Environmental Microbiological Risk Assessment and Management

EMRAM
Current Awareness 2008-2009
Cyanobacteria and Cyanotoxins

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by

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Client Report
(FW09081)

Current Awareness 2008-2009

Cyanobacteria and Cyanotoxins

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SUMMARY

We are reporting on the current literature relevant to cyanobacteria and cyanotoxins to capture changes in knowledge that can then be incorporated into how to best manage harmful algal blooms in freshwater.

This report introduces cyanobacteria, the toxins they may produce and the potential health consequences of exposure to the toxins. Then, recent Australasian research is summarised as part of maintaining current awareness from 2008-2009 in areas relevant to the management of cyanobacteria and cyanotoxins in New Zealand's freshwater systems, including drinking-water sources.

It is planned that this new output from the *Environmental Microbiological Risk Assessment and Management* (EMRAM) Service Description will become an annual feature of the service description to ensure continuity of science informing policy and that, should it be required, relevant information from the literature that is consistent and rapidly accessible between the Ministry of Health and ESR.

The key points are:

- The review confirms that conditions influencing cyanobacterial growth are location-specific and that remediation and/or monitoring strategies for water quality need to be determined for the conditions in a particular location and modified over time as conditions may change.

- Despite problems associated with cyanobacteria from a human perspective, cyanobacteria provide important ecosystem functions. Cyanobacteria are beneficial components of soil crusts in unvegetated areas or areas of sparse vegetation. Soil crusts help reduce dust and conserve soil during drought. Disruption of these fragile crusts due to animal or human activity can increase levels of dust and material flushed into water during storms;

- Aeolian dust (fine wind-blown soil, slit and clay material) contributes significant nutrients, including micro-nutrients such as iron required for nitrogen fixation by cyanobacteria, to surface waters. The dust then contributes to rapid cyanobacterial growth. Management options to limit landuse that generates dust or disrupts the fine crust of dry soils near shallow surface water could reduce these inputs and may help manage cyanobacterial blooms.

- The effect of global climate change on potential micronutrient levels in drinking-water sources should also be considered in catchment management. There may be the potential to manage cyanobacterial bloom severity by managing micronutrient levels.

- Reservoir depth seemed to be the causal link for the onset of cyanobacterial blooms in this system and should be considered as a part of water management strategies as increased drawdown events are likely with global weather changes.

- Water remediation strategies that focus on removal of the cyanobacterial cells may not remove these toxins;

- The presence of copper-based algicides in the water inhibited the biodegradation of cylindrospermopsin. This suggests that the use of copper-based algicides to control cyanobacterial blooms in water sources may be detrimental on two counts: they have the propensity to lyse the algal cell, releasing toxins into the water, but they may also inhibit the biodegradation of these toxins.

- The effect of cylindrospermopsin on the growth of the aquatic plant *Hydrilla verticillata*, or water thyme, showed that root growth was significantly increased, suggesting a possible bioremediation opportunity to manage cylindrospermopsin toxicity in water sources;

- Retention of microcystins in crop plants indicates that irrigation with water containing microcystins has the potential to move these toxins into farm animals and human food chains at concentrations that can exceed recommended tolerable limits. This is of significance to human health if irrigation waters are sourced from sites susceptible to cyanobacterial blooms;

- Reptiles trying to avoid consumption of the cyanobacterium may have consumed less food overall or a substandard diet leading to malnutrition. This type of sub-acute effect of cyanobacterial blooms on organisms in the food-web could have long-term impacts on reproductive outputs of ecosystems.

- Increasingly, molecular diagnostic tests are used to differentiate toxic from non-toxic strains of cyanobacteria genera that should in the future offer high throughput analyses for water samples.

- There are no new data available that would support a move from provisional maximum allowable values (PMAVs) for the cyanotoxin determinands to defensible MAVs, therefore the current PMAVs appear to be the best estimate of risk to public health.

- Benthic cyanobacteria, which include *Phormidium sp.*, have been recently recognised as a potential hazard. This novelty means that the health impacts are not yet understood for New Zealand and therefore this cyanobacterial genus, and other benthic cyanobacteria are not yet regularly monitored in many recreational waters. Changes to reflect the increase in benthic cyanobacterial problems in freshwater have been suggested in recent revisions of the *Draft Guidelines for Drinking-Water Quality Management for New Zealand*.

- Based on the studies in this literature review, one area where increased research would be of value is in toxin biosynthesis and determination of environmental factors that may stimulate this biosynthesis. This research would provide useful data to inform water source management and minimise cyanotoxin production.

1. INTRODUCTION TO CURRENT AWARENESS REPORTING

This report has been produced as a mechanism to collate information that is, or maybe in the future, important towards the management of freshwater cyanobacteria, their blooms and their toxins. Every year hundreds of scientific articles about cyanobacteria are published internationally. The aim of current awareness is to review this plethora of information and identify components that may be useful to effectively and efficiently manage cyanobacteria. Management of cyanobacterial blooms is necessary because some blooms produce toxins that adversely affect human health and unless well managed, cyanotoxins may enter drinking-water distribution systems.

The objectives of maintaining current awareness are long-term:

- To highlight emerging problems associated with cyanobacteria;
- To summarise research on cyanobacteria and cyanotoxins, focusing mainly on areas relevant to their management;
- To identify improvements in the way cyanobacteria are monitored and the way data are collected, collated and managed;
- To identify new tools available for managers of water bodies susceptible to cyanobacterial blooms.

As this is the first current awareness report for cyanobacteria, the scope has been limited to Australasia during 2008-2009. Future reports will extend the coverage to the international literature.

2. INTRODUCTION TO CYANOBACTERIA

Cyanobacteria, or blue-green algae as they are still sometimes referred to, are prokaryotic (no defined nucleus), Gram-negative photosynthetic eubacteria that are normal inhabitants soils and marine and freshwater ecosystems. Typically, they are not found in groundwater ecosystems, although their toxins could be carried from surface water to shallow groundwater or flow into unprotected wells from surface ponds. While cyanobacteria are unicellular, several species join to form robust filamentous multi-organism colonies, which may be visible to the naked eye (e.g. *Anabaena* spp., *Nostoc* spp., and *Oscillatoria* spp.; Figure 1). While cyanobacteria are endemic to surface waters, they can cause problems for recreational water users and drinking-water sources if a bloom forms that is dominated by species that produce cyanotoxins. Such blooms are referred to be several names, including harmful algal blooms, toxic algal blooms or cyanoblooms. If cyanotoxins are produced in a drinking-water source it is necessary to stop these entering the water distribution zone. The need to manage cyanobacteria, and thereby their cyanotoxins, is recognised in the DWSNZ.

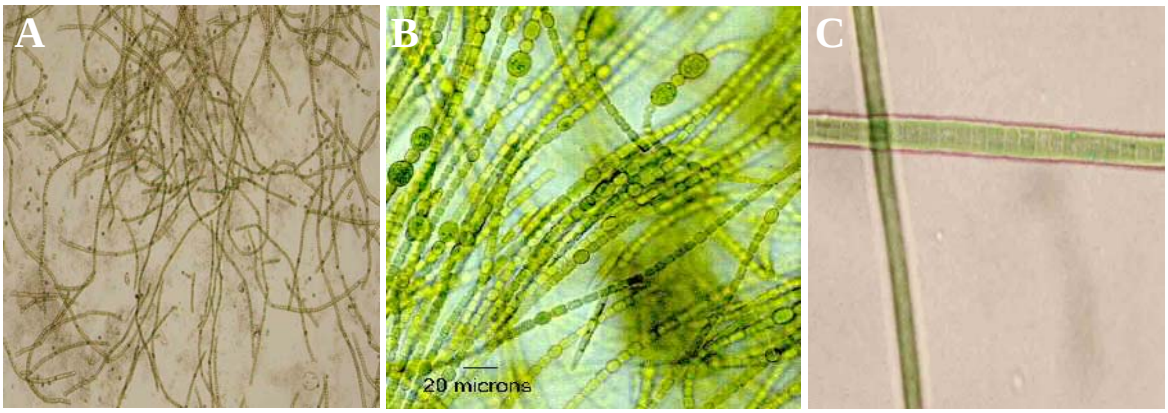

Figure 1 Some filamentous cyanobacteria. (A) *Anabaena*, (B) *Nostoc*, and (C) *Oscillatoria*. Images from www.marietta.edu, www.ebc.uu.se, and www.marietta.edu, respectively.

The DWSNZ:2005 (revised 2008) includes seven cyanotoxins of concern: anatoxin-a, anatoxin-a(S), homoanatoxin, cylindrospermopsin, microcystins, nodularin, and saxitoxin. This is in contrast to guidelines developed for the management of cyanobacteria and their toxins by other jurisdictions, such as the World Health Organization (*WHO) Guidelines for Drinking-water Quality (WHO, 2006)* and *Australian Drinking Water Guidelines*, 2004, which provide guideline values for microcystins only. The additional cyanotoxins included in DWSNZ:2005 (revised 2008) represent a New Zealand-specific precautionary approach, with the suite of cyanotoxins listed either found in New Zealand waters or produced by cyanobacterial genera present in New Zealand waters.

In the DWSNZ:2005 (revised 2008), cyanotoxins are classified as chemical determinands, but, unlike most chemicals listed in DWSNZ, cyanotoxins can kill quickly and at very low doses.

Table **1** lists the cyanotoxins of significance to New Zealand drinking-waters, their target tissues or organs, the cyanobacterial species currently known to produce these toxins and the assays that were used to derive the maximum allowable values (MAVs). For differences in the chemical structures of anatoxin-a, anatoxin-a(S), and homoanatoxin, see Figure 2 below.

