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# New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters

## Interim Guidelines

New Zealand Government

**Acknowledgements**

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# Contents

# Decision Charts



# Tables



# Figures

# Boxes

# How to use this document

This document is divided into four main sections, plus 14 appendices.

**Section 1. Introduction** provides an overview of the purpose and status of the document as well as advice on who should use it.

**Section 2. Framework** provides a background to the overall guidelines approach, recommendations on agency roles and responsibilities, and information on the condition of use of this document.

**Section 3. Guidelines** describes the recommended three-tier monitoring and action sequence for planktonic and benthic cyanobacteria.

**Section 4. Sampling** provides advice on sampling planktonic and benthic cyanobacteria.

The **appendices** give further background information and include templates for data collection and reporting, including:

- background information on known cyanotoxins and their distribution in New Zealand
- information on the derivation of guideline values
- photographs of typical bloom events
- a list of biovolumes for common New Zealand cyanobacteria
- templates for field assessments
- suggested media releases and warning sign templates.

A **glossary** provides definitions for abbreviations and terms used in these guidelines.

# Section 1. Introduction

## 1.1 What is the purpose of these guidelines?

These guidelines provide advice on how public health risk associated with cyanobacteria in recreational waters can be managed. They have been developed in response to requests for best-practice advice from regional resource management and health agencies, and are intended to:

- help these agencies develop monitoring protocols appropriate for local conditions and circumstances
- encourage the adoption of a nationally unified approach to managing cyanobacterial risk in water used for recreational purposes.

## 1.2 What does this document cover?

These guidelines set out a monitoring framework for establishing the public health risk from cyanobacteria associated with contact recreation in lakes (mainly planktonic cyanobacteria) and rivers (mainly benthic cyanobacteria).

They do not cover the public health risk associated with recreation in coastal or estuarine waters, food gathering (eg, shellfish) or drinking waters. Further information on cyanobacteria in drinking water supplies can be found in the Ministry of Health's *Drinking-water Standards for New Zealand 2005* (Ministry of Health, 2005b). The *Drinking-water Standards* set out provisional maximum allowable values for seven cyanotoxins. The accompanying *Draft Guidelines for Drinking-water Quality Management for New Zealand* (Ministry of Health, 2005a) provide information to water supply authorities on how to monitor and manage water supplies for cyanobacteria and their toxins, including sampling requirements, recommended actions in response to threshold breaches, and treatment options. These documents are available at: www.moh.govt.nz

For further advice on levels of cyanotoxins in aquatic organisms (eg, shellfish), contact the New Zealand Food Safety Authority: www.nzfsa.govt.nz

Finally, this document does not provide explicit guidance for managing the impacts of cyanobacterial events on other environmental values such as ecosystem health and amenity. There are guidelines available that are more appropriate for this, such as the *New Zealand Periphyton Guideline* (Biggs, 2000).

## 1.3 Who should use these guidelines?

The *New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters* have been developed for staff and agencies involved in monitoring and reporting water quality for human recreation. Specifically, these are:

- science staff within regional councils and unitary authorities who routinely monitor the state of the environment
- public health officers in district health boards and public health units who assess and communicate environmental health risks to the public

- operational staff within territorial authorities who are responsible for alerting people to dangers in public spaces.

More guidance on the roles and responsibilities of these staff and agencies is provided in Section 2.4.

These guidelines may also be of interest to the wider environmental science and resource management community. For example, policy and planning staff within councils may find some of the background material on the environmental causes of bloom occurrence useful for fresh water policy development (eg, the setting of environmental flows). However, the guidelines should not be used for resource consenting work. For example, guideline thresholds should not be used as the basis for establishing conditions for discharge consents, although they may be used as a component of the decision-making process.

The guidelines are a companion to the existing *Microbiological Water Quality Guidelines for Marine and Freshwater Recreational Areas* (Ministry for the Environment and Ministry of Health, 2002).

## 1.4 Status of this guidance

This document is an *interim* version of the guidelines. This version has been released for trial use by monitoring and health agencies until the end of the 2011/12 summer, at which point it will be revised, based on feedback from practitioners. The guidelines will subsequently be released as a final version.

It is important to note that the guidelines are not mandatory. They constitute a recommended approach that is considered best practice for many management circumstances, given current understanding of cyanobacterial risks in New Zealand fresh waters. However, there are still gaps in scientific knowledge and health risk management that require local judgements to fill. Also, local decisions about whether to follow the guidelines' approach should ultimately result from consideration of site-specific factors (such as resource availability, historical understanding of local bloom conditions), as well as the guidance offered in this document.

The word 'should' has been used throughout the guidelines to describe recommended actions by monitoring and health agencies. This is intended to convey that the action being described is considered best practice as a *general rule*. Local knowledge and historical data should be used when establishing monitoring programmes.

# Section 2. Framework

## 2.1 Why monitor for cyanobacteria in fresh water?

Cyanobacteria (commonly known as blue-green algae) are photosynthetic prokaryotic organisms that are integral parts of many terrestrial and aquatic ecosystems. In aquatic environments, under favourable conditions, cyanobacterial cells can multiply and form planktonic (suspended in the water column) blooms or dense benthic (attached to the substrate) mats. An increasing number of cyanobacterial species are known to include toxin-producing strains. These natural toxins, known as *cyanotoxins*, are a threat to humans and animals when consumed in drinking water or by contact during recreational activities. The mechanisms of toxicity for cyanotoxins are very diverse, ranging from acute unspecified intoxication symptoms (eg, rapid onset of nausea and diarrhoea), to gastroenteritis and other specific effects, such as hepatotoxicity (liver damage) and possibly carcinogenesis.

## 2.2 What is contact recreation?

For the purposes of applying these guidelines, contact recreation covers all of those activities that bring people physically in contact with water and involve a risk of involuntary ingestion or inhalation of water. Swimming, whether partially or fully immersed, is perhaps the most obvious one, but others include kayaking, white-water rafting, water skiing, sailing and diving. This definition is consistent with that used for the *Microbiological Water Quality Guidelines for Marine and Freshwater Recreational Areas* (Ministry for the Environment and Ministry of Health, 2002).

## 2.3 The overall approach

These guidelines are based on the multi-tiered approach recommended by the World Health Organization (WHO, 2003) and the Australian National Health and Medical Research Council (NHMRC, 2008). These organisations recommend that when developing guidelines for cyanobacteria in fresh water, the following should be considered:

- the particular hazard caused by the well-characterised microcystin (a common hepatotoxin produced by cyanobacteria) toxins
- the occurrence of cyanobacteria in general (in addition to known toxins) as part of the hazard, because not all known toxic components have been identified and irritation symptoms reported may be caused by these unknown substances
- the hazard associated with the patchy and often unpredictable distribution of cyanobacterial populations.

### 2.3.1 A three-tier surveillance, alert and action sequence

The WHO and NHMRC have found that a single guideline value is not appropriate, and both use a series of guideline values associated with incremental severity and probability. The New Zealand guidelines use a similar approach and are based on a three-tier alert-level framework. This framework incorporates a monitoring and management action sequence which

regulators can use for a graduated response to the onset and progress of a cyanobacterial bloom or benthic proliferation in the water body. The thresholds can also be applied when responding to an unexpected cyanobacterial bloom event. Two separate frameworks are given: one for planktonic (water column) cyanobacteria and the second for benthic (attached to substrate) cyanobacteria.

### 2.3.2 Change from current practice

A major change in these guidelines (for planktonic cyanobacteria only) from current standard practice is the use of estimates of biovolume as thresholds in the alert-level framework instead of cell concentrations. This is in response to a recent increase in the reported high concentrations of picocyanobacteria (small cyanobacteria, less than 2 µm in diameter) in some New Zealand water bodies. Biovolume takes into account the variability in size between cyanobacterial species and thus allows for a more accurate assessment of health risk.

The health risks associated with benthic cyanobacteria are less well known than the risks for their planktonic counterparts. There has been little international research in this area and no attempts to develop quantitative guidelines. Yet benthic, mat-forming cyanobacteria are widespread throughout New Zealand rivers, and recent investigations have revealed the widespread distribution of toxic species commonly linked to dog poisonings (Wood, Selwood, Rueckert et al, 2007; Heath et al, 2009a, 2009b; Wood, Heath, McGregor et al, 2009; Wood, Heath and Ryan, 2009). Research also indicates the presence of uncharacterised toxins within benthic mats (Wood, unpublished data). In this document, guidelines based on preliminary research are given, but it is anticipated that these will require further refining as knowledge and monitoring tools improve.

These guidelines also suggest that cyanotoxin testing (ie, measuring the concentration of toxin produced by the cyanobacteria in a sample) should be considered as a useful addition to biovolume and mat coverage assessments when surveillance indicates that potentially toxic species are present. Cyanotoxin testing is useful to:

- provide further confidence on the potential health risks when a health alert is being considered
- enable the use of a higher biovolume threshold (ie, to show that no toxins are present, Section 3.2)
- demonstrate that residual cyanotoxins are not present when a bloom subsides.

### 2.3.3 Where and when should monitoring be done?

People are generally free to swim or undertake other water-based activities wherever they like in and around New Zealand's many rivers and lakes, but it would be impossible to monitor them all. Criteria for identifying which areas to monitor will vary from region to region, but will generally be based on the type and frequency of human recreational usage, the available information, and the resources available to the monitoring agency.

In general, blooms are much more common in summer months, and this is when routine monitoring should occur. However, the causes of cyanobacterial blooms are many, varied and often interrelated (see Section 4.6). Blooms and benthic proliferations may undergo rapid changes in extent and toxicity. These complexities mean deciding when and where to monitor can be challenging. Information is provided in Section 4 to assist agencies through a decision-making process for monitoring and sampling.

The New Zealand Ministry for the Environment and the Ministry of Health recommend that the general areas to be included in any routine monitoring programme be agreed by the regional council, territorial authority and public health unit, and documented in a regional protocol. However, in recognition of the monitoring challenges, there will be a need to retain flexibility about sites and the timing of visits.

## 2.4 Roles and responsibilities

Determining which agency is responsible for which roles in monitoring and reporting for public health protection needs to be established before developing a sampling and reporting programme. Roles and responsibilities should be agreed for both routine monitoring programmes as well as management responses to bloom events at locations that are not routinely monitored.

Roles and responsibilities are best tailored to suit each region, and decisions will depend on many factors, such as institutional arrangements and available expertise. However, as a general guide the Ministry for the Environment and Ministry of Health recommend the following framework.

### 2.4.1 Recommended framework for routinely monitored sites

The recommended framework below is largely consistent with recommendations for the routine monitoring and reporting of microbiological health risks provided in the *Microbiological Water Quality Guidelines for Marine and Freshwater Recreational Areas* (Ministry for the Environment and Ministry of Health, 2002).

1. The regional council coordinates the monitoring, sample analysis and reporting strategy.

2. The regional council implements surveillance and alert-level monitoring.

3. The public health unit reviews the effectiveness of the monitoring and reporting strategy.

4. The regional council informs the public health unit and territorial authority if alert or action levels are reached.

5. The public health unit ensures that the territorial authority is informed.

6. The public health unit or territorial authority informs the public when the action level is exceeded (eg, through media releases). The public health unit requests that territorial authorities erect warning signs at affected water bodies.

7. If the action level is reached, the territorial authority undertakes nuisance monitoring and causes all proper steps to be taken to remove or abate the nuisance. (On occasion it may be more appropriate for the regional council to undertake this duty.) The public health unit should provide advice to help territorial authorities and/or regional councils undertake necessary actions.

8. It is the responsibility of the public health unit to downgrade alert levels in accordance with these guidelines and in consultation with territorial authorities and regional councils.

9. The regional council collates the information for state of the environment reporting and a review of management policies.

### 2.4.2 Responding to bloom events at un-monitored locations

Occasionally cyanobacterial blooms will occur at locations that are not part of routine monitoring programmes. By default, steps 4 to 9 above could be applied, but the ultimate decision about who takes the lead role may be determined by the extent to which the event is considered a public health risk management issue or a wider resource management issue.

### 2.4.3 Regional protocols

Regional councils, territorial authorities and public health units should clearly identify and agree on a lead agency to develop a monitoring protocol covering both scenarios just described. This protocol should be based on each agency's respective legislative functions relating to recreational water-quality monitoring and reporting. The protocol should specify details of:

- which agency is responsible for which roles (ie, steps 1 to 9 in the framework above)
- how the monitoring programme will be implemented
- what the management and communication/education responses will be to exceedance events.

Consideration should also be given to the role of non-regulatory groups, such as community groups and/or iwi. Interactions between communities, authorities and organisations are a key requirement in monitoring, reporting and resolving water-quality issues.

### 2.4.4 State of environment reporting

Regional councils and the Ministry for the Environment have responsibilities under the Resource Management Act (RMA) to monitor the state (ie, condition or health) of the environment. Reporting on the state of the environment, and how it is changing over time, is undertaken at both regional and national scales by these agencies. The purpose of state of the environment monitoring and reporting is to measure how well our management practices, policies and laws are working, and whether environmental outcomes are being achieved.

There are many factors relating to human land uses and activities that cause cyanobacterial blooms and mats to form, or that exacerbate naturally occurring blooms and mats (eg, flow alteration, shade reduction, nutrient input). It is therefore important to capture information about bloom occurrence to assist with the interpretation of the impacts of catchment land uses (in addition to managing health risks).

At the time of writing these interim guidelines (2009) there are no national environmental indicators that relate to cyanobacterial bloom and mat events. However, some regional councils report qualitatively on cyanobacterial blooms and mats, and whether cyanotoxins were found. Collating data on the number of sites per region per year where cyanotoxins have been identified, along with the toxin type and algae species, could provide a useful regional and national summary of cyanotoxin occurrence. Once risk thresholds are embedded in regional programmes in a standardised way, it may be possible to begin collating exceedance data on biovolumes, cell counts and toxin concentrations.

As with any monitoring network, caution is needed when drawing conclusions about overall bloom and mat occurrence and risk across New Zealand based on limited sample points.

## 2.5 Cost and resource implications

Undertaking monitoring in accordance with these guidelines has cost and resource implications for the agencies involved. In particular, there are costs associated with increasing the frequency of sampling and/or introducing toxin testing. A number of agencies in New Zealand provide toxin testing services (see Appendix 8) and can provide information to help resource managers assess the feasibility of introducing these services.

## 2.6 Conditions of using these guidelines: a disclaimer

Compliance with these guidelines *does not* guarantee that a water body is safe. Sampling may miss or under-represent a toxic bloom event, or there may be other water-quality problems, such as microbiological, chemical and physical quality, that pose a health risk. It is important that water managers use these guidelines judiciously and consider carefully how they can best be applied.

See Sections 1.2 and 1.3 for more detail on what these guidelines should and should not be used for.

# Section 3. The guidelines

**Important note: interpreting the guidelines framework**

The following guidelines provide a recommended approach that is considered best practice for many management circumstances given current understanding of cyanobacterial risks in New Zealand fresh waters. However, local decisions about whether to follow the guidelines' approach should ultimately result from consideration of site-specific factors (such as resource availability, management priorities and historical understanding of local bloom conditions) as well as the guidance offered in this document. Monitoring agencies may have reason to depart from the methodologies suggested in these guidelines.

# Part A: Planktonic cyanobacteria

## 3.1 Planktonic cyanobacteria: an introduction

Cyanobacteria inhabit all natural waters and usually only become a problem when they increase to high concentrations, forming 'blooms'. Cyanobacterial blooms have been a regular occurrence in many New Zealand lakes since the 1970s. However, they have become increasingly prominent in recent decades, possibly in association with anthropogenic eutrophication and climate change (Wood, Jentzsch et al, 2008). The growth of cyanobacteria and the formation of blooms are influenced by a variety of physical, chemical and biological factors. Key variables within New Zealand water bodies that can lead to bloom formation are detailed in Section 4.6.

Planktonic cyanobacteria in New Zealand are now known to produce the following cyanotoxins: microcystins, nodularin, anatoxin-a, cylindrospermopsin, deoxycylindrospermopsin and saxitoxins (Stirling and Quilliam, 2001; Wood, Stirling et al, 2006; Wood, Selwood et al, 2007; Wood, Rasmussen et al, 2007). (See Appendix 1 for information on cyanotoxin distribution in New Zealand). The health risks associated with cyanotoxins are greatest during bloom events. The highest concentrations of cyanotoxins are usually contained within the cells (intracellular), and toxin concentrations dissolved in the water (extracellular toxins) are rarely reported above a few parts per billion (Chorus and Bartram, 1999). People using water bodies for recreational purposes are most likely to experience maximum exposure when a cyanobacterial bloom develops or forms surface scums near water entry points. Wind-driven accumulations of surface scums can result in toxin concentrations increasing by a factor of 1000 or more, and such situations can change within very short time periods (hours).

In New Zealand, research is still underway to determine which species produce the various cyanotoxins. It is therefore recommended that when a species is known to be a toxin producer elsewhere in the world, it should be regarded as potentially toxic in New Zealand until proven otherwise. These guidelines are aimed at protecting human health during recreational activities. Details on the methods use to derive the action alert-level values are given in Appendix 2. For a detailed risk assessment of the risks posed by frequent occupational exposure (eg, daily contact), see de Wet, 2008.

# 3.2 Alert-level framework: planktonic cyanobacteria

### Decision Chart 1: Alert-level framework for planktonic cyanobacteria

| Alert level | Actions<br><br>(See section 2.4 for the recommended framework for roles and responsibilities relating to actions, and the text box at the beginning of Section 3 for advice on interpreting the guidance in this table.) |
|---|---|
| ***Surveillance (green mode)***<br><br>*Situation 1:* The cell concentration of total cyanobacteria does not exceed 500 cells/mL.[a]<br><br>*Situation 2:* The biovolume equivalent for the combined total of all cyanobacteria does not exceed 0.5 $mm^3$/L. | • Undertake weekly or fortnightly visual inspection[b] and sampling of water bodies where cyanobacteria are known to proliferate between spring and autumn. |
| ***Alert (amber mode)***<br><br>*Situation 1:* Biovolume equivalent of 0.5 to < 1.8 $mm^3$/L of potentially toxic cyanobacteria (see Tables 1 and 2); **or**<br><br>*Situation 2*[c]*:* 0.5 to < 10 $mm^3$/L total biovolume of all cyanobacterial material. | • Increase sampling frequency to at least weekly.[d]<br>• Notify the public health unit.<br>• Multiple sites should be inspected and sampled. |
| ***Action (red mode)***<br><br>*Situation 1:* ≥ 12 µg/L total microcystins; **or** biovolume equivalent of ≥ 1.8 $mm^3$/L of potentially toxic cyanobacteria (see Tables 1 and 2); **or**<br><br>*Situation 2*[c]*:* ≥ 10 $mm^3$/L total biovolume of all cyanobacterial material; **or**<br><br>*Situation 3*[e]*:* cyanobacterial scums consistently present. | • Continue monitoring as for alert (amber mode).[d]<br>• If potentially toxic taxa are present (see Table 1), then consider testing samples for cyanotoxins.[f]<br>• Notify the public of a potential risk to health. |

a) A cell count threshold is included at this level because many samples may contain very low concentrations of cyanobacteria and it is not necessary to convert these to a biovolume estimate.

b) In high concentrations planktonic cyanobacteria are often visible as buoyant green globules, which can accumulate along shorelines, forming thick scums (see Appendix 3). In these instances, visual inspections of water bodies can provide some distribution data. However, not all species form visible blooms or scums; for example, dense concentrations of *Cylindrospermopsis raciborskii* and *Aphanizomenon issatschenkoi* are not visible to the naked eye (see Appendix 3).

c) This applies where high cell densities or scums of 'non-toxigenic' cyanobacteria taxa are present (ie, where the cyanobacterial population has been tested and shown not to contain known toxins).

d) Bloom characteristics are known to change rapidly in some water bodies, hence the recommended weekly sampling regime. However, there may be circumstances (eg, if good historical data/knowledge is available) when bloom conditions are sufficiently predictable that longer interval sampling is satisfactory.

e) This refers to the situation where scums occur at the recreation site for more than several days in a row.

f) Cyanotoxin testing is useful to: provide further confidence on potential health risks when a health alert is being considered; enable the use of the action level 10 $mm^3$/L biovolume threshold (ie, show that no toxins are present; and show that residual cyanotoxins are not present when a bloom subsides).

