5	8.6	7.69	7.22	7.5	7.2	7.5	7.44	NS	NS
Total <i>Anabaena</i> sp. (cells/ml)	4460	12500	7290	4	0	140	0	0	0	NS	NS

Table 2. Chemical, physical and biological data for the Lower Karori Reservoir February 2001 – September 2001 - Site 1 (surface, north end near dam).

Date	22/2/01	07/03/01	26/03/01	09/04/01	26/04/01	08/05/01	21/05/01	06/06/01	25/06/01	03/08/01	13/9/01
Water Temp (°C)	19.9	19.1	18	15.5	14	13.8	12.7	9.5	9.4	7.8	11.4
Secchi Disk (at surface) (m)	0.5	0.8	0.4	3	2.9	3.4	3.2	3	3.4	2.8	2.8
Turbidity (NTU's)	36	32.6	21.2	1.8	1.8	1.4	1.4	2.05	0.6	1.4	1.1
Nitrate (mg l ⁻¹)	0.3	0.2	0.5	0	0.3	0.3	0.2	0.7	0.9	0.6	0.2
Nitrite (mg l ⁻¹)	0.007	0.003	0	0	0.006	0.018	0.011	0.014	0.003	0.002	0.002
Tot. Phosphorus (mg l ⁻¹)	1.4	2.41	1.2	0.93	1.37	0.03	1.2	0.83	1.1	1.65	1.18
Diss. Phosphorous (mg l ⁻¹)	NS	0.85	NS	1.18	1.24	0.34	1.1	1.77	1.18	1.35	1.12
Diss. Oxygen (mg l ⁻¹)	6.7	6.05	4.3	1.7	4.6	4.5	5.2	5.8	7.1	NS	6.2
Ammonia (mg l ⁻¹)	0.03	0.03	0.17	1.16	0.75	1.16	0.76	0.49	0.1	0.01	0.03
pH	9.58	7.5	7.3	7.42	7.18	7.22	7.2	7.45	7.4	7.3	7.24
Total <i>Anabaena</i> sp. (cells/ml)	5270	250	24400	90	110	0	0	0	0	0	0

Table 3. Chemical, physical and biological data for the Lower Karori Reservoir February 2001 – September 2001 - Site 2 (mid-lake 1 – 6 m).

Date	22/2/01	07/03/01	26/03/01	09/04/01	26/04/01	08/05/01	21/05/01	06/06/01	25/06/01	03/08/01	13/09/01
Water Temp (°C)	NS	NS	NS	15.5	13.8	13.5	12.7	9.5	9.3	7.5	9.7
Secchi Disk (at surface) (m)	NS	NS	NS	2.1	2.6	1.6	1.2	1.6	0.7	1.6	1.8
Turbidity (NTU's)	NS	NS	NS	0	0.3	0.3	0.2	0.5	0.9	0.6	0.3
Nitrate (mg l ⁻¹)	NS	NS	NS	0	0.008	0.015	0.011	0.012	0.002	0.002	0.002
Nitrite (mg l ⁻¹)	NS	NS	NS	1.64	0.8	0.11	1.11	0.98	1.1	1.64	1.41
Tot. Phosphorus (mg l ⁻¹)	NS	NS	NS	1.19	1.3	0.99	1.26	1.75	1.22	1.4	1.36
Diss. Phosphorous (mg l ⁻¹)	NS	NS	NS	3.06	3.87	NS	5.2	5.8	7.4	NS	5.4
Diss. Oxygen (mg l ⁻¹)	NS	NS	NS	1.04	1	0.9	0.82	0.46	0.08	0.004	0.14
Ammonia (mg l ⁻¹)	NS	NS	NS	7.25	7.17	7.12	7.15	7.4	7.2	7.39	7.03
pH	NS	NS	NS	90	0	0	0	0	0	0	0

Table 4. Chemical, physical and biological data for the Lower Karori Reservoir February 2001 – September 2001 - Site 2 (mid-lake 6 – 11 m).

Date	22/2/01	07/03/01	26/03/01	09/04/01	26/04/01	08/05/01	21/05/01	06/06/01	25/06/01	03/08/01	13/9/01
Water Temp (°C)	17.1	18.9	17.7	15.4	13.8	13.5	12.7	9.4	9.3	7.5	9.4
Secchi Disk (at surface) (m)	NS	20	10.6	18.1	2	7.5	2.2	2.31	6.6	8.7	6.2
Turbidity (NTU's)	0.6	0.5	0.1	0	0.3	0.4	0.2	0.4	1.1	0.6	0.1
Nitrate (mg l ⁻¹)	0	0	0	0	0.007	0.016	0.009	0.012	0.004	0.002	0.002
Nitrite (mg l ⁻¹)	2.99	4.38	1.3	1.25	1.1	0.2	1.52	0.89	2.72	1.68	2.36
Tot. Phosphorus (mg l ⁻¹)	NS	2.46	NS	1.48	1.23	0.35	1.27	1.87	1.23	1.19	1.29
Diss. Phosphorous (mg l ⁻¹)	1.8	NS	1.7	3.6	4.05	NS	NS	NS	NS	NS	4.2
Diss. Oxygen (mg l ⁻¹)	1.5	1.75	1.07	1.08	1.06	0.95	0.9	0.47	0.09	0.03	0.14
Ammonia (mg l ⁻¹)	NS	6.5	7.23	7.12	7.16	7.14	7.3	7.35	7.51	7.53	7.12
pH	100	520	5650	0	24000	0	0	0	0	0	0

Table 5. Chemical, physical and biological data for the Lower Karori Reservoir February 2001 – September 2001 - Site 2 (mid-lake 11 – 15 m).