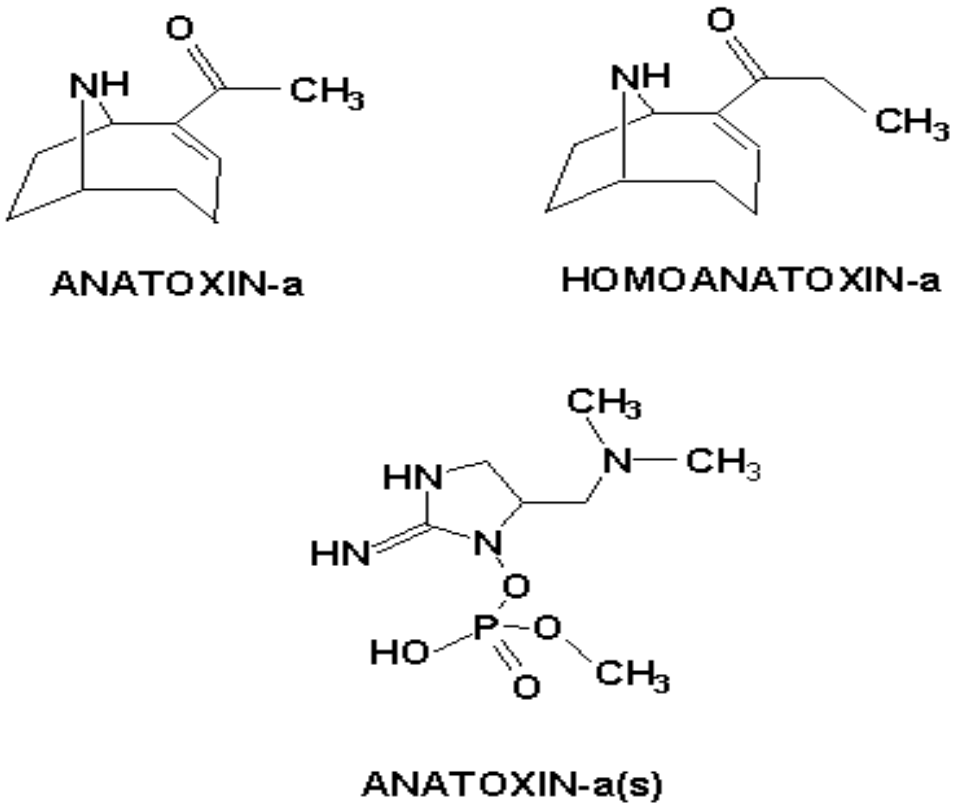

Figure 2 Chemical structures of the cyanobacterial alkaloid neurotoxins anatoxin-a, homoanatoxin-a, and anatoxin-a(S). Note that despite the names suggesting similarity, the structure of anatoxin-a(S) is unrelated to that of anatoxin-a.

2.1. Cyanobacterial bloom formation

Cyanobacteria can replicate sufficiently to create the phenomenon known as a "bloom" (Figure 3) under certain conditions, which can include readily available nutrients (particularly phosphorus), certain light intensities, molecular oxygen, and low water turbulence and flow. The algal blooms in Figure 3 are 2–3 cm below the water surface and the spatial arrangement of the bloom organisms in Figure 3 is most likely to be mediated by gas vesicles, which help the cells orientate themselves to achieve an optimal light intensity in this pond. Cyanobacteria use several mechanisms to compete with eukaryotic algae. For example, they conserve energy excellently, contain additional light-capturing pigments that significantly expand the range of photosynthetically-available light compared to eukaryotic algae (Mur et al., 1999), and they tightly regulate synthesis of gas vesicle buoyancy mechanisms, which enable cyanobacteria to remain at optimal light conditions for each species (Etheredge and Pridmore, 1984) (Figure 4). In addition to light, cyanobacteria use gravitational, chemical and thermal gradients as cues for their position in the water column (Mur et al., 1999). By using these mechanisms, freshwater "algal" blooms are often dominated by cyanobacteria rather than eukaryotic algae (such as diatoms), with the latter tending to dominate marine ecosystems.

Unfortunately, in addition to taste and odour problems caused by some cyanobacterial blooms, several cyanobacterial species produce toxic secondary metabolites (cyanotoxins), and some of these cyanotoxins have potent adverse effects on human, animal and fish health. It is estimated that at least 50–75% of cyanobacterial blooms are dominated by species that have the molecular capacity to synthesise toxins (Broady, 2007; and references within). It is not yet clear which environmental cues trigger activation of cyanotoxin-related genes.

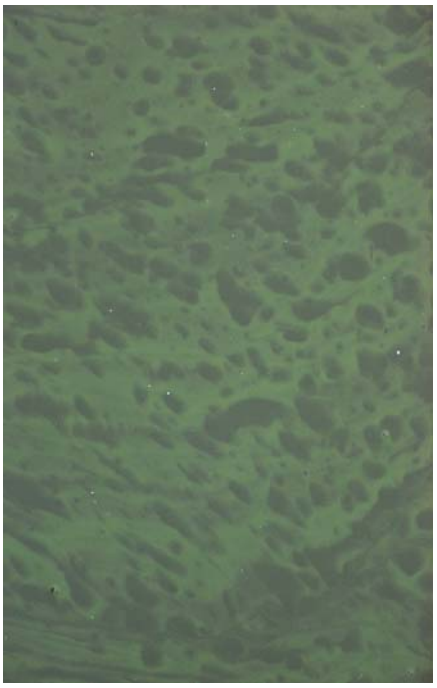

Figure 3 The "spill of green paint" appearance of an unidentified cyanobacterial bloom in freshwater ornamental ponds. The bloom organisms were 2–3 cm below the water surface.

2.2. Ecology of cyanobacteria and their toxins

So far it has not been possible to predict when a cyanobacterial bloom will form, and while some triggers are understood, their presence does not always result in a bloom, nor are all cyanobacterial blooms toxic. Toxic blooms arise only when the toxin-producing organisms dominate the bloom. Benthic cyanobacterial blooms, which also include species that are toxin-producers, may detach and float or wash to the margins of rivers and lakes. If these cyanobacteria contain cyanotoxins, they represent a hazard to humans and animals, such as dogs, which may eat toxic scum near shores or banks (Edwards et al., 1992; Hamill, 2001; Kroger et al., 2006; Milne and Watts, 2007).

In addition to light and nutrients as triggers of cyanobacterial blooms, it is plausible that biotic interactions such as protozoan grazing and viral infection can trigger bloom formation, and such a postulation is well supported by evidence from biotic interactions for eukaryotic algae (Long et al., 2007) and bacteria (Matz et al., 2004; Matz and Kjelleberg, 2005).

Cyanotoxins are classified according to their basic structure and mode of action: cyclic peptide hepatotoxins (microcystins and nodularins), alkaloid neurotoxins (saxitoxins,

anatoxin-a, homoanatoxin-a, anatoxin-a(s), alkaloid hepatotoxins (cylindrospermopsins), and the endotoxins (lipopolysaccharides). The cyanotoxins produced are diverse, even within a class, with more than 70 different microcystins identified so far (Orr and Schneider, 2006). There are slight differences in the nomenclature of some algal-toxins depending on whether they are produced in fresh or marine waters; for example, neurotoxic alkaloids from marine dinoflagellates are termed paralytic shellfish poisons, whereas when they are produced by freshwater cyanobacteria they are generically referred to as saxitoxins (Orr and Schneider, 2006).

For most (but not all) of the toxin-producing cyanobacterial strains, the toxins are retained intracellularly, with little released into the water body while the cell remains viable. Not all species of cyanobacteria produce toxins, and some produce several different cyanotoxins (Pridmore and Etheredge, 1987; Edwards et al., 1992; Stirling and Quilliam, 2001; Ryan and Hamilton, 2003; Wood and Stirling, 2003; Kouzminov et al., 2007). When cell death occurs in those species that produce cyanotoxins, whether by normal senescence or cellular rupture, the intracellular toxins are released into the surrounding water and may be hazardous either to the health of recreational water users or to drinking-water quality if an abstraction point is downstream.

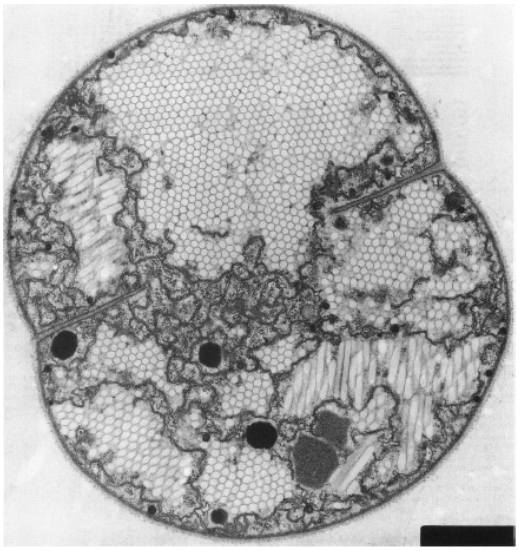

Figure 4 Transverse section of a dividing cell of the cyanobacterium *Microcystis* sp. showing the hexagonal stacking of the cylindrical gas vesicles, which are used for buoyancy by cyanobacteria to help maintain their position in the water column for optimum light and nutrient conditions for photosynthesis and growth. The gas vesicles are made of protein, which while homologous between all prokaryotes, including cyanobacteria, shows considerable interspecific variation of vesicle length and diameter (from (Walsby, 1994)).

Protozoan grazing pressure on marine eukaryotic micro-algae (small cells of 4–6 μm) can induce considerable changes in algal cellular morphology with the micro-algae forming large colonies (30,000 μm in diameter), and this change can be driven by protozoan species-specific grazing habits (Long et al., 2007). Similarly, an understanding of cyanobacterial bloom responses to biotic interactions could be gained if a similar mechanism were employed by cyanobacteria as is used by the bacterium *Chromobacterium violaceum* in response to protozoan grazing. The bacterium *C. violaceum* produces an alkaloid compound, violacein (Figure 5), and like the cyanobacterial alkaloid compound saxitoxin (Figure 5), violacein is an intracellular secondary metabolite toxic to many other organisms. Violacein synthesis is induced by the grazing activity of flagellate protozoa, and violacein is only released upon cellular rupture during protozoan grazing (Matz et al., 2004; Matz and Kjelleberg, 2005). Violacein is

acutely toxic to protozoa and its induction, via the acylhomoserine lactone (AHL) quorum sensing metabolic pathway, is responsive to increasing *C. violaceum* cellular density and grazing pressure (Matz et al., 2004; Matz and Kjelleberg, 2005). These are just two examples of biotic interactions that may explain at least some cyanobacterial population dynamics. However, other reports suggest that protozoa are not adversely affected by cyanotoxins, so alternative ecological roles for the secondary metabolites of cyanobacteria, some of which are toxic to humans, are possible (Lukac and Aegerter, 1993).

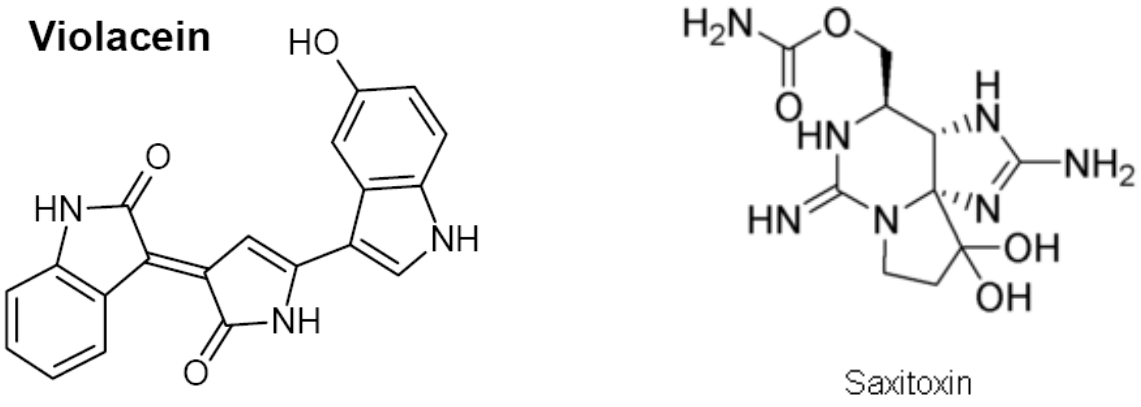

Figure 5 The chemical structure of the indole-alkaloid violacein produced by the bacterium *Chromobacterium violacein*, and the chemical structure of the amino acid-alkaloid saxitoxin produced by cyanobacteria including *Anabaena circinalis* and *Cylindrospermopsin raciborskii*.

