Aotearoa New Zealand guidelines for cyanobacteria in recreational freshwaters

Ngā Aratohu o Aotearoa mō ngā Huakita-kārikiōrangi i rō Wai Māori ā-Rēhia

Ministry for the Environment
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Cover image: *Tasman District Council's drinking water team conducting a benthic cyanobacteria survey at the Wai-iti River.*

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

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

Section 5. Communications provides advice on communicating with the public about the risks from toxic algae in recreational waterways.

References provides citations for sources referred to in the body of the guidelines and the appendices.

Glossary provides definitions for abbreviations and terms used in the guidelines.

Appendices give further background information and include templates for data collection and reporting, including:

- background information on known cyanotoxins and their distribution in Aotearoa New Zealand, climate-change effects on cyanobacteria, management of freshwater cyanobacteria, impacts on marine environments, and benthic cyanobacteria in lakes and ponds
- information on the derivation of guideline values
- photographs of typical bloom events
- a list of biovolumes for common cyanobacteria observed in Aotearoa
- suggested media releases and examples of information and warning signs for cyanobacteria
- templates for field assessments
- examples of frequently asked questions and responses.

Section 1. Introduction

1.1 What is the purpose of these guidelines?

These guidelines provide advice on how to manage public health risk associated with cyanobacteria in recreational freshwaters in Aotearoa New Zealand (hereafter, Aotearoa). They have been developed in response to requests from regional resource management and health agencies for best-practice advice, and are meant to:

- help these agencies develop monitoring protocols suitable for local conditions and circumstances
- encourage a nationally unified approach to managing cyanobacterial risk in waterways used for recreational purposes.

1.2 What do these guidelines cover?

These guidelines set out a monitoring framework for establishing the public health risk from cyanobacteria associated with recreational activities 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 (for example, shellfish), or drinking water. The maximum acceptable values for four classes of cyanotoxins (anatoxins, cylindrospermopsins, microcystins, nodularins and saxitoxins) in drinking-water supplies in Aotearoa can be found in the *Water Services (Drinking-Water Standards for New Zealand) Regulations 2022*[1]. The accompanying 'cyanobacterial compliance' chapter of the *Guidelines for Drinking-Water Quality Management for New Zealand*[2] informs drinking-water suppliers on how to monitor and manage drinking-water supplies for cyanobacteria and their toxins, including sampling requirements, recommended actions in response to threshold breaches, and treatment options.

For further advice on cyanotoxins in aquatic organisms (shellfish and others), contact New Zealand Food Safety at the Ministry for Primary Industries. Appendix 1 provides high-level information.

Finally, this document does not provide explicit guidance for managing the impacts of cyanobacterial events on other environmental values; there are more appropriate guidelines available for this, such as the *New Zealand Periphyton Guideline*[3].

1.3 Who should use these guidelines?

The *Aotearoa New Zealand Guidelines for Cyanobacteria in Recreational Freshwaters* are for staff and agencies involved in the monitoring of and reporting on recreational water. Specifically, these are:

- science and policy professionals within regional councils and unitary authorities who routinely monitor the state of the environment
- public health professionals and practitioners in the National Public Health Service of Health New Zealand (HNZ) | Te Whatu Ora 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.

Section 2.4 contains more about the roles and responsibilities of these staff and agencies.

These guidelines may also interest the wider environmental science and environmental management community. For example, within councils, policy and planning staff who are developing freshwater policy may find useful some of the background material on the environmental causes of blooms. These guidelines cannot, however, be used for resource consenting work. For example, guideline thresholds cannot be used as the basis for establishing conditions for discharge consents, although they could be 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*[4].

1.4 Changes in cyanobacterial taxonomy

Since the introduction of the *New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters: Interim Guidelines* in 2009, several significant changes in cyanobacterial taxonomy and nomenclature have occurred (see Table 1). Throughout this revision, the authors use the updated taxonomy to maintain consistency with current taxonomic nomenclature used by the international community; usually, the authors also include a reference to the previous taxonomy to assist with the change in species names.

Table 1: Revised taxonomy of cyanobacteria frequently encountered in Aotearoa New Zealand

Old name	New name
All planktonic *Anabaena* #	*Dolichospermum*
Anabaena circinalis	*Dolichospermum circinale*
Anabaena planctonica	*Dolichospermum planctonicum*
Aphanizomenon issatschenkoi	*Cuspidothrix issatschenkoi*
Aphanothece clathrata	*Anathece clathrata*
Cylindrospermopsis raciborskii	*Raphidiopsis raciborskii*
Lyngbya wollei	*Microseira wollei*
Phormidium autumnale	*Microcoleus autumnalis*
Planktolyngbya subtilis	*Planktolyngbya limnetica*

Only benthic strains are now considered as Anabaena

1.5 Status of this guidance

The *New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters: Interim Guidelines* were released by the Ministry for the Environment (MfE) / Manatū mō te Taiao and the Ministry of Health (MoH) / Manatū Hauora in 2009. Knowledge of cyanotoxin-producing species, cyanotoxin production and toxicity, and methods for detection and monitoring, have advanced markedly since this initial publication. In addition, new issues – related to potentially toxic cyanobacteria not covered by the 2009 guidelines – have arisen and will continue to emerge. These revised guidelines are designed to be a living document that will be updated to present the best advice available.

Although the guidelines are not mandatory, we strongly recommend that freshwater managers use them as a robust guide for protecting human health from toxic cyanobacteria in recreational freshwaters. They provide a best-practice approach for many management circumstances, given the current understanding of cyanobacterial risks in recreational freshwaters in Aotearoa. However, local judgement is still required to fill gaps in scientific knowledge and health-risk management. Local decisions about whether to follow the guidelines' approach should be made while considering site-specific factors (for example, resource availability, and historical understanding of local bloom conditions and toxin production), as well as the guidance offered in these guidelines. We have developed the management approach described here to be widely applicable around Aotearoa, but that should not limit regional authorities from incorporating new technologies into their management strategies (for example, satellite imagery, drones, and phycocyanin fluorometers).