Date	22/2/01	07/03/01	26/03/01	09/04/01	26/04/01	08/05/01	21/05/01	06/06/01	25/06/01	03/08/01	13/9/01
Water Temp (°C)	23.4	23.2	20.9	15.8	15.9	14.5	14.4	10.2	10.5	NS	NS
Secchi Disk (at surface) (m)	0.5	0.4	0.2	*	*	*	*	*	*		
Turbidity (NTU's)	58.5	74.5	822	15.6	10.6	3.6	6	5.2	4.2	NS	NS
Nitrate (mg l ⁻¹)	0.2	0.2	0.4	0	0.3	0.4	0.1	0.2	0.9	NS	NS
Nitrite (mg l ⁻¹)	0.007	0.002	0	0	0.009	0.018	0.008	0.005	0.004	NS	NS
Tot. Phosphorus (mg l ⁻¹)	1.66	3.01	15.59	2.34	0.9	0.3	1.07	1.03	1.5	NS	NS
Diss. Phosphorous (mg l ⁻¹)	NS	0.63	NS	0.66	1.06	0.47	1.13	1.89	1.32	NS	NS
Diss. Oxygen (mg l ⁻¹)	7.6	10.46	8.5	2.5	4.9	4.5	4.5	5.63	6.36	NS	NS
Ammonia (mg l ⁻¹)	0.06	0.47	0.15	1.16	0.9	1.16	0.66	0.23	0.22	NS	NS
pH	9.6	8	10.6	7.15	7.2	7.13	7.18	7.2	7.6	NS	NS
Total <i>Anabaena</i> sp. (cells/ml)	27100	27500	231000	4100	80	80	40	6	0	NS	NS

*Clear to bottom (approx 0.7m)

Table 6. Chemical, physical and biological data for the Lower Karori Reservoir February 2001 – September 2001 - Site 3 (surface, south end).

Date	22/2/01	07/03/01	26/03/01	09/04/01	26/04/01	08/05/01	21/05/01	06/06/01	25/06/01	03/08/01	13/9/01
Water Temp (°C)	20.4	21	18.4	14.4	12.6	14.5	11.5	8.2	8.8	7.6	11.2
Air Temp (°C)	21	22	19	16	15	18	15	10	12	11	11.6
Secchi Disk (at surface) (m)	2.2	2.4	2.5	2.2	2.4	3	2.4	2.6	2.8	2	3.2
Turbidity (NTU's)	3.6	2	7.3	2.5	3.6	1.1	1.2	5.2	2.2	1.8	0.6
Nitrate (mg l ⁻¹)	0.1	0.2	0.1	0	0.1	0.1	0	0.2	0.3	0.2	0.1
Nitrite (mg l ⁻¹)	0.007	0.003	0	0	0.003	0	0.004	0.001	0.001	0.003	0.002
Tot. Phosphorus (mg l ⁻¹)	1.44	1.52	1.69	1.32	1.1	0.23	2.67	0.94	1.99	1.69	2.33
Diss. Phosphorous (mg l ⁻¹)	NS	0.49		1.2	1.71	0.42	1.1	1.73	1.47	1.09	1.48
Diss. Oxygen (mg l ⁻¹)	6.33	6.3	6.21	7.33	6.54	7	7.2	7.59	8.37		6.2
Ammonia (mg l ⁻¹)	0.04	0.09	0.04	0.1	0.04	0.22	0.047	0.03	0.38	0.05	0.03
pH	8.06	6.5	7.5	7.3	7.14	7.25	7.75	7.2	7.57	7.36	7.17
Total <i>Anabaena</i> sp. (cells/ml)	0	2000	5000	20	0	0	0	0	0	0	0

Table 7. Chemical, physical and biological data for the Upper Karori Reservoirs February 2001 – September 2001 - Site 4 (surface).

4.8 Sediment Analysis

The results of the sediment analysis are given in Table 8. The amounts of each element did not differ conspicuously between samples except for the carbon content (LOI%) which was noticeably lower in the 0 – 100 mm sample from the Upper Reservoir.

Element	Lower Karori Reservoir		Upper Karori Reservoir	
	0-100 mm	100-200 m	0-100 mm	100-200 mm
C	5.8	5.0	5.4	10.9
Na	0.957	0.976	0.951	0.791
Mg	1.136	1.247	1.232	1.089
Al	8.529	8.983	8.394	7.743
Si	28.470	28.986	27.946	24.87
P	0.184	0.177	0.178	0.216
S	0.285	0.205	0.237	0.362
K	1.988	2.029	2.142	1.778
Ca	0.7	0.649	0.619	0.719
Ti	0.501	0.569	0.435	0.505
Fe	3.803	3.898	2.902	3.288
LOI (%)	14.73	14.10	24.38	13.21

Table 8. Sediment analysis for the Upper and Lower Karori Reservoirs.

5.0 Discussion

The cyanobacteria species responsible for the bloom (summer 2000 – 2001) were identified as *Anabaena lemmermannii* and *A. circinalis*. No cyanotoxins were detected in the two samples analysed, however both species are known to produce cyanotoxins overseas and this limited testing does not rule out the possibility of cyanotoxins been produced in the future. If cyanotoxins are produced they could pose a threat to wildlife in the reserve, in particular waterfowl as cyanotoxins have been linked to the deaths of such birds elsewhere (Onodera et al. 1997). The species composition and cyanotoxins should continually be monitored as there is the possibility that the cyanobacteria species composition in the Karori Reservoir could change and that cyanotoxins could be produced by other strains/species in the future. The Karori reservoirs are no longer used as potable water supplies for Wellington and are not used for recreational activities thus potential human health risks associated with these cyanobacterial blooms are low. However, the blooms may cause a number of other problems. For example dense blooms can create low dissolved oxygen which can result in fish deaths and this may have been the cause of the reported fish deaths during the 1998 bloom.