2.3. Health risks

Both the acute and chronic health effects of cyanotoxins are discussed in the *Guidelines*. Acute effects can result in rapid death at low doses of cyanotoxins, while chronic exposure is relevant to people who live or work close to water bodies, and may lead to progressive debilitation from liver and nerve disease. There are no toxicological data for humans. The mouse assay is the usual model used to estimate probable toxic exposure levels for humans and the consequences of that exposure.

Table **2** shows how the PMAV for anatoxin-A was derived. The data are adapted from the *Guidelines* Volume III section 1.3 (page 70), and show the derivation in tabular form, rather than the equation form of *Draft Guidelines for Drinking-Water Quality Management for New Zealand* (the DW-*Guidelines*). A similar approach was taken for each of the other cyanotoxins, except nodularin, in deriving their PMAVs. There are few toxicological data available for nodularin, although some studies for nodularin suggest an LD_{50} for mice of 0.05-0.06 mg kg^{-1} (by intraperitoneal (i.p.) administration; (Eriksson et al., 1988; Towner et al., 2002). Several of the cyanotoxin PMAVs are based on data for other toxins that have

structural similarity, but for several cyanotoxins, the toxicological association is not well supported experimentally.

An additional cyanobacterial genus has been suggested for DWSNZ revisions, *Phormidium*. *Phormidium* spp. have been associated with taste and odour problems of water due to the production of 2-methylisoborneol (Baker et al., 2001). However, the presence of anatoxin-a and homoanatoxin-a has also been found to originate from *Phormidium* spp. (Cadel-Six et al., 2007). In New Zealand, a dog died in November 2007, after eating "algae" from the Rangitaki River and analysis of the dog's stomach contents showed elevated levels of anatoxin-a (230 $\mu g\ kg^{-1}$) and the presence of *Phormidium* sp (laboratory reports P93656 and P93672 are available from Cawthron Institute). In February 2008 algal mats were sampled from the Mangatainoka River, south of Woodville, and found to be dominated by *Phormidium* sp. and to have homoanatoxin-a present at 55 and 100 $\mu g\ kg^{-1}$, for the mid-river and river edge samples, respectively (laboratory report SO1440 is available from Cawthron Institute). However, the presence of *Phormidium* sp. does not necessarily mean that anatoxin-a or homoanatoxin-a is present. For example, several water samples also analysed in November 2007 from Bay of Plenty catchments showed that while *Phormidium* sp. dominated the water sample, no cyanotoxins were present above the detection limit (Cawthron Institute Report P94008). Similarly, in January 2008 samples from the Tukituki River and the drinking-water intake at Herbert, Otago showed the presence of *Phormidium* sp. but no cyanotoxins (laboratory reports S00312 and S0034 are available from Cawthron Institute).

2.4. Methods for cyanotoxin analyses

A review of cyanobacteria in New Zealand was published in 2006 (Wood et al., 2006). A literature search on methods for cyanotoxin analysis from 2006 onwards did not indicate any advances on the existing referee or alternative methods, and indicates that the methods prescribed by the DWSNZ:2005 are sufficient for detection of cyanotoxins in New Zealand drinking-water sources. However, the limit of detection required may not be easily achieved.

An excellent review on cyanobacterial toxins has been published by (van Apeldoorn et al., 2007), and several comments are relevant to DWSNZ including: no acute reference doses have formally been derived so far; bioassays and biochemical assays are non-specific and so can only be used for screening purposes; solid-phase extraction (SPE), especially with immunoaffinity columns, can be used for sample concentration and clean-up prior to high performance liquid chromatography (HPLC), and HPLC is appropriate for the separation of several cyanotoxins from complex mixes.

The complexity of analysing an array of cyanotoxins is illustrated by the different methods necessary, depending on the toxin. For example, cylindrospermopsin and saxitoxin, which due to their hydrophilic nature cannot be extracted and concentrated from water using SPE-C18 columns, require concentration procedures such as graphitised carbon-based sorbents. A variety of detectors for separated cyanotoxins is required for toxin identification and quantification (e.g. UV-spectroscopy for fluorescent detection and mass spectroscopy (MS). For microcystin-separation by HPLC, the amide C16 column had the best overall performance. A comment that common additives in plastics could

contaminate water samples and coelute with microcystins to have interfering absorbance at 238 nm, indicates that collection vessels need to be checked and standardised to ensure erroneous results are not reported. The use of tandem mass spectroscopy for improving detection sensitivity and for identifying novel cyanotoxins is increasing, but this technique is still expensive. For anatoxin-a and homoanatoxin, LC-MS is preferred because derivatisation is not required, and when coupled with additional MS and ion-spray techniques, currently known degradation products of anatoxin-a and homoanatoxin can be determined and identified.

Table 1 Cyanotoxins of health significance for drinking-water in New Zealand; modified from DWSNZ:2005, the Guidelines Volume III section 1.3, and (Sivonen and Jones, 1999). Limits of detection (LOD) values are from Cawthron Institute reports of recent cyanotoxin testing from a variety of matrices. The provision maximum acceptable values (PMAV) are for treated (finished) water. Toxicology assays are from (Kuiper-Goodman et al., 1999).

Name (PMAV ug L^{-1}) (LOD)	**Primary target organ in mammals**	**Cyanobacterial genera production**	**Toxicological assay**
Anatoxin-a (PMAV 6) LOD 0.2 μg L^{-1} water 2.0 μg kg^{-1} solid	Nerve synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon Phormidium*	Neurotoxic alkaloid LD_{50} 0.38 mg kg^{-1} Mouse i.p.
Anatoxin-a(S) (PMAV 1)	Nerve synapse	*Anabaena*	Organphosphate Neurotoxin LD_{50} 0.02 mg kg^{-1} Mouse i.p.
Cylindrospermopsin (PMAV 1) LOD 0.2 μg L^{-1} water 2.0 μg kg^{-1} solid	Liver and lymphoid tissue	*Cylindrospermopsis, Cylindrospermum, Aphanizomenon, Raphidiopsis , Umezakia,*	Hepatoxin LD_{50} 0.18–0.30 mg kg^{-1} Mouse i.p.
Homoanatoxin-a (PMAV 2) LOD 0.2 μg L^{-1} water 2.0 μg kg^{-1} solid	Muscles-Respiratory failure	*Planktothrix, Raphidiopsis,*	Neuromuscular block LD_{50} 0.25 mg kg^{-1} Mouse i.p.
Microcystins (PMAV 1) (expressed as MC-LR toxicity equivalents) LOD LR 2 μg L^{-1} water RR 1 μg L^{-1} water YR 2 μg L^{-1} water LR 10 μg kg^{-1} solid RR 5 μg kg^{-1} solid YR 10 μg kg^{-1} solid	Liver	*Anabaena, Anabaenopsis, Aphanocapsa, Aphanizomenon, Arthrospira,Hapalosiphon, Microcystis, Nostoc, Planktothrix (Oscillatoria), Phormidium, Pseudanabaena, Raphidiopsis, Snowella, Synechocystis, Woronichinia*	Enzyme inhibitor LD_{50} 0.05–0.06 mg kg^{-1} Mouse i.p.
Nodularin (PMAV 1) LOD 1 μg L^{-1} water	Liver	*Nodularia*	Enzyme inhibitor LD_{50} Not available

Saxitoxins (PMAV 3) (as STX-eq) LOD Presence/absence	Nerve axons	*Anabaena, Aphanizomenon, Cylindrospermopsis , Lyngbya, Microcystis, Planktothrix (Oscillatoria)*	Neuromuscular block LD_{50} <0.01 mg kg^{-1} Mouse i.p.

i.p. = intraperitoneal route for toxicity assay application

Table 2 Parameters used to derive the PMAV for anatoxin-A in the DWSNZ 2005.

Parameter	**Value**	**Comment**
Toxicity Parameter (NOAEL, LD_{50} etc)	200 μg kg^{-1}	LD50 mice; pure compound by i.p. injection.
Fraction of TDI allocated to drinking-water	0.8	Using the same factor as WHO for microcystin
Assumed body weight	70 kg	Standard body weight for NZ MAVs
Assumed daily drinking-water consumption	2 L	Standard volume used for NZ MAVs
Uncertainty factor		
Interspecies variation	10	
Intraspecies variation	10	
Database adequacy	10	
Calculated PMAV	5.6 μg L^{-1} DW μg L^{-1}	
Rounded PMAV to be used	≡ 0.006 mg L^{-1} DW	

i.p. = intraperitoneal route for toxicity assay application; DW = drinking-water; TDI = tolerable daily intake

3. MAINTAINING AWARENESS

3.1. Introduction

Every year hundreds of scientific articles about cyanobacteria are published internationally. The aim of current awareness is to review this plethora of information and identify components that may be useful to effectively and efficiently manage cyanobacteria. As this is the first current awareness report for cyanobacteria, the scope has been limited to Australasia during 2008-2009. Future reports will extend the coverage to the international literature. The primary focus is on freshwater, however some relevant advances may come from the marine setting, so the overall scope is quite broad.

During the 2008/09 reporting period, 39 papers relating to cyanobacteria and/or cyanotoxins were published in peer-reviewed journals and were authored by New Zealand and/or Australian researchers. The majority of these publications describe studies to determine factors influencing the growth of cyanobacteria in the environment. While not specifically addressing the quality of drinking-water, the results of these studies can be extrapolated to include drinking-water sources. Other publications cover aspects of persistence of cyanobacteria and/or cyanotoxins in the environment, detection and monitoring of cyanobacteria, as well as the use of cyanobacteria as markers for geological

profiling and as model organisms to study the mechanisms of photosynthesis and nutrient utilisation.

As a broad concept of how databases on harmful algal blooms may be managed, the web site of the Intergovernmental Oceanographic Commission of UNESCO is a good example (even though it is marine rather freshwater) of an approach to manage several databases. It includes an international directory of experts in harmful algae and their effects on fisheries and public health, harmful algae monitoring systems, taxonomy, images and harmful algal events: http://www.iocunesco.org/hab/index.php?option=com_content&task=view&id=16&Itemid=0.