When modifications are made to the approach described in the guidelines, public health staff *must* engage in that decision-making process, and *must* consider whether public health is still protected under the revised risk-management system. They may need to seek expert advice as part of this process.

The word 'should' has been used throughout the guidelines to describe recommended actions by monitoring and health agencies. This is not prescriptive but 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 recreational freshwaters?

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*, can harm humans and animals when consumed in drinking water, or by ingestion and inhalation during recreational activities. The mechanisms of toxicity for cyanotoxins include hepatotoxicity (liver damage), neurotoxicity (ultimately causing suffocation through paralysis of nerve transmission), and carcinogenesis (tumour promotion). Other negative health effects from exposure to high levels of cyanobacteria include respiratory irritations, skin rashes, and stomach discomfort, although these symptoms do not necessarily arise from the main classes of cyanotoxins (anatoxins, cylindrospermopsins, microcystins, nodularins and saxitoxins).

2.2 What is contact recreation?

For the purposes of applying these guidelines, 'contact recreation' covers all activities that bring people physically into contact with water and that involve a risk of involuntary ingestion or inhalation of water. Swimming (whether partially or fully immersed) is perhaps the most obvious activity, but others include kayaking, white-water rafting, water skiing, wakeboarding, sailing and diving.

2.3 The overall approach

These guidelines are based on the multi-tiered approach recommended by the World Health Organization (WHO)[5] and the Australian National Health and the Medical Research Council (NHMRC)[6]. These organisations recommend that, during the development of guidelines for dealing with cyanobacteria in recreational freshwaters, the following should be considered:

- the particular hazard caused by the well-characterised cyanotoxins: anatoxins, cylindrospermopsins, microcystins and saxitoxins
- 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 may be caused by currently unknown compounds
- the patchy and often unpredictable distribution of cyanobacterial populations.

2.3.1 A three-tier Surveillance, Alert and Action sequence

The World Health Organization and the NHMRC have found that a single guideline value is not appropriate, so we recommend using a series of guideline values associated with incremental severity and probability. These guidelines for Aotearoa use a similar approach and are based

on a three-tier alert-level framework. This framework incorporates a monitoring and management action sequence that regulators can use for a graduated response to the onset and progress of a cyanobacterial bloom or benthic proliferation in a water body:

- In Surveillance Level (green mode), cyanobacteria levels are low.
- In Alert Level (amber mode), cyanobacteria are present in a water body and regular monitoring is required to evaluate the level of risk.
- In Action Level (red mode), cyanobacteria are present at health-adverse levels, and access to the water body needs to be restricted to protect human health or the risk level needs to be more accurately assessed through toxin testing.

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 from those set out in the 2009 version is the alert-level framework for planktonic cyanobacteria. The cyanobacterial biovolume threshold for potentially toxic cyanobacteria – based on international observations – has been replaced with thresholds for toxin-producing cyanobacteria confirmed in Aotearoa. Another change is that these thresholds are based on cell concentration measurements rather than cyanobacterial biovolume measurements. The thresholds were developed using toxin quota data and the WHO guideline documents for anatoxins[7], cylindrospermopsins[8], microcystins[9] and saxitoxins[10]. The intention is to avoid unnecessary escalations into the Action Level (red mode) when non-toxic cyanobacteria are dominant.

A threshold based on total cyanobacterial biovolumes has been retained in the alert-level framework for situations where high cyanobacteria concentrations are observed (which can lead to respiratory effects, or skin irritations in sensitive individuals) but levels of confirmed toxin-producing taxa are low. The process of developing the revised thresholds for planktonic cyanobacteria in recreational freshwaters, and the end-user consultation undertaken as a part of it, is documented in Puddick et al (2022)[11]. Due to the increased complexity of the alert-level framework for planktonic cyanobacteria, a flow diagram to assist decision making has also been developed (see box 1 at the end of section 3.3).

The health risks associated with benthic cyanobacteria are understood less fully than the risks for their planktonic counterparts. There has been little international research in this area – and no attempts have been made to develop quantitative guidelines – yet benthic mat-forming cyanobacteria are widespread throughout rivers in Aotearoa[12]. Investigations have revealed the widespread distribution of toxic species commonly linked to dog poisonings[13-18]. This document provides guidelines based on preliminary research.

These guidelines also suggest that cyanotoxin testing (measuring the concentration of toxins produced by the cyanobacteria in a sample) should be considered as a useful addition to cell concentration, 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

- show that toxins are not present at health-adverse levels and remain in Alert Level (amber mode) – see section 3.2
- demonstrate that residual cyanotoxins are not present when mass lysis of a bloom has occurred
- develop greater understanding on toxin-producing cyanobacteria in Aotearoa.

2.3.3 Where and when should monitoring be done?

People are generally free to swim or undertake water-based activities wherever they like in and around rivers and lakes in Aotearoa, and it would be impossible to monitor all of our waterways. 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 use, the likelihood of cyanobacteria growth in the water body, and the resources available to the monitoring agency.

In general, blooms (sometimes called benthic proliferations for benthic species) are much more common in summer months, so this is when routine monitoring should occur. However, the causes of cyanobacterial blooms are many, varied and often inter-related. Cyanobacterial blooms may undergo rapid changes in extent and toxicity. These complexities mean that deciding when and where to monitor can be challenging. Section 4 provides information to assist agencies through a decision-making process for monitoring and sampling.

The Ministry for the Environment and Health New Zealand (HNZ) recommend that the general areas to include in any routine monitoring programme are agreed upon by the regional council, territorial authority and public health staff, and are 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

Before a sampling and reporting programme is developed, decisions must be made about which agency or agencies are responsible for which roles in monitoring and reporting for public health protection. Roles and responsibilities should be agreed upon for both routine monitoring programmes, as well as who will be responsible for responding to bloom events at other locations that are not routinely monitored. Close collaboration between council and public health staff will be mutually beneficial and lead to better outcomes (see the case study in box 2 of section 5).