## 3.3 Details of the framework: planktonic cyanobacteria

Three levels of monitoring have been identified: surveillance (green mode), alert (amber mode) and action (red mode) (see Decision Chart 1). There are some important points to note in relation to sampling and cell concentrations for these different alert levels.

For a start, the cell concentrations, or biovolumes, that define the levels apply to samples of the recommended type (ie, composite 50 cm hose-pipes, see Section 4.3.2) that are taken at a representative location(s) in the water body (ie, the likely or designated recreational areas). A single site that is representative of the recreational area is the absolute minimum, but multiple sites are warranted if the area is large, due to the potential for large spatial variations from buoyant cyanobacteria that aggregate under specific physical conditions (eg, calm and still water). Cyanobacteria can still form surface scums at low population densities, particularly if the wind pushes the cyanobacteria to one side of a water body. It is good practice to visually inspect waters regularly under calm conditions from multiple viewpoints. The number of samples taken depends on factors such as the size of the water body and the degree of use of different recreational sites (see Section 4.3.1).

The rationale for the use of biovolumes (as opposed to cell concentrations) is given in Appendix 4. Table A4.1 in Appendix 4 gives biovolumes for common problematic New Zealand species. In many instances this will enable a direct conversion of cell concentrations to biovolumes. For species not listed in Appendix A4.1 it will be necessary to establish their biovolume by undertaking cell measurements. Formulas for calculating biovolume for common geometries of cyanobacteria cells are given in Table A4.2 in Appendix 4.

It is also important to note that in some circumstances monitoring agencies may have good reasons to depart from some of the recommended actions in the three-tier framework. For example, if there is a long history of monitoring and management for a particular water body, a monitoring agency may not consider it necessary to adopt high-frequency sampling (eg, weekly) or to undertake toxin testing in order to confidently characterise recreational health risks.

### 3.3.1 Surveillance: green mode

Surveillance (green mode) is triggered when cyanobacteria are first detected at low levels in water samples, signalling the early stages of a possible bloom. A lower limit of up to 0.5 $mm^3/L$ or 500 cells/mL is given, because the presence of some cyanobacteria in water samples is common and does not indicate the early stages of bloom formation. Sampling and cell counts should be undertaken weekly or fortnightly (from spring to autumn) where cyanobacteria are known to proliferate. Fortnightly sampling frequency may be appropriate for the surveillance level where non-toxigenic species are present and the risk is perceived to be lower (eg, in a low-usage recreational water body).

### 3.3.2 Alert: amber mode

Alert (amber mode) is triggered when either there is a biovolume equivalent of 0.5 to < 1.8 $mm^3/L$ of potentially toxic cyanobacteria (see Tables 1 and 2 below) or the biovolume range is 0.5 to < 10 $mm^3/L$ for the combined total of all cyanobacterial material where known toxins are not present. This accommodates the transition to the action level (red mode) – situation 2 (see Decision Chart 1, ie, > 10 $mm^3/L$ biovolume). (See Appendix 2 for further explanation on how the values are derived.)

The alert level (amber mode) requires notification and consultation with public health units and ongoing assessment of the status of the bloom (see Section 2.4). This consultation should start as early as possible and continue after the results of toxin analysis become available (if used). The requirement for information on toxins will depend on advice and discussion with public health units, and on circumstances such as whether the cyanobacteria are known toxigenic species or whether there is a past history of toxin production. The sampling frequency depends to some extent on the sensitivity and usage of the area, as well as historical knowledge of the site. For example, twice-weekly sampling may be justified where there is a pressing need to issue advice for ongoing use if the site is being used heavily by recreational users or a special event is imminent. In most circumstances weekly sampling provides sufficient information to assess the rate of change of cyanobacterial populations, and to judge the population growth rate and spatial variability – and therefore the hazard.

### 3.3.3 Action: red mode

The action level (red mode) is triggered when representative samples exceed either:

- situation 1: a concentration of > 12 μg/L total microcystins, or a biovolume estimate of 1.8 $mm^3$/L of potentially toxigenic cyanobacteria (see Tables 1 and 2 below); or
- situation 2: > 10 $mm^3$/L for total biovolume of all cyanobacteria where known toxins are not present; or
- situation 3: cyanobacterial scums are consistently present.

(See Appendix 2 for further explanation on how the values are derived.)

In the action level (red mode), public health units (see Section 2.4 for details on the role and responsibilities of each agency) should warn the public of the existence of potential health risks. This should be done (although not exclusively) by media releases and by requesting territorial authorities to erect signs at affected water bodies. An example of information that should be included in a media release is given in Appendix 5. Appendix 6 provides a warning sign template. Warning signs should provide the public with information that enables them to make informed decisions about appropriate use of the water body. Also, local doctors should be encouraged to report any illness that may be linked to contact with water containing cyanobacteria to the public health unit.

The action level (red mode) situation 1 guideline is designed to protect against health effects of repeated exposure to cyanobacterial toxins ingested during recreational activity. Situations 2 and 3 guidelines apply where there is an increased probability of respiratory, irritation and allergy symptoms from exposure to very high cell densities of cyanobacterial material, irrespective of the presence of toxicity or known toxins.

The biovolume threshold of action level (red mode) situation 1 may be used to trigger the action level (red mode). If subsequent toxin analysis is undertaken and is negative, the mode may revert to alert (amber mode) in the biovolume range 0.5 to < 10 $mm^3$/L. If cell numbers continue to increase to < 10 $mm^3$/L, action (red mode) situations 2 and 3 guidelines definitions apply; in other words, either the total biovolume of all cyanobacterial material exceeds 10 $mm^3$/L or cyanobacterial scums are consistently present.

### 3.3.4 Changes in alert levels over time

In slow-moving water bodies toxin testing is usually only warranted at 7- to 10-day intervals. Research has shown that toxin concentrations in a cyanobacterial population can change, but that it is unlikely to become completely non-toxic within a few days. It is therefore recommended that the alert level not be changed from a higher to a lower level (eg, from action to alert) until two successive results (biovolumes) from representative samples have been recorded. The sampling interval between these should be greater than seven days.

Note that cell counts and biovolumes may not give a true indication of toxin levels in a water body. As cyanobacteria die their cells break open, releasing the toxins contained in them. It is therefore possible to have elevated levels of dissolved cyanotoxins corresponding with low cell counts. This was shown in a study at Lake Rotoiti (Rotorua), where biovolumes were below 1.8 $mm^3/L$ but total microcystin concentrations were over 12 µg/L (Wood, Briggs, Sprosen et al, 2006). Microcystins have been shown to persist dissolved in water (ie, extracellularly) for up to 21 days in a water body during post-bloom decline (Jones and Orr, 1994). For greatest confidence in decisions to downgrade alert levels, toxin testing should be considered to ensure concentrations are below the recommended thresholds. Some species, particularly *Cylindrospermopsis raciborskii*, actively transport toxins out of their cells (Chiswell et al, 1999), resulting in high extracellular toxin content, and so toxin testing is recommended when *C. raciborskii* is present.

**Table 1: Toxic cyanobacterial species and their habitat***

| Toxic species | Habitat |
|---|---|
| *Anabaena bergii* | Planktonic |
| *Anabaena circinalis* | Planktonic |
| *Anabaena flos-aquae* | Planktonic |
| *Anabaena lemmermannii* | Planktonic |
| *Anabaena planktonica* | Planktonic |
| *Anabaena* spp. | Planktonic |
| *Anabaenopsis millenii* | Planktonic |
| *Aphanizomenon flos-aquae* | Planktonic |
| *Aphanizomenon issatchenkoi* | Planktonic |
| *Aphanizomenon ovalisporum* | Planktonic |
| *Aphanizomenon* sp. | Planktonic |
| *Aphanocapsa cumulus* | Planktonic |
| *Arthrospira* spp. | Planktonic |
| *Cylindrospermopsis raciborskii* | Planktonic |
| *Cylindrospermum* sp. | Planktonic/benthic |
| *Fischerella* sp. | Benthic |
| *Haphalosiphon* spp. | Soil |
| *Lyngbya wollei* | Benthic |
| *Microcystis aeruginosa* | Planktonic |
| *Microcystis botrys* | Planktonic |
| *Microcystis flos-aquae* | Planktonic |
| *Microcystis* spp. | Planktonic |
| *Microcystis viridis* | Planktonic |
| *Microcystis wesenbergii* | Planktonic |
| *Nodularia spumigena* | Planktonic |
| *Nostoc* spp. | Benthic |
| *Oscillatoria limosa* | Planktonic |
| *Oscillatoria* spp. | Benthic |
| *Phormidium autumnale* | Benthic |
| *Planktothrix agardhii* | Planktonic |
| *Planktothrix formosa* | Planktonic |
| *Planktothrix mougeotii* | Planktonic |
| *Planktothrix* spp. | Planktonic/benthic |
| *Pseudanabaena* sp. | Planktonic |
| *Raphidiopsis curvata* | Planktonic |
| *Snowella* spp. | Planktonic |
| *Synechocystis* spp. | Planktonic |
| *Umezakia natans* | Planktonic |
| *Woronichinia* spp. | Planktonic |

* New toxic species continue to be identified, and all cyanobacteria should be regarded as potentially toxic until proven otherwise.

**Table 2: Summary of known cyanobacteria genera that produce each cyanotoxin***

| Cyanobacteria genus | Cyanotoxin |
|---|---|
| ***Anabaena*[1]**** | **Anatoxin-a**, anatoxin-a(S), cylindrospermopsins, **microcystins**, **saxitoxins** |
| *Anabaenopsis* | Microcystins |
| ***Aphanizomenon*[2]** | **Anatoxin-a**, cylindrospermopsins, microcystins, saxitoxins |
| *Aphanocapsa* | Microcystins |
| *Arthrospira* | Microcystins |
| ***Cylindrospermopsis*[3]** | **Cylindrospermopsins,** microcystins, saxitoxins |
| *Cylindrospermum* | Anatoxin-a |
| *Fischerella* sp. | Microcystins |
| *Haphalosiphon* sp. | Microcystins |
| *Lyngbya* sp. | Anatoxin-a, cylindrospermopsins, saxitoxins |
| ***Microcystis*[1,4]** | Anatoxin-a, **microcystins,** saxitoxins |
| ***Nodularia*[5]** | **Nodularin** |
| ***Nostoc*[1]** | **Microcystins** |
| ***Oscillatoria*[6]**** | **Anatoxin-a, anatoxin-a(S), microcystins** |
| ***Phormidium*[1,7]**** | **Anatoxin-a, homoanatoxin-a, microcystins** |
| ***Planktothrix*[1,8]**** | Anatoxin-a, homoanatoxin-a, **microcystins,** saxitoxins |
| *Pseudanabaena* | Microcystins |
| *Raphidiopsis* | Anatoxin-a, cylindrospermopsins, homoanatoxin-a |
| *Snowella* | Microcystins |
| *Synechocystis* | Microcystins |
| *Woronichinia* | Microcystins |
| *Umezakia* | Cylindrospermopsins |

* This is a compilation of worldwide information, and the toxins are not produced by all species of the particular genus. Species from the genera in **bold type** are known to produce the associated toxin (in bold type) in New Zealand.

** The results of cyanotoxin testing on environmental samples indicate that species from this genus produce the associated cyanotoxin in New Zealand. 1. Wood, Stirling et al, 2006; 2. Wood, Rasmussen et al, 2007; 3. Wood and Stirling, 2003; 4. Christoffersen and Burns, 2000; 5. Carmichael et al, 1988; 6. Hamill, 2001; 7. Wood, Selwood et al, 2007; Wood, Heath et al, in press.

# Part B: Benthic cyanobacteria

## 3.4 Benthic cyanobacteria: introduction

The guidelines are designed to manage risks to *recreational users*. They have been designed to protect users from the risks associated with ingestion of and contact with water and mats. The levels given in the guidelines are not relevant for addressing risks to dogs that actively seek out and consume cyanobacterial mats. (Appendix 2 provides further explanation on how the values are derived.)

Benthic, mat-forming cyanobacteria are widespread throughout New Zealand rivers and are found in a wide range of water-quality conditions, including oligotrophic waters (Biggs and Kilroy, 2000). The most common mat-forming benthic cyanobacteria genus in New Zealand is *Phormidium*. During stable flow conditions *Phormidium* mats can proliferate, at times forming expansive black-brown leathery mats across large expanses of river substrate (see Appendix 7). Flow conditions, substrate, water chemistry and species composition can influence the

macroscopic appearance of benthic cyanobacterial mats (see Appendix 7), and at times they may easily be confused with other algal groups (eg, diatoms or green algae). Microscopic confirmation should be undertaken by either competent regional council staff or a laboratory with micro-algae identification expertise (see Appendix 8).

Dog deaths associated with the consumption of benthic cyanobacteria have become increasingly common around New Zealand (eg, Hamill, 2001; Wood, Selwood et al, 2007; Heath et al, in press (a), in press (b)). In most instances these deaths have been associated with the presence of the neurotoxins anatoxin-a and/or homoanatoxin-a (Wood, Selwood et al, 2007), and this often results in the rapid death of the animal. The production of microcystins by benthic cyanobacteria (*Nostoc*. sp. and *Pankthothrix* sp.) in New Zealand has now been confirmed (Wood, Stirling et al, 2006; Wood. Heath, McGregor et al, in press), and in at least once instance a dog death was caused by microcystins (Wood, Heath, McGregor et al, in press). In other parts of the world benthic species are known to produce saxitoxins and cylindrospermopsins (Carmichael et al, 1997; Seifert et al, 2006).

Recent research suggests the presence of cytotoxic (toxic to cells) compounds affecting mammalian cells from multiple *Phormidium* species collected around New Zealand (Wood, Froscio and Campbell, unpublished data). Therefore health warnings should not rely solely on the presence of known toxins. In an in-depth study of the spatial and temporal distribution of *Phormidium* mats in the Hutt River (Lower Hutt) it has been shown that toxin concentrations within mats can vary markedly among sampling sites and over short time frames (eg, a week; Heath et al, in press(b)). It has also been demonstrated that the presence and concentrations of anatoxins within the mat are not related to the abundance of the *Phormidium* mats (Heath et al, in press (b); Wood, Heath, et al, in press). Therefore a negative toxin test does not guarantee the absence of toxins within a water body.

Under certain environmental conditions, or as they become thicker (and bubbles of oxygen gas become entrapped within them), mats will detach from the substrate and may accumulate along river edges (see Appendix 7). During these events the risk to human and animal health is higher due to the accessibility of the cyanobacterial mats to river users. The highest risks to users is likely to be via ingestion of and/or direct contact with these cyanobacterial mats. The risk associated with both types of contact is likely to rise as the abundance and/or number of detachment events increases.

It is unclear whether extracellular toxins (toxins in the water column) are released in substantial quantities from cyanobacterial mats, but these are likely to be rapidly diluted and pose a lesser risk. Traditional water-column sampling (ie, taking a grab sample) only provides a snap-shot from the flow continuum and may underestimate the risk posed by benthic cyanobacteria. A passive *in situ* methodology known as solid-phase adsorption toxin tracking technology (SPATT) is currently under development to assist in sampling benthic cyanobacterial toxins (Wood, Holland et al, 2008). This has the potential to be a useful and economical tool for early warning and for monitoring the presence of extracellular toxins in rivers. This methodology has yet to be validated in rivers.

Although not specifically covered in these guidelines, benthic cyanobacteria do occur in lakes and ponds, where they have caused animal fatalities (Naegeli et al, 1997). These can detach and accumulate on shorelines (see Appendix 7). Where this occurs in recreational areas it is recommended that samples be collected for microscopic identification and cyanotoxin analysis, and the percentage of affected shoreline estimated.

# 3.5 Alert-level framework: benthic cyanobacteria

**Decision Chart 2: Alert-level framework for benthic cyanobacteria**

| **Alert level**[a] | **Actions**<br>(See section 2.4 for the recommended framework for roles and responsibilities relating to actions, and the text box at the beginning of Section 3 for advice on interpreting the guidance in this table.) |
|---|---|
| ***Surveillance (green mode)***<br>Up to 20% coverage[b] of potentially toxigenic cyanobacteria (see Table 1) attached to substrate. | • Undertake fortnightly surveys between spring and autumn at representative locations in the water body where known mat proliferations occur and where there is recreational use. |
| ***Alert (amber mode)***<br>20−50% coverage of potentially toxigenic cyanobacteria (see Table 1) attached to substrate. | • Notify the public health unit.<br>• Increase sampling to weekly.<br>• Recommend erecting an information sign that provides the public with information on the appearance of mats and the potential risks.<br>• Consider increasing the number of survey sites to enable risks to recreational users to be more accurately assessed.<br>• If toxigenic cyanobacteria (see Table 2) dominate the samples, testing for cyanotoxins is advised. If cyanotoxins are detected in mats or water samples, consult the testing laboratory to determine if levels are hazardous. |
| ***Action (red mode)***<br>*Situation 1:* Greater than 50% coverage of potentially toxigenic cyanobacteria (see Table 1) attached to substrate; **or**<br>*Situation 2:* up to 50% where potentially toxigenic cyanobacteria are visibly detaching from the substrate, accumulating as scums along the river's edge or becoming exposed on the river's edge as the river level drops. | • Immediately notify the public health unit.<br>• If potentially toxic taxa are present (see Table 2) then consider testing samples for cyanotoxins..<br>• Notify the public of the potential risk to health. |

a The alert-level framework is based on an assessment of the percentage of river bed that a cyanobacterial mat covers at each site. However, local knowledge of other factors that indicate an increased risk of toxic cyanobacteria (eg, human health effects, animal illnesses, prolonged low flows) should be taken into account when assessing a site status and may, in some cases, lead to an elevation of site status (eg, from surveillance to action), irrespective of mat coverage.

b This should be assessed by undertaking a site survey as documented in Section 4.4.