Cyanobacterial blooms are often the result of a multitude of chemical, biological and physical factors interacting (Oliver & Ganf 2000). The results of this preliminary investigation indicate that a number of factors may play a part in the formation of blooms in the Lower Reservoir.

Stratification of the Lower Reservoir appears to be an important factor in the formation of cyanobacterial blooms. The highest cyanobacteria cell concentrations were recorded in February and March during stratification. The ability of cyanobacteria to regulate their buoyancy gives them a competitive advantage over other phytoplankton species during stratification. Cyanobacteria are able to move up and down in the water column. They use deeper water to obtain nutrients and upper well-mixed layer for photosynthesis. Both cyanobacteria species identified in the Lower Reservoir are able to fix atmospheric nitrogen and thus low levels of nitrogen would not affect their growing potential. When a lake becomes stratified only the upper epilimnion is aerated by oxygen from the atmosphere. Bacteria in the hypolimnion use what oxygen there is in the hypolimnion in their processes of breaking down organic matter and other substances. Thus the

hypolimnion can become anaerobic. An anaerobic hypolimnion can promote the release of phosphorus, toxic metals, ammonia and hydrogen sulfide from sediments. The release of ammonia and dissolved phosphorus was seen in this study when elevated levels were recorded at Site 2 (11- 15m) when the Lower Reservoir was stratified during February and March. Phosphorus released to the hypolimnion usually remains in the deep water during the period of oxygen depletion. At overturn when the water is re-aerated, the phosphorus is mixed throughout the water column – thus phosphorus regenerated from bottom sediments is made available for the subsequent algal growth cycle. Further research into phosphorous levels and the role of sediment nutrients are recommended for the Lower Reservoir.

The Lower Reservoir contains both brown trout and red fin perch (*Perca fluviatilis*). Early life stages of the red fin perch are known to feed on zooplankton - an important consumer of cyanobacteria. Department of Conservation survey's indicate high numbers of red perch in the Lower Reservoir. Further research into the effect of these fish on zooplankton and cyanobacteria populations in the Lower Reservoir are recommended.

The cyanobacterial species present in the Lower Reservoir are also present in the Upper Reservoir and concentrations of 5 000 cells ml⁻¹ were recorded in March 2001. There are some differences between the reservoirs that may account for the lack of cyanobacterial blooms in the Upper Reservoir. These differences include:

- The Upper Reservoir is shallower. Despite some stratification the hypolimnion is not becoming anaerobic.
- There are some macrophytes in the Upper Reservoir (native & introduced species). Studies have shown that macrophytes use up substantial amounts of dissolved phosphorous; they also reduce water movement and sediment re-suspension.
- The Upper Reservoir is several degrees colder than the Lower Reservoir. It also receives less sunlight.
- The Upper Reservoir contains only brown trout. The Lower Reservoir contains both brown trout and red fin perch (*Perca fluviatilis*) – see above.
- There is a thin layer of concrete on the bottom of Lower Reservoir.

- In the Upper Reservoir the outflow is subsurface and may help prevent stratification. In the Lower Reservoirs water flows out from the surface down a spillway.

It is likely that if no management actions are taken the cyanobacterial problems will continue in the Lower Reservoir and may worsen due to the large amount of biomass being produced each year. This is returned to the sediment when the bloom dies and releases nutrients back into the sediment and water column which in turn aids the growth of the following year's bloom.

Because of the controlled environment of the Karori Sanctuary and relatively small size of the Lower Reservoir, careful planning and management could lead to remedial measures being taken to reduce or eliminate the blooms. Further research would be required before any major management actions are put in place. Ideally intervention should provide a long term solution, be cost effective and have a low environmental impact. Below are some possible management options:

- Removal of red finned perch to increase zooplankton populations. Removal options could involve using poisons such as Rotenone or nets. Care would need to be taken to ensure that all fish were removed from all waterbodies in the catchment.
- Hypolimnetic withdrawal. This requires that the depth at which the water leaves the lake be changed from the surface to near the maximum depth. This reduces the retention time of the water in the hypolimnion and thus decreases the chances of anaerobic conditions developing. Caution needs to be taken to prevent de-stratification of the reservoir i.e.; epilimnetic water being drawn down. Advantages of hypolimnetic withdrawal include; relatively low capital and operational costs, evidence of effectiveness in a number of case studies and potentially long term and even permanent effectiveness (Cooke et al. 1993, Nurnberg 1987). In a number of studies it has been found that hypolimnetic dissolved oxygen increased, resulting in a decrease in the anaerobic volume and the days of anoxia. There are two possible options for hypolimnetic withdrawal in the Lower Karori Reservoir. The first would involve using existing structures, i.e. the water gate at the base of the water tower. However this carries high risk because of the age of the valves. There is

also little control of water flow. The second option involves setting up a siphon system using plastic pipe (Figure 19). The pipe would remove water from approximately 14 m below the surface and release it into the stream below. It would be relatively cheap to establish and maintain.

Figure 17. Diagram showing how a siphon system could be applied in the Lower Karori Reservoir.

- Artificial mixing preventing stratification. As stratification appears to be a major factor in the cause of cyanobacterial blooms in the Lower Reservoir this management option may prove successful. A number of methods can be used to prevent stratification. Aeration tubes which bubble oxygen into the water are one method that has proved successful (Cooke et al. 1993). For this method to be successful studies have shown that at least 80% of the water body needs to be mixed. High installation and running costs would be associated with such systems.
- Dilution or flushing. This would involve using water from other sources which are low in nutrients and has proved successful in some lakes (Oglesby 1969). This option is likely to be expensive due to the amount of water required and it may need to continue for a reasonably long length of time. Nutrient levels in the Lower Reservoir are not particularly high and are unlikely to be the main cause of the blooms.
- Algaecides e.g. Copper Sulphate. This maybe effective only in the short term and care would need to be taken to avoid adverse environmental effects. It would probably be most successful if it was applied in the early stage of a bloom development. If the cyanobacteria contain cyanotoxins, algaecides may

cause the cells to die and release a sudden pulse of cyanotoxins into the waterbody.