Roles and responsibilities are best tailored to suit each region, and decisions will depend on many factors, such as institutional arrangements, and available expertise and resources. It may help to review the roles and responsibilities for the region every year to compensate for any changes in expertise or resourcing – and to act as a 'refresher' for those involved in any aspect of cyanobacteria monitoring and assessment. As a general guide, the Ministry for the Environment and HNZ recommend the following strategy.

2.4.1 Recommended strategy for routinely monitored sites

The recommended strategy described below for monitoring and responding to cyanobacteria in recreational water bodies 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[4] (note that in some regions the role of a regional council will be undertaken by a territory authority).

1. The regional council coordinates the monitoring, sample analysis and reporting strategy.
2. The regional council implements surveillance monitoring and the increased monitoring frequency required during the Alert and Action Levels.
3. The National Public Health Service reviews the effectiveness of the monitoring and reporting strategy.
4. The regional council informs public health staff and the territorial authority if Alert or Action Levels are reached.
5. The National Public Health Service ensures that the territorial authority is informed.
6. The National Public Health Service or territorial authority informs the public when the Action Level is exceeded (for example, through media releases). Public health staff should request 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 (occasionally it may be more appropriate for the regional council to undertake this duty). The National Public Health Service should provide advice to help territorial authorities and regional councils undertake necessary actions.
8. It is the responsibility of the National Public Health Service to lower 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 unmonitored locations

Occasionally, cyanobacterial blooms will occur at locations that are not part of routine monitoring programmes. By default, Points 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, a wider resource management issue, location of the bloom (private versus public land) or legal jurisdiction.

2.4.3 Regional protocols

Regional councils, territorial authorities and the National Public Health Service should clearly identify and agree on a lead agency to develop a monitoring protocol that covers both monitored and unmonitored locations. This protocol should be based on each agency's respective legislative functions relating to the monitoring and reporting of recreational water quality. The protocol should specify details of:

- which agency is responsible for which roles (that is, Points 1 to 9 in the framework above)
- how the monitoring programme will be implemented
- what the management and communication or education responses will be to exceedance events, or reports of associated human or animal illness.

Proactive consideration should also be given to the role or inclusion of non-regulatory groups, such as lake or river Kaitiaki (iwi or hapū groups who are mana whenua in a region) or community groups, in monitoring for cyanobacteria and communicating information to their communities. 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 some territorial authorities) and Ministry for the Environment have responsibilities under the Resource Management Act to monitor the state (that is, the condition or health) of the environment. These agencies undertake reporting on the state of the environment, and how it is changing over time, at both regional and national scales. The purpose of monitoring and reporting the state of the environment is to measure how well the agencies' management practices, policies and laws are working, and whether environmental outcomes are being achieved.

Many factors arising from human uses of and activities on land can cause cyanobacterial blooms and mats to form, or exacerbate naturally occurring blooms and mats (including flow alteration, shade reduction and 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).

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 introducing toxin testing or both.

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 underrepresent a toxic bloom event, or there may be other water-quality problems that pose a health risk (for example, microbiological, chemical or physical qualities). It is important that water managers use these guidelines judiciously and consider carefully how to best apply them.

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

The following guidelines are a recommended best-practice approach for many management circumstances, given our current understanding of cyanobacterial risks in freshwaters around Aotearoa. But local decisions about whether to follow every aspect of this 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 are only problematic when they increase to high concentrations, forming 'blooms'. Cyanobacterial blooms have occurred in many lakes in Aotearoa since the 1970s; however, they have become increasingly frequent in recent decades, possibly influenced by anthropogenic eutrophication and climate change[19]. Appendix 2 compiles information on the effects of climate change on cyanobacterial blooms. A variety of physical, chemical and biological factors, which can vary between water bodies, influence the growth of cyanobacteria and the formation of bloomswater bodies. Appendix 3 reviews multiple strategies to reduce cyanobacterial blooms that have been developed in the past decade.

Although these guidelines have been produced for safeguarding recreational users of freshwater environments, we acknowledge that cyanobacterial blooms have the potential to migrate downstream and impact coastal environments. Appendix 4 outlines information on this topic.

Planktonic cyanobacteria in Aotearoa are now known to produce the following cyanotoxins: anatoxin-a, cylindrospermopsins, microcystins and nodularins[13, 20-22] (see appendix 5 for information on cyanotoxin distribution and diversity in Aotearoa). The health risks associated with cyanotoxins are greatest during bloom events when high cell concentrations are present in a water body. 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 µg/L[23]. The exception to this is *Raphidiopsis raciborskii*, which can actively transport toxins outside their cells[24]. 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 (should they come in contact with or accidentally ingest contaminated water). Wind-driven accumulations of surface scums can result in toxin concentrations increasing by a factor of 1,000 or more, and such situations can change within very short periods, even just hours.

These guidelines aim to protect human health during recreational activities. Appendix 6 provides details on the methods used to derive the threshold values of Surveillance Level

(green mode), Alert Level (amber mode) and Action Level (red mode). For a detailed assessment of the risks posed by frequent occupational exposure (for example, daily contact), see de Wet 2008[25].

3.2 Alert-level framework: Planktonic cyanobacteria

Research undertaken over the past decade has led to the identification of four confirmed toxin-producing planktonic cyanobacteria in Aotearoa: *Cuspidothrix issatschenkoi*, *Microcystis* spp, *Nodularia spumigena* and *Raphidiopsis raciborskii* (table 2). Microcystin-producing *Microcystis* species have been found in a range of lakes in Aotearoa[26, 27], and nodularin-producing *Nodularia spumigena* has been reported in brackish lakes and lagoons[26, 28]. Anatoxin-producing *Cuspidothrix issatschenkoi* has been observed in a small number of lakes, primarily in the central North Island[19, 22]. Whereas *Raphidiopsis raciborskii* is commonly found in lakes in the Waikato region, cylindrospermopsin has only been detected on one occasion in 2003, in Lake Waahi[29]. For these four taxa, we recommend the use of taxon-specific cell concentration thresholds, based on taxon-specific toxin quotas and the WHO recreational guideline values for anatoxins, cylindrospermopsins and microcystins[11].