# 3.6 Details of the framework: benthic cyanobacteria

## 3.6.1 Surveillance: green mode

This is triggered when cyanobacteria are first detected at low abundance (up to 20 per cent coverage), signalling the early stages of possible mat proliferation. Site surveys should be conducted as described in Section 4.4. Microscopic identification should be undertaken on samples to confirm the presence of cyanobacteria. Perform weekly surveys at representative

locations along the river from spring to autumn and during peak recreational use periods. A single site that is representative of the recreational area may be acceptable, but multiple sites are warranted if the area is large. Fortnightly or monthly sampling frequency may be appropriate during cooler months and low use periods. Flow alerts (Section 3.7) can be used to trigger the surveillance level.

### 3.6.2 Alert: amber mode

The alert level (amber mode) is triggered when there is 20−50 per cent coverage of potentially toxic cyanobacteria (see Table 1) attached to substrate. The alert level requires notification and consultation with public health units for ongoing assessment of the status of the cyanobacterial proliferation (see Section 2.4). This consultation should start as early as possible and continue after the results of toxin analysis become available (if used). Testing for toxins can be undertaken to obtain a clearer indication of the health risks at a site. For example, if coverage is below 50 per cent and high concentration of cyanotoxins are detected, the risk level may be increased to action.

Weekly sampling should be undertaken. In most circumstances this will provide sufficient information to assess the rate of change of cyanobacterial populations, and to judge the population growth rate and spatial variability and therefore the hazard. The number of survey sites depends on factors such as the length of the water body and the degree of use of different recreational sites.

The alert level is also a good time to raise public awareness of the potential risk to water uses. Media releases (see Appendix 10) and information pamphlets (see Appendix 11) left at veterinary clinics, for example, are useful publicity methods. Information signs that provide the public with information on the appearance of mats and potential risk should be erected (see Appendix 9).

### 3.6.3 Action: red mode

The action level (red mode) is triggered when representative site surveys and sampling reveal either greater than 50 per cent coverage of potentially toxigenic cyanobacteria attached to substrate, or where up to 50 per cent of the available substrate is covered by potentially toxigenic cyanobacteria taxa (Tables 1 and 2) and these are visibly detaching from substrate, accumulating as scums along the river's edge or becoming exposed on the river's edge as river levels drop.

In action level (red mode), public health units (see Section 2.4 for details on the role and responsibilities of each agency) should warn the public of the potential health risks. This should be done (although not exclusively) through media releases and by requesting territorial authorities to erect signs at affected water bodies (Appendix 6 and Appendix 9).

Benthic cyanobacteria in New Zealand are known to produce toxic substances that have not yet been characterised. Also, based on data from planktonic cyanobacteria (eg, Pilotto et al, 2004; Stewart et al, 2006), there is an increased likelihood of respiratory, irritation and allergy symptoms from exposure to high abundances of cyanobacterial material, irrespective of toxicity or of the presence of known toxins. This is the rationale for action level (red mode) situation 1. As benthic cyanobacterial mats detach, they can accumulate along a river edge. Because of the increased availability of these mats, this is considered to be a period of high risk regardless of the percentage coverage in a water body (see action level − situation 2, Decision Chart 2, page 17).

### 3.6.4 Changes in alert levels over time

It is recommended that the action level (red mode) not be changed from a higher to a lower level (eg, from action to alert) until the percentage cover falls below the action level on two successive surveying occasions (collected at weekly intervals). The regularity of flushing flow (Decision Chart 3) should also be considered when downgrading health alerts.

## 3.7 Benthic cyanobacteria and river flows

A correlation between benthic cyanobacterial mat abundance, water temperature and a lack of 'flushing flow' conditions has been observed in some rivers (Milne and Watts, 2007; Wood, Selwood, Rueckert et al, 2007; Heath et al, in press (b)). In some instances, the length of time since a flushing flow event can be used as an early warning of elevated risk of benthic cyanobacterial proliferations. However, the flow velocity required to shift cyanobacteria from the river bed will vary depending on factors such as the river bed substrate type and size. For example, a river with a sandy substrate will require a markedly smaller flow to flush benthic cyanobacteria compared to a river with a large cobble substrate. In addition, the length of time required for cyanobacteria to proliferate following a flushing flow event will vary. So, although there is no 'one size fits all' warning system, on a regional basis experts could use periphyton coverage records, flow data and local knowledge to develop warning systems for cyanobacterial proliferation risk.

The following is an example of an automated river flow-based warning system for benthic cyanobacterial proliferation risk that is currently used by the Greater Wellington Regional Council for selected rivers within their region (Milne and Watts, 2007). Such a system is recommended for recreational use rivers within New Zealand known to experience potentially toxic benthic cyanobacterial proliferations. Some in-depth research is needed to establish the appropriate 'flushing flows' in each river. For example, in the Otaki, Waikanae, Hutt, Mangaroa and Wainuiomata rivers a 'flushing flow' is defined as "three times the median flow", consistent with field observations and the findings of Clausen and Biggs (1997).

The automated river warning system for cyanobacterial proliferation risk has two complementary alert levels based on flow conditions (see Decision Chart 3).

**Decision Chart 3: Flushing flow alert framework for benthic cyanobacteria**

| Flow alert level | Monitoring suggestion |
|---|---|
| ***Alert mode 1***<br>No flushing flow[1] for 2 weeks.[2] | Survey known problematic sites to assess cyanobacterial cover, as per surveillance level (green mode) (see Section 3.6.1). |
| ***Alert mode 2***<br>No flushing flow for 2 weeks and river flows are low (set at lowest 10$^{th}$ percentile flow for each river).[3] | Increase frequency (eg, to weekly) of surveys of known problematic sites to assess cyanobacterial cover, as per alert level (amber mode) (see Section 3.6.2). |

1 In the Wellington region a flushing flow is defined as three times the median flow.

2 Following an assessment of the events of spring 2005, two weeks was determined to be an appropriate (and conservative) duration.

3 The justification for a low flow alarm is that water temperatures may be elevated (promoting algal growth), and any cyanobacterial growths may become exposed or near exposed at the river edges.

# Part C: Cyanotoxin accumulation

## 3.8 Cyanotoxin accumulation in aquatic organisms

Cylindrospermopsins, microcystins, nodularin and saxitoxins can accumulate in a variety of fresh water and marine organisms (Kotak et al, 1996; Vasconcelos, 1999; Saker and Eaglesham, 1999; Sipia et al, 2002). When this occurs, warnings to avoid consuming aquatic organisms should be included in media releases and on warning signs (Appendices 5, 6, 9 and 10).

Wood, Briggs et al (2006) showed that microcystins accumulate in rainbow trout (*Oncorhynchus mykiss*) and freshwater mussels (*Hyridella menziesii*) in Lakes Rotoiti and Rotoehu (Rotorua). Based on the microcystin levels found in their study, it is considered unlikely that eating trout flesh as part of a regular balanced diet would result in adverse health effects. However, concentrations of microcystins were significantly higher in rainbow trout liver, so it is recommended that fish be gutted and thoroughly washed in clean tap water before eating.

The downstream effects of water bodies containing cyanobacterial blooms should also be considered. For example, Lake Omapere (Northland) experiences blooms containing microcystins, and the outlet of the lake flows into the Hokianga harbour, where microcystins have been found in shellfish.

For further advice on appropriate levels of cyanotoxins in aquatic organisms, contact the New Zealand Food Safety Authority (www.nzfsa.govt.nz).

# Section 4. Sampling

## 4.1 Health and safety

When sampling cyanobacteria in lakes or rivers, consideration needs to be given to protecting the sampler. Samplers should wear gloves and rubber waders (rather than neoprene) or gumboots to reduce the risk of skin contact. If sampling when there is excessive foam present and windy conditions, a dust/surgical face mask should be worn. When wading into swift-flowing rivers and streams, standard water-quality sampling procedures (held by most regional councils) should be observed to identify hazards and reduce the risk of being swept downstream.

## 4.2 Biosecurity

The procedures detailed in this section involve entering water bodies that may contain the introduced invasive diatom didymo (*Didymosphenia geminata*). Therefore, all equipment and clothing should be decontaminated when leaving a water body where there is any chance of didymo being present. Decontamination protocols for didymo can be downloaded from www.biosecurity.govt.nz

## 4.3 Planktonic cyanobacteria

The design of monitoring programmes for planktonic cyanobacteria is challenging due to factors such as:

- their ability to grow in open waters
- the ability of some species to regulate their buoyancy
- their ability to form scums that may be shifted and concentrated by wind
- the interactions of buoyant cells with the surface drift currents created by wind
- the ability of some species to produce toxins that may be contained in their cells or dissolved in water.

Due to these factors, monitoring programmes for planktonic cyanobacteria should be tailored to the characteristics of each water body. They also need to be flexible to take account of changes in the risk posed by rapid changes in the cyanobacterial populations with time and location, which should be recorded along with the sample depth and type. Collection of historical information on blooms and growth conditions, and the identification of patterns of cyanobacterial growth, can be used to help focus the monitoring programme on critical periods and locations in the water body of interest. The aims of the sampling protocols outlined below are to enable an assessment of health hazards caused by planktonic cyanobacteria and their toxins in recreational use waters. Detailed protocols for sampling drinking water are provided by the Ministry of Health (2005a), and protocols for sampling for ecological and other studies are provided by Pridmore (1987) and Codd et al, (1999).

### 4.3.1 Site selection

The heterogeneous (mixed) and dynamic nature of many cyanobacterial populations can make selecting a sampling site difficult. A flexible response when choosing the sampling sites may, at times, be more appropriate than following a rigid programme. Alternatively, fixed sites can be sampled within a broader monitoring programme to provide linear time series, supplemented by sampling of sites currently harbouring cyanobacterial scums.

The selection of sampling sites is a key factor in collecting representative samples. The following should be considered.

#### A. Use of the site for contact recreation

- Sampling sites should include shoreline areas frequented by recreational users, perhaps with a focus on public bathing sites.
- Make use of local logistical resources, and consider accessibility and safety factors.

#### B. Risk of a site having cyanobacterial blooms/mats

- The history, if available, of cyanobacterial population development and the occurrence of toxins in the water body is useful. This information may indicate sites most likely to harbour scums/mats.
- Specific incidents, such as animal deaths or human illness, may provide indications of 'high risk' sites.
- Morphometric and hydrophysical characteristics of the water body (eg, exposure to wind or thermal stratification) may help identify sites that are prone to scum accumulation.
- Prevailing weather conditions, particularly wind direction, can lead to scum accumulation along certain shorelines.

### 4.3.2 Sample collection

An entry-point or near-shore sample should consist of a composite sample comprising five 50 cm depth-integrated column (hosepipe) sub-samples collected relatively randomly along an approximately 20–30 m transect (parallel to the lake shore) and mixed into a single container (eg, a bucket). From this, a composite sample is taken for the cell counts and/or toxin analysis.

The rationale for this sample type is that:

- the 50 cm integrated column or tube covers the surface zone that recreational users are most likely to be exposed to
- the sampling of this shallow 0–50 cm zone also covers the accumulation of buoyant cyanobacteria near the surface under calm conditions
- the recommendation for five pooled sub-samples accounts for spatial variability within a single site.

The volume of the composite sample required will vary. When sampling eutrophic lakes, 100 mL is usually sufficient for cyanobacterial identification and 500 mL for cyanotoxin analysis. In oligotrophic lakes, two 500 mL samples are required: one for identification and one for cyanotoxins (see Sections 4.5.1 and 4.5.2).

Integrated samples can be collected using a rigid or flexible plastic hosepipe with an inner diameter of at least 2.5 cm; a rigid polyvinylchloride (PVC) or acrylic plastic pipe is more practical than a flexible pipe.

Where wading or boat access is not available, the alternative is to collect a pooled surface-grab (ie, dipped bucket samples). Additional individual, non-composite samples should also be collected where scums or obvious discoloured water are encountered. These individual 'grab' samples represent the maximum hazard at the time of inspection and may assist in the overall health risk assessment.

It is advisable to collect samples in the morning because cyanobacterial blooms are usually at their densest at the surface in the early morning. For comparative purposes, the sampling time should be consistent between sampling trips, where practical.

The frequency of samples collected at any one location is dictated by the alert-level framework (see Section 3.2).

### 4.3.3 Field data records

It is important to record all relevant details about the sampling site, sampling methods and prevailing conditions. The following should be noted, where possible:

- weather conditions at the time of sampling and 24 hours prior to sampling (including wind direction and strength)
- water transparency (use a Secchi disc if available)
- any discolouration of the water or signs of blooms or mats
- water temperature
- dissolved oxygen.

Integration of sampling with a more comprehensive water-quality sampling programme will help to develop an understanding of the causal factors promoting cyanobacterial growth for each specific water body (see Section 4.6).

Interpretation of the significance of a particular cyanobacterial cell concentration in relation to others may require an examination of the field sheet to verify the type of sample collected (ie, surface, depth or integrated depth) or the place or time of collection. An example of a typical field sheet is provided in Appendix 12.

## 4.4 Benthic cyanobacteria

The method described below is intended for use in rivers where cyanobacterial mats are likely to occur and is recommended as a quick, easy and reproducible way of keeping a record of benthic cyanobacterial coverage. These records are designed to help assess the risk posed by cyanobacteria in rivers under recreational use. Routine sampling is recommended under low coverage (< 20%) if there is any doubt about the identity of observed algal mats.

At established sites it should be possible to complete the survey procedure in 15–20 minutes (ie, completion of the survey form, Appendix 13). The greatest investment in time will occur during site selection and collecting background information on the site.

### 4.4.1 Site selection and collecting background site information

Refer to Section 4.3.1 for key factors that should be considered when selecting sampling sites. Cyanobacterial mats tend to proliferate initially in riffles, then runs, so priority should be given to examining these habitat types.

On the first visit to the site choose a 40–60 m reach where a survey can be undertaken on a regular basis. Where possible, collect the following background information for each site:

- reach length and river width (measure or estimate – photos are useful)
- substrate composition (ie, bed substrate type – cobbles, gravels, sand-silt)
- water velocity
- amount of shade at each survey reach
- bank vegetation (descriptive – this could be captured photographically)
- hydrology (eg, time since last flood, 2x and 3x median flow, see Section 3.7).

Integration within a broader programme of water-quality monitoring may be useful.

### 4.4.2 Site surveying and sample collection

The following equipment is required to undertake a benthic cyanobacterial assessment:

- Underwater viewer (Figure 1): this is constructed from clear perspex or bought (eg, a Nuova Rade viewer, available from www.marisafe.com/store/viewItem.asp?ID=506050907). These viewers allow a clear view of the stream bed with no interference from surface turbulence and reflection. They also enable a more-or-less standard area of the stream bed to be defined at each survey point (equivalent to a quadrat in terrestrial ecology). Photographs can be taken through these viewers for improved documentation of mat coverage.
- Clipboard, pencils and monitoring forms (see Appendix 13): forms should preferably be printed on waterproof paper.
- Sampling containers and permanent marker or equivalent (for labelling).

**Figure 1: Using an underwater viewer**

Photos: S Wood, Cawthron Institute

Ideally the survey should be undertaken in teams of two: one observer and one scribe. However, some tips are provided for one-person surveys below.

All monitoring should be undertaken under similar flow conditions (eg, at no more than median flow). This ensures the surveys always cover the permanently wetted channel. Surveys in very low flows are acceptable, but higher flows should be avoided due to associated safety issues and reduced water clarity.

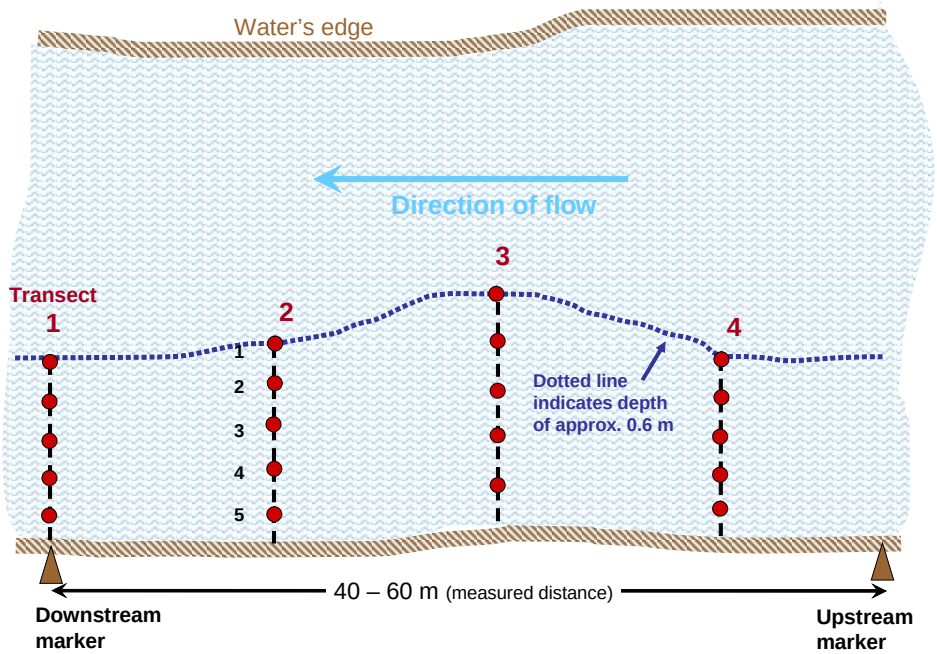
### 4.4.3 Monitoring procedure

1. After arriving at a survey area, spend approximately five minutes looking along a 30−60 m section of river bed for the presence of cyanobacterial mats. Ensure this section includes some riffles and runs. Mark out four transects in the selected area by placing marker rocks along the water's edge, approximately 10–15 m apart.

2. Complete the first section of the monitoring form (Appendix 13) with site, date, time, etc, and note the general presence/absence of cyanobacterial mat and the presence of any detached mat along the shoreline.