New Zealand has a broad multi-organisational research programme under FRST, *Lake Ecosystem Restoration New Zealand* (http://www.lernz.co.nz/index.html) that includes cyanobacteria in the *Harmful Algal Bloom* section (http://www.lernz.co.nz/harmful_algal_blooms.shtml).

The following review summarises the findings of the 2008-2009 publications.

3.2. Factors influencing the growth of cyanobacteria in the environment

Human health is at risk when exposed to cyanotoxins and an increase in cyanobacterial biomass can lead to an increase in cyanotoxins. Therefore, the ideal would be for water suppliers to have tools to predict cyanobacterial blooms and then to take steps to prevent them or, if that is not possible, to prevent cyanotoxins entering the distribution zone. Therefore, factors that influence the growth of cyanobacteria in the environment (rather than in laboratory studies) are important because it is the increase in cyanobacterial biomass that can lead to harmful algal blooms. Identifying new contexts that promote the growth of cyanobacterial blooms will give drinking-water suppliers a more in-depth knowledge of these conditions, and this will to help them manage cyanobacteria in the context of their particular drinking-water supplies. Accumulating knowledge of the factors common to cyanobacterial blooms will highlight potential monitoring and/or trigger points.

The recent literature is reviewed next; however, the salient points are:

- Increases in the macronutrients N and P and increases in micronutrients, e.g. iron, promote algal growth. These have a strong positive effect on increasing N-fixing cyanobacteria and changing the species of cyanobacteria present, which gives some cyanobacteria a competitive advantage and may promote domination by toxin-producing species;

- Macro- and micro- nutrients enter surface water from multiple sources including groundwater, run-off during floods after drought, and in windborne soil (dust). This adds to the nutrients already available to support cyanobacterial growth and will aid bloom formation;

- Aeolian dust (fine wind-blown soil, slit and clay material) contributes significant nutrients, including micro-nutrients such as iron required for nitrogen fixation by

cyanobacteria, to surface waters. The dust then strongly stimulates the formation of cyanobacterial blooms, and does so very rapidly. Management options to limit landuse that generates dust or disrupts the fine crust of dry soils near shallow surface water could reduce these inputs and may help manage cyanobacterial blooms.

- Nutrients entrained in sediments mean that any positive results arising from changes in catchment management protocols (such as decreasing external nutrient inputs) may be delayed. Preventing nutrients entering drinking-water sources is of primary importance for long-term management of cyanobacterial bloom development;

- Cyanobacteria favour freshwater over saline water, and small decreases in salinity levels increase the proportion of cyanobacteria in a water body. This may be important during droughts when salinity levels typically increase in freshwater or if seawater incursions occur due to heavy extraction of groundwater. Thus, when a drought is broken a bloom may occur as salinity decreases;

- Climate change, with increasing periods of droughts punctuated by intense rain storms, is likely to promote cyanobacterial growth through increased pulses of macro- and, especially, micro- nutrients entering water bodies, including pulses into groundwater.

A number of studies were reported by both Australian and New Zealand researchers that aimed to determine the factors that influence the growth of cyanobacteria in the environment. Studies were undertaken in a range of locations including water storage reservoirs, freshwater lakes, estuarine lake systems and marine environments. Most studies examined aspects of nutrient status, water temperature, water stratification and anthropogenic activity in the catchment area. Some of these studies specifically focused on cyanobacterial growth, while others looked at total phytoplankton and/or algal growth, with cyanobacteria as a subset of this. While few of these publications focused directly on drinking-water sources, the general environmental results can be extrapolated and raise issues that should be considered for drinking-water sources.

The importance of nitrogen and phosphorus levels was highlighted in several publications. (Bayer et al., 2008) used Lakes Hayes in the South Island of New Zealand as a case study. This lake is fed by both surface- and ground- water inflows and recent environmental management strategies (e.g. Otago Regional Council Management Plan) at this lake have aimed to reduce algal blooms by reducing external nutrient influxes. Despite catchment nutrient management, algal blooms still occur at this site. The study showed potential for periods of nitrogen limitation in this lake, which may limit algal growth. There has been a recent shift in composition of algal blooms from dominance by cyanobacteria to dinoflagellates, which suggests changes in nutrient loads are affecting cyanobacterial bloom formation. However, a strong internal phosphorous cycling also occurred in this lake, which may explain the continued occurrence of algal blooms despite improved catchment nutrient management. This study also showed that groundwater can be an important source of nutrients that may support cyanobacterial growth: almost 30% of the nitrate entering Lakes Hayes was from a groundwater-fed spring and the groundwater contained trace elements silicon, boron and selenium. These observations highlight the

importance of groundwater-derived nutrients as a potential driver of algal proliferation in freshwater lakes.

The influence of nutrients stored in surrounding catchments on phytoplankton growth was also illustrated in a study of an Australian floodplain environment (Kobayashi et al., 2008). It was demonstrated that floodplain sediments in a marsh environment, which had been exposed to dry conditions, released nutrients and carbon to overlying floodwater following inundation. Nitrogen release was rapid, followed by more gradual increases in phosphorous and dissolved organic carbon. Concomitant with this release in nutrients was the rapid development of phytoplankton communities.

Along with the importance of nitrogen and phosphorus levels for the growth of cyanobacteria, is the role of micronutrients. Micronutrients are important for cyanobacterial growth as a number are involved as co-factors for enzymes in the photosynthetic process. For those cyanobacteria that are also diazotrophic[1], iron and molybdenum are important in the nitrogen-fixation process. A specific study on micronutrient importance to the growth of the nitrogen fixing, potential toxin-producing cyanobacteria *Anabaena flos-aquae* was undertaken by Downs et al. (2008) in a mesotrophic[2] and a eutrophic[3] lake system. Both lakes are located in Otago, NZ and have agriculture-dominant land-use in the catchment area. Downs et al. (2008) found that nitrogen and phosphorus limitation was the main factor affecting cyanobacterial growth in the mesotrophic situation; however, micronutrient levels become the limiting factor where there was no macronutrient limitation. These results agree with those from an extensive literature study, undertaken by the authors, indicating 71% of lakes tested internationally had increased phytoplankton productivity when micronutrients were added. In the eutrophic lake situation the study by Downs et al. (2008), it was also seen that cyanobacterial bloom severity was influenced by micronutrient availability. Agricultural practices may well include the addition of micronutrients to pasture in inorganic fertilisers, resulting in the potential for anthropogenic influence on water micronutrient levels.

Micronutrients may enter an environment by a number of routes. In the northern hemisphere, aeolian dust from Saharan dust storms has stimulated blooms of nitrogen-fixing cyanobacteria in the North Atlantic Ocean. Australia is the major contributor of atmospheric dust to the southern hemisphere; a phenomenon that is likely to increase with global climate change. (Shaw et al., 2008) used satellite imagery to link the deposition of aeolian dust in coastal Queensland waters, which resulted from a severe dust storm in 2002, with increased phytoplankton levels. Phytoplankton responses to this injection of nutrients were rapid, with increased growth within one day. It is suggested that aeolian

[1] Diazotrophs are bacteria that fix atmospheric nitrogen gas into a more usable form such as ammonia.
[2] Mesotrophic lakes are lakes with an intermediate level of productivity.
[3] Eutrophic lakes have a high primary productivity, resulting from high nutrient content.

dust may be a critical source of iron for nitrogen-fixing cyanobacteria. Extrapolation of the effect of this micronutrient loading event would be an increase in water nitrogen levels by nitrogen-fixing cyanobacteria that would then enable growth of other nitrogen-limited cyanobacterial species. The importance of iron for cyanobacterial growth was also demonstrated by (Ahern et al., 2008) in a targeted study of the nitrogen-fixing, toxin producing, cyanobacterium *Lyngbya majuscula* in a marine environment. Chelated iron was added to *in vivo* environmental samples along with nitrogen and phosphorous. Each of these nutrients stimulated growth of *L. majuscula*, with by far the greatest effect from iron. There was also some evidence that increased availability of iron and phosphorous stimulated nitrogen fixation in this cyanobacterium. *L. majuscule* has been identified in NZ freshwaters and so results of this study may also apply to growth of this cyanobacterium in drinking-water sources.

The work on micronutrients suggests that the potential contribution of micronutrient enrichment of water sources should not be overlooked in the management of water catchments for cyanobacterial growth. Ahern *et al*. (2008) suggested that a "Precautionary Principle" approach should be taken in limiting anthropogenic sources of micronutrients in drinking-water sources. The effect of global climate change on potential micronutrient levels in drinking-water sources should also be considered in catchment management. There may be the potential to manage cyanobacterial bloom severity by managing micronutrient levels.

The effect of water salinity on cyanobacterial growth was discussed in several publications. Two studies were undertaken in the Myall Lakes area of NSW, Australia (Redden and Rukminasari, 2008; Ryan et al., 2008). This is a system of four interconnecting lakes that includes both variable and stable physicochemical environments. Freshwater inflows from rivers can invert the water column, while salt-water incursion into river mouth regions can occur during drought. Both studies showed that the variability in phytoplankton communities in the lake system was influenced by nutrient availability and water salinity. In particular, the growth of *A. circinalis* positively correlated with ammonia concentration (increased nitrogen availability) and negatively correlated with increased salinity of the water. These results provided an explanation for cyanobacterial blooms observed in the lake system following heavy rain after a period of drought, a situation that would result in a decrease in water salinity.

It was suggested that a correlation between increased salinity and increased micronutrient availability in the eutrophic lake situation may indicate a future global climate change issue, as increased salinity in river mouths may influence micronutrient availability (Downs et al., 2008). This increase in micronutrient availability from increased water salinity would need to be balanced against direct influences of increased salinity on cyanobacterial growth. This is likely to be cyanobacterial species-specific. Increased

salinity due to increased drought episodes may cause a shift in the composition of cyanobacterial communities in specific regions.

In NZ and Australia[4] a number of catchment management systems have been established to reduce the potential for cyanobacteria blooms in water systems. These include the 1995 Otago Regional Council management plan for Lake Hayes, NZ (Otago Regional Council and Queenstown Lakes District Council, 1995), discussed before, and a plan established in 1991 to remove treated wastewater inputs from Lake Rotorua in NZ. Despite these plans, cyanobacterial blooms continue to occur in these lakes. Lake Rotorua is a large, shallow, eutrophic lake with a number of inflow points; including nine major and nine minor cold water inflows and eight major geothermal inflows. External nutrient loads of nitrogen and phosphorus are known and were used by researchers from the University of Waikato to model the relative importance of internal and external nutrient loads on water column nutrient concentrations and phytoplankton biomass in this lake system (Burger et al., 2008). A sophisticated modelling program was utilised that accounted for external nutrient loads, discharge from the single outflow point, meteorological data and measurements of sediment nutrient release. Model simulations indicated that cyanobacterial blooms coincided with large sediment nutrient releases and periods of increased temperature and high irradiance. The model also showed that nutrient release from lake sediment had a greater influence on water column nutrient levels than external nutrient influxes. This may explain why measures to reduce external nutrient influxes have not yet reduced cyanobacterial blooms in this lake. It is suggested that only a significant and prolonged reduction in external nutrient loads, that will eventually reduce internal sediment nutrient levels, will ultimately reduce cyanobacterial biomass.