- Sediment dredging. Sediment can be a substantial source of phosphorus particularly if the sediment is becoming anoxic. However, removal of sediment can be costly and the sediment needs to be dumped away from the waterbody (Cooke et al. 1993). Although this option could be costly it might be feasible in Karori because the water level could be lowered to help facilitate sediment removal. Further studies should investigate the effect nutrients and their release from the sediment play in causing the blooms.
- Ultrasound. This has been trialed in the Lower Karori Reservoir and results indicate that it was not successful. It is possible that the area of the Lower Reservoir could not be covered by the range of the two machines installed.

6. Conclusion

Because of the small size of the Lower Karori Reservoir it is likely that with careful planning and management the condition of the water in the reservoir can be improved. Further research should be carried out into the effect of stratification, nutrients, zooplankton and fish should be carried out before making decisions regarding the management of the reservoirs. In the interim, cyanobacteria species composition and concentrations in both reservoirs should be monitored on a regular basis.

7. References

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Appendix 11. Recipes Used For Culture Media

BG-11

Reference: Rippka, R.; Deruelles, J.; Waterbury, J.; Herdman, M.; Stanier, R. 1979: Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* 111: 1-61.

Stock Solutions	Per Litre Distilled Water	To Prepare BG-11 Medium Add to 983 ml Sterile Distilled Water
NaNO ₃	150 g	10 ml
K ₂ HPO ₄ ·3H ₂ O	40 g	1 ml
MgSO ₄ ·7H ₂ O	75 g	1 ml
CaCl ₂ ·2H ₂ O	36 g	1 ml
Citric Acid combined with Ferric Ammonium Citrate	6 g	1 ml
EDTA	1 g	1 ml
Na ₂ CO ₃	20 g	1 ml
Trace Metal solution	See below	1 ml

Adjust pH to approximately 7.5. (Initial pH is approximately 8.5.)

Trace Metal Solution

Stock Solutions	Per Litre Distilled Water
H ₃ BO ₃	2.86 g
MnCl ₂ ·4H ₂ O	1.81 g
ZnSO ₄ ·7H ₂ O	0.222 g
Na MoO ₄ ·5H ₂ O	0.390 g
CuSO ₄ ·5H ₂ O	0.079 g
Co(NO ₃) ₂ ·6H ₂ O	0.0494 g

Dissolve each of the above substances separately prior to adding the next on the list.

Jaworski's (JM) Medium

Stock Solutions	Per Litre Distilled Water	To Prepare JM Medium Add to 986 ml Sterile Distilled Water
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	20 g	1.0 ml
KH_2PO_4	12.4 g	1.0 ml
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	50 g	1.0 ml
NaHCO_3	15.9 g	1.0 ml
EDTAFeNa	2.25 g	1.0 ml
EDTANa ₂	2.25 g	1.0 ml
H_3BO_3	2.48 g	1.0 ml
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.39 g	1.0 ml
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	1.0 g	1.0 ml
Cyanocobalamin	0.04 g	1.0 ml
Thiamine HCl	0.04 g	1.0 ml
Biotin	0.04 g	1.0 ml
NaNO_3	80 g	1.0 ml
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	36 g	1.0 ml

Appendix 12. Human Health Risk Posed by Microcystins in Samples

Amount of sample (containing microcystins) that would be required to be consumed to possibly kill and cause health impairment in a 10 kg toddler.

Sample No.	Sample Location	Microcystins/ Nodularins (mg l ⁻¹)	Amount of Sample (litres) Required to Kill a 10kg Toddler	Amount of Sample (ml) That Could Cause Health Impairment in a 10 kg Toddler
L82	Horowhenua	36.5000	1.37	0.011
L178	Waitawa	32.0000	1.56	0.013
L150	Wiritoa	20.0000	2.50	0.020
L143	Dudding	20.0000	2.50	0.020
L160	Taupo*	10.8700	4.60	0.037
L162	Hakanoa	0.4550	110	1
L118	Ngaroto	0.4230	118	1
L141	Rotoiti	0.3500	143	1
L156	Rotoehu	0.0430	1163	9
L149	Westmere	0.0131	3817	31
L139	Forsyth	0.0090	5556	44
L58	Omapere	0.0083	6024	48
L17	Papaitonga	0.0050	10000	80
L27	Orakai	0.0040	12500	100
L30	Hatuma	0.0027	18519	148
L121	Te Ko Utu	0.0021	23364	187
L147	Virginia	0.0021	23810	190
L6	Henly	0.0020	25000	200
L15	Waiholā	0.0017	29412	235
L155	Ngahewa	0.0015	33738	270
L97	Kuwakatai	0.0011	47259	378
L145	Kaitoke	0.0006	81301	650
L131	Kainui	0.0005	92593	741
L100	Ngaopouri	0.0004	132275	1058
L91	Rotorua	0.0003	166667	1333
L43	Okaro	0.0002	333333	2667

Table continued....