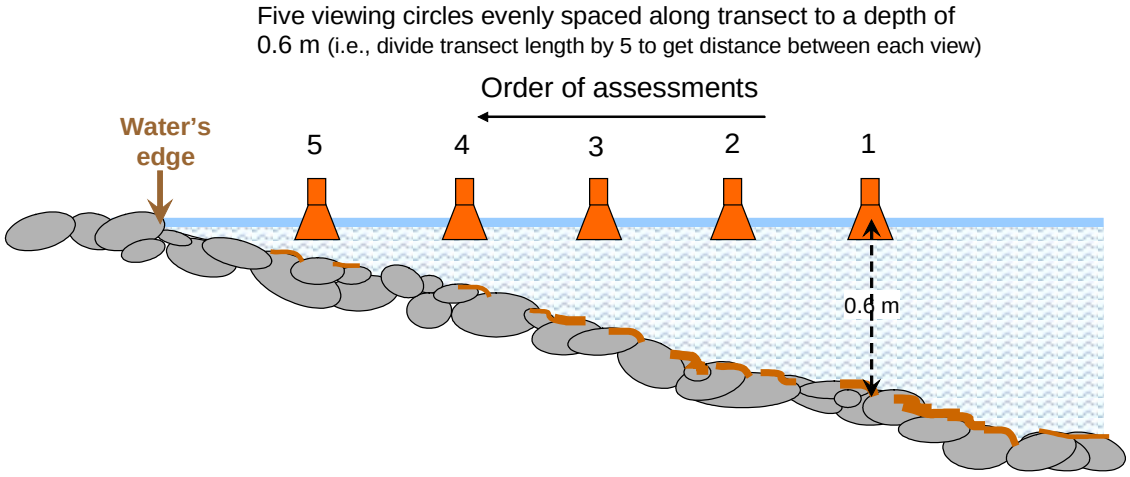
3. Assemble the underwater viewer and, starting at the *downstream end*, wade into the stream at right angles to the water's edge. Go out to a depth of approximately 0.6 m (see Figure 2 and Figure 3) A standard maximum depth of 0.6 m should be used at all sites, where possible. In shallow rivers the transects may span the entire width. Wading into fast-flowing water can be dangerous and *extreme care* should be taken.

**Figure 2: Schematic of layout of transects (numbered in red) and survey areas (red circles, numbered in black) at a site (not to scale)**




Notes: The numbering indicates the order in which assessments are made, and corresponds to the numbers on the monitoring form (Appendix 13). The transects are spaced evenly along the survey reach. It may not always be possible to have five viewer results (eg, in steep-sided rivers). In these circumstances, take as many views as practical per transect. If the river does exceed 0.6 m in depth, the transect should span its entire width. Source: C Kilroy, NIWA

**Figure 3: Schematic of transect cross-section showing arrangement of sampling points (not to scale)**




Notes: Assessment 1 will cover a greater area than assessment 5 because of the greater water depth. However, this will be the case at all sites. Therefore assessments should be comparable. Source: C Kilroy, NIWA

4 Record the maximum distance and depth in the boxes at the top of the column for transect 1.

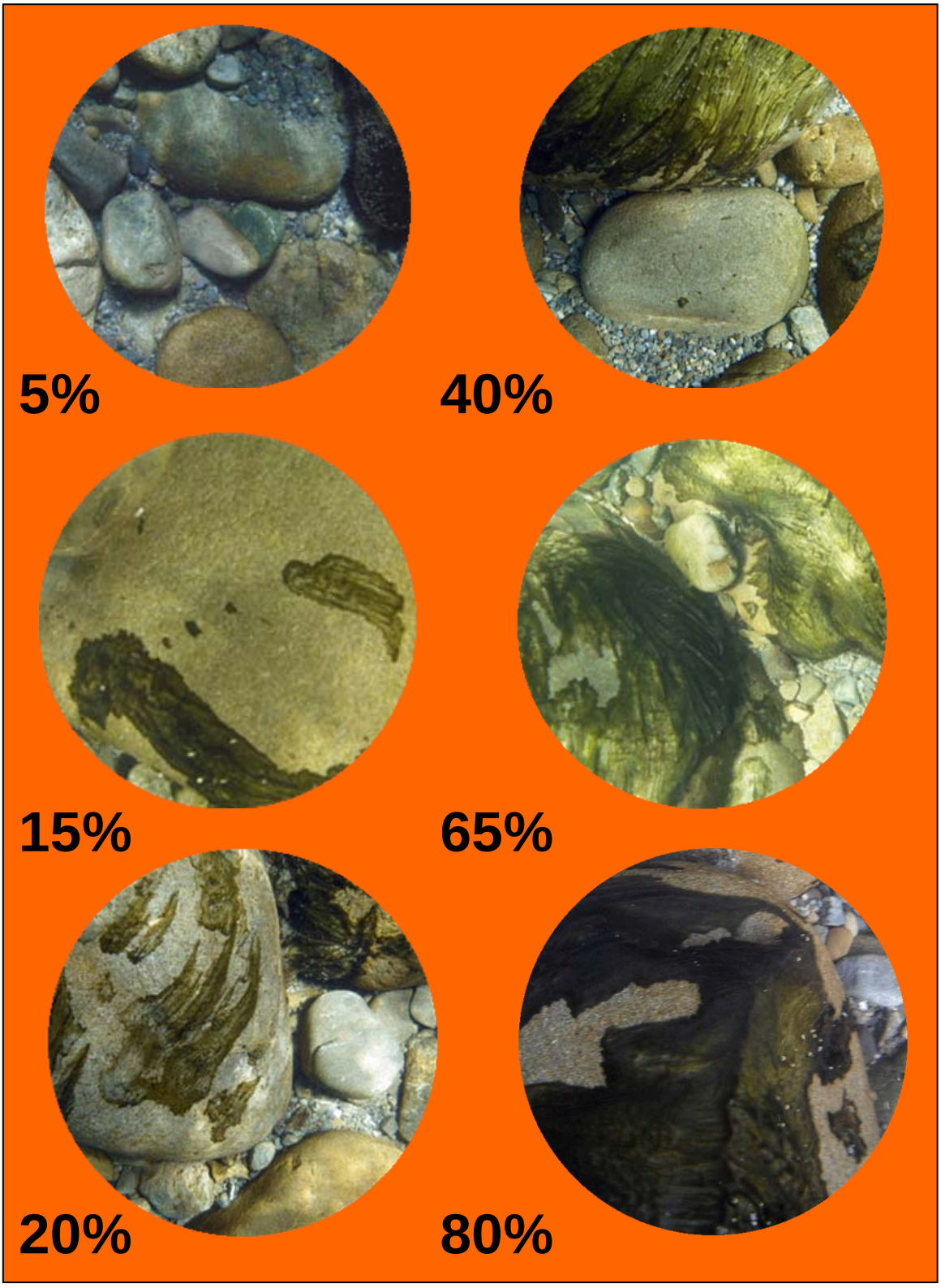
5. Hold the underwater viewer about 20 cm under the water, more or less on the transect line. The area of view should not be one that has just been walked over. Holding the viewer steady and as vertical as possible, estimate to the nearest 5 per cent the proportion of the area you see that is occupied by the cyanobacterial mat**.** Some examples are shown in Figure 4. Cyanobacterial mats are usually dark black, dark brown or dark green in colour, leathery, and have an earthy, musty odour. Refer to Appendix 7 for a photographic guide to benthic algae/cyanobacteria. Coverage should only be recorded if mats are greater than 1 mm thick, although it is useful to record the presence of thin mats.

6. If there is any doubt about the identity of mat cover (ie, whether it is cyanobacteria) at any sampling point, take a sample for microscopic identification. Samples should be collected by scraping an egg-sized clump of mat into a sampling pottle. Samples for microscopy should be preserved with Lugol's iodine (see Appendix 14), whereas samples for cyanotoxin analysis **SHOULD NOT** be preserved (see Section 4.3.2). Toxin content can vary markedly between rocks within a site (Wood, Heath et al, in press), so where possible take 10 samples from separate rocks and pool these for toxin analysis. To enable the amount of toxin within an area to be estimated, the sample should be taken from a known area on the rock (eg, use the top of a sampling pottle to mark out the sampling area) by scraping all periphyton from that defined area into a sampling pottle. Label the sampling container with the site name, area sampled and transect number.

7. Record the percentage cover in the appropriate boxes for each transect. Ideally, be consistent with the order of survey points on each transect (eg, point 1 is always the deepest into the water and 5 is always closest to the waters' edge, see Figures 2 and 3). Indicate at which (if any) sites samples were taken. Record any notes regarding other algal cover (eg, green filaments overgrowing cyanobacterial mats).

8. Space the points evenly along the transect to a depth of 0.1–0.15 m nearest to the water's edge, although this depth will vary according to the type of river. For example, if the river bank is incised (channelised), the closest survey point will be deeper.

**Figure 4: Examples of different levels of cyanobacterial cover viewed through an underwater viewer**




Photos: M Heath, Victoria University.

9. Move upstream to transects 2, 3 and 4, and repeat steps 5 to 9 to complete the survey at this site.

10. Calculate the average percentage cover per transect and then the average percentage cover per site. Average percentage cover results for each site should be interpreted via the alert-level framework (Section 3.5) and the appropriate actions taken.

The frequency of samples collected at any one location is dictated by the alert-level framework (Section 3.5).

For health and safety reasons it is usually advisable to work in teams of two or more. However, there may be occasions when only one person is available. In this case, a single person must handle the equipment for both observing and recording. Here are some tips to make this easier.

- Always use data sheets copied onto waterproof paper (eg, Rite-in-the-Rain paper).
- Reduce the size of the data sheet to A5 to create a smaller, more manageable clipboard.
- Tether the viewer securely to your waist so that both your hands are free for writing.
- Secure the clipboard and pencil to your waders so that, if dropped the items can be retrieved without damage.
- Tie a small towel to the wader shoulder strap so that it is possible to dry your hands before writing.
- For estimating water depth, mark a scale on the side of the waders in, for example, 5 cm intervals.

## 4.5 Sample storage and transport

The following are standard protocols for sample preservation, storage and transport. Analytical laboratories may have specific requirements and it is strongly recommended that you contact the relevant laboratory (see Appendix 8) well before sample collection.

### 4.5.1 Cyanobacterial identification and enumeration

Sub-samples should be preserved as soon as possible after collection by the addition of 1 per cent acid Lugol's iodine preservative (Appendix 14). Lugol's iodine is added drop by drop until the sample is the colour of beer or weak tea (approximately 4 drops per 100 mL in water). Dense samples (eg, scum material or benthic mats) will absorb Lugol's and may require additional Lugol's if long-term storage is required.

Samples should be stored in the dark. Some plastic bottles (polyethylene) tend to absorb iodine very quickly into the plastic, so care should be taken with any samples requiring longer term storage. It is useful to retain a portion of sample in a live (unpreserved) state, as cyanobacteria are often easier to identify in this way. Live samples degrade quickly, however, and a small amount of material should be collected and covered with water. Ensure there is plenty of air space above the sample and refrigerate. Examine as soon as possible after collection. Each bottle should be labelled clearly with the site name and location, approximate depth, date, sample type (integrated or grab), sampler's name, and indication of whether Lugol's has been added.

### 4.5.2 Cyanobacterial toxins

Samples for toxin analysis should be stored in glass bottles, where possible, because plastics may absorb cyanotoxins. The volume of sample required depends on the type of analysis. For planktonic samples, at least 500 mL of water should be collected. Benthic samples should be collected as described in Section 4.4.3 (point 6).

Cyanotoxins are readily degraded, both photochemically (in light) and microbially. Samples should be transported in dark, cold conditions and kept refrigerated prior to analysis. Where samples for toxin analysis won't reach the analytical laboratory within 24 hours, samples can be stored frozen. However, note that freezing releases cyanotoxins from the cells and so only the total amount of toxins in a sample can be determined.

## 4.6 Susceptibility of a water body to a cyanobacterial bloom or benthic mat event

In some regions it may not be practical to monitor phytoplankton abundance in all water bodies where there is recreational use. The decision on which water bodies to monitor should be based on a combination of:

- the amount of recreational use
- pre-existing knowledge of the characteristics of the water body
- any prior monitoring data on cyanobacterial events.

Aerial and satellite photography can be used to derive information on water clarity and phytoplankton biomass of larger surface waters, which then provides a basis for comparative assessments between different water bodies.

A decision support tree can also be useful to help assess the likelihood of a cyanobacterial bloom occurring in a water body. Figure 5 shows such an example, which has been extended to include the risk of formation of a benthic cyanobacterial mat as well as the occurrence of a cyanobacterial bloom. The logic proposed for cyanobacterial blooms is similar to that given by Oliver and Ganf (2000), but it has been adapted on the basis of New Zealand observations and simplified, as follows.

- A prerequisite for blooms in Oliver and Ganf's model is an available phosphorus concentration in excess of 10 mg/$m^3$. Blooms of cyanobacteria have been observed in many New Zealand lakes when available phosphorus concentrations are close to detection limits, and in Lake Taupo, for example, when total phosphorus concentrations are below 10 mg/$m^3$.
- Some variables in Oliver and Ganf's model that may not be easy to measure (eg, grazing, turbulent water velocity and cell floating velocity) are not included in the model presented in Figure 5.
- A model to assist with the prediction of benthic mats of cyanobacteria is included for both lakes and rivers.
- The inclusion in the decision tree for bloom development of stratified lakes in which bottom waters become anoxic does not reflect a direct process or causal linkage to bloom development. It is simply an observation that this category of lakes appears to be susceptible to cyanobacterial blooms.

Because of the complexity of interactions among nutrients, wind and lake size, probabilistic functions have been added to the decision support tree for a subset of water bodies (see Figure 6). These functions are intended to represent the fact that simple 'yes' or 'no' decisions are not always possible and that knowledge of the causal factors of cyanobacterial blooms is also imperfect. The subset of water bodies for which these probabilistic functions applies includes deeper lakes that undergo seasonal stratification but whose bottom waters do not become anoxic.

**Feedback requested on Figures 5 and 6**

Note that the decision support tree presented in Figure 5 is intended for testing and feedback, to provide a basis for iterative improvement and development on the basis of regional and local information. Your feedback would be appreciated.

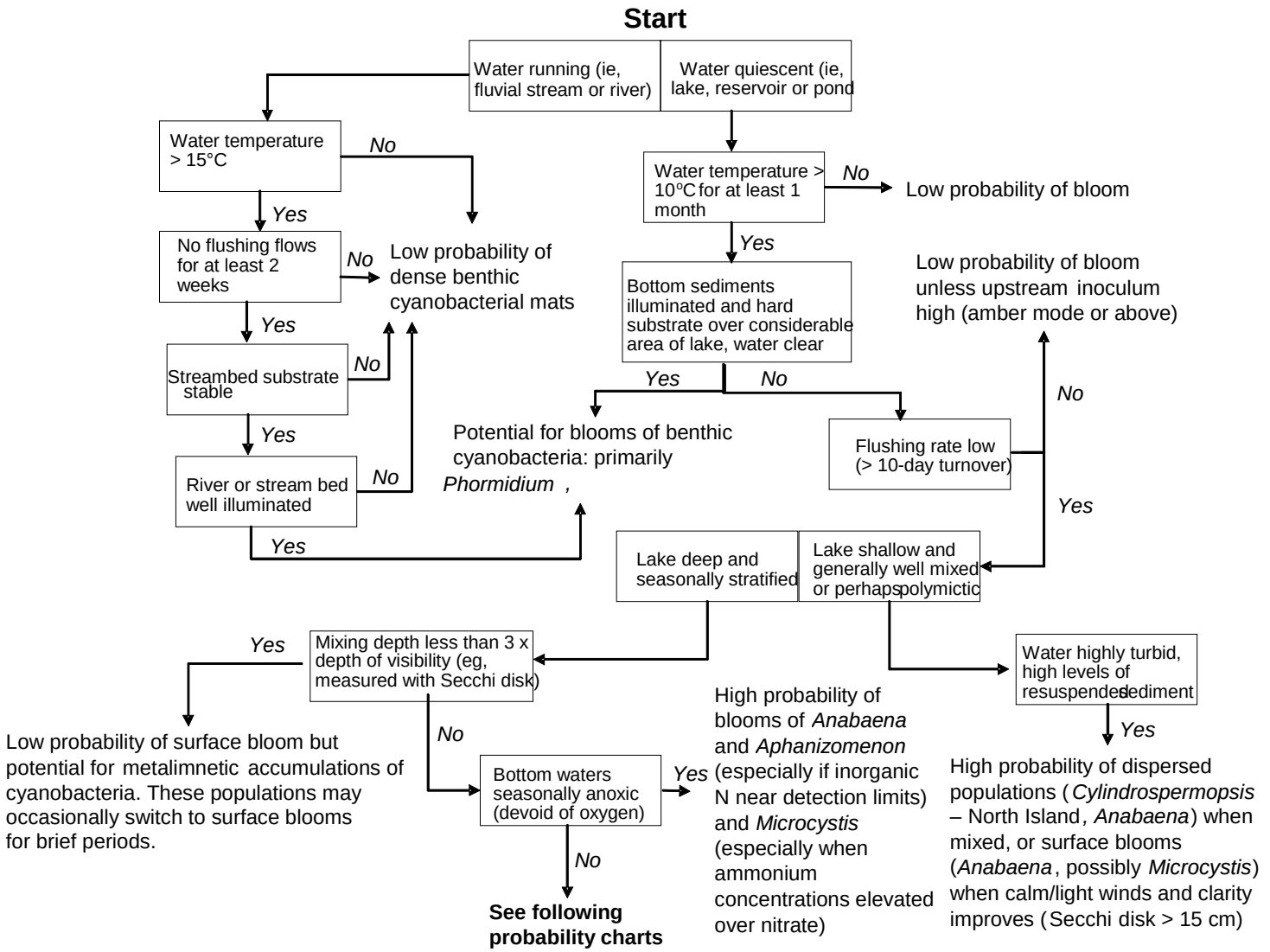
The probability charts shown in Figure 6 are a first attempt to apply numerical values of probability for the occurrence of a cyanobacterial bloom. The inclusion of the variables lake area, total phosphorus concentration, dissolved inorganic nitrogen concentration ($NO_3$-N + $NO_2$-N + $NH_4$-N) and wind speed reflect the fact that there is a gradation in the response of cyanobacterial biomass to these variables rather than an abrupt transition denoted by 'yes' or 'no'. The probability charts given in Figure 6 provide, like the decision support tree in Figure 5, an opportunity for testing and iterative refinement and development as information is accumulated.