The effect of sediment-derived nutrients on cyanobacterial growth was also shown in a study of a large water storage reservoir in Australia during a period of extreme drawdown (Baldwin et al., 2008). A combination of low water levels due to drawdown, along with wind-driven events were seen to result in turnover of the water thermocline and periodic pulses of sediment-derived nutrients into the warm surface water layer. This stimulated cyanobacterial growth. Reservoir depth seemed to be the causal link for the onset of cyanobacterial blooms in this system and should be considered as a part of water management strategies as increased drawdown events are likely with global weather changes.

Therefore, it seems that the relative contribution of external and internal nutrient levels needs to be considered when establishing catchment management strategies to reduce cyanobacterial growth. As internal, sediment-derived, nutrient levels will relate to both

[4] see http://www.water.gov.au/ and http://www.waterquality.crc.org.au/ for information on Australian water management schemes.

geographical factors and to historical anthropogenic activities at a site, nutrient effects need to be addressed in a site-specific manner.

3.3. Persistence of cyanobacteria and cyanotoxins in the environment

The ways in which cyanobacteria and their toxins persist in the environment are important because factors that promote the persistence of cyanobacteria will contribute to the reoccurrence of blooms, while factors that aid toxin persistence will increase the magnitude of cyanotoxin problems. If we have a better understanding of toxin persistence, then tools to reduce persistence will aid cyanobacterial management.

The recent literature is given below; however, the salient points are:

- Despite problems associated with cyanobacteria from a human perspective, cyanobacteria provide important ecosystem functions. Cyanobacteria are beneficial components of soil crusts in unvegetated areas or areas of sparse vegetation. Soil crusts help reduce dust and conserve soil during drought. Disruption of these fragile crusts due to animal or human activity can increase levels of dust and material flushed into water during storms;

- Cyanobacterial spores aid their environmental survival. Spores are induced by, and allow cells to survive desiccation and nutrient limitation. Spores return to a vegetative state when conditions improve;

- Cyanobacterial spores can remain viable in sediments for over 100 years, providing considerable stability to cyanobacterial populations;

- Cyanobacteria that associate with biofilms persist for longer during desiccating events – either as vegetative cells or spores. This makes biofilms a key feature of the environment for the survival of cyanobacteria;

- Cyanotoxins are degraded by endemic bacteria. Such biodegradation increases as exposure increases, and decreases when microbial processes are inhibited, for example by copper. Managing cyanotoxins may include promoting those bacteria that degrade the toxins;

- Cylindrospermopsin is released from cyanobacteria without cell lysis. Cylindrospermopsin concentrates in deeper water than the cells, which means that lowering water abstraction points to exclude cyanobacterial cells may inadvertently coolect more of the toxin;

- Microcystin can be retained by plants when it becomes incorporated into irrigation water. Consequently, cyanotoxins may enter the food chain.

The persistence of cyanobacteria/toxins in the environment was the subject of several studies and they fall into three broad themes: (i) environmental persistence influenced by climate events, (ii) persistence and breakdown of cyanotoxins in the environment, and (iii) persistence related to movement through the biosphere.

3.3.1. Environmental persistence of cyanobacteria and influence by climate events

Cyanobacteria are an important ecological component in soil-crusts. The ability of some species to fix atmospheric nitrogen gives them an advantage as a successional coloniser of unvegetated soils. Increased grazing pressure during drought episodes is likely to impact on the integrity of soil crusts in rangeland. Williams and colleagues undertook a study of cyanobacterial crust composition in a drought-affected area of Australian rangeland (Williams et al., 2008). Stock grazing during a drought period had a major impact on soil crusts associated with water sources, and crust composition did not recover concomitant with the cessation of drought. While not directly related to drinking-water issues *per se*, this research adds to the body of data on the persistence of cyanobacteria in the environment and how this may be affected by global climate change. In particular these data indicate how catchment management during drought periods may impact on re-vegetation and ground-cover colonisation, which in turn may impact on nutrient loading in water sources as a result of catchment run-off.

A number of cyanobacterial species, including the toxin-producing *A. circinalis,* form resting spores known as akinetes. Akinetes are essentially a thick-walled spore that can survive harsh conditions and functions as an asexual resting stage. These akinetes are important for survival during a range of adverse conditions including desiccation. Phosphate limitation, carbon to nitrogen ratio, anaerobiosis and low temperature have been individually shown to trigger akinete formation in different cyanobacterial species. Irradiance also appears to be a major trigger for akinete differentiation in many cyanobacteria. To study this effect Thompson and collaborators studied the influence of light quality on akinete formation and subsequent germination in *A. circinalis* (Thompson *et al*., 2009). *A. circinalis* cultures were subjected to a range of light spectra *in vitro*. The degree of akinete production was measured along with subsequent akinete germination and growth rate of germlings. Akinete production followed the trend fluora-white light = red>cool-white>green>blue. It was seen that cultures subjected to blue light showed a marked reduction in production of akinetes, approximately 3000-times fewer than produced under red light treatment. Reduction in the growth rate of germlings was also seen with blue light irradiance. The researchers were unable to test if blue light at akinete formation reduced subsequent germination as too few akinetes were produced under blue light. These results are environmentally significant. Pure water is a strong absorber of red light (long wavelengths), while the coloured dissolved organic matter in water absorbs more blue light (shorter wavelengths).

In Australian freshwaters blue-light spectra reduce with increasing depth. Therefore factors such as water flow, vertical mixing, the degree and persistence of stratification are all likely to affect the proportion of blue light that is intercepted by cyanobacteria and the subsequent differentiation of akinetes. In situations with reduced blue-light spectra akinete encystment may be favoured, potentially leading to seeding of subsequent cyanobacterial blooms under more favourable conditions.

Persistence of cyanobacteria was also studied in a system of seasonally flowing streams in Australia (Robson et al., 2008). Water flow in these streams was either unregulated, and dependant on climactic conditions, or was regulated. The latter situation results in more rapid rates of water level change and potential for algal desiccation. This study aimed to determine the relative influence of different refuges within this system on algal recolonisation. Refuges included dry biofilm on stones, dry biofilm on leaf packs and

living algal biofilm on stones in pools. Cyanobacteria appeared to be more dependent on recolonisation from dry biofilm in regulated flow streams than from drift from pool refuges. This likely reflects the ability of cyanobacteria to tolerate rapid decreases in water depth and subsequent desiccation. Therefore, when considering the management of cyanobacterial recolonisation of seasonally flowing streams, the incidence of pool refuges is less important than the incidence of dry algal biofilms.

The persistence of cyanobacteria in the environment was also the subject of a study undertaken at Lake Okaro, NZ, to determine the historical composition of cyanobacterial communities at this site. Currently sparse historical data on cyanobacterial blooms means it can be difficult to assess whether current blooms reflect historical events, or an increase in blooms due to climate change and anthropogenic effects, or a succession of new arrivals to a site. Wood and colleagues used a combination of germination experiments and molecular typing to identify cyanobacteria in a sediment core from Lake Okaro (Wood et al., 2009). Akinetes were able to germinate from sediments that were laid down *c*. 120 years ago. Overall, results from the study indicated that there has not been a dramatic change in cyanobacterial species composition in the lake over the past 100 years. However, several cyanobacterial species were identified that were not previously documented at this site. Of particular note is the identification of *Aphanizomemnon issatschenkoi*, which can produce the potent toxin anatoxin-a. This cyanobacterial species has recently become dominant in a number of NZ lakes and should be closely monitored due to its potential threat to human health.

3.3.2. Persistence and breakdown of cyanotoxins in the environment

During the 2008-2009 period, two studies focused directly on the persistence and breakdown of cyanotoxins in the environment (Smith et al., 2008; Everson et al., 2009). The studies were of the toxins cylindrospermopsin and deoxycylindrospermopsin. These cyanotoxins are potent alkaloid toxins that inhibit protein synthesis. Cyanobacterial species that can produce cylindrospermopsin and deoxycylindrospermopsin have been identified in NZ freshwaters [5] and so have the potential to contaminate NZ drinking-water sources. These cyanotoxins are of particular importance to drinking-water sources as, unlike most cyanotoxins, they are released by the cyanobacteria into the surrounding media during cyanobacterial growth. Therefore, water remediation strategies that focus on removal of the cyanobacterial cells may not remove these toxins.

[5] See Appendix 1 for Table 9.2 in the Draft Guidelines for Drinking-water Quality Management for New Zealand, October 2005, for cyanobacterial species known to occur in NZ freshwater, and the toxins they can produce.

Several studies have examined the conditions that influence growth of cyanobacterial species that produce cylindrospermopsin and deoxycylindrospermopsin; however, little is known about the effect of environmental conditions on the relative proportions of the two toxins that may be produced and how these toxins are distributed in the water column. To address this, a study was undertaken in a stratified lake in NE New South Wales, Australia, to determine the distribution of cylindrospermopsin and deoxycylindrospermopsin (Everson et al., 2009). Water quality analysis indicated that stratification and oxygenation of the water column were both significant in the distribution of the cyanobacterial populations and their toxins. As expected, cyanobacterial cell concentrations were highest in the warm, oxygenated and low conductivity surface waters, and cyanobacterial species succession was associated with nutrient and trace metal depletion in this surface layer. The two cyanotoxins, however, were distributed throughout the water column, with the highest concentrations recorded in the hypolimnion[6]. The relative distribution of cylindrospermopsin and deoxycylindrospermopsin paralleled the distribution of ammoniacal nitrogen and oxidised nitrogen, with the oxygenated toxin, cylindrospermopsin, dominating above 15 metres and the deoxygenated toxin, deoxycylindrospermopsin, dominating below 15 metres. Therefore, in this lake the highest toxin concentrations occurred where cyanobacterial cell concentration was the lowest. This has direct implications for the management of water supplies, particularly with respect to the placement of off-take devices. These are often placed deeper in the water column in order to avoid surface layers where cyanobacterial cells are likely to be concentrated. The observations from this study indicate that before changing the abstraction depth, determination of the toxins present at the new depth may be required if the bloom contains cyanobacteria capable of producing cylindrospermopsin and deoxycylindrospermopsin.