Anatoxin-a was detected in one environmental sample containing *Dolichospermum lemmermannii* (table 2); however, more data are needed before this can be included in the revised threshold. Likewise, low levels of saxitoxins were detected in samples dominated by *Dolichospermum planctonicum* (table 2), but the method of toxin detection was not considered robust and further research is needed.

Table 2: Summary of confirmed and suspected toxin-producing cyanobacteria identified in Aotearoa New Zealand

Cyanobacterial taxon	Habitat	Cyanotoxin(s)	Refs
Confirmed toxin-producing cyanobacteria			
Cuspidothrix issatschenkoi	Planktonic	Anatoxin-a	22, 30
Microcoleus autumnalis #	Benthic	Anatoxins	14, 18
Microcystis spp	Planktonic	Microcystins	31, 32
Nodularia spumigena	Planktonic	Nodularin	33
Nostoc sp	Benthic	Microcystins	27
Planktothrix sp	Benthic	Microcystins	16
Raphidiopsis raciborskii	Planktonic	Cylindrospermopsins	20, 29
Scytonema cf. *crispum*	Benthic	Saxitoxins	34
Suspected toxin-producing cyanobacteria			
Dolichospermum lemmermannii	Planktonic	Anatoxin-a *	21, 35
Dolichospermum planctonicum	Planktonic	Saxitoxins *	36
Oscillatoria sp	Benthic	Anatoxin-a *, Microcystins *	37

Note: New toxic species continue to be identified, and all cyanobacteria should be regarded as potentially toxic until proven otherwise. # Previously *Phormidium autumnale*. * This result was obtained from testing environmental samples dominated by this species rather than an isolated culture.

The alert-level framework for planktonic cyanobacteria (Decision Chart 1) incorporates a combination of cell counts for confirmed toxin-producing cyanobacteria from Aotearoa (*Cuspidothrix issatschenkoi*, *Raphidiopsis raciborskii*, *Microcystis* spp and *Nodularia spumigena*), and biovolumes for others. But there are probably other planktonic toxin-producing species that have not yet been identified or confirmed through toxin testing. When a species is known to be a toxin producer in another country (for example, *Dolichospermum lemmermannii*; see appendix 5 – table A5.1 for a more complete list), it is recommended that toxin gene screening or toxin testing should be undertaken in parallel with cell counts until there is more comprehensive knowledge of the toxin-producing capabilities of taxa within a specific lake. Total cyanobacterial biovolumes are still incorporated into the guidelines, as high-level exposure to many cyanobacterial taxa (even those that do not produce cyanotoxins) can lead to a range of negative health effects (including respiratory irritations, skin rashes and stomach discomfort[38-40]; see box 10 of appendix 6 for more information). Appendix 7 describes the rationale for the use of biovolumes. Section 3.3 describes details on the alert-level framework, and a flow diagram to assist decision making can be found at the end of the section (box 1).

Decision Chart 1: Alert-level framework for planktonic cyanobacteria in recreational freshwaters (see section 2.4 for the recommended framework for roles and responsibilities relating to actions, and the information provided in section 3.3 for advice on interpreting the guidance in this table).

Alert level	Action
Surveillance Level (green mode) **Situation 1:** The cell concentration for toxin-producing cyanobacteria observed in Aotearoa (Cuspidothrix issatschenkoi, Raphidiopsis raciborskii, Microcystis spp and Nodularia spumigena)[a] are **< 500 cells/mL**, or **Situation 2:** The biovolume equivalent for the combined total of all cyanobacteria is **< 0.5 mm³/L.**	Undertake weekly or fortnightly visual inspections[b] and sampling of water bodies where cyanobacteria are known to proliferate between spring and autumn.
Alert Level (amber mode) **Situation 1:** The cell concentration for toxin-producing cyanobacteria observed in Aotearoa is;[a,c] Cuspidothrix issatschenkoi **500 to < 100,000 cells/mL** Raphidiopsis raciborskii **500 to < 5,000 cells/mL** Microcystis spp **500 to < 30,000 cells/mL** Nodularia spumigena **500 to < 10,000 cells/mL**, or **Situation 2: 0.5 to < 10 mm3/L** total biovolume of all cyanobacteria.[d]	Increase sampling frequency to at least weekly.[e] If possible, multiple sites should be inspected and sampled. If potentially toxic cyanobacterial taxa (see table A5.1) are present at levels ≥ 0.5 mm³/L, then consider testing samples for toxin-production genes[f] or cyanotoxins.[g] Notify public health staff. Consider erecting information signs.[h]
Action Level (red mode) **Situation 1:** Cell concentration thresholds for toxin-producing cyanobacteria observed in Aotearoa);[a,c] Cuspidothrix issatschenkoi **≥ 100,000 cells/mL** Raphidiopsis raciborskii **≥ 5,000 cells/mL**[i] Microcystis spp **≥ 30,000 cells/mL** Nodularia spumigena **≥ 10,000 cells/mL**, or **Situation 2: ≥ 10 mm³/L** total biovolume of all cyanobacteria,[d] or Situation 3: Cyanobacterial scums consistently present,[j] or Situation 4: Cyanotoxin concentration thresholds;[g] Anatoxins **≥ 60 µg/L** Cylindrospermopsins **≥ 6 µg/L** Microcystins / Nodularins **≥ 24 µg/L** Saxitoxins **≥ 30 µg/L.**	Continue monitoring as for Alert Level (amber mode).[e] Notify the public of a potential risk to human health (see section 5.5 for more information). Samples should be tested for toxin-production genes[f] or cyanotoxins[g] to continue growing our knowledge on toxin-producing cyanobacteria in Aotearoa.

a. The cell concentrations of different species of *Microcystis* should be summed to determine if the threshold is breached (for example, *Microcystis* sp, *Microcystis aeruginosa*, and *Microcystis flos-aquae*). When cyanobacteria from the genera *Cuspidothrix*, *Raphidiopsis* and *Nodularia* are not identified to species level, they should also be summed (for example, *Cuspidothrix* sp, and *Cuspidothrix issatschenkoi* cell concentrations should be summed).