The individual probabilities (with values from zero to 1) for bloom occurrence are denoted as follows:

| | |
|---|---|
| P(lake area) | = 0.0816 x Ln(A) + 0.41, where A is lake area in $km^2$, and Ln is the natural logarithm |
| P(total phosphorus) | = 0.17 x Ln(TP), where TP is total phosphorus concentration ($mg/m^3$) |
| P(IN) | = -0.0965 x Ln(IN) +0.88, where IN is the total dissolved inorganic nitrogen concentration ($mg/m^3$) |
| P(U) | = $0.0032 \times U^4 - 0.037 \times U^3 + 0.1084 \times U^2 - 0.1818 \times U + 1.00$, where U is wind speed (m/s) averaged over a period of six hours. |

The basis of these functions is that increasing lake area leads to an increased likelihood that blooms will be 'magnified' at the water surface. The function relating to total phosphorus concentrations is intended to reflect the fact that an increased supply of phosphorus will increase cyanobacterial biomass as this group is generally a poor competitor under conditions of strong phosphorus limitation. High levels of inorganically bound phosphorus are more likely to occur under other options given in the flow chart of Figure 5 (eg, shallow, turbid lakes). A decreasing probability of cyanobacterial blooms with increasing inorganic nitrogen is intended to reflect the predominance of nitrogen-fixing cyanobacteria (especially *Anabaena*) as inorganic nitrogen becomes strongly limiting. The general trend of increasing probability of blooms with increasing nutrient concentrations is already reflected in the P(TP) function. Finally, the probability of a cyanobacterial bloom increases when wind speed decreases. A duration of six hours was chosen for this function, but there will inevitably be some interaction of duration and lake size: large lakes will have greater inertia and therefore respond more slowly than small lakes to changes in wind speed. This is not reflected in the current model.

**Figure 5: Decision tree summarising the major environmental variables important in the development of cyanobacterial blooms and benthic mats, and the selection of specific genera**

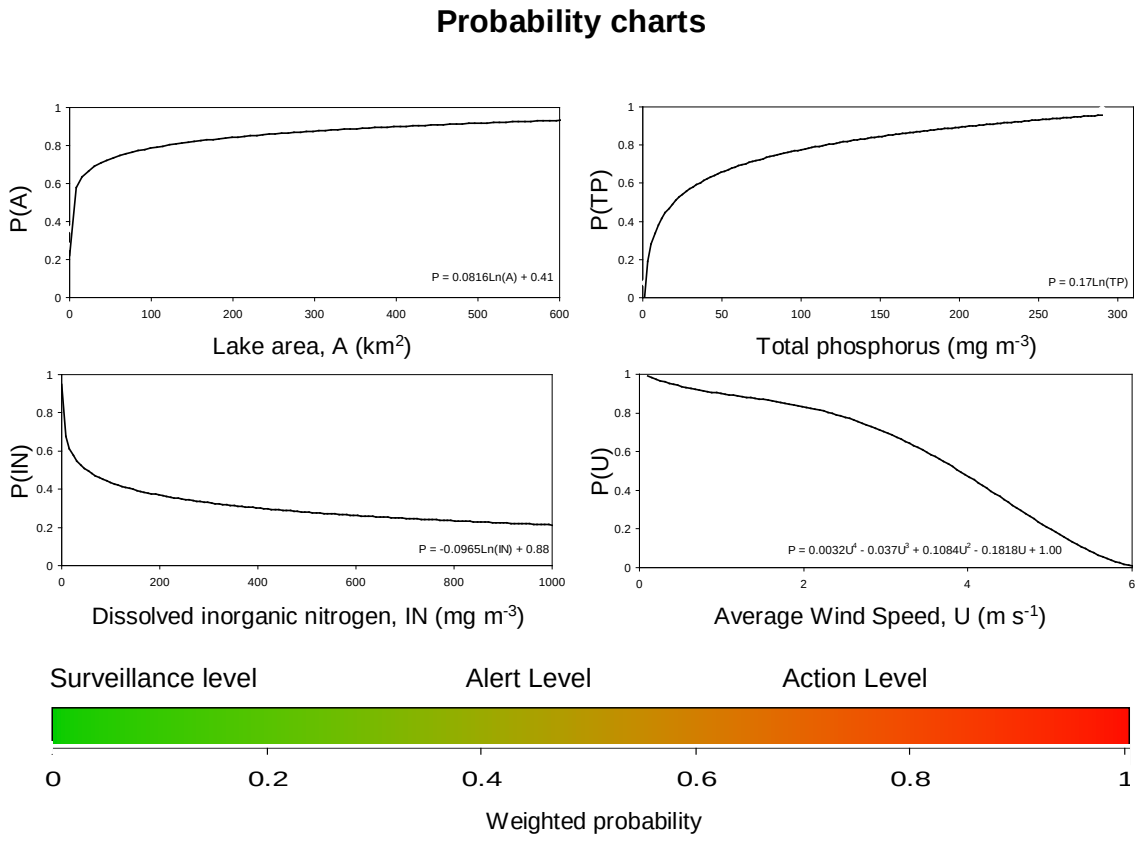


To determine an overall weighted probability, the individual functions are weighted as follows:

P(weighted) = 0.2 x P(lake area) + 0.4 x P(TP) + 0.15 x P(IN) +0.25 x P(U).

This weighted probability function P (weighted) can then be interpreted according to the surveillance level (green mode), alert level (amber mode) and action level (red mode), as shown in Figure 6. Excel spreadsheets of the different functions (P(A), P(TP), P(IN), P(U) and P(weighted)) can readily be created or made available if required, and feedback from applications will allow this model to be refined and adapted to a wide range of conditions.

**Figure 6: Probability charts demonstrating the individual probability contributions of lake area, total phosphorus, total dissolved inorganic nitrogen and 6-hour average wind speed to the weighted probability, assessed as P(weighted) = 0.2 x P(lake area) + 0.4 x P(TP) + 0.15 x P(IN) + 0.25 x P(U), and interpreted in terms of surveillance, alert and action levels with a colour bar**



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# Glossary

| | |
|---|---|
| Anatoxin | a group of neurotoxic alkaloids produced by a number of cyanobacterial genera. |
| Anoxic waters | an area of water that is depleted of dissolved oxygen. |
| Benthic | the lowest level of a body of water. |
| Cyanobacteria | (also known as blue-green algae), are a phylum of bacteria that obtain their energy through photosynthesis. |
| Cylindrospermopsin | a hepatotoxic alkaloid produced by a variety of cyanobacterial genera. |
| Cytotoxic | toxic to cells. |
| Dermatotoxic | affects the skin. |
| Eutrophication | degradation of water quality due to enrichment by nutrients such as nitrogen and phosphorus, resulting in excessive algal growth and decay, and often associated with low dissolved oxygen in the water. |
| Exposure | contact of a chemical, physical or biological agent with the outer boundary of an organism (eg, through inhalation, ingestion or dermal contact). |
| Hazard | a biological, chemical, physical or radiological agent that has the potential to cause harm. |
| Hepatotoxic | toxic to the liver. |
| Hydrophilic | literally 'water loving' − the capacity of a molecule to interact with polar solvents, in particular with water. |
| Neurotoxic | toxic to nerves or nerve tissue. |
| Macroscopic | large enough to be seen by the unaided eye. |
| Microcystin | a hepatotoxic cyanotoxin produced by a range of cyanobacteria. |
| Monomictic | mixing only once a year. |
| Nodularin | a hepatotoxic cyanotoxin produced by the planktonic cyanobacterium *Nodularia spumigena.* |
| Oligotrophic | a water body with low primary productivity, the result of low nutrient content. These water bodies often have low algal production, and consequently often have very clear water of high quality. |

| | |
|---|---|
| Periphyton | the mixture of algae, cyanobacteria, heterotrophic microbes, and detritus found attached to submerged surfaces in most aquatic ecosystems. |
| Planktonic cyanobacteria | cyanobacteria that are free-floating (drifting) in the water body. |
| Polymictic | lakes in which the water column undergoes frequent periods of stratification and remixing. |
| Pool | a deep, slow-moving region of a river, usually with fine substrate, often containing eddies. |
| Procaryote | an organism whose nucleus is not clearly defined (bacteria and cyanobacteria, but not animals, plants or fungi). |
| Riffle | shallow water where the surface is broken into ripples or waves by totally or partially submerged obstructions. |
| Run | swiftly flowing region of river (deeper than a riffle) with a relatively smooth surface. |
| Saxitoxin | a neurotoxin produced by cyanobacteria and some marine algae. Also know as paralytic shellfish poison. |
| Stratification | the formation of separate layers (of temperature, plant or animal life) in a water body. Each layer has similar characteristics (eg, all water in the layer has the same temperature). |
| Toxigenic | producing toxin. |

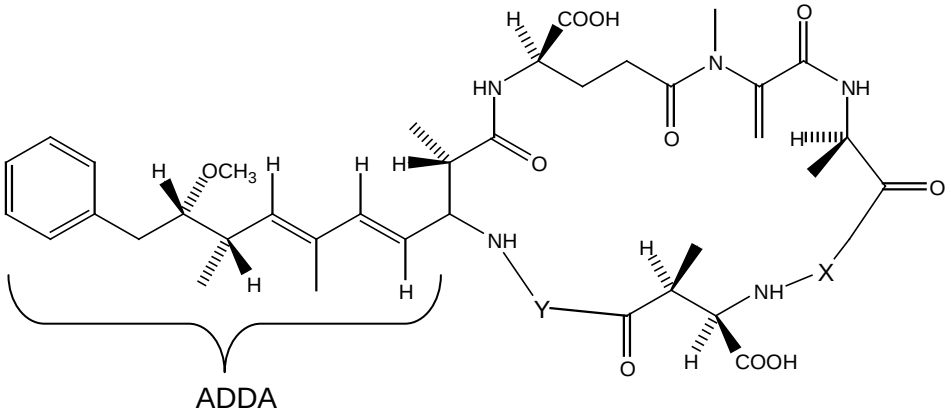
# Appendix 1: Cyanotoxins and their distribution in New Zealand

Cyanotoxins are a diverse assemblage of natural toxins that have a very broad range of toxicity mechanisms, ranging from hepatotoxicity (toxic to the liver) and neurotoxicity (toxic to nerves or nerve tissue), to dermatotoxicity (affects the skin). Some cyanotoxins have also been shown to promote liver tumour growth when ingested in low doses over extended periods (Falconer and Humpage, 1996; Kuiper-Goodman et al, 1999). Based on their chemical structure, cyanotoxins can be divided into the following three groups: cyclic peptides (microcystins and nodularins), alkaloids (cylindrospermopsins, anatoxins and saxitoxins) and lipopolysaccharides (LPS).

## Microcystins

Globally, microcystins are the most frequently found cyanotoxin (Chorus and Bartram, 1999). Microcystins are cyclic peptides, and more than 80 microcystin variants have been isolated and characterised (Spoof, 2004). Each variant differs with respect to the methyl groups and two amino acids within the ring (see Figure A1.1). This results in pronounced differences in toxicity among the variants. The amino acid ADDA (see Figure A1.1) is unique to microcystins and nodularins, and is required for their biological activity (Sivonen and Jones, 1999).

**Figure A1.1: General structure of microcystins (X and Y are the common variable amino acids)**




Microcystins are hepatotoxins that block protein phosphatase 1 and 2a in affected organisms (MacKintosh et al, 1990). This binding is inhibitory, highly specific and irreversible. The chief pathway into cells for microcystins is the bile acid carrier, which is found in liver cells and, to a lesser extent, in intestinal epithelia (Falconer, 1983; Runnegar et al, 1993). Most microcystins are highly toxic, with intra-peritoneal (ip) mouse toxicities ranging between 50 and 300 µg/kg body weight (mouse) (Sivonen and Jones, 1999). In vertebrates, a lethal dose of microcystin causes death by liver necrosis (premature death of cells) within hours to a few days. In addition, Fitzgeorge et al, (1994) published evidence for disruption of nasal tissues by the common hydrophilic variant microcystin-LR. Toxicity by oral uptake is generally at least an order of magnitude lower than toxicity by intra-peritoneal injection. However, intra-nasal application in these experiments was as toxic as intra-peritoneal injection, and membrane damage by microcystin enhanced the toxicity of anatoxin-a. This uptake route may be relevant for water sports such as waterskiing that lead to inhalation of spray and droplets.

Fitzgeorge et al, (1994) demonstrated that microcystin toxicity is cumulative. A single oral dose showed no increase in liver weight (a measure of liver damage), whereas the same dose applied daily over seven days caused an increase in liver weight of 84 per cent and thus had the same effect as a single oral dose 16 times as large. This may be explained by the irreversible covalent bond between microcystin and the protein phosphatases, which leads to subsequent damage to cell structure (Goldberg et al, 1995; Maynes et al, 2006). Sub-acute liver injury is likely to go unnoticed for two reasons:

- liver injury shows externally noticeable symptoms only when it is severe
- acute dose-response curves for microcystins are steep, so little acute liver damage may be observed up to levels close to severe acute toxicity.

The two potential mechanisms for chronic microcystin damage to the liver are progressive active liver injury (as described above) and the promotion of tumour growth. The tumour-promoting activity of microcystins is well documented in animals, although microcystins alone have not been shown to be carcinogenic. 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. The International Agency for Research on Cancer (IARC) has recently classified microcystin-LR as a group 2B carcinogen (Grosse et al, 2006). Group 2B compounds are considered possible carcinogens to humans.

Numerous incidents of animal and human poisonings have been attributed to microcystins (Ressom et al, 1994). One of the most severe cases occurred in 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 microcystins. This resulted in over 50 fatalities at the dialysis treatment clinic (Azevedo et al, 2002). In a further case from Brazil, the death of 88 people, mostly children, was associated with drinking water from a newly constructed reservoir, which contained a bloom of *Microcystis* spp. (Teixeira et al, 1993).

## Microcystins in New Zealand

Microcystins are the most commonly found cyanotoxin in planktonic cyanobacteria in New Zealand. They have been identified in over 60 water bodies (Christoffersen and Burns, 2000; Wilding, 2000; Hamill, 2001; Stirling and Quilliam, 2001; Wood, Briggs et al, 2006; Wood, Stirling et al, 2006). The highest concentrations recorded (36.5 mg/L) were found in cyanobacterial scum material from Lake Horowhenua (Wood, Stirling et al, 2006).

Of New Zealand planktonic species, *Microcystis* spp. are the only confirmed microcystin producers. The production of microcystins by *Anabaena* spp. and *Planktothrix* spp. is suspected due to the detection of microcystins in environmental samples dominated by these species and/or via the detection of genus-specific genes involved in microcystin production from environmental samples (Christoffersen and Burns, 2000; Wood, Stirling et al, 2006; Wood, unpublished data).

Analysis of environmental samples indicated that microcystins are also produced by benthic species (Hamill, 2001; Wood, Stirling et al, 2006). The production of microcystins by a benthic species (*Planktothrix* sp.) has recently been confirmed (Wood, Heath, McGregor et al, in press). In March 2003 the eastern shore of Lake Taupo was lined with thick gelatinous mats of *Nostoc commune* (Appendix 7) that contained high levels of microcystins (708 mg/kg). Gelatinous colonies accumulated along the shoreline following a storm event in which waves had dislodged the *Nostoc commune* from rocks. A water sample collected close to the shoreline at Lake Taupo also contained microcystins and demonstrated that some of the microcystins were being released from these mats back into the water body (Wood, Stirling et al, 2006).

# Nodularins

Nodularins have a very similar structure to microcystins and are produced by *Nodularia spumigena*, which is primarily a brackish-water species. Nodularin is also a potent inhibitor of protein phosphatases 1 and 2a (Honkanen et al, 1991; Maynes et al, 2006). Nodularin has an ip LD50 of 60 µg/kg body weight (mouse) (Carmichael et al, 1988).

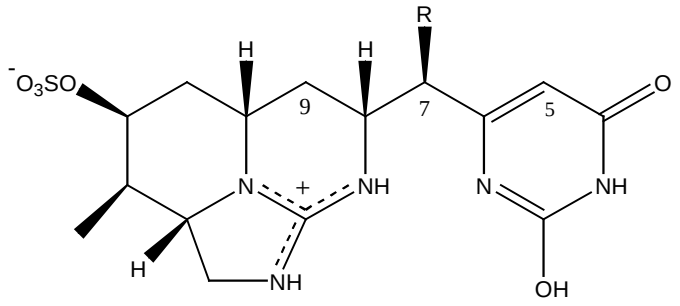
## Nodularins in New Zealand

Nodularin has been identified from blooms of *Nodularia spumigena* in Lake Ellesmere (Carmichael et al, 1988) and Lake Forsyth. There is long history of stock deaths around both of these lakes (Connor, 1977). *Nodularia spumigena* is known to occur in other brackish lakes around New Zealand (Etheredge and Pridmore, 1987), although the occurrence of nodularin has not been investigated.

# Cylindrospermopsins

Cylindrospermopsin causes extensive damage to the liver and kidney and is a potent inhibitor of protein synthesis (Terao et al, 1994; Falconer et al, 1999; Froscio et al, 2003). Clinical symptoms may appear several days after exposure, so it is often difficult to determine a cause–effect relationship. Falconer and Humpage (2001) suggest that cylindrospermopsin may also act directly as a tumour initiator, which has implications for both short- and long-term exposure. Crude extracts of *Cylindrospermopsis raciborskii* (a common cylindrospermopsin producer) injected or given orally to mice also induce pathological symptoms in the kidneys, spleen, thymus and heart (Seawright et al, 2000). Two variants of cylindrospermopsin exist (see Figure A1.2): 7-epicylindrospermopsin, a toxic minor metabolite of the cyanobacterium *Aphanizomenon ovalisporum* (Banker et al, 2000), and deoxy-cylindrospermopsin (Norris et al, 1999), which is now thought to be as toxic as cylindrospermopsin. Pure CYN and 7-epi-CYN exhibits an LD50 of 2.0 mg/kg body weight (mouse) after 24 hours, but 0.2 mg/kg body weight after five days (Ohtani et al, 1992).

**Figure A1.2: General structure of cylindrospermopsin**




| | R | |
|---|---|---|
| cylindrospermopsin(CYN) | OH | |
| 7-epicylindrospermopsin | OH | epimer at C7 |
| deoxycylindrospermopsin (do-CYN) | H | |

*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 (Byth 1980; Bourke et al, 1983). The copper sulphate caused the cells to break apart and resulted in the release of cyanotoxins into the water supply (Hawkins et al, 1985). Recent cattle deaths in Queensland (Australia) have been attributed to cylindrospermopsin (Saker et al, 1999).

## Cylindrospermopsins in New Zealand

Cylindrospermopsin was first identified in New Zealand in Lake Waitawa (Otaki) in 1999, although the species responsible for its production was not confirmed (Stirling and Quilliam, 2001). *Cylindrospermopsis raciborskii* was identified for the first time in a bloom in Lake Waahi (in March 2003), during which liquid chromatography-mass spectrometry (LC-MS) confirmed the presence of cylindrospermopsin and deoxy-cylindrospermopsin (Wood and Stirling, 2003). These two incidents remain the only detections of cylindrospermopsin in New Zealand. Multiple samples with high concentrations of *C. raciborskii* have been analysed, but with no cylindrospermopsin detected (Wood, unpublished data), indicating that not all strains of *C. raciborskii* in New Zealand produce this toxin.