Sample No.	Sample Location	Microcystins/ Nodularins (mg l ⁻¹)	Amount of Sample (litres) Required to Kill a 10kg Toddler	Amount of Sample (ml) That Could Cause Health Impairment in a 10 kg Toddler
L116	Arapuni	0.0000	1250000	10000
L151	Grassmere*	0.0000	1666667	13333
DP61	Neuman Pond	11.6220	4	0
DP50	Avalon Park Pond	0.0260	1923	15
DP13	Torrington Farm - Lower Dam	0.0040	12500	100
DP14	Richardson Farm Pond	0.0035	14286	114
DP58	Witherhills Park Pond	0.0034	14706	118
DP42	Taylors Dam - Blenheim	0.0032	15625	125
DP47	Winchester Farm - Pond	0.0008	62500	500
DP60	Lindale Pond - Nth Pond	0.0008	63291	506
DP56	Welt St Culvet - Blenheim	0.0005	105485	844
DP8	Elizabeth Park Pond - Masterton	0.0005	111111	889
DP1	Pond - Blenheim	0.0004	125000	1000
DP3	Waihopa Cemetery Pond	0.0002	250000	2000
OP3	Kuratau	0.1000	500	4
OP8	Kaitaia (Pond 1)	0.0200	2500	20
OP5	Masterton (Pond 3)	0.0170	2941	24
OP1	Blenheim (Nth Pond)	0.0066	7576	61
OP4	Kaitaia (Pond 2)	0.0020	25000	200
OP7	Kaitaia (Pond 3)	0.0015	33333	267
OP2	Blenheim (Sth Pond)	0.0005	100000	800
R5	Motueka River - Alexanders Bridge*	0.2000	250	2
R6	Motupiko River - Quinneys Bush*	0.0440	1136	9
R7	Tikotu Creek*	0.0260	1923	15
R10	Utakura River*	0.0127	3937	31
R4	Hutt River - Kennedy Good Bridge*	0.0048	10417	83
R8	Waikanae River - Edge Park*	0.0021	23810	190

Appendix 13. Cyanobacteria Cell Counts for each Sample During Enclosure Experiment

Date	Depth	Aphanocapsa holicalica	Anabaena sp.	Anabaena planktonica	Anabaena leimnermannii	Anabaena torsulosa	Coelosphaerium cf kutzingianum	Gellierinema splendillum	Limnothrix aff. planktonica	Microcystis aeruginosa / M. Botrys	Microcystis protocystis	Plankthotrix aff. planktonica	Pseud- anabaena galeata	Sphaerocavernum brasiliensis	TOTAL cells/ml
07/03/04	0m			1637						744			74	3385	5840
	0m			112	2009					5022				9114	16256
	1 m			2809						1748			186	930	5673
	2 m			7347						223			558		8128
	3 m	1860	37	13526						558				11160	27141
08/03/04	0m			1183	856					2790					4829
	0m			1774						54089				12722	68586
	1 m			8035						744				3348	12127
	2 m			4085						3646					7730
	3 m	558		5357				1116				595			7626
09/03/04	0m			2176						3720				3422	9319
	0m			1934						372			223	1786	4315
	1 m			4843	74					7812					12730
	2 m			6108						4390				4390	14887
	3 m			4390					1116	223				10044	15773
10/03/04	0m			2522						8630				2232	13385
	0m			1481						5059				6770	13310
	1 m			1481						4464				1339	7284
	2 m			1339			446			2083				4836	8705
	3 m			1741					372	2381				521	5015
11/03/04	0m			3125	223		1190			149			74	4687	9449
	0m			2753	744		744			6696				8779	19716
	1 m			4352	1190					446				6696	12685
	2 m			3757	1190		595			1190	670		112	8333	15847
	3 m			7031	3720					3125	670		283	2232	17060

Table continued....

Date	Depth	Aphanocapsa holsatica	Anabaena sp.	Anabaena plan-htonica	Anabaena lemmer- mannii	Anabaena torsulsoa	Coelosphaerium cf kutzingianum	Gastriernema splendidum	Limnolnrix aff. planctonica	Microcystis aeruginosa/ M. Botrys	Microcystis protocystis	Planktothrix aff. planctonica	Pseud- anabaena galeata	Sphaerocavum brasilensis	TOTAL cells/ml
12/03/04	0m			5096	56					707	74			1972	7905
	0m			4966	1972							558		8258	15754
	1 m	1488		7998						4464	521			3050	17521
	2 m			1004					223				149	1116	2492
	3 m			3117						1339			223	4464	9144
13/03/04	0m			11398						7440	670			5952	25460
	0m			8333			298			4910	298			10118	23957
	1 m			3527						8928	74				12529
	2 m	1488		8749			298		223	3497	1042	372		1414	17082
	3 m			13719						3720	223				17663
14/03/04	0m			372						1510				2976	4858
	0m			2344	521					2083	372		149	223	5692
	1 m			2969	521					298	74			2232	6093
	2 m			3646			298			149			149	5208	9449
	3 m			3073						5134		372	298	5208	14084
15/03/04	0m			5468			818			2158	298			2381	11123
	0m			2083	112					28644	1414			372	32624
	1 m			409	37					3125				149	3720
	2 m			565						2530				1116	4211
	3 m			201						5134	74			744	6153
16/03/04	0m			3541						1860	521			5729	11651
	0m			1354						11755	744			3050	16904
	1 m			3594						10639	1339			2158	17730
	2 m			3073						4538	1339			9151	18102
	3 m			11093					446	2678					14218

Table continued....