b. In high concentrations, planktonic cyanobacteria are often visible as buoyant green globules, which can accumulate along shorelines, forming thick scums (see appendix 8). 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 *Raphidiopsis raciborskii* and *Cuspidothrix issatschenkoi* are not necessarily visible to the naked eye and when visible could easily be confused with other types of water discolouration (see appendix 8).

c. Cell concentration thresholds for planktonic toxin-producing cyanobacteria found in Aotearoa were developed using toxin quotas and the 2020 World Health Organization guideline values for cyanotoxins in recreational waters (anatoxins, cylindrospermopsins and microcystins; see boxes 3–9 in appendix 6 for more details). When multiple toxin-producing cyanobacteria are present in a water body at the same time, the combined risk of cyanotoxins with the same mode of action should be accounted for by using the

ratio of each cell concentration to the relevant 'Action Level' thresholds and summing the ratios; if the sum exceeds 1, then the 'Action Level' is triggered (see section 3.3.3 for example calculations).

d. Situation 2 applies where high concentrations of 'non-toxigenic' cyanobacteria taxa are present and the 10 mm^3/L threshold is to protect human health from the risks associated with other agents produced by or co-occurring with cyanobacteria (see box 10 in appendix 6 for more details).

e. Blooms can change rapidly in some water bodies, hence the recommended weekly sampling regime.

f. Testing for toxin-production genes provides information on the potential for cyanotoxin production and the types of cyanotoxins that might be present. When a health alert has been issued using the cell concentration thresholds for Action Level (red mode) – Situation 1, but subsequent analysis is negative for toxin-production genes, the mode may revert to Alert Level (amber mode) if the total cyanobacterial biovolume is < 10 mm^3/L.

g. Cyanotoxin testing is useful to provide further confidence on potential health risks when a health alert is being considered, and to show that residual cyanotoxins are not present when a toxic cyanobacteria bloom subsides. Toxin concentration thresholds are based on the 2020 World Health Organization guideline values for cyanotoxins in recreational waters (anatoxins, cylindrospermopsins, microcystins and saxitoxins; see boxes 3–6 in appendix 6 for more details). When multiple cyanotoxins are present in a water body at the same time, the combined risk of cyanotoxins with the same mode of action should be accounted for using the ratio of each toxin concentration to the relevant 'Action Level' thresholds and summing the ratios – if the sum exceeds 1, then the 'Action Level' is triggered (see section 3.3.3 for example calculations). When a health alert has been issued using the cell concentration thresholds for Action Level (red mode) – Situation 1 but subsequent analysis shows that toxin concentrations are lower than the Situation 4 thresholds, the mode may revert to Alert Level (amber mode) if the total cyanobacterial biovolume is < 10 mm^3/L.

h. To avoid desensitisation and unnecessary avoidance of water bodies that do not pose a human health risk, information signs should not contain the 'danger' signals featured in warning signs (see section 5.5 for more information).

i. Toxin testing is recommended when *Raphidiopsis raciborskii* is at high levels as cylindrospermopsin can be exported out of the cells and persist in the environment.

j. As scums will likely contain cyanobacterial biovolumes greater than 10 mm^3/L, Situation 3 allows for quick enactment of measures to protect human health, if desired (see section 3.3.3). Photos of cyanobacterial scums in lakes can be found in Photos B–I of appendix 8.

3.3 Details of the framework: Planktonic cyanobacteria

The framework involves three levels of monitoring: Surveillance Level (green mode), Alert Level (amber mode) and Action Level (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.

The cell concentrations, or biovolumes, that define the levels apply to samples of the recommended type (composite 50-centimetre hose-pipes, see section 4.3.2) that are taken at a representative location or locations in the recreational water body (likely or designated recreational areas). A single site, 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 (for example, 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 use of biovolumes (as opposed to cell concentrations) for taxa where toxin production has not been established in Aotearoa (the Situation 2 thresholds) compensates for high concentrations of picocyanobacteria that would otherwise cause many water bodies to breach an alert-level framework that was based solely on cell concentrations. This rationale is fully described in appendix 7. Table A7.1 in appendix 7 gives cell volumes for common problematic

species observed in Aotearoa; in many instances this will enable a direct conversion of cell concentrations to biovolumes. For species not listed in table A7.1 in appendix 7, it will be necessary to establish their mean cell volume by undertaking cell measurements. Dimensions to measure and formulas for calculating cell volumes for common geometries of cyanobacteria cells are given in table A7.2 in appendix 7.

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 (for example, weekly) or to undertake toxin testing to confidently characterise recreational health risks.

3.3.1 Surveillance level (green mode)

Surveillance Level (green mode) is the lowest alert-level. Cyanobacterial cell concentrations (< 500 cells/mL; Situation 1) and biovolumes (< 0.5 mm^3/L; Situation 2) observed in Surveillance Level (green mode) will be common in many lakes around Aotearoa and are highly unlikely to pose human health risks in recreational settings. section 2.3.3 provides advice on which water bodies should be monitored and section 3.4 provides advice on how modern technologies might be used to determine this.

From spring to autumn, sampling and cell counts should be carried out weekly or fortnightly in lakes where cyanobacteria are known to proliferate and where recreational activity occurs (see section 2.3.3 for more information on site selection). A fortnightly sampling frequency may be appropriate for the Surveillance Level (green mode) where non-toxigenic species are present and where the risk is perceived to be lower (such as a low-usage recreational water body).