A second study determined factors that influence the biodegradation of cylindrospermopsin by endemic organisms in drinking-water sources (Smith et al., 2008). Biodegradation was only evident in water supplies that had a history of cylindrospermopsin-producing cyanobacterial blooms. A lag period was evident prior to the onset of biodegradation, however, this lag period decreased in time after repeat exposure of the endemic organism to cylindrospermopsin. The concentration of the toxin influences biodegradation, with a near linear relationship between biodegradation rate and initial toxin concentration. Lag times seen for biodegradation are likely to relate to the concentration of toxin reaching a lower threshold limit before biodegradation occurs. Temperature was also shown to effect biodegradation. Degradation occurred between 20^{o}C and 35^{o}C, with a maximum rate of degradation at 25^{o}C. Temperature effects likely relate to the range in which microorganism are able to grow and may reflect temperatures

[6] The hypolimnion is the colder, dense, deep water layer in a thermally stratified lake, lying below the epilimnion and metalimnion, and isolated from surface influences.

favourable to activity of toxin-degrading enzymes. These temperature effects on the biodegradation of cylindrospermopsin are important as the cyanobacterial species able to produce these toxins are becoming more evident in temperate waters. The presence of copper-based algicides in the water inhibited the biodegradation of cylindrospermopsin. This suggests that the use of copper-based algicides to control cyanobacterial blooms in water sources may be detrimental on two counts. Not only do they have the propensity to lyse the algal cell, releasing toxins into the water, but they may also inhibit the biodegradation of these toxins. The suggestion is that these algicides be only applied early in bloom development when cyanobacterial cell numbers and toxin levels are low. While this study was unable to isolate particular organisms able to degrade cylindrospermopsin, it has shown that environmental degradation of this toxin can occur in water sources and highlights that previous toxin exposure at the site, toxin concentration and water temperature are important factors that influence this biodegradation.

3.3.3. Persistence and effect of cyanotoxins in the biosphere

During toxic algal blooms organisms in the biosphere can be exposed to the cyanobacteria and/or their toxins. Several studies during the 2008-2009 period have reported the effects of such exposure on plants, crustaceans and marine animals. Aquatic plants, in particular, can experience continual exposure to algal blooms, compared with other biota that may be more mobile and/or land-based. Kinnear and colleagues undertook a study on the effect of whole-cell extracts of the cyanobacteria *Cylindrospermopsis raciborshii* on the growth of the aquatic plant *Hydrilla verticillata*, or water thyme (Kinnear et al., 2008). The whole-cell extracts contained the cyanotoxin, cylindrospermopsin. The responses of *Hydrilla* to the treatments varied depending on the concentration of the toxin and the length of exposure, with significant growth stimulation and redistribution of plant resources seen. Root growth was significantly increased by exposure to whole-cell extracts containing the maximum toxin concentration of 400 μg/L. *Hydrilla* does not usually invest many resources in root volume compared to shoot volume and the increased root growth was considered to be a toxin specific response. Speculation was that perhaps a greater root volume leads to an overall decrease in toxin concentration per cell, enabling the plant to survive in the presence of high toxin concentrations. As plant death, necrosis nor chlorosis were not seen at the highest toxin concentration used, the results of this study suggest that *Hydrilla* root production could aid in reducing cylindrospermopsin toxicity in waters. As mentioned previously, cylindrospermopsin is a cyanotoxin that is released into the extracellular environment during cyanobacterial growth. The results of this study identify a possible bioremediation opportunity to manage cylindrospermopsin toxicity in water sources.

Aquatic plants have also been shown to absorb the microcystin class of cyanotoxins and microcystin application has been shown to cause malformations in some terrestrial plants. Microcystins are strong inhibitors of protein phosphatase enzymes and could be toxic to plant cells based on this mode of action. Crush and colleagues have extended these studies to determine the effect of irrigation of crop and forage plants with water containing microcystins (Crush et al., 2008). Experiments were undertaken with ryegrass, clover, forage rape and lettuce, with the plants irrigated by either shoot application, or by root application by direct watering of the surface of the growing media. Microcystins in the irrigation water were retained in the plants in a plant-specific manner. In particular, shoot application showed low retention for forage rape and ryegrass; where shoot morphology resulted in water running off the shoots and little visible shoot wetting. Conversely, clover

and lettuce shoots were visibly wet with shoot irrigation and both retained microcystins. Following root irrigation, microcystin retention was highest in clover roots, intermediate in lettuce and low in ryegrass and forage rape. No translocation of microcystins was seen between roots and shoots. The effect of irrigation with microcystins on plant growth was variable. Repeat applications of microcystins in irrigation water applied to the roots increased the root dry weight of ryegrass and lettuce (analogous to the results seen by Kinnear *et al*. for *Hydrilla* and cylindrospermopsin) and decreased the shoot dry weight of ryegrass, forage rape and lettuce. Overall, lettuce showed the most consistent growth inhibition in the presence of microcystins. These results indicate that microcystins applied to terrestrial plants at naturally occurring concentrations can be retained by the plants, and can affect plant productivity. Retention of microcystins in these crop plants indicates that irrigation with water containing microcystins has the potential to move these toxins into farm animals and human food chains at concentrations that can exceed recommended tolerable limits. This is of significance to human health if irrigation waters are sourced from sites susceptible to cyanobacterial blooms.

Two studies from Australia looked at the retention and effect of exposure to cyanobacteria on higher aquatic organisms. A study at Myalls Lakes in Australia showed that freshwater shrimps (*Paratya australiensis*) derive a considerable portion of their dietary carbon and nitrogen requirements from cyanobacterial mat accumulations (Piola et al., 2008). That these shrimps could digest the cyanobacteria was in contrast to studies on other crustaceans that have shown large portions of cyanobacteria to be passed through the gut undigested. This study did not, however, address the question of whether the freshwater shrimp was a vector for the passage of cyanobacterially-derived toxins through the food web. The second study looked at blood profiles of marine turtles exposed to blooms of the toxin-producing cyanobacterium *L. majuscule* (Arthur et al., 2008). No acute impact was seen on exposed turtles compared to turtles in areas where they were unlikely to have had exposure, however significant differences were seen in blood profiles of exposed individuals. In particular, there was a decrease in plasma low density lipoproteins and in glucose concentrations in exposed turtles. These decreases were likely related to malnutrition and it is suggested by the authors that this may indicate that in trying to avoid consumption of the cyanobacterium the reptiles may have consumed less food overall or a substandard diet. This type of sub-acute effect of cyanobacterial blooms on organisms in the food-web could have long-term impacts on reproductive outputs of ecosystems. Although not directly related to drinking-water safety, these studies add to the body of data on the persistence of and effect of cyanobacteria and their toxins in the environment.

3.4. Cyanotoxin toxicity studies

Toxicity studies are important because they inform the MAVs for drinking-water and help to identify chronic effects of a toxin, which are additional to the acute effects of cyanotoxins that are the usual focus of studies.

The recent literature is given below; however, the salient points are:

- No new concentrations are available to justify altering the current PMAVs or moving to MAVs;

- Bioassays for cyanotoxin effects can identify new toxins, with recent ones showing chronic and sub-clinical effects, such as dermal irritation.

Australian researchers Osborne and Shaw published two studies related to the dermal toxicity of cyanotoxins from the cyanobacterium *L. majuscula*. Both studies concentrated on *L. majuscula* from marine environments, however, this cyanobacterium species has been identified in NZ freshwaters and so the results may also be relevant to freshwater systems.

Toxic factors have been identified from *L. majuscula* following injection into the abdominal skin of mice and guinea pigs. Two toxins – lungybya toxin a (LA) and debromoaplysiatoxin (DAT) – have been isolated and identified that show similarity to known dermal toxins. Oral toxicity of *L. majuscula* and its toxins has also been shown in mice. The first paper by Osborne et al. (2008) aimed to establish an animal model for dermal toxicity that could be used to elucidate toxicity of *L. majuscula* strains from Queensland. A mouse ear swelling bioassay was established. No toxin related deaths were seen during the study. The effect of extracts from *L. majuscula* was compared to that of purified LA and DAT. Additional potency was observed using crude extracts of *L. majuscula*, with known LA content, compared to the purified toxin alone. Also, some samples of *L. majuscula* containing no measurable quantities of LA or DAT were found to exert an inflammatory response in the bioassay. Together these results suggest that the *L. majuscula* strains tested in the assay contained compounds in addition to the known, measured toxins that also exerted an irritant effect. While confirming the dermal irritant activity of LA and DAT toxins produced by the cyanobacterium *L. majuscula*, this result also highlights the importance of bioassays that can detect toxicity from previously unidentified compounds.

The second paper, by Osborne and Shaw, examined first aid records from Fraser Island, off the Queensland coast, to determine if deleterious health consequences correlated with known blooms of *L. majuscula* (Osborne and Shaw, 2008). Presentation of individuals to the first aid station with symptoms synonymous with exposure to toxic *L. majuscula* was concentrated into a seven-week period in the summer of 1998. This was the only period in which blooms of *L. majuscula* were identified on the island. Environmental conditions at the time of the bloom, particularly water temperature and exposure of the sites to wind, along with accessibility of the bloom locations to the public, may have resulted in the correlation between the cyanobacterial bloom and the reporting of symptoms of toxin exposure. These reports on dermal toxicity of cyanotoxins from *L. majuscula* add important data to the information of cyanotoxins and their potential health effects.

3.5. Methods to detect and monitor cyanobacteria in water sources

It is important to maintain awareness about the methods to detect cyanobacteria because the literature pool will generate the future methods to be incorporated into the DWSNZ.

The recent literature is given below; however, the salient points are:

- Molecular biology tools, such as 16S ribosomal genes and DNA micro-arrays, are being used more frequently to increase the specificity of species identified and to

provide greater throughput than the more traditional microscopy and immuno-enzyme techniques. However, as with most molecular biology tools, considerable *a priori* knowledge is required;

- The Cawthron Institute has recently developed cryopreservation techniques that allow long-term cyanobacterial storage without loss of cyanotoxin production ability, which had been a problem associated with other storage methods;

- Context-specific monitoring plans are needed to obtain samples that can capture the dynamics of cyanobacteria, indicating that tailored regimes will return better data to help manage bloom potential. However, these programmes are likely to increase the frequency of monitoring and also increase the number of samples required for each sampling event, which will increase management costs;

- Tailored plans are showing that managing cyanobacteria well in advance of a bloom is the best long-term strategy, not only to reduce bloom magnitude but also to decrease bloom frequency – a long-term approach.