Date	Depth	Aphanocapsa holsatica	Anabaena sp.	Anabaena planktonica	Anabaena leimmermannii	Anabaena torsulosa	Coelosphaerium cf. kuetzingianum	Geitlerinema splendidum	Limnolthrix aff. planctonica	Microcystis aeruginosa / M. Borrys	Microcystis protocystis	Planktothrix aff. planctonica	Pseud- anabaena galeata	Sphaerocavum brasiliensis	TOTAL cells/ml
17/03/04	0m			747						7142	223			1637	16450
	0m			417						6696	818			372	8303
	1 m			201			446			5134	818			2232	8831
	2 m			677						2083	446			3497	6703
	3 m			625						4464	223			2232	7544
18/03/04	0m			1094						1637	223			2604	5558
	0m			2135						62645	2232				67012
	1 m			1823	521						744			9300	12388
	2 m			2239						40399				372	43011
	3 m			30	1004						223			744	2001
19/03/04	0m	744		2239							372			2232	5587
	0m			543						61008	1860			1488	64899
	1 m			1458						5654	744			223	8080
	2 m			2239						17633	298			1042	21211
	3 m			3958						21204	223			5208	30593
20/03/04	0m			2187	30					6919	967			223	10327
	0m			223						8333	967			5208	14731
	1 m			1927						6919	2232			744	11822
	2 m			2135						6696	744			1562	11138
	3 m			3906						3497	223			1860	9486
21/03/04	0m			2344						1190	372			372	4278
	0m			4062			446	223		372				4464	9568
	1 m			2916						5357	446			1190	9910
	2 m			2604						1562	372			6026	10565
	3 m			781						372				1488	2641

Table continued....

Date	Depth	Aphanocapsa holsatica	Anabaena sp.	Anabaena planktonica	Anabaena lemmermannii	Anabaena torsulosa	Coelosphaerium cf kutzingianum	Gaillardinella splendendum	Limnithrix aff. planktonica	Microcystis aeruginosa / M. Botrys	Microcystis protocystis	Plankton hrix aff. planktonica	Pseud- anabaena galeata	Sphaerocolum brasilensis	TOTAL cells/ml
22/03/04	0m			2448			149			13392	744			744	17477
	0m			1927						16368	1042			1042	20378
	1 m			506						1488	446			4836	7276
	2 m			1823						2158			74	1488	5543
	3 m	2232		3802			10			7738	744		446	5952	20924
23/03/04	0m			5781			744			2158				7440	16122
	0m			2604						7738	744			4762	15847
	1 m			1406						10416	1637			744	14203
	2 m			260						2976				1860	5096
	3 m			4323						372				372	5067
24/03/04	0m			2292						967	223			2976	6458
	0m			27915						2232	744			2232	33123
	1 m			8802	223				149	2753	1190			1860	14977
	2 m			1406			298			670	1934			2232	6540
	3 m			937					372	1116	372		74	2604	5476
25/03/04	0m			2292			446			1488	372			6026	10624
	0m	1302		1406						2232	223			1860	7023
	1 m			2812						3422	1265			967	8467
	2 m			2031						5803	893			2009	10736
	3 m			625			298			595				3720	5238
26/03/04	0m			1927						3348	818			1116	7209
	0m			2448						10342	1190		149	2232	16361
	1 m			4427						446			149	372	5394
	2 m			1719			298			2604			409	2009	7038
	3 m			3125						7440	893		52		11510
27/03/03	0m			4062						1934	446			744	7187

Appendix 14. Supporting Paper

This published paper; “Wood, S. A.; Stirling, D. J. 2003: First identification of the cylindrospermopsin producing cyanobacteria *Cylindrospermopsis raciborskii* in New Zealand. *New Zealand Journal of Marine & Freshwater Research* 37: 821-828” which arose from this doctoral study is included as supporting material. The results published in this paper are covered in detail in Chapters 2, 3 and 4.

First identification of the cylindrospermopsin-producing cyanobacterium *Cylindrospermopsis raciborskii* in New Zealand

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Abstract *Cylindrospermopsis raciborskii* is a planktonic freshwater cyanobacterium that is becoming increasingly prevalent in water bodies worldwide. During a survey of toxic cyanobacteria in New Zealand, *C. raciborskii* was identified in a sample collected from Lake Waahi (Waikato). This is the first identification of this species in New Zealand. Liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry were used to confirm the presence of the cyanotoxins cylindrospermopsin (CYN) and deoxy-cylindrospermopsin (do-CYN). Detection of CYN and do-CYN demonstrates that *C. raciborskii* is now a species of concern in recreational, stock drinking, and potable water supplies in New Zealand.

Keywords *Cylindrospermopsis raciborskii*; cylindrospermopsin; deoxy-cylindrospermopsin; cyanobacteria; cyanotoxin; New Zealand

INTRODUCTION

Cylindrospermopsis raciborskii (Woloszyńska) Seenayya et Subba Raju is a planktonic freshwater cyanobacterium that was initially reported as found only in tropical and subtropical areas (Komárek & Kling 1991). However, a later review of the worldwide distribution of *C. raciborskii* (Padisák 1997) has reported this species in an expanding range, including temperate regions of Australia, Europe, and North and South America.

Cylindrospermopsis raciborskii was implicated in one of the most significant cases of human poisoning from exposure to a cyanobacterial toxin. In 1979, 148 people required hospitalisation with symptoms of gastro-enteritis after a local water supply on Palm Island (Australia) was dosed with copper sulfate to control a dense algal bloom (Byth 1980; Bourke et al. 1983). Later studies identified, isolated, and cultured a highly toxic strain of *C. raciborskii* from the water supply (Hawkins et al. 1985).

Cylindrospermopsis raciborskii is known to produce the tri-cyclic alkaloid cylindrospermopsin (CYN, Fig. 1) (Ohtani et al. 1992; Hawkins et al. 1997; Heintzelman et al. 2001). CYN causes extensive damage to the liver and is a potent protein synthesis inhibitor (Hawkins et al. 1985, 1987; Falconer et al. 1999; Griffiths & Saker 2003). Saker et al. (1999) reported on the suspected role of CYN in cattle mortality and Humpage et al. (2000) suggest that CYN may increase carcinogenic activity.