# Anatoxin-a and homoanatoxin-a

Anatoxin-a and homoanatoxin-a are neurotoxic poisons. They are powerful depolarising neuromuscular blocking agents acting through the nicotinic acetylcholine receptor (Carmichael et al, 1979). 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 is often linked with animal and wildfowl poisonings (Ressom et al, 1994), but there have been no reported human fatalities from anatoxin-a. Anatoxin-a and homoanatoxin-a have ip LD50 of 200−250 μg/kg body weight (mouse) (Devlin et al, 1977; Skulberg et al, 1992).

## Anatoxin-a and homoanatoxin-a in New Zealand

Following the rapid deaths of dogs near the Waikanae River (Lower North Island) in 1998, the toxicity of a benthic mat of *Oscillatoria* sp. mat was investigated using a mouse bioassay and high-performance liquid chromatography with fluorescence detection (HPLC-FLD). The presence of natural degradation products of anatoxin-a was confirmed (Hamill, 2001). Further sudden deaths of dogs were reported at the Mataura River (Lower South Island) in 1999 and 2000. Benthic *Oscillatoria*-like sp. mats were collected and their toxicity confirmed (Hamill, 2001). Wood, Stirling et al (2006) identified anatoxin-a in three planktonic samples collected from Lake Rotoehu (Rotorua), Lake Henley (Masterton) and Lower Karori Reservoir (Wellington); all three samples were dominated by *Anabaena* spp.

*Aphanizomenon issatschenkoi* was identified for the first time in New Zealand in 2003, and LC-MS analysis of a strain isolated from Lake Hakanoa (Waikato) confirmed it was producing anatoxin-a (Wood, Rasmussen et al, 2007). Interestingly, despite the absence of cylindrospermopsin production, genes implicated in the biosynthesis of cylindrospermopsin were successfully amplified from the *Aph. issatschenkoi* strain. *Aph. issatschenkoi* is now a common bloom-forming species in the Waikato region, and blooms have been reported elsewhere in the North Island (Wood, unpublished data).

In November 2005 at least five dogs died rapidly after contact with water from the Hutt River (Wellington). Extensive mats of benthic material were present in the river at the time of the poisonings. Subsequent LC-MS analysis identified anatoxin-a, homoanatoxin-a and their

degradation products, dihydro-anatoxin-a and dihydro-homoanatoxin-a (Wood, Selwood et al, 2007). The causative species was identified as *Phormidium autumnale* (Wood, Selwood et al, 2007). Since this incident, mats of *P. autumnale* have commonly been linked to dog poisoning events in other parts of New Zealand (eg, Canterbury, Bay of Plenty) and anatoxin-a and homoanatoxin-a have been detected on multiple occasions (Wood, unpublished data). Examination of stomach contents from dead dogs has revealed copious amounts of 'algal' material, suggesting the dogs had ingested cyanobacterial material rather than being exposed directly to toxins that are free in the water column (Wood, Selwood et al, 2007). It is unknown whether dogs are more susceptible to anatoxin poisoning than other organisms.

# Anatoxin-a(S)

Anatoxin-a(S) is structurally different and up to 10 times more potent (towards mice) than anatoxin-a. It is a cholinesterase inhibitor that induces hypersalivation, diarrhoea, shaking and nasal mucus discharge in mammals (Carmichael, 1992; Mahmood and Carmichael, 1987). It is thought to be produced only by *Anabaena lemmermannii* (Henriksen et al, 1997) and *A. flos-aquae* (Mahmood and Carmichael, 1987). Anatoxin-a(S) has an ip LD50 of 20 µg/kg body weight (mouse) (Carmichael, 1992). Anatoxin-a(S) has not been detected in New Zealand.

# Saxitoxins

Saxitoxins are fast-acting neurotoxins that inhibit nerve conduction by blocking sodium channels (Adelman et al, 1982). Saxitoxins are also produced by various marine dinoflagellates under the name of paralytic shellfish poisons (PSPs), and the human health effects caused by saxitoxins are well described from numerous reports of human toxicity associated with the consumption of shellfish containing relatively high concentrations of PSPs. No PSP-like illnesses have been reported in humans from the consumption of drinking water or contact with recreational water containing saxitoxins (Chorus and Bartram, 1999). More than 30 saxitoxin variants have been isolated and characterised. Saxitoxin has an ip LD50 of 10 µg/kg body weight (mouse). Other analogues are mostly less toxic than saxitoxin.

Saxitoxins have caused sheep mortalities in Australia (Negri et al, 1995) and were identified in an extensive bloom of *A. circinalis* in 1990 on the Murray Darling River (Australia), which resulted in the death of over 1600 stock (Bowling and Baker, 1996).

## Saxitoxins in New Zealand

A cyanobacterial bloom (predominantly *Anabaena planktonica*) in the Waikato River in 2003 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 (via ELISA and neuroblastoma assay) in water samples taken from the water treatment intake and throughout the water treatment process (Kouzminov et al, 2007), but levels were well below the provisional maximum acceptable values set out in the *Drinking-water Standards for New Zealand 2005* (Ministry of Health, 2005b). The saxitoxin-producing organism/s were not identified.

Using an ELISA and neuroblastoma assay, Wood, Stirling et al (2006) detected low levels of saxitoxins in 38 different water bodies. Although only low levels of saxitoxins were detected, the results imply that saxitoxins may be more prevalent in New Zealand water bodies than previously assumed. Further investigation and chemical analysis are required to confirm which species are responsible for saxitoxin production and to determine the variants produced.

# Lipopolysaccharides

Lipopolysaccharides (LPS) are an integral component of the cell wall of all gram-negative bacteria, including cyanobacteria. Found in the outer cell membrane, LPS form complexes with proteins and phospholipids (Chorus and Bartram, 1999). Lipopolysaccharides can elicit irritant and allergenic responses in humans and animals tissue (Torokne et al, 2001; Pilotto et al, 1997). Cyanobacterial LPS are considerably less potent than LPS from pathogenic gram-negative bacteria such as *Salmonella* (Keleti and Sykora, 1982). Although comparatively poorly studied, LPS from cyanobacteria have been implicated in human health problems associated with exposure to cyanobacteria (Ressom et al, 1994). Recent studies (eg, Pilotto et al, 2004) showed no correlation between dermatological reactions experienced by exposed individuals and the presence/absence of other cyanotoxins in the samples tested.

## Lipopolysaccharides in New Zealand

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, personal communication; D Hood, Waikato District Health, Hamilton, personal communication).

# β-N-methylamino-l-alanine (BMAA)

β-N-methylamino-l-alanine (BMAA) is a non-protein amino acid produced by cyanobacteria. BMAA is considered a possible cause of the amyotrophic lateral sclerosis/Parkinsonism–dementia complex that has an extremely high rate of incidence among the Chamorro people of Guam. It has been suggested that BMAA biomagnifies through the food web. In the Chamorro case, a root symbiont of the genus *Nostoc* is found on cycad trees. The Chamorro eat fruit bats, which feed on cycad seeds (all of which contain BMAA; Cox and Sacks, 2002). There is current debate on the occurrence of BMAA in other cyanobacterial genera (eg, Cox et al, 2005; Rumsby et al, 2008). Current international research should provide further information on sources of BMAA and guidance on acceptable levels.

# Cyanotoxin and toxicity testing

For health assessments in recreational use water bodies it is recommended that total (ie, combined intracellular and intracellular) toxin content and/or toxicity concentrations in samples are measured.

A range of methods has been developed to detect and identify cyanotoxins and their toxicity (see Lawton et al, 1999 for an in-depth review). In New Zealand, five methods are currently commercially available for cyanotoxin or toxicity analysis (Appendix 8).

## Enzyme-linked immuno sorbent assay (ELISA)

An ELISA is available for detecting total ADDA containing microcystins and nodularins (Fischer et al, 2001). It uses antibodies raised against ADDA (an amino acid unique to microcystins/nodularin; see Figure A1.1) and should detect over 80 per cent of all known microcystin variants and nodularin. 'Free' ADDA may also be detected in some instances, potentially overestimating total microcystin load in a sample. This method cannot distinguish

between microcystins and nodularin. However, nodularin is only produced by *Nodularia spumigena*, a brackish water species, and so this is unlikely to be problematic. The ELISA offers a rapid and cost-effective method of determining the total microcystin/nodularin content of a sample. A 'dip-stick' method based on the same technology is available commercially (www.abraxiskits.com). This enables users to rapidly screen samples for microcystins or nodularin and requires no additional equipment. An ELISA kit is also available through ABRAXIS for cylindrospermopsin (www.abraxiskits.com).

## Liquid chromatography mass spectrometry (LC-MS)

Liquid chromatography mass spectrometry methods are available for microcystins, nodularin, anatoxin-a, homoanatoxin-a and cylindrospermopsins in New Zealand. This method detects the specific mass of individual toxins in a sample and thus provides information on which variants are present. This is particularly relevant for microcystins, where over 80 variants exist, each varying in its toxicity. Routine LC-MS screens may miss unusual microcystin variants and therefore underestimate the total microcystin load. Recent research in New Zealand has demonstrated a high correlation between the ADDA-ELISA and LC-MS for microcystin detection (Mountfort et al, 2004; Wood, Mountfort et al, 2008).

## RAPID TEST™

This is an ELISA-based technique that detects all saxitoxin variants to varying degrees (www.jellet.ca). The test determines the presence or absence of saxitoxins. It is not truly quantitative, nor does it provide information on which variants are present.

## Acetylcholinesterase assay

The biochemical activity of anatoxin-a(S) can be exploited in an enzyme-based assay to detect the inhibition of acetylcholinesterase (AChE), thereby providing an indication of the presence of this toxin. Although this assay is available, it has not yet been validated for the detection of anatoxin-a(S) in New Zealand.

## Toxicity tests

In the strict sense, toxicity refers only to animal-testing data and is expressed as the amount of cyanobacteria lethal to an animal (usually normalised per kilogram of body weight). Although not routinely used in New Zealand, mouse bioassays are available. A mouse bioassay may be used when animal or human poisonings indicate the presence of toxic substances but results for known toxins are negative. However, a positive mouse test does not definitively demonstrate that the cyanobacteria or toxin being tested is also acutely toxic to humans, although a positive result does provide strong evidence that an active toxin is present.

# Appendix 2: Derivation of guideline values

## Planktonic cyanobacteria

Based on animal toxicological studies, guidelines for exposure to microcystins via ingestion have been developed for the action level (red mode) – situation 1. The guideline values are extrapolated from animal experiments and make various assumptions about exposure, and also include uncertainty factors. Uncertainty factors are used to account for safety margins, errors in extrapolation from animal experiments to human risk, and other limitations associated with experiments or limited data.

In this document tolerable daily intakes (TDIs) for microcystins are calculated based on data from two separate animal toxicological studies: a 13-week mouse study (Fawell et al, 1999) conducted with purified microcystin-LR via gavage, and a 44-day pig study (Falconer et al, 1994; see Box A2.1) carried out with cyanobacterial bloom material in the drinking water, containing nine microcystin congeners but no microcystin-LR. A TDI is defined as an estimate of the intake of a substance over a lifetime that is considered acceptable without appreciable health risk. The TDIs were used to calculate maximum allowable values (MAVs) of microcystins in recreational water under the following assumptions: the proportion of microcystin intake from recreational water is 100 per cent (ie, there are no other microcystin sources or exposures), and there is an average consumption of 100 mL of water per day (see Box A2.1).

Usually only a small proportion (less than 20 per cent) of the total microcystin load in a water body is extracellular (outside the cells) when cells are healthy (Orr and Jones 1998; Park et al, 1998). Therefore cells can only provide an approximate measure of microcystin concentrations present in a sample. The microcystin MAVs can be translated to an equivalent worst-case cell density of *Microcystis* sp. based on toxin quota data (Wood, Rhodes et al, 2008; Wood unpublished data; Box A2.1). This cell density is then converted into an equivalent biovolume of total cyanobacterial material (see Box A2.1) to gauge the potential hazard of other cyanobacteria, irrespective of whether toxin status is known.

The rationale for using cell counts, which are converted to biovolume estimates, rather than toxin concentrations to prompt management actions is that for most practical purposes cell counting is still primarily used by water managers to detect algae/cyanobacterial-related water-quality problems. This is because the testing is widely available and provides relatively rapid and cost-effective information. Cyanotoxin testing is offered commercially by a few organisations within New Zealand (see Appendix 8), although cost may be prohibitive for large sample numbers. Biovolumes must, however, be regarded as an indicator or 'surrogate' for a potential toxin hazard. These should be used to prompt actions, such as toxin monitoring, that are outlined in the alert levels framework (Section 3).

**Box A2.1: Derivation of a guideline for microcystin and cyanobacterial exposure during recreational activities over a lifetime**

Tolerable daily intakes for recreational exposure to microcystins were calculated (Table A2.1) using data from a 13-week mouse study (Fawell et al, 1999) and a 44-day pig study (Falconer et al, 1994) and the following equation:

$$\text{TDI} = \frac{\text{NOAEL or LOAEL}}{\text{uncertainty factors}} \quad (1)$$

**Table A2.1: Summary of data and uncertainty factors used to calculate TDIs for microcystins**

| **Study** | Falconer et al, 1994 | Fawell et al,1999 |
|---|---|---|
| **Test animal** | Pigs | Mouse |
| **Duration** | 44 days | 13 weeks |
| **Material/toxin** | Cyanobacterial bloom material, containing nine microcystin congeners but no microcystin-LR, via drinking water | Purified microcystin-LR via gavage |
| **LOAEL**[a] | 88[c] | |
| **NOAEL**[b] | | 40 |
| ***Uncertainty factors*** | | |
| **Intraspecies variability** | 10 | 10 |
| **Interspecies variability** | 10 | 10 |
| **LOAEL to NOAEL** | 2 | _ |
| **Lifetime exposure** | 5 | 5 |
| ***Sum of uncertainty factors*** | 1000[d] | 500[d] |
| **TDI (µg/kg bw/day)** | 0.088 | 0.08 |

[a] LOAEL = lowest observed adverse effect level – the lowest dose at which adverse health effects are observed.

[b] NOAEL = no observed adverse effect level – the highest dose at which no adverse health effects are observed.

[c] As measured by PP2A assay (worst case scenario).

[d] These safety factors do not incorporate an additional safety factor for tumour promotion. The risk scenario of a swimmer, kayaker, sailor, etc, whether adult or child, being repeatedly but discontinuously exposed during a short visit, should only incorporate liver damage as the endpoint. Incorporation of tumour promotion, and thus an additional safety factor, would suggest continuous exposure (eg, via drinking water or food).

The TDIs are used to calculate maximum allowable values (MAVs) for microcystins during recreational exposure.

**MAVs based on TDIs, derived from Falconer et al, 1994 (Table A2.1)**

**Child** = 0.088 µg/kg/day x 15 kg ×10 = 13.2 µg/L total microcystins (2)

**Adult** = 0.088 µg/kg/day ×70 kg ×10 = 61.6 µg/L total microcystins (3)

where:

- 0.088 μg/kg body weight per day is the TDI (Table A2.1)
- 15 is the average weight of a child in kg (equation 2) and 70 is the average weight of an adult in kg (equation 3)
- 10 is the conversion from the amount of water accidentally swallowed per day (approximately 100 mL) to litres.

**MAVs based on TDIs derived from Fawell et al, 1999 (Table A2.1)**

**Child =** 0.08 μg/kg/day x 15 kg ×10 = 12 μg/L total microcystins (4)

**Adult =** 0.08 μg/kg/day ×70 kg ×10 = 56 μg/L total microcystins (5)

where:

- 0.08 μg/kg body weight per day is the TDI (Table A2.1)
- 15 is the average weight of a child in kg (equation 4) and 70 is the average weight of an adult in kg (equation 5)
- 10 is the conversion from the amount of water accidentally swallowed per day (approximately 100 mL) to litres.

The child exposure guideline derived for microcystins (measured as total microcystins and expressed as microcystin-LR toxicity equivalents),[1] from the Fawell et al, (1999) study, provided the lowest MAV (12 μg/L) and is used as the action level (red mode) – situation 1 guideline.

To derive a cell number that is equivalent to this toxin hazard, a microcystin cell quota of $6.3 \times 10^{-7}$ μg total microcystins/cell is assumed. This data was obtained from five *Microcystis* sp. isolates from Lake Horowhenua. *Microcystis* sp. material analysed from this lake produces the highest values recorded in New Zealand (Wood, Stirling et al, 2006). Toxin quotas can vary under culture conditions. Wood and Dietrich (unpublished data) recently measured similar microcystin toxin quotas in environmental samples collected from Lake Rotoura (Kaikoura, South Island). Maximum microcystin cell quotas of $0.9 \times 10^{-7}$ μg total microcystins/cell were recorded in their study.

**Table A2.2: Toxin quotas of *Microcystis* sp. isolated from Lake Horowhenua**

| Strain no. | Genus | Microcystins/cell (pg/cell) |
|---|---|---|
| CYN08 | *Microcystis* sp. | 0.27 |
| CYN09 | *Microcystis* sp. | 0.21 |
| CYN10 | *Microcystis* sp. | 0.81 |
| CYN11 | *Microcystis* sp. | 0.57 |
| CYN12 | *Microcystis* sp. | 1.27 |
| **Average toxin quota** | | **0.63** |

Source: Wood, Rhodes et al, 2008; Wood, unpublished data

[1] The guidelines developed in this document are based on microcystin-LR only. There is insufficient data to derive TDI values for the other microcystins and their toxicity should be expressed in microcystin-LR equivalents.

Therefore, the equivalent concentrations of toxic cells of *Microcystis* sp. that are tolerable for a small child and an adult during recreational activities are:

$$\textbf{Child} = \frac{12\ \mu g/L \times 10^{-3}\ L/mL}{6.3 \times 10^{-7}\ \mu g\ /cell} = 19{,}000\ cells/mL \quad (6)$$

$$\textbf{Adult} = \frac{56\ \mu g/L \times 10^{-3}\ L/mL}{6.3 \times 10^{-7}\ \mu g\ /cell} = 90{,}000 cells/mL \quad (7)$$

where:

- 12 µg/L is the MAV guideline (equation 4) for cyanobacterial exposure in children (equation 6), and 56 µg/L is the MAV guideline (equation 5) for cyanobacterial exposure in adults (equation 7)
- $10^{-3}$ is the conversion from litres to millilitres
- $6.3 \times 10^{-7}$ is the toxin cell quota for total microcystins/cell.

The approximate biovolume equivalent to 19,000 cells/mL of *Microcystis* sp. is 1.8 $mm^3$/L (see Appendix 4).