3.3.2 Alert level (amber mode)

In Situation 1, Alert Level (amber mode) is triggered when the cell concentration for known planktonic toxin-producing cyanobacteria from Aotearoa (*Cuspidothrix issatschenkoi*, *Raphidiopsis raciborskii*, *Microcystis* spp and *Nodularia spumigena*) are ≥ 500 cells/mL but are lower than the Action Level (red mode) threshold (100,000 cells/mL for *Cuspidothrix issatschenkoi*, 5,000 cells/mL for *Raphidiopsis raciborskii*, 30,000 cells/mL for *Microcystis* spp and 10,000 cells/mL for *Nodularia spumigena*). The cell concentrations of different species of *Microcystis, Cuspidothrix*, *Raphidiopsis* and *Nodularia* should be summed to determine if the threshold is breached (for example, *Microcystis* sp, *Microcystis aeruginosa*, and *Microcystis flos-aquae* should all be combined to *Microcystis* spp).

When known toxin-producing cyanobacteria are < 500 cells/mL, but the biovolume for the combined total of all cyanobacterial taxa is 0.5 to 10 mm^3/L, then Alert Level (amber mode) is triggered via Situation 2.

See appendix 6 for further explanation on how the threshold values are derived.

The Alert Level (amber mode) requires notification of and consultation with public health staff, and an ongoing assessment of the status of the bloom (see section 2.4 for guidance on roles and responsibilities). 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 staff, and on circumstances such as whether the cyanobacteria are confirmed planktonic toxin-producing species in Aotearoa (*Cuspidothrix issatschenkoi*, *Raphidiopsis raciborskii*, *Microcystis* spp and *Nodularia*

spumigena); whether there is a history of toxin production; and whether the cyanobacteria observed in the water body have been reported to produce toxins overseas (see appendix 5 – table A5.1).

Assessment of the potential for cyanotoxins in the water body might include testing for the genes involved in toxin production or for the toxins themselves (for example, by liquid chromatography-mass spectrometry). The tests used to detect genes involved in cyanotoxin production can yield false positives when only a portion of the genes necessary for toxin production are present and do not provide a toxin concentration in the water body. Positive results for cyanotoxin production genes should be followed up with toxin testing for the relevant toxins (the toxins associated with the cyanotoxin production genes that were detected). When a potentially toxic cyanobacteria species (those listed in appendix 5 – table A5.1) is present in a water body at biovolumes ≥ 0.5 mm^3/L, we recommend undertaking toxin testing, as this will help to improve our understanding on toxin-producing cyanobacteria in Aotearoa.

The sampling frequency also depends 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 – for example, if the site is being used heavily for recreation, or a special event is imminent. In most circumstances, however, weekly sampling provides enough information to assess the rate of change of cyanobacterial populations and to judge the population growth rate. Inspecting multiple sites around the water body allows for better understanding on spatial variability. If resourcing is restricted, then monitoring should focus on the areas of greatest risk: places where people commonly access the water and at the downwind end of the lake (on that day).

If it is not there already, consider erecting signage that provides information on the cyanobacteria and that communicates the potential health risks. Sections 5.4 and 5.5 provide further guidance on this.

3.3.3 Action level (red mode)

The Action Level (red mode) is triggered when representative samples exceed either:

- Situation 1: a concentration of ≥ 100,000 cells/mL for *Cuspidothrix issatschenkoi*, ≥ 5,000 cells/mL for *Raphidiopsis raciborskii*, ≥ 30,000 cells/mL for *Microcystis* spp, ≥ 10,000 cells/mL for *Nodularia spumigena*; or
- Situation 2: ≥ 10 mm^3/L for the total biovolume of all cyanobacteria where confirmed toxin-producing taxa are not present at concentrations over the Situation 1 thresholds; or
- Situation 3: cyanobacterial scums are consistently present and quick enactment of human health protection measures is desired, or
- Situation 4: cyanotoxin concentrations are ≥ 60 µg/L for anatoxins, ≥ 6 µg/L for cylindrospermopsins, ≥ 24 µg/L for microcystins / nodularins, ≥ 30 µg/L for saxitoxins.

See appendix 6 for further explanation on how the values are derived.

In Situations 1 and 4, when there are multiple toxin-producing cyanobacterial taxa present (Situation 1), or different types of cyanotoxins present (Situation 4), the combined risk needs to be evaluated when determining if the Action Level (red mode) is triggered.

The combined risk only needs to be evaluated for cyanotoxins (and the cyanobacteria that produce them) with the same target organ (the liver or the nervous system).

- Microcystins, nodularins and cylindrospermopsins should be evaluated together as they all accumulate in the liver. Note that the molecular target for cylindrospermopsins, and for microcystins and nodularins, is different, but this is a precautionary approach since robust toxicology work evaluating combined exposure to both toxin classes has not been undertaken.
- Anatoxins and saxitoxins should be evaluated together as they are both neurotoxins that affect nerve transmission resulting in asphyxiation (note that the molecular receptor for each toxin is different, but this is a precautionary approach since robust toxicology work evaluating combined exposure to both toxin classes has not been undertaken).
- *Microcystis* spp, *Nodularia spumigena* and *Raphidiopsis raciborskii* should be evaluated together as, respectively, they produce microcystins, nodularins and cylindrospermopsins, which all accumulate in the liver (note that the molecular target for cylindrospermopsins, and microcystins and nodularins, is different, but this is a precautionary approach since robust toxicology work evaluating combined exposure to both toxin classes has not been undertaken.

The combined risk from multiple cyanotoxins should be accounted for using the ratio of the cell concentration for each toxin-producing taxon or each toxin concentration to the relevant threshold and summing the ratios. If the ratio exceeds 1, then Action Level (red mode) is triggered. Hypothetical example calculations using cell concentration thresholds (Situation 1) and cyanotoxin concentrations (Situation 4) are provided below:

Situation 1 example: if 7,500 cells/mL for *Microcystis* spp and 6,000 cells/mL for *Nodularia spumigena* were detected in a lake, they should be evaluated together as microcystins and nodularins both affect the liver. The ratio for these toxin-producing cyanobacteria would be:

Microcystis spp	7,500 cells/mL ÷ 30,000 cells/mL = 0.25
Nodularia spumigena	6,000 cells/mL ÷ 10,000 cells/mL = 0.6
giving a combined ratio of	0.25 + 0.6 = <u>0.85</u>

As this value is < 1, the Action Level (red mode) threshold is not breached, and this lake would remain at Alert Level (amber mode).