Two analogues of CYN exist—7-epicylindrospermopsin (Banker et al. 2000), a toxic minor metabolite of the cyanobacterium *Aphanizomenon ovalisporum*, and the non-toxic deoxycylindrospermopsin (do-CYN) (Norris et al. 1999).

In 1999, Stirling & Quilliam (2001) reported the first occurrence of the cyanotoxin CYN in a cyanobacterial bloom sample from Lake Waitawa, a recreational lake near Wellington. The dominant species was preliminarily identified as *Cylindrospermum* sp., however a rigorous identification of the causative organism was not carried out.

Single ion monitoring (SIM) and selected reaction monitoring (SRM) experiments for the detection of the cylindrospermopsins were performed in a similar manner to that previously described by Eaglesham et al. (1999).

A 10 ml aliquot of the frozen sample was freeze-thawed twice and filtered through a glass fibre filter (Whatman GF/C, 25 mm diam.). The sample was then analysed by high performance liquid chromatography with fluorescence detection for anatoxin-a and homoanatoxin-a following derivatisation as described in the method of James et al. (1998).

The total microcystin content of the sample was analysed with a competitive indirect ELISA using the methods of Fischer et al. (2001). The sample was freeze-thawed twice then filtered through a glass fibre filter (Whatman GF/C, 25 mm diam.) and used for the ELISA assay. An ELISA plate (NUNC MaxiSorp 1 no. 439454, Denmark) was coated overnight with OVA-Adda-hemiglutaryl, in 0.05M sodium carbonate buffer at pH 9.6 (100 µl, 20°C). After being washed twice with phosphate buffered saline (PBS), additional binding sites were blocked by incubation with Ovalbumin (Inovatech, BC, Canada) (1% in PBS, 300 µl, 1 h, 20°C). The plate was washed twice with PBS. The samples and Microcystin-LR standard (Calbiochem, Germany, 50 µl) were added to the wells together with antiserum (Cat-BSA-Adda antiserum, AgResearch, Hamilton, New Zealand, 50 µl). After incubation at 20°C for 2 h, the wells were washed twice with PBS containing 0.05% Tween 20 (PBST) and twice with PBS. Anti-sheep secondary antibody (Anti-sheep IgG-HRP, Lifescience NZ Ltd, 100 µl) was then added to the wells and the plate incubated for 2 h. The wells were then washed twice with PBST and twice with PBS. BioFX (BioFX Laboratories, Maryland, United States) substrate solution (100 µl) was added and the plate incubated for 15 min. The reaction was stopped by addition of 2M H₂SO₄ (50 µl) and the absorbance at 450 nm was determined with a microplate spectrophotometer (VERSAmax, Molecular Devices, CA, United States).

Lyophilised cell material (0.5 g) was extracted in 5% acetic acid and the extract tested for PSP toxins by the neuroblastoma assay (Truman et al. 2002).

RESULTS

Morphological

The concentration of *C. raciborskii* in the sample collected on 13 March 2003 was 3.5×10^5 cells/ml. The Lake Waahi strain conforms to species

descriptions from natural populations described by Baker (1991) and Komárek & Kling (1991). However the Lake Waahi strain produced only straight trichomes and the vegetative cell and heterocyte dimensions are slightly narrower than those in Baker (1991) but within the range of those described in Komárek & Kling (1991). Morphologically, the *C. raciborskii* from Lake Waahi showed the following characteristics (Fig. 2): trichomes solitary and straight with no mucilaginous sheath evident; heterocytes elongated and tapered to a rounded point. These were always terminal and present at one or occasionally both ends of the trichome, length 3–9 µm, breadth 1.5–2.5 µm; akinetes oblong-ovate to cylindrical, solitary or in pairs, usually but not exclusively adjacent to heterocyte, length 5–19 µm, breadth 2–4 µm; vegetative cells quadrate to cylindrical and not constricted at the cross walls, length 4–10 µm, breadth 1.5–2.5 µm; and apical cells often slightly attenuated at apices when heterocytes were not present.

Toxicity and toxin characterisation

The toxicity of the Lake Waahi sample was confirmed by mouse bioassay, which resulted in a time-to-death of 9.5 h.

No evidence of anatoxin-a or homoanatoxin-a was detected by HPLC-FLD analysis of the Lake Waahi sample. The ELISA did not detect any Adda-containing microcystins in the sample. This ELISA uses antibodies raised to the Adda moiety that is present in most (>80%) of the known toxic pentapeptide and heptapeptide toxin congeners (Fischer et al. 2001). The neuroblastoma assay did not detect any PSP toxins in the sample.

The analysis method for CYN was successfully developed and tested using a cylindrospermopsin containing Australian *C. raciborskii* sample gifted by Dr Gary Jones (CSIRO, Australia). Do-CYN ($M + H^+$ m/z 400.1) and CYN ($M + H^+$ m/z 416.1) were detected by LC-MS at 5.6 and 6.3 min respectively in the Lake Waahi sample. These retention times corresponded well to those for the Australian sample (Fig. 3). To provide further unequivocal evidence for the presence of CYN, a selected reaction monitoring (SRM) experiment was carried out under identical chromatographic conditions to the SIM experiment. CYN was positively identified by transitions m/z 416→194 and 416→176 at identical retention times to those obtained in the SIM experiment.

Quantification of cylindrospermopsin in the sample was not possible as no commercial calibration standard exists.

Fig. 2 Light photomicrographs of *Cylindrospermopsis raciborskii*. **A**, Terminal heterocytes in solitary straight trichomes. **B**, Akinete adjacent to terminal heterocyte. (A, akinete; H, heterocyte. Scale bars represent 10 μ m).