One of the major issues for the control of cyanotoxins in water sources is the detection of cyanobacterial species that may produce toxins. Cyanobacterial morphology and physiological traits do not correlate well with toxicity, which makes the distinction between toxin-producing algal blooms and non-toxin-producing events difficult. Traditional methods for detection of toxic cyanobacteria include use of animal bioassays, for example the mouse peritoneal bioassay for saxitoxins. While these assays have the advantage of detecting toxicity from known and potentially unknown compounds in an environmental sample, as described for the mouse ear inflammation assay of *L. majuscula*, they have ethical issues associated with their use, are often insensitive and have associated high costs. Sensitive analytical methods have been developed for analysis of toxins, such as HPLC. However, these detection/identification methods often require laborious sample preparation, use of purified toxin standards and availability of expensive analytical equipment. Immunological and biochemical tests have been established for detecting cyanotoxins in laboratory and field samples. For example, enzyme-linked immunoabsorbent assays (ELISA) tests and the colorimetric protein phosphatase inhibition assay. These assays have the advantage of being highly sensitive, but usually require *a priori* knowledge of the toxin and its mode of action.

Molecular detection methods are becoming increasingly popular as a means of detecting toxic cyanobacteria. These methods are highly sensitive and can detect very low levels of target in environmental samples containing complex communities of organisms. During the 2008-2009 period, the Australian researchers Pearson and Neilan published a review of molecular methods used for monitoring water quality (Pearson and Neilan, 2008). Detection of ribosomal RNA gene sequences using the polymerase chain reaction (PCR) has been used to differentiate toxic and non-toxic members of some genera of cyanobacteria e.g. planktonic nodularin-producing strains of *Nodularia*, from benthic, non-toxic strains. However, the genetic variation in this gene target is not enough to distinguish between toxin and non-toxin producing strains for all genera of cyanobacteria. Recent characterisation of biosynthetic genes that encode enzymes involved in the pathways for synthesis of cyanotoxins has been exploited to develop new molecular diagnostic markers. Adaptation of some of these tests into quantitative tests has enabled the monitoring of dynamic bloom populations and the identification of particularly

problematic species. DNA microarray technologies have also been adapted to provide high throughput methods for detecting and differentiating toxic strains in complex samples. The speed, economy and high sensitivity of these molecular methods for identification and discrimination of toxin-producing cyanobacteria make them highly suited for testing environmental samples. The methods are also highly amenable to automation for high throughput and are anticipated to become increasingly popular for water quality assessment.

Detailed studies and characterisation of cyanobacterial species and the development of testing methods, relies on the availability of known species with known toxin-producing characteristics. The Cawthron Institute in Nelson, NZ, has established a collection of marine and fresh-water planktonic and benthic cyanobacteria from sites across New Zealand. There have been problems with the genetic stability of genera when they are kept in long-term culture, including changes in morphology and loss of toxin production. A method has been developed to cryopreserve cyanobacteria for long-term storage (Wood et al., 2008). Cryopreservation was shown to be a viable method for long-term storage, with 17 out of 20 strains successfully cryopreserved. All strains tested that were known to produce cyanotoxins before preservation retained their ability to produce these toxins following recovery from cryopreservation. As well as enabling better long-term preservation of cyanobacterial genotypes, this long-term storage method will reduce the cost and time involved in the maintenance of the NZ cyanobacterial collection and will allow for continued expansion of the collection.

Monitoring water sources for cyanobacteria is an important part of water quality assessment for human health and is routinely undertaken in many parts of the world. Australian researchers Baldwin and Boulding have published a revised monitoring strategy for a large water-storage reservoir in SE Australia, Lake Mulwala (Baldwin and Boulding, 2008; Baldwin et al., 2008). The salient feature of this water-storage lake is the highly variable distribution of cyanobacteria, both in time and space. It was also noted that there was an apparent disparity between phytoplankton counts taken at similar times, but at different locations, and by different jurisdictions. The paper describes a study to determine the spatial distribution of cyanobacteria in this lake, both with respect to surface distribution and distribution within the water column. These data were then used to design a monitoring strategy with enough statistical power to determine changes in overall phytoplankton community structure over time and to fulfil statutory cyanobacterial biovolume monitoring obligations. The monitoring strategy developed resulted in more sampling sites and higher sampling frequency than the previously established strategy for this lake and for other water reservoirs of similar size. The study highlights the need to assess water storage systems individually to determine optimum monitoring strategies to inform public health decisions.

Traditionally, water quality management strategies rely on monitoring for cyanobacterial blooms in conditions favourable to bloom formation. An alternative approach could be remediation of water conditions to reduce bloom formation, as seen in a number of water remediation programmes. As an example of this approach, low-dose alum (aluminium sulphate) application was trialled, by Paul and colleagues, as a potential management tool for internal nutrient loads in a NZ lake (Paul et al., 2008). Alum application has been shown in other systems to facilitate flocculation and sedimentation of phosphorous from the water column, followed by binding of phosphorus in bottom sediments. It should be noted, however, that this treatment has the potential to elevate ionic aluminium

concentrations in the water column to potentially toxic levels. Lake Okaro is a eutrophic lake with high nutrient loads, low alkalinity and frequent cyanobacterial blooms. Alum was applied to the surface (epilimnion) layer of the lake. In this study a low dose of alum was used and its short-term effect monitored. A number of problems were encountered in using this approach in this lake system, some of which related to the naturally low alkalinity of the lake and to the availability and concentration of phosphorous species. The study authors concluded that further research, using discrete alum application and better timing of application, are needed to determine optimum effectiveness of applications.

4. CONCLUSION

It can be seen from the preceding literature review that researchers in NZ and Australia are reporting a wide range of studies on cyanobacteria and cyanotoxins. While most publications do not address drinking-water quality issues directly, the majority of publications are in the area of environmental factors that affect cyanobacterial growth and persistence of cyanobacteria in the Australasian environment, which can be extrapolated to include drinking-water sources. It can be seen from these studies that macro- and micro-nutrient levels are critical to the development of cyanobacterial blooms and these can be influenced by both geographical situation and anthropogenic activities in water catchments. A number of issues that may result from global climate change were identified, including changes to water conductivity, changes to water flow patterns and deposition of nutrients from atmospheric dust associated with increased drought events. Perhaps the most important conclusion from all of the environmental studies is that conditions that influence cyanobacterial growth are highly location-specific and that remediation and/or monitoring strategies for water quality need to be determined for the conditions in a particular location and modified over time as conditions may change.

The increasing use of molecular diagnostic tests to differentiate toxic from non-toxic strains of cyanobacteria genera should enable better species identification than is currently possible by microscopy methods, as well as high throughput analyses to be undertaken for water samples. Continuing studies on cyanobacterial metabolism should inform the better management of water sources by determining critical parameters for nutrient utilisation. Based on the studies in this literature review, one area where increased research would be of value is the area of toxin biosynthesis and determination of environmental factors that may stimulate this biosynthesis. This research would provide useful data to inform water source management and minimise cyanotoxin production.

5. REFERENCES

Ahern, K.S., Ahern, C.R., and Udy, J.W. (2008) In situ field experiment shows Lyngbya majuscula (cyanobacterium) growth stimulated by added iron, phosphorus and nitrogen. *Harmful Algae* **7**: 389-404.

Arthur, K.E., Limpus, C.J., and Whittier, J.M. (2008) Baseline blood biochemistry of Australian green turtles (Chelonia mydas) and effects of exposure to the toxic cyanobacterium Lyngbya majuscula. *Australian Journal of Zoology* **56**: 23-32.

Baker, P.D., Steffensen, D.A., Humpage, A.R., Nicholson, B.C., Falconer, I.R., Lanthois, B. et al. (2001) Preliminary evidence of toxicity associated with the benthic cyanobacterium Phormidium in South Australia. *Environmental Toxicology* **16**: 506-511.

Baldwin, D.S., and Boulding, A.M. (2008) Developing a monitoring strategy for phytoplankton community structure in a large water-storage reservoir: Lake Mulwala, Australia. *Lakes & Reservoirs: Research and Management* **13**: 221-229.

Baldwin, D.S., Gigney, H., Wilson, J.S., Watson, G., and Boulding, A.N. (2008) Drivers of water quality in a large water storage reservoir during a period of extreme drawdown. *Water Research* **42**: 4711-4724.

Bayer, T.K., Schallenberg, M., and Martin, C.E. (2008) Investigation of nutrient limitation status and nutrient pathways in Lake Hayes, Otago, New Zealand: a case study for integrated lake assessment. *New Zealand Journal of Marine and Freshwater Research* **42**: 285-295.

Burger, D.F., Hamilton, D.P., and Pilditch, C.A. (2008) Modelling the relative importance of internal and external nutrient loads on water column nutrient concentrations and phytoplankton biomass in a shallow polymictic lake. *Ecological Modelling* **211**: 411-423.

Cadel-Six, S., Peyraud-Thomas, C., Brient, L., de Marsac, N.T., Rippka, R., and Mejean, A. (2007) Different Genotypes of Anatoxin-Producing Cyanobacteria Coexist in the Tarn River, France. *Applied and Environmental Microbiology* **73**: 7605-7614.

Cox, P. A., S. A. Banask and S. J. Murch (2003). Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proc. Natl. Acad. Sci. USA, **100**, pp 13380 – 13383.Crush, J.R., Briggs, L.R., Sprosen, J.M., and Nichols, S.N. (2008) Effect of irrigation with lake water containing microcystins on microcystin content and growth of ryegrass, clover, rape, and lettuce. *Environmental Toxicology* **23**: 246-252.

Downs, T., Schallenberg, M., and Burns, C. (2008) Responses of lake phytoplankton to micronutrient enrichment: a study in two New Zealand lakes and an analysis of published data. *Aquatic Sciences* **70**: 347-360.

Edwards, C., Beattie, K.A., Scrimgeour, C.M., and Codd, G.A. (1992) Identification of anatoxin-A in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland. *Toxicon* **30**: 1165–1175.

Eriksson, J.E., Meriluoto, J.A.O., Kujari, H.P., Osterlund, K., Fagerlund, K., and Hallbom, L. (1988) Preliminary characterization of a toxin isolated from the cyanobacterium *Nodularia spumigena*. *Toxicon* **26**: 161–166.

Etheredge, M.K., and Pridmore, R.D. (1984) New records of planktonic blue-green algae (Cyanophyceae/Cyanobacteria) in New Zealand freshwaters. *New Zealand Journal of Botany* **22**: 539–544.

Everson, S., Fabbro, L., Kinnear, S., Eaglesham, G., and Wright, P. (2009) Distribution of the cyanobacterial toxins cylindrospermopsin and deoxycylindrospermopsin in a stratified lake in north-eastern New South Wales, Australia. *Marine and Freshwater Research* **60**: 25-33.

Hamill, K.D. (2001) Toxicity in benthic freshwater cyanobacteria (blue-green algae): first observations in New Zealand. *New Zealand Journal of Marine and Freshwater Research* **35**: 1057–1059.