Situation 4 example: if 15 µg/L of saxitoxins and 45 µg/L of anatoxins were detected in a lake, they should be evaluated together as both toxins affect the nervous system. The ratio for these cyanotoxins would be:

saxitoxins	15 µg/L ÷ 30 µg/L = 0.5
anatoxins	45 µg/L ÷ 60 µg/L = 0.75
giving a combined ratio of	0.5 + 0.75 = <u>1.25</u>

As this value is > 1, the Action Level (red mode) threshold is breached for this lake.

Situation 1 example with multiple toxin classes: if 18,000 cells/mL for *Microcystis* spp, 1,000 cells/mL for *Raphidiopsis raciborskii* and 50,000 cells/mL for *Cuspidothrix issatschenkoi* were detected in a lake, the ratio for these toxin-producing cyanobacteria would be:

Microcystis spp	18,000 cells/mL ÷ 30,000 cells/mL = 0.6
Raphidiopsis raciborskii	1,000 cells/mL ÷ 5,000 cells/mL = 0.2

giving a ratio of 0.8 for toxins that primarily affect the liver

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Cuspidothrix issatschenkoi	50,000 cells/mL ÷ 100,000 cells/mL = 0.5

giving a ratio of 0.5 for toxins that affect the nervous system.

The Action Level (red mode) threshold is not breached and this lake would remain at Alert Level (amber mode) because *Cuspidothrix issatschenkoi* does not produce cyanotoxins that primarily impact the same organ as *Microcystis* spp and *Raphidiopsis raciborskii*, and the ratio for *Microcystis* spp / *Raphidiopsis raciborskii* is < 1.

At Action Level (red mode), public health staff should warn the public of the existence of potential health risks. In some regions, local and regional councils might play a role in communicating the health risk with the public (see section 2.4 for suggestions on the roles and responsibilities of each agency). This should be done with (although not exclusively with) media releases, notifications through the communications channels of the responsible agencies in the region, and requesting territorial authorities to erect signs at affected water bodies. Appendix 9 gives an example of information that should be included in a media release, and appendix 10 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, as it is a notifiable disease under the Health Act – Chemical poisoning arising from contamination of the environment. Section 5 provides more information on communicating with the public about toxic algae.

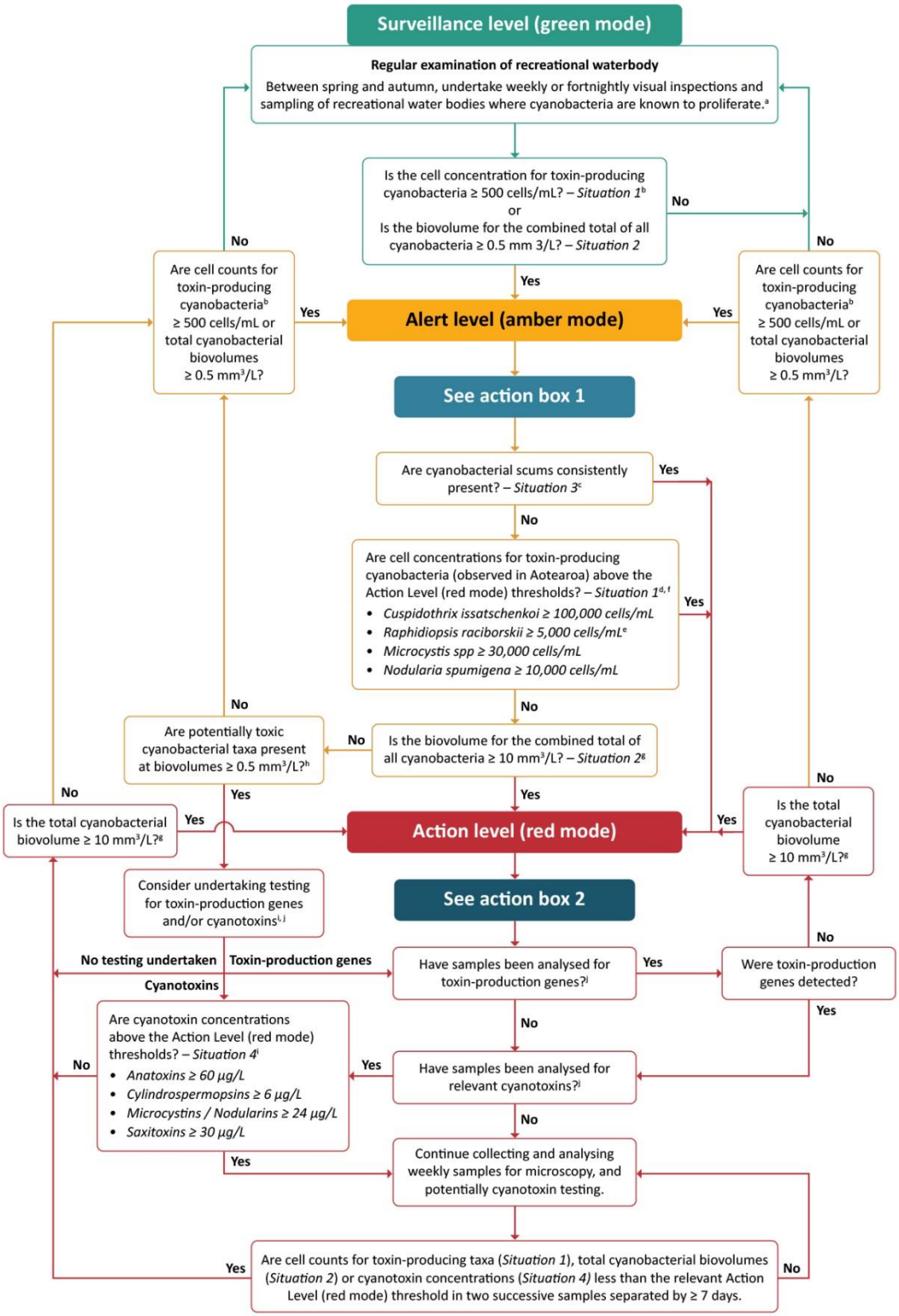
The Action Level (red mode) Situations 1 and 4 guidelines are designed to protect against adverse health effects from repeated exposure to cyanobacterial toxins accidentally ingested during recreational activity. Situation 2 guidelines apply where there is an increased possibility of respiratory, irritation and allergy symptoms from exposure to very high cell densities of cyanobacterial material, irrespective of the presence of toxicity or known toxins. Situation 3 allows for quick measures to protect human health through visual observation of consistent cyanobacterial scums (surface blooms) in a water body, rather than waiting for microscopy analyses before issuing public health warnings. The Situation 3 threshold is linked to the negative human health effects described for Situation 2, as cyanobacterial scums will likely contain cyanobacterial biovolumes > 10 mm^3/L. When the cell concentration thresholds for Action Level (red mode) Situation 1 are used to trigger the Action Level (red mode) – but subsequent analysis undertaken is either negative for cyanotoxin production genes or toxin concentrations are lower than the Situation 4 thresholds – then the mode may revert to Alert Level (amber mode) if the total cyanobacterial biovolume is < 10 mm^3/L.