DISCUSSION

Blooms of cyanobacteria have been reported in many water bodies around New Zealand especially in the North Island (Pridmore & Etheredge 1987). The most frequent bloom-forming species are *Microcystis* sp. and *Anabaena* sp., with *Nodularia* sp., *Planktothrix* sp., and *Aphanizomenon* sp. recorded in a lesser number of water bodies (Pridmore & Etheredge 1987; Wood unpubl. data). Until recently, information on the toxin content of New Zealand blooms of cyanobacteria has been sparse. Flint (1966) published the first report of

animal poisonings in New Zealand caused by suspected ingestion of toxic cyanobacteria from a farm pond in Waipukurau. Carmichael et al. (1988) first identified the cyanotoxin nodularin from a bloom of *Nodularia spumigena* in Lake Forsyth (Canterbury) and there is also a long history of unexplained stock deaths from around both Lake Forsyth and nearby Lake Ellesmere. These stock deaths are thought to be caused by ingestion of lake water contaminated with *Nodularia* sp. The cyanotoxin microcystin has now been identified in over 40 water bodies in New Zealand (Christoffersen & Burns 2000; Hamill 2001; Stirling & Quilliam 2001;

Fig. 3 Single ion monitoring analysis of **A**, Australian *Cylindrospermopsis raciborskii* and comparison to **B**, a sample of *C. raciborskii* from Lake Waahi, New Zealand.

Wood unpubl. data). Degradation products of anatoxin-a from a species of benthic cyanobacterium have been found in the Waikanae River (Hamill 2001; Kevin James pers. comm.).

Over the last 10 years *C. raciborskii* has been identified as a component of the phytoplankton of many overseas water bodies where it was not previously thought to be present (Chapman & Schelske 1997; Padisák 1997; Saker & Neilan 2001). This identification of *C. raciborskii* from Lake Waahi is the first report of this species in New Zealand. Because *C. raciborskii* is not described in the commonly used New Zealand taxonomic algal literature (Etheredge & Pridmore 1987) it seems plausible that this species has been previously misidentified. When CYN was detected in Lake

Waitawa (Stirling & Quillam 2001) the causative species was identified as *Cylindrospermum* sp. *Cylindrospermum* sp. is not known to produce CYN (Chorus & Bartram 1999) and is primarily a benthic species (Baker & Fabbro 2003).

Following the discovery of *C. raciborskii* in Lake Waahi, this species has been identified in other water bodies in the lower Waikato River basin region (Ryan et al. 2003). The detection of CYN in the Lake Waahi sample is only the second recorded incidence of this cyanotoxin in New Zealand and the first record of the non-toxic analogue do-CYN. Microcystins, anatoxin-a, and homoanatoxin-a were not detected in this sample.

Several studies have indicated a substantially increased toxicity for extracts of *C. raciborskii* that

cannot be explained solely by the presence of CYN (Norris et al. 1999; Metcalf et al. 2002).

Two strains of *C. raciborskii* isolated from a water supply in Brazil have been shown to produce the paralytic shellfish poisoning (PSP) toxins neosaxitoxin, saxitoxin, and the gonyautotoxin 2/3 isomers (Lagos et al. 1999). However, analysis for cylindrospermopsin was not carried out even though there was a marked discrepancy between the mouse bioassay result and the HPLC-FLD result. Another Brazilian strain of *C. raciborskii* has been found to produce further PSP toxins with no evidence of CYN production (Molica et al. 2002). These findings prompted the investigation of the potential for the New Zealand strain to contain the PSP compound, but none was detected. Four strains from reservoirs and a river in Portugal have been found to produce a yet-to-be-identified toxic compound(s) (Saker et al. 2003).

Saker & Eaglesham (1999) have shown that cylindrospermopsin accumulates in the flesh of a commercially cultured freshwater crayfish, demonstrating possible risks from the consumption of these aquatic organisms during times of *C. raciborskii* blooms. Lake Waahi provides an important habitat to the shortfinned eel (*Anguilla australis*), and historically eels from Lake Waahi have provided a significant contribution to the wild eel fishery in the Waikato region (Chisnall 1996; Jellyman & Chisnall 1999). The possibility of bioaccumulation in this species and other aquatic organisms harvested from the lake is of concern, especially as this particular toxin is distributed through body tissues and organs (Norris et al. 2001).

CYN has been shown to degrade rapidly when exposed to sunlight and in the dark with the presence of plant pigments (Chiswell et al. 1999). However, McGregor & Fabbro (2000) found that CYN could be detected up to 6 weeks after cells of *C. raciborskii* were below detectable levels. Factors such as turbidity, mixing depth, and depth of the photic zone may affect the rate of CYN degradation. CYN degradation kinetics remains unknown for New Zealand water bodies.

Chiswell et al. (1999) found that at different stages of a bloom of *C. raciborskii*, extracellular CYN varied between 19% and 98% of the total toxin content. Cell counts are routinely used in New Zealand as a measure of the risk posed from a cyanobacterial bloom. However, cell counts do not take into consideration the extracellular toxin content and therefore may not be a true indicator of the

potential risk to human and animal health when *C. raciborskii* is present in a water body.

The identification of *C. raciborskii* from Lake Waahi along with the finding of *C. raciborskii* in other locations (Ryan et al. 2003) demonstrates that *C. raciborskii* is now a species of concern in both recreational, stock drinking, and potable water bodies in New Zealand. The detection of CYN and do-CYN by LC-MS and CYN by LC-MS/MS provide unequivocal evidence for the presence of these toxins. Recent research worldwide on the toxicity and adaptive nature of this species highlights the need for further investigation into the toxin production, toxicity, genetics, ecology, health risk, and distribution of the New Zealand strain of *C. raciborskii*.

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