Kinnear, S.H.W., Fabbro, L.D., and Duivenvoorden, L.J. (2008) Variable growth responses of water thyme (Hydrilla verticillata) to whole-cell extracts of Cylindrospermopsis raciborskii. *Archives of Environmental Contamination and Toxicology* **54**: 187-194.

Kobayashi, T., Ryder, D.S., Gordon, G., Shannon, I., Ingleton, T., Carpenter, M., and Jacobs, S.J. (2008) Short-term response of nutrients, carbon and planktonic microbial communities to floodplain wetland inundation. *Aquatic Ecology* **10.1007/s10452-008-9219-2**: 1-16.

Kouzminov, A. (2001): personal communication from Dr David Stirling and Dr Penny Truman, Biotoxin Research Scientists, ESR, Kenepuru Science Centre.

Kouzminov, A., Ruck, J.G., and Wood, S.A. (2007) New Zealand risk management approach for toxic cyanobacteria in drinking water. *Australian and New Zealand Journal of Public Health* **31**: 275–281.

Kroger, K., Gardner, J.P.A., Rowden, A.A., and Wear, R.G. (2006) Long-term effects of a toxic algal bloom on subtidal soft-sediment macroinvertebrate communities in Wellington Harbour, New Zealand. *Estuarine Coastal and Shelf Science* **67**: 589–604.

Kuiper-Goodman, T., Falconer, I., and Fitzgerald, J. (1999) Human Health Aspects. In *A Guide to their Public Health Consequences, Monitoring and Management*. Chorus, I., and Bartram, J. (eds). London: E & FN Spon, pp. 113–153.

Long, J.D., Smalley, G.W., Barsby, T., Anderson, J.T., and Hay, M.E. (2007) Chemical cues induce consumer-specific defenses in a bloom-forming marine phytoplankton. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 10512–10517.

Lukac, M., and Aegerter, R. (1993) Influence of trace metals on growth and toxin production of *Microcystis aeruginosa*. *Toxicon* **31**: 293–305.

Matz, C., and Kjelleberg, S. (2005) Off the hook - how bacteria survive protozoan grazing. *Trends in Microbiology* **13**: 302-307.

Matz, C., Deines, P., Boenigk, J., Arndt, H., Eberl, L., Kjelleberg, S., and Jurgens, K. (2004) Impact of violacein-producing bacteria on survival and feeding of bacterivorous nanoflagellates. *Applied and Environmental Microbiology* **70**: 1593–1599.

Milne, J., and Watts, L. (2007) Toxic benthic cyanobacteria proliferations in Wellington's rivers 2005/06. In. Council, G.W.R. (ed). Wellington: Greater Wellington Regional Council, p. 45.

Mur, L.R., Skulberg, O.M., and Utkilen, H. (1999) Cyanobacteria in the environment. In *Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management*. Chorus, I., and Bartram, J. (eds). London: E & FN Spon, pp. 15–40.

Orr, P.T., and Schneider, P.M. (2006) *Toxic cyanobacteria risk assessment - Reservoir vulnerability and water use best practice*. Brisbane, Australia: South-East Queensland Water Corporation.

Osborne, N., and Shaw, G. (2008) Dermatitis associated with exposure to a marine cyanobacterium during recreational water exposure. *BMC Dermatology* **8**: 5.

Otago Regional Council and Queenstown Lakes District Council (1995) Lake Hayes Management Strategy. In. Dunedin: Otago Regional Council and Queenstown Lakes District Council.

Paul, W.J., Hamilton, D.P., and Gibbs, M.M. (2008) Low-dose alum application trialled as a management tool for internal nutrient loads in Lake Okaro, New Zealand. *NZ J Marine and Freshwater Research* **42**: 207-217.

Pearson, L.A., and Neilan, B.A. (2008) The molecular genetics of cyanobacterial toxicity as a basis for monitoring water quality and public health risk. *Current Opinion in Biotechnology* **19**: 281-288.

Piola, R.F., Suthers, I.M., and Rissik, D. (2008) Carbon and nitrogen stable isotope analysis indicates freshwater shrimp Paratya australiensis Kemp, 1917 (Atyidae) assimilate cyanobacterial accumulations. *Hydrobiologia* **608**: 121-132.

Pridmore, R.D., and Etheredge, M.K. (1987) Planktonic cyanobacteria in New Zealand inland waters: distribution and population dynamics. *New Zealand Journal of Marine and Freshwater Research* **21**: 491–502.

Redden, A.M., and Rukminasari, N. (2008) Effects of increases in salinity on phytoplankton in the Broadwater of the Myall Lakes, NSW, Australia. *Hydrobiologia* **608**: 87-97.

Robson, B.J., Matthews, T.G., Lind, P.R., and Thomas, N.A. (2008) Pathways for algal recolonization in seasonally-flowing streams. *Freshwater Biology* **53**: 2385-2401.

Ryan, E.F., and Hamilton, D.P. (2003) Recent occurrence of Cylindrospermopsis raciborskii in Waikato Lakes of New Zealand. *New Zealand Journal of Marine and Freshwater Research* **37**: 829–836.

Ryan, N.J., Mitrovic, S.M., and Bowling, L.C. (2008) Temporal and spatial variability in the phytoplankton community of Myall Lakes, Australia, and influences of salinity. *Hydrobiologia* **608**: 69-86.

Shaw, E.C., Gabric, A.J., and McTainsh, G.H. (2008) Impacts of aeolian dust deposition on phytoplankton dynamics in Queensland coastal waters. *Marine and Freshwater Research* **59**: 951-962.

Sivonen, K., and Jones, G. (1999) Cyanobacterial toxins. In *A Guide to their Public Health Consequences, Monitoring and Management*. Chorus, I., and Bartram, J. (eds). London: E & FN Spon, pp. 41–111.

Smith, M.J., Shaw, G.R., Eaglesham, G.K., Ho, L., and Brookes, J.D. (2008) Elucidating the factors influencing the biodegradation of cylindrospermopsin in drinking water sources. *Environmental Toxicology* **23**: 413-421.

Stirling, D.J., and Quilliam, M.A. (2001) First report of the cyanobacterial toxin cylindrospermopsin in New Zealand. *Toxicon* **39**: 1219.

Thompson, P.A., Jameson, I., and Blackburn, S.I. (2009) The influence of light quality on akinete formation and germination in the toxic cyanobacterium Anabaena circinalis. *Harmful Algae* **8**: 504-512.

Towner, R.A., Sturgeon, S.A., Khan, N., Hou, H., and Swartz, H.M. (2002) In vivo assessment of nodularin-induced hepatotoxicity in the rat using magnetic resonance techniques (MRI, MRS and EPR oximetry). *Chemico-Biological Interactions* **139**: 231.

van Apeldoorn, M.E., van Egmond, H.P., Speijers, G.J.A., and Bakker, G.J.I. (2007) Toxins of cyanobacteria. *Molecular Nutrition & Food Research* **51**: 7–60.

Walsby, A.E. (1994) Gas vesicles. *Microbiological Reviews* **58**: 94–144.

WHO (2006) Guidelines for drinking-water quality. In. http://www.who.int/water_sanitation_health/dwq/gdwq0506.pdf (ed): World Health Organization, p. 595.

Williams, W.J., Eldridge, D.J., and Alchin, B.M. (2008) Grazing and drought reduce cyanobacterial soil crusts in an Australian Acacia woodland. *Journal of Arid Environments* **72**: 1064-1075.

Wood S. A. (2005). Bloom forming and toxic cyanobacteria in New Zealand: species diversity, distribution, cyanotoxin production and accumulation of microcystins in selected freshwater organisms. [PhD thesis]. Wellington: Victoria University and Massey University. 310 pp.

Wood, S.A., and Stirling, D.J. (2003) First identification of the cylindrospermopsin-producing cyanobacterium *Cylindrospermopsis raciborskii* in New Zealand. *New Zealand Journal of Marine and Freshwater Research* **37**: 821–828.

Wood, S.A., Jentzsch, K., Rueckert, A., Hamilton, D.P., and Cary, S.C. (2009) Hindcasting cyanobacterial communities in Lake Okaro with germination experiments and genetic analyses. *Fems Microbiology Ecology* **67**: 252-260.

Wood, S.A., Holland, P.T., Stirling, D.J., Briggs, L.R., Sprosen, J., Ruck, J.G., and Wear, R.G. (2006) Survey of cyanotoxins in New Zealand water bodies between 2001 and 2004. *New Zealand Journal of Marine and Freshwater Research* **40**: 585–597.

Wood, S.A., Rhodes, L.L., Adams, S.L., Adamson, J.E., Smith, K.F., Smith, J.F. et al. (2008) Maintenance if cyanotoxin production by cryopreserved cyanobacteria in the New Zealand culture collection. *New Zealand Journal of Marine and Freshwater Research* **42**: 277-283.

APPENDIX 1

From DWSNZ 2005 (revision 2008)

Table 9.2: Cyanobacteria genera known to occur in New Zealand fresh waters and the toxins they are known to produce

Genera	Cyanotoxins known to be produced
Anabaena	anatoxin-a*, anatoxin-a(S), LPS, microcystins*, saxitoxins, cylindrospermopsin
Anabaenopsis	microcystins
Aphanocapsa	microcystins
Aphanizomenon	anatoxin-a, cylindrospermopsin, LPS, saxitoxins, microcystins
Arthrospira	microcystins
Cylindrospermum	**cylindrospermopsin**[1], LPS
Cylindrospermopsis	**cylindrospermopsin**[2]**,** saxitoxins
Lyngbya	aplysiatoxins, antillatoxins, kalkitoxin, **lyngbyatoxin-a**, saxitoxins
Microcystis	anatoxin-a, cylindrospermopsin, **microcystins**, LPS, saxitoxins
Nodularia	5.1.1. nodularin
Nostoc	microcystins*, BMAA (beta-methylamino-L-alanine)[3]
Oscillatoria	**anatoxin-a**[4], aplysiatoxins, LPS, microcystins*, anatoxin-a(S)
Phormidium	microcystin*, anatoxin-a and other toxin(s) have yet to be defined
Planktothrix	microcystins*, homoanatoxin-a, anatoxin-a, aplysiatoxins, saxitoxins, homoanatoxin-a
Pseudanabaena	microcystins
Raphidiopsis	cylindrospermopsin, anatoxin-a* homoanatoxin-a, microcystins*
Snowella	microcystins
Synechocystis	microcystins
Woronichinia	microcystins

Data source: Kouzminov (2001), Wood (2005)

1 Stirling and Quilliam (2001). Rigorous identification of the causative species not carried out. This taxon is likely to have been *Cylindrospermopsis* given the habitat sampled.
2 Wood and Stirling (2003)
3 Cox et al. (2003)
4 Hamill (2001)

***** The results of cyanotoxin testing on environmental samples indicate this toxin is produced by species from the associated genera in New Zealand, (Wood 2005).

REPORT DISTRIBUTION

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