Of the New Zealand planktonic species, *Microcystis* spp. are the only confirmed microcystin producers. The production of microcystins by *Anabaena* spp. and *Planktothrix* spp. is suspected due to the detection of microcystins in environmental samples dominated by these species and/or via the detection of genus-specific genes involved in microcystin production in environmental samples (Wood, Stirling et al, 2006; Wood, unpublished data).

It is recommended that the biovolume of greater than 1.8 mm3/L be applied as an 'equivalent' guideline for populations of known potentially toxic cyanobacteria (see Tables 1 and 2) other than Microcystis sp. The rationale is that the hazard from toxicity is unlikely to exceed the worst case for an equivalent biovolume of highly toxic Microcystis spp. containing microcystins. This should allow protection from significant risk while further health risk assessments are made.

It is generally accepted that there is insufficient toxicological data available to calculate quantitative guidelines for cyanotoxins other than microcystins (eg, anatoxins and saxitoxins). In New Zealand there is a lack of information on toxin quotas from other toxin-producing species. There is substantial data on the anatoxin-a producer *Aphanizomenon issatschenkoi* (Wood, Rasmussen et al, 2007; Selwood et al, 2007). This data was used to calculate the concentration of anatoxin-a that would be present if the 1.8 mm3/L biovolume threshold was applied to a bloom of *Aph. issatschenkoi*. Although there is little data available on the toxicity of anatoxin-a, the value of 8.9 µg/L is only slightly higher than the PMAV of 6 µg/L developed for the Drinking-water Standards for New Zealand 2005 (Ministry of Health, 2005b) seems acceptable for a recreational-use water body if the assumptions used in the calculation of the microcystin TDI are adopted.

**Box A2.2: Calculation of anatoxin-a for a sample containing 1.8 $mm^3$/L of *Aphanizomenon issatschenkoi***

Biovolume of *Aphanizomenon issatschenkoi* (see Appendix 4):

= 89 $\mu m^3$

Concentration of cells to obtain a biovolume of 1.8 $mm^3$/L:

= (1.8 $mm^3$/L x $10^{-3}$ L/mL)/(89 $\mu m^3$/cell x $10^{-9}$ $mm^3/\mu m^3$)

= 20,200 cells/mL

Amount of toxin for this cell concentration:

= 22,200 cell/mL x 0.4 pg/mL

**= 8.9 ug/L**

A second guideline (ie, action level (red mode – situation 2) is required for circumstances where high cell densities, or scums, of 'non-toxic' cyanobacteria are present; that is, where the cyanobacterial population has been tested and shown not to contain known toxins (anatoxins, cylindrospermopsins, microcystins, nodularins or saxitoxins). Where the microcystin-related biovolume guideline is exceeded and no known cyanotoxins are present, it is appropriate to issue warnings if either the total biovolume of all cyanobacterial material exceeds 10 $mm^3$/L or scums are consistently present (ie, scums are seen at some time each day at the recreational site).

This guideline recommendation is based on the work of Stewart et al, (2006), where it was shown that there was an increase in the likelihood of symptom reporting in bathers above a cyanobacterial cell surface area equivalent to this approximate biovolume. The potential symptoms reported above this cell surface area are primarily mild respiratory complaints. The biovolume represents a conversion from the surface area units given by Stewart et al, (2006), where total surface area of 12 $mm^2$/mL is given as being equivalent to a total biovolume of approximately 12.5 $mm^3$/L. This value is rounded here to a more conservative value of 10 $mm^3$/L (two significant figures) to account for the uncertainties associated with sampling cyanobacterial populations in typical water bodies and with estimating cell densities from cell counting, which are subsequently used to derive biovolumes.

The action level (red mode) – situation 3 guideline accounts for protection from health hazards associated with the occurrence of cyanobacteria at high levels in general, demonstrated in particular by the consistent presence of scums (ie, where scums occur daily at a number of sites in a water body). This is consistent with the WHO Level 3 guideline for the occurrence of scums (WHO, 2003).

## Benthic cyanobacteria

The benthic alert-level framework is based on three tiers of alert levels, with cyanobacterial abundance and attachment the triggers for changes in threshold levels. Methods for conducting a site survey are given in Section 4.2.3. The percentage coverage thresholds are based on preliminary observations. For example, the surveillance level (green mode), with less than 20 per cent coverage, is common in many rivers in New Zealand and does not necessarily indicate that a proliferation event is likely. If these mats do detach they are likely to be quickly

washed away. When abundance is over 50 per cent (action level – red mode), mats commonly detach from the substrate and are more likely to accumulate along shorelines or catch in vegetation. Once mats become easily accessible the health risks are higher.

The framework is based on preliminary research and observations, and it is anticipated that these will require further refining as knowledge and monitoring tools improve. Note that the alert-level framework is designed to manage risks to *recreational users*. The levels given in the framework are not relevant for addressing risks to dogs that actively seek out and consume cyanobacterial mats. Raising public awareness (eg, through information/warning signs, see Appendix 9), media releases (see Appendix 10) and information pamphlets and signs (see Appendices 9 and 11), are recommended to reduce human exposure and dog poisonings.

Toxin testing should be used to help further define the health risk at sampling sites. However, quantitatively measuring toxin levels (even if samples are collected quantitatively) in benthic cyanobacteria can be problematic. This is due to the requirement for short sample turnaround times: in most commercially analysed samples, toxin concentrations are reported in micrograms of toxin per kilogram of wet weight, and it is difficult to standardise the volume of liquid and inorganic material within a mat. There is also a lack of toxicological data available, making it difficult to determine appropriate threshold values for anatoxins. For these reasons, no toxin concentrations have been recommended within the framework. It is anticipated that future research will enable the inclusion of toxin thresholds.

# Appendix 3: Photographs of planktonic blooms

This appendix compares the blooms/scums of different cyanobacterial species.


(A) Toxic bloom of *Cylindrospermopsis raciborskii* (Lake Waahi, Waikato) of approximately 200,000 cells/mL. Cells were not visible macroscopically. The water had a slight brown tinge, but was not markedly different from the usual colour of this peat lake. Photo: S Wood, Cawthron.

(B) Toxic bloom of *Microcystis panniformis* (Lake Rotoehu, Rotorua). Colonies were visible in the water and scum had formed along the shoreline. Photo: S Wood, Cawthron.

(C) A non-toxic bloom of *Anabaena circinalis* and *A. lemmermannii* (Lower Karori Reservoir, Wellington). A thick surface scum covered large areas of the reservoir and filaments were clearly visible in the water. The light blue/white streak is decaying cells that are lysing and releasing their pigments. This is a common occurrence during bloom decline. Photo: S Wood, Cawthron.

(D) Toxic bloom of *Microcystis* spp. (Lake Horowhenua, Levin). A thick scum had accumulated along the downwind shoreline. Photo: S Wood, Cawthron.




(E) A non-toxic bloom of *Anabaena planktonica* (Lower Karori Reservoir, Wellington). This species rarely forms scums. Photo: S Wood, Cawthron.

(F) Shoreline scum of toxic *Microcystis* spp. (Lake Wiritoa, Levin). Photo: S Wood, Cawthron.

(G) Shoreline scum of toxic *Microcystis panniformis* (Lake Rotoehu, Rotorua). Photo: S Wood, Cawthron.



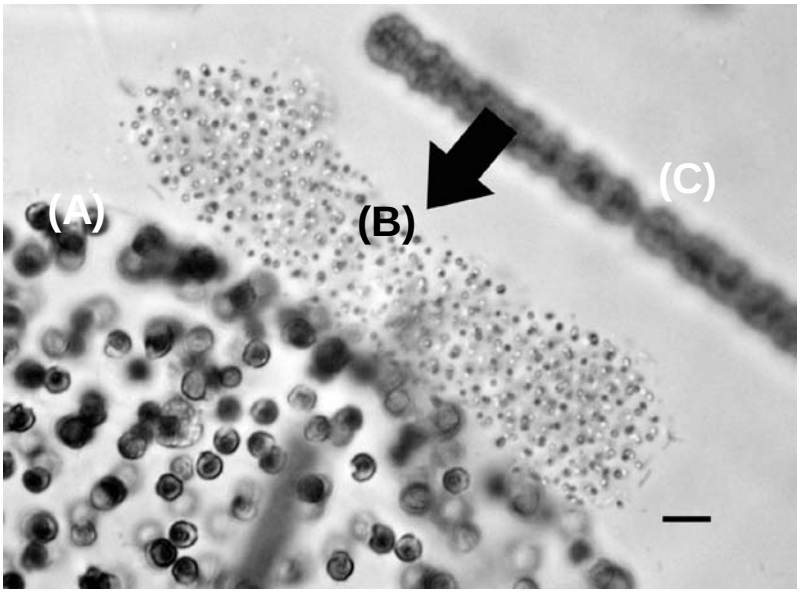
(H) Bloom of *A. lemmermannii* (Lake Waihola, Dunedin). Photo: J Milne, Greater Wellington Regional Council.

(I) Bloom of *A. lemmermannii* (Lake Waihola, Dunedin). Photo: J Milne, Greater Wellington Regional Council.

# Appendix 4: Biovolumes explained

Health warnings have traditionally been issued based on cell concentration thresholds; for example, over 15,000 cells/mL. However, cell concentrations do not account for the variability in size of cyanobacteria (see Figure A4.1). This is particularly relevant when there are high concentrations of cyanobacteria that are very small. Also, toxin concentration per cell is more closely related to cyanobacteria biovolume than to total cell number. Thus, simply relying on cell concentrations as an indicator of health risk may give biased results on the cyanobacterial taxa that are abundant in the water body. In the last three years there has been an increase in reports of pico-cyanobacteria (< 2 µm; eg, *Aphanothece* sp. and *Aphanocapsa* sp.) in some regions of New Zealand (eg, the Rotorua lakes), and basing health warning solely on cell counts has in some instances resulted in the unnecessary issuing of health warnings.

**Figure A4.1: Light photomicrographs demonstrating the difference in cell size among A. *Microcystis* sp.; B. *Aphanocapsa holsatica*; and C. *Anabaena planktonica* (arrow points towards *Ap holsatica*)**




Note: Scale bar = 10 µm.

It is time consuming and impractical to measure and calculate a biovolume for every individual in routine counting, so it is recommended that standardised species lists with fixed biovolumes be used. Where possible these should be specific to the water bodies being monitored. In conjunction with the development of these guidelines, biovolumes for 22 of the most problematic species in New Zealand lakes have been established (Table A4.1).

However, there are several caveats that need to be considered when using biovolumes.

1. In taxa that contain specialised cells such as akinetes and heterocytes, volume measurements are of vegetative cells only. Specialised cells usually make up a very small proportion of all cells, and this is unlikely to have a significant effect on overall biovolume.

2. Hawkins et al, (2005), showed that preservation of samples with Lugol's iodine (a preservative commonly used in New Zealand) causes shrinkage rates of up to 40 per cent, depending on the Lugol's concentration, the species and the length of time in Lugol's iodine. Using a low concentration of Lugol's iodine and analysing samples within

24−48 hours of collection will minimise shrinkage. The cell biovolumes produced for this document were obtained on Lugol's-preserved samples that had been stored for several months.

The biovolume (BV) in $mm^3/L$ of each species in a sample can be calculated using the following formula:

$$BV = (n \times vol) / 1 \times 10^6$$

where:

n = number cells in a sample (cells/mL)

vol = volume of each cell ($\mu m^3$)

$1 \times 10^6$ is a units conversion from $\mu m^3/mL$ to $mm^3/L$.

The total biovolume (TBV) of each sample is calculated by combining the individual totals for each species. For example, the total biovolume in a sample containing 10,200 cells/mL of *Anabaena planktonica* and 5600 cells/mL of *Microcystis wesenbergii* is calculated as follows:

| | |
|---|---|
| BV (*A. planktonica*) | = (10,200 x 399*) / $1 \times 10^6$ |
| | = 4.07 $mm^3/L$ |
| BV (*M. wesenbergii*) | = 5600 x 182* / $1 \times 10^6$ |
| | = 1.02 $mm^3/L$ |
| Total BV | = 4.07 + 1.02 |
| | **= 5.09 $mm^3/L$** |

* Using values from Table A4.1.

**Table A4.1: Volumes of common cyanobacteria in New Zealand**

| | Average volume (μm$^3$) | Length (μm) | Width (μm) | Diameter (μm) | Source | Shape | Count (n) |
|---|---|---|---|---|---|---|---|
| *Anabaena circinalis* | 208 | 5.9 (4, 8.2) | 8.2 (6.1,10.9) | | Kainui, Maraetai | OVO | 39 |
| *Anabaena lemmermannii* | 116 | 5.5 (3.1, 8.5) | 6.3 (4,8.5) | | Karapiro, Okareka, Rotoehu, Rotoiti, Rotorua, Tarawera, | OVO | 50 |
| *Anabaena planktonica* | 399 | 6.8 (3.9, 10.2) | 10.5 (7.3,13.3) | | Kaituna River, Karapiro, Ngaroto, Okaro, Rotoiti, Rotorua, Tarawera | OVO | 75 |
| *Aphanocapsa holsatica* | 1.7 | | | 1.4 (0.8,2.3) | Kaituna River, Okareka, Okaro, Rotoiti, Waahi | OVOR | 48 |
| *Aphanizomenon gracile* | 32 | 4.4 (2, 11.3) | 2.8 (1,5) | | Rotoiti, Tarawera, Waikare | CYL | 50 |
| *Aphanizomenon issatschenkoi* | 89 | 10.7 (6.5, 264.1) | 3.2 (1.6, 4.7) | | Kainui | CYL | 30 |
| *Aphanothece clathrata* | 2.1 | 2.3 (1.8, 3.2) | 1 (0.7,1.4) | | Okareka, Okaro, Tarawera | CYL | 30 |
| *Chroococcus* cf. *minutus* | 35 | 2.7 (2.1, 3.4) | 4.9 (3.6, 6.1) | | Waahi | OVO | 30 |
| *Coelosphaerium kuetzingianum* | 8.9 | 2.0 (1.2, 2.9) | 2.7 (2, 4) | | Kaituna River, Rotoiti | OVO | 32 |
| *Cylindrospermnopsis raciborskii* | 15 | 6.5 (3.6, 9.9) | 1.7 (1, 3.9) | | Whangapehe | CYL | 30 |
| *Leptolyngbya* cf. *subtilis* | 8.6 | 3.2 (2, 5) | 1.8 (1.2, 2.6) | | Kainui | CYL | 30 |
| *Merismopedia punctata* | 6.4 | 2.8 (2, 3.8) | 2 (1.5, 2.8) | | Forsyth | OVO | 30 |
| *Microcystis* sp. *(*small) | 19 | | | 3.2 (2.4, 4.2) | Ngaroto, Okareka, Okaro, Rotoehu, Rotoiti, Tarawera, | OVOR | 60 |
| *Microcystis* sp. (large) | 93 | | | 5.5 (4.1,7.4) | Kaituna River, Rotoehu, Rotoiti, Rotorua, Tarawera | OVOR | 54 |
| *Microcystis wesenbergii* | 182 | | | 6.9 (4.6,9) | Ngaroto, Rotoiti, Rotorua, | OVOR | 60 |
| *Nodularia spumigena* | 355 | 5.2 (3.2, 8.3) | 9.3 (7.3, 10.7) | | Lake Forsyth | CYL | 30 |
| *Planktolyngbya* cf *tallingii** | 1 | 3 (2, 4.4) | 0.6 (0.5, 0.8) | | Waikare | CYL | 30 |
| *Planktolyngbya subtilis* | 3 | 3 (2, 4.5) | 1.1 (0.8, 1.6) | | Waahi, Waikare | CYL | 42 |
| *Planktothrix* cf *agardhi* | 28 | 3 (2, 4.8) | 3.4 (2.9, 4.1) | | Oxidation pond (Horowhenua) | CYL | 30 |

| | Average volume (μm$^3$) | Length (μm) | Width (μm) | Diameter (μm) | Source | Shape | Count (n) |
|---|---|---|---|---|---|---|---|
| *Pseudanabaena limnetica* | 8.3 | 3.7 (1.9, 6.8) | 1.6 (1.1, 2.2) | | Karapiro, Okareka, Rotoehu, Rotoiti, | CYL | 30 |
| *Snowella lacustris* | 99 | 5 (3.5, 7.7) | 6.0 (4.2, 8.6) | | Rotoiti, Rotorua | OVO | 48 |
| *Trichodesmium iwanoffiana* | 102 | 4.3 (3.4, 5.3) | 5.4 (4.4, 7.1) | | Okareka, Okaro, Rotoiti, Tarawera | CYL | 30 |

Notes: Equations given by the United States Environmental Protection Agency (2007) were used to calculate volumes

CYL = cylinder; OVOR = ovoid (round); OVO = ovoid. Minimum and maximum dimensions are given in brackets.

* This species is commonly identified as *Planktolyngbya* cf *contorta* in New Zealand.

**Table A4.2: Volume equations for common cyanobacteria cell shapes**

| Cell shape | Formula |
|---|---|
| Ovoid (round) | $V = ((4 / 3)*\pi * (diam / 2)^3)$ |
| Ovoid | $V = (4 / 3) * \pi * (width / 2)^2 * (length / 2)$ |
| Cylinder | $V = (\pi * (width / 2)^2 * (length))$ |

Source: US Environmental Protection Agency, 2007

# Appendix 5: Media release – planktonic cyanobacteria

The following is an example of text for inclusion in media releases for recreational water bodies affected by planktonic cyanobacteria

**Health Warning Issued for < name of water body >**

**<Day, Month, Year>**

**<Time>**

For Immediate Release:

Tests carried out by < **agency** > have shown high concentrations of cyanobacteria (blue-green algae) at < **name of water body** > and a health warning has now been issued. Visitors to < **name of water body** > are advised not to use the water body for recreational purposes until health warnings are removed.

Cyanobacteria produce toxins that are harmful to humans and animals if swallowed or through contact with skin (such as may occur when swimming, water skiing or kayaking). Exposure to cyanobacteria may cause symptoms such as skin rashes, nausea, tummy upset and tingling and numbness around the mouth or tips of fingers. If you experience health symptoms after contact with contaminated water, contact < **name of agency** > and visit a doctor immediately. Boiling water does not remove toxins and drinking of the water should be avoided at all times.

Fish and shellfish can concentrate toxins and their consumption should be avoided. If fish are eaten, remove the gut and liver and wash in clean water.

Cyanobacteria occur naturally but can increase rapidly during summer months. If the water is cloudy, discoloured, or has small globules suspended in it, avoid all contact. Not all cyanobacterial blooms are visible to the naked eye and toxins can persist after the bloom has disappeared. Cyanobacterial concentrations can change quickly with changing environmental conditions (eg, wind). If a health warning is in place, avoid contact with the water.

< **agency** > monitors cyanobacteria weekly at < **name of water body** > during summer and the public will be advised of any changes in water quality that are of public health significance.

For further information, visit < **website address** > or contact < **name and telephone number** >

Press release ends.

# Appendix 6: Health warning sign for planktonic cyanobacteria

The following is an example of the text for a health warning sign for planktonic cyanobacteria. A health warning sign should provide enough information to inform the public of the potential health risks and enable them to make an informed decision. It should be clearly dated to inform the public when the warning was issued.

## WARNING

**Toxic cyanobacteria (blue-green algae) health hazard**

All persons are warned that potentially toxic cyanobacteria are present in this water body and may affect the health of persons and animals coming into contact with the water.

Swimming, sailing, water skiing, or any other activity involving body contact with the water may cause skin and eye irritation.

Drinking or accidentally swallowing water may result in illness.

Toxins can accumulate in the internal organs of fish and shellfish. Remove the internal organs of fish before cooking and avoid eating shellfish.

**NOTICE POSTED ON:** **<Date>**

**EFFECTIVE UNTIL :** **<Date>**

**NOTICE POSTED BY:** **<Name of organisation>**

**<Contact>**

**<Website>**

# Appendix 7: Benthic cyanobacterial and other benthic algae photos

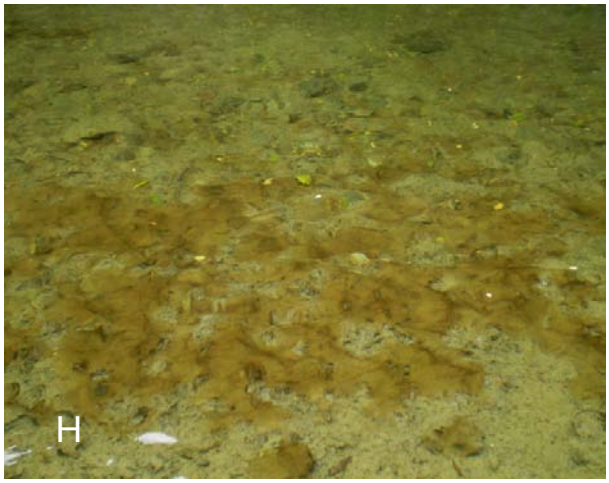

(A) *Phormidium* sp. mat (Hokitika River, West Coast). Photo: K Shearer, Cawthron.

(B) *Phormidium* sp. mat (Hokitika River, West Coast). Photo: K Shearer, Cawthron.

(C) *Phormidium* sp. mats drying out on the river's edge. (Hokitika River, West Coast). Photo: K Shearer, Cawthron.

(D) Detached *Phormidium* sp. mat drying out on the river's edge. Photo: K Shearer, Cawthron.




(E) Dark brown *Phormidium* sp. mats (Hutt River, Wellington). Tire tracks run through the centre of the mats Photo: M Heath, Victoria University.

(F) Detached *Phormidium* sp. mat drying on the river's edge. Photo: S Wood, Cawthron.

(G) Detached *Phormidium* sp. mat (Hutt River, Wellington) on the river's edge. Photo: S Wood, Cawthron.

(H) Benthic cyanobacterial mat (Hutt River, Wellington) growing on fine and sandy sediment. Note the lighter brown colour. Photo: M Heath, Victoria University.


(I) Thick *Phormidium* sp. mat growing on a rock taken from the Makarewa River (Southland). Photo: K Meijer, Environment Southland.

(J) Thick *Phormidium* sp. mat growing on a rock taken from the Whakatane River (Whakatane). Photo: S Wood, Cawthron.

(K) *Phormidium* sp. attached to a large boulder, Hutt River, Wellington. Photo: M Heath, Victoria University.

(L) *Phormidium* sp. mats in the Wakapuaka River (Nelson). Photo: A Crowe, Cawthron.



(M) *Phormidium* sp. mats at the Silverstream Bridge in the Hutt River (Lower Hutt, Wellington). Photo: J Milne, Greater Wellington Regional Council.

(N) *Phormidium* sp. mats at the Silverstream Bridge in the Hutt River (Lower Hutt, Wellington). Photo: J Milne, Greater Wellington Regional Council.




(O) *Nostoc commune* mats along the shores of Lake Taupo. Photo: S Wood, Cawthron.

(P) An enlargement of the mat from (P) showing the individual colonies of *N. commune* that had been detached from the rocks. Photo: S Wood, Cawthron.

(Q) A pebble with attached *N. commune*. Low lake levels and high winds caused *N. commune* colonies to be dislodged from rocks in the lake and these accumulated along the shoreline as shown in (O). Photo: S Wood, Cawthron.







**Non-cyanobacterial benthic mats**

(R) Native diatom mats. Photo: A Crowe, Cawthron.

(S) Native diatom mats. Photo: A Crowe, Cawthron.

(T) Filamentous green algae. Photo: A Crowe, Cawthron.

(U) Filamentous green algae. Photo: A Crowe, Cawthron.

(V) Mats of the invasive diatom didymo. Photo: S Wood, Cawthron.

(W) Mat of the invasive diatom didymo. Photo: S Wood, Cawthron.

# Appendix 8: Cyanobacteria and cyanotoxin capabilities in New Zealand

**Table A8.1: Freshwater micro-algae/cyanobacterial analysis capabilities in New Zealand**

| | IANZ accred.* | Location | Contact |
|---|---|---|---|
| Cawthron | Yes | Nelson | Stef Naldi<br>Ph: 03 548 2319 ext. 266<br>Email: stef.naldi@cawthron.org.nz<br>Web: www.cawthron.org.nz/analytical-laboratory/natural-toxins.html |
| Landcare Research | No | Auckland | Stephen Moore<br>Ph: 09 574 4100<br>Email: MooreS@landcareresearch.co.nz |
| NIWA | Yes | Hamilton | Karl Safi<br>Ph: 07 856 7026<br>Email: algalservices@niwa.co.nz<br>Web: www.niwa.cri.nz/ncwr/tools/algae |
| Ryder Consulting | No | Dunedin | Ben Ludgate<br>Ph: 03 477 2113<br>Email: b.ludgate@ryderconsulting.co.nz |
| University of Canterbury | No | Christ-church | Dr Paul Broady, School of Biological Sciences<br>Ph: 03 364 2525<br>Email: paul.broady@canterbury.ac.nz |
| Watercare Laboratory Services | Yes | Auckland | Lynette Ronberg<br>Ph: 09 539 7784<br>Email: clientsupport@water.co.nz |
| University of Waikato | No | Hamilton | Prof. David Hamilton<br>Ph: 07 858 5046<br>Email: d.hamilton@waikato.ac.nz |

* International Accreditation New Zealand (IANZ) is the national authority for the accreditation of testing and calibration laboratories. IANZ accreditation is not required to undertake analysis of cyanobacteria/algae in recreational use water bodies. It is required for water used for drinking.

## Table A8.2: Cyanotoxin analysis capabilities in New Zealand

| Laboratory | Cyanotoxins | Method | IANZ accred. | Contact |
|---|---|---|---|---|
| AgResearch Hamilton | Total MC/NOD | ADDA- ELISA | No | Jan Sprosen<br>Ph: 07 838 5203<br>Email: jan.sprosen@agresearch.co.nz |
| Cawthron Nelson | MC/NOD | LC-MS | Yes | Catherine Moisan<br>Section Head, Natural Toxins<br>Ph: 03 548 2319 ext 268<br>Email: catherine.moisan@cawthron.org.nz<br>Web: www.cawthron.org.nz/analytical-laboratory/natural-toxins.html |
| | ATX, HTX, CYN | LC-MS | Yes | |
| | SAX | Rapid Kit | No (but plan to obtain) | |
| | ATX-a(S) | AchE assay* | No | |
| | General toxicity | Mouse bioassay* | No | |
| Watercare Laboratory Services | MC/NOD | LC-MS | No | Dr Peter Boniface<br>Head of Organic Chemistry Services<br>Ph: 09 539 7760<br>Email: clientsupport@water.co.nz |

Notes: LC-MS = liquid chromatography mass spectrometry; ELISA = enzyme-linked immuno sorbent assay; AchE = acetylcholinesterases inhibition assay; MC = microcystin; NOD = nodularin; ATX = anatoxin-a; HTX = homoanatoxin-a, SAX = saxitoxin; ATX-a(S) = anatoxin-a(S).

* Not routinely used but available on request.

# Appendix 9: Health warning sign for benthic cyanobacteria

Below is an example of the text for a health warning sign for benthic cyanobacteria. A health warning sign should provide enough information to inform the public of the potential health risks and enable them to make an informed decision. It should be clearly dated to inform the public when the warning was issued. Photographs on signs should show the public what to look for. For benthic cyanobacteria it may be useful to have a 'medium risk' sign which advises the public on what to look for at sites with known problems. 'High risk' signs can then be put in place when the action level (red mode) threshold is exceeded. Examples of medium risk and high risk signs used by the Greater Wellington Regional Council are given on the following two pages.

**WARNING**

**Toxic cyanobacteria (blue-green algae) health hazard**

Warning: potentially toxic cyanobacteria (blue-green algae) are present in this river/stream and may affect the health of persons or animals coming into contact with the water.

Contact with the water may cause skin and eye irritation. Drinking or accidentally swallowing water may result in illness.

Cyanobacteria usually occur as dark brown/black mats attached to rocks. These mats can detach and accumulate along the riverbank.

Don't let your dog eat anything from the riverbank or come in contact with the water. Contact your vet or doctor immediately if illness occurs.

**NOTICE POSTED ON: <Date>**

**EFFECTIVE UNTIL : <Date>**

**NOTICE POSTED BY: <Name of organisation>**

**<Contact>**

**<Website>**

**Medium risk sign – Greater Wellington Regional Council**

# Warning! kia tupato!

**Toxic algae may be in this part of the river during warm weather and low river flows**

Toxins produced by blue-green algae (cyanobacteria) can kill dogs and make humans and other animals sick.

## If you see toxic algae

- **Don't touch it**
- **Don't let your dog scavenge in or near the river**

## What to look out for

Thick **dark brown** or **black** algal mats attached to rocks on the river bed

Live or dead mats washed up on the river bank

Live or dead mats floating on the surface in shallow waters

If you, your dog or other animals are sick after being in or near the river consult your doctor or vet **immediately**.

More information about toxic algae and any current warnings can be found at www.gw.govt.nz/toxic-algae or from an Environmental Health Officer at Upper Hutt City Council - **04 527 2169**

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Regional Public Health
Better Health For The Greater Wellington Region

greater WELLINGTON
REGIONAL COUNCIL

**High risk sign – Greater Wellington Regional Council**

# Appendix 10: Media release – benthic cyanobacteria

The following is an example of text for inclusion in media releases for a recreational water bodies affected by benthic cyanobacteria.

<**Day, Month, Year**>

<**Time**>

**For Immediate Release:**

Tests carried out by < **agency** > have shown high abundance of benthic cyanobacteria (blue-green algae) at < **name of water body** > and a health warning has now been issued. Humans and animals (in particular dogs) should avoid contact with < **name of water body** > until health warnings are removed.

Cyanobacteria produce toxins that are harmful to humans and animals if swallowed or through contact with skin. Exposure to cyanobacteria may cause symptoms such as skin rashes, nausea, tummy upset and tingling and numbness around the mouth or tips of fingers. If you experience health symptoms after contact with contaminated water, contact < **name of agency** > and visit a doctor immediately. Boiling water does not remove toxins and drinking of the water should be avoided at all times. Animals that consume cyanobacteria should be taken to a vet immediately.

Cyanobacteria occur naturally but can increase rapidly during warmer periods of the year. Benthic cyanobacteria usually occur as dark brown/black mats which grow attached to rocks in the river or accumulate on the surface in shallow, slow-flowing areas. They often have a strong, musty smell. Cyanobacterial concentrations can vary quickly with changing environmental conditions; for example, high river levels will remove cyanobacteria. If a health warning is in place, avoid contact with the water.

< **agency** > monitors cyanobacteria weekly at < **name of water body** > during summer and the public will be advised of any changes in water quality that are of public health significance.

For further information, visit < **website address** > or contact < **name and telephone number** >

Press release ends.

# Appendix 11: Example pamphlet – Greater Wellington Regional Council

## Where can I get more information?

- Your local council with queries relating to animals and water supply.
- Regional Public Health (ph (04) 570 9002) or Wairarapa Public Health (06 370 5020) with queries relating to human health.
- Greater Wellington's website: **www.gw.govt.nz/toxic-algae** where you will find information on toxic blue-green algae and a list of any rivers with current 'toxic algae alerts' on them. Regular monitoring of water quality occurs at selected freshwater swimming sites across the Wellington region.
- Published January 2008
  GW/EMI-G-08/04

info@gw.govt.nz
www.gw.govt.nz

Blue-green algal mats at the water's edge

# Toxic blue-green algae

**Take care when swimming in rivers or walking your dog near water**

greater WELLINGTON
REGIONAL COUNCIL

HUTT CITY

Regional Public Health

greater WELLINGTON
REGIONAL COUNCIL

## What are blue-green algae?

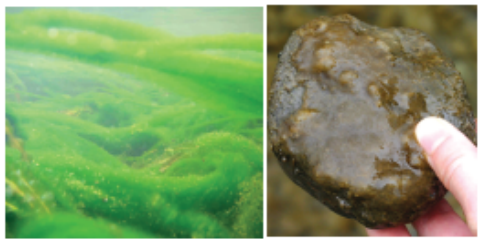
Blue-green algae (or cyanobacteria) are microscopic organisms that are naturally present in many New Zealand waters, including relatively 'clean' waters. Like other algae, growth of blue-green algae is encouraged when river flows are low and stable and temperatures consistently warm.

## What do they look like?

Toxic Blue-green algae

Blue-green algal mats are actually **dark brown or black** and grow attached to rocks on the river bed. Mats that come loose from the river bed can wash up on the river bank or form floating 'rafts' in shallow areas. Where exposed, the mats may dry out and turn a light brown or white colour. They may also produce a strong musty odour.

Blue-green algae differ from harmless bright green algae, which often form long filaments. Mats of light brown or olive green algae are also harmless.



Harmless algae

## What is the problem with blue-green algae?

Some algal mats produce toxins that can be harmful to humans, dogs, livestock and wildlife. Five dogs died in the summer of 2005/06 and several died in the summer of 2007/08 after ingesting algal mats along the edge of the Hutt River.

## What are the possible health effects?

Swallowing water containing blue-green algae toxins can lead to vomiting, diarrhoea, abdominal pain, cramps, nausea and other effects in humans. Skin contact can cause irritation of the skin, eyes, nose and mouth. Exposure to high levels of toxins can result in serious illness or death. Dogs are particularly susceptible to poisoning from blue-green algae as they love to scavenge and play near water. Livestock are also at risk from poisoning.

## What should I do if I find blue-green algae?

Rivers users, particularly those with dogs, or those taking water for livestock or human consumption, should avoid contact with any **thick** dark brown-black algal mats (see photo), particularly those that are easily accessible, exposed on river edges or floating in shallow areas of riverbanks or near rocks. If blue-green algal mats are widespread in a river you should presume that the water may be unsafe for bathing or drinking.

## Who should I call if I experience a reaction?

If you think you are experiencing a serious reaction, seek urgent medical attention. Advise your doctor of your potential exposure to toxic algae. Your GP has been asked to notify Regional Public Health or Wairarapa Public Health of any people with possible reactions.

## Who should I call if I think my animal is sick?

If you are concerned about your animal(s), contact a vet immediately (the toxins can affect dogs within minutes). You or your vet can report any animal illness resulting from contact with the blue-green algae to your local council.

## Who should I call if I think I've seen toxic blue-green algal mats?

If you see thick blue-green algal mats, please contact Greater Wellington on **04 384 5708** or your local council.

Blue-green algal mats forming a floating 'raft' on the water's surface

# Appendix 12: Example field sampling sheet for planktonic cyanobacteria

**Contact Information:**

Name of sampler:..... Ph: ..... Fax:.....

Email: ..... Address: .....

**Sample Information:**

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

Sample location (Please be as detailed as possible; eg, north end of Green Bay, Lake Karori.

If possible, provide a sketch on the back of sampling sheet): .....

.....

.....

Sampling method (no. of samples, composite or grab): .....

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

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

Human drinking water Stock drinking water Irrigation Oxidation pond

Recreation (please state activities; eg, 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 24 hours prior to sampling: .....

**Bloom Information:**

What percentage of the water body does the bloom cover:.....

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

Are there any signs of animal/human poisonings (eg, dead birds, fish, stock, rashes on swimmers): .....

.....

**Limnological Data:**

Max. water depth:..... Ave. water depth: ..... ..... Secchi depth: .....

Area of water body: .....Water temperature: .....

Predominant catchment cover (eg, farmland):... .....

What wildlife is present on or in water body (eg, trout/ducks):.....

.....

**Samples Collected for:**

□ Cyanobacterial identification (Lugol's preserved) □ Cyanobacterial identification (unpreserved)
□ Cyanotoxin identification (unpreserved)

**Additional Comments** .....

.....

.....

.....

# Appendix 13: Example field sampling sheet for benthic cyanobacteria

**Contact Information:**

Name of sampler:..... Ph: ..... Fax:.....

Email: ..... Address: .....

**Sample Information:**

Date:...../...../.....Time: ..... Bank of river: TLB TRB

Sample location (please be as detailed as possible): .....

### Method:

- Select an area of river bed (40−60 m long) suitable for 4 transects. It should include areas of riffle and run.
- Take each transect across the river, or to a maximum depth of **0.6 m** for larger, deeper rivers.
- Start at the most downstream transect and work upstream to avoid disturbance to areas not yet surveyed.
- Divide transect into 5 points. To do this, estimate the distance between viewing points by counting paces across the river, or to 0.6 m depth, then dividing by 5; work back to your starting point.
- Estimate % cover occupied by benthic cyanobacterial mats at each viewing point. Only record mats if they are greater than 1 mm thick
- Note presence or absence of detached or detaching mats on each transect and exposed mats on the river bed.
- Note bed substrate type (cobbles, gravels, sand-silt, macrophytes).

| | **Transect 1** | **Transect 2** | **Transect 3** | **Transect 4** | **Comments** |
|---|---|---|---|---|---|
| Transect length | | | | | |
| Riffle or run? | | | | | |
| Substrate | | | | | |
| Detached or detaching mats? | | | | | |
| Exposed mats on river's edge? | | | | | |
| Sample taken? | | | | | |
| **% cover by benthic cyanobacteria** (to nearest 5%) | | | | | |
| View 1 | | | | | |
| View 2 | | | | | |
| View 3 | | | | | |
| View 4 | | | | | |
| View 5 | | | | | |
| Mean % cover/ transect | | | | | Average % cover at site |

# Appendix 14: Lugol's solution

Dissolve 10 g pure iodine crystals, 20 g of potassium iodide (KI) and 20 g of glacial acetic acid in 200 mL distilled water (Pridmore, 1987).