3.3.4 Changes in alert levels over time

Research has shown that toxin concentrations in a cyanobacterial population can change but it is unlikely to become completely non-toxic within a few days. It is, therefore, recommended that the alert-level is not changed from a higher to a lower level – for example, from Action Level (red mode) to Alert Level (amber mode) – until two successive results (cell counts, biovolumes or toxin measurements) 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 off, their cells break open and release the toxins contained in them. It is, therefore, possible to have elevated levels of dissolved cyanotoxins corresponding with low cell counts. Dissolved microcystins have been shown to persist in water (that is, extracellularly) for up to 21 days in a water body following the decline of a cyanobacterial bloom[41]. When mass lysis has occurred in a water body, toxin testing should be undertaken to ensure that toxin concentrations are below the recommended thresholds. For most toxin-producing cyanobacteria, the cell concentration is a good indicator of expected toxin concentrations; however, this is not necessarily the case for *Raphidiopsis raciborskii* and other cylindrospermopsin producers, as they can actively transport cylindrospermopsin out of the cells[24], which can then persist in the environment. Therefore, where cylindrospermopsin producers such as *Raphidiopsis raciborskii* are observed, toxin testing for cylindrospermopsin should be undertaken even after the cyanobacterial population has declined.

Box 1: Flow diagram for navigating decision making related to the alert-leel framework for planktonic cyanobacteria in recreational freshwaters (Aotearoa)



Flow Chart Notes:

a) In high concentrations, planktonic cyanobacteria are often visible as buoyant green globules, which can accumulate along shorelines, forming thick scums (see appendix 8). 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 *Raphidiopsis raciborskii* and *Cuspidothrix issatschenkoi* are not necessarily visible to the naked eye, and when visible could easily be confused with other types of water discolouration (see appendix 8).

b) Toxin-producing planktonic cyanoabcteria confirmed in Aotearoa: *Cuspidothrix issatschenkoi*. *Raphidiopsis raciborskii*, *Microcystis* spp and *Nodularia spumigena*.

c) As scums will likely contain cyanobacterial biovolumes greater than 10 mm^3/L, Situation 3 allows for quick enactment of measures to protect human health, if desired (see section 3.3.3). Photos of cyanobacterial scums in lakes can be found in Photos B–I of appendix 8.

d) Cell concentration thresholds for planktonic toxin-producing cyanobacteria found in Aotearoa were developed using toxin quotas and the 2020 World Health Organisation guideline values for cyanotoxins in recreational waters (anatoxins, cylindrospermopsins and microcystins; see boxes 3–9 in appendix 6 for more details). When multiple toxin-producing cyanobacteria are present in a water body at the same time, the combined risk of cyanotoxins with the same mode of action should be accounted for using the ratio of each cell concentration to the relevant 'Action Level' thresholds and summing the ratios; if the sum exceeds 1, then the 'Action Level' is triggered (see section 3.3.3 for example calculations).

e) Toxin testing is recommended when *Raphidiopsis raciborskii* is at high levels as cylindrospermopsin can be exported out of the cells and persist in the environment.

f) The cell concentrations of different species of *Microcystis* should be summed to determine if the threshold is breached (for example, *Microcystis* sp, *Microcystis aeruginosa* and *Microcystis flos-aquae*). When cyanobacteria from the genera *Cuspidothrix*, *Raphidiopsis* and *Nodularia* are not identified to species level, they should also be summed (for example, *Cuspidothrix* sp and *Cuspidothrix issatschenkoi* cell concentrations should be summed).

g) Situation 2 applies where high cell concentrations of 'non-toxigenic' cyanobacteria taxa are present and the 10 mm^3/L threshold is to protect human health from the risks associated with other agents produced by or co-occurring with cyanobacteria (see box 10 in appendix 6 for more details).

h) If potentially toxic cyanobacterial taxa (see table A5.1) are present at levels ≥ 0.5 mm^3/L, then samples should be tested for toxin-production genes or cyanotoxins to evaluate the potential risk they pose to human health and to continue growing our knowledge on toxin-producing cyanobacteria in Aotearoa.

i) Cyanotoxin testing is useful to provide further confidence on potential health risks when a health alert is being considered, and to show that residual cyanotoxins are not present when a toxic cyanobacteria bloom subsides. Toxin concentration thresholds are based on the 2020 World Health Organisation guideline values for cyanotoxins in recreational waters (anatoxins, cylindrospermopsins, microcystins and saxitoxins; see boxes 3–6 in appendix 6 for more details). When multiple cyanotoxins are present in a water body at the same time, the combined risk of cyanotoxins with the same mode of action should be accounted for using the ratio of each toxin concentration to the relevant 'Action Level' thresholds and summing the ratios; if the sum exceeds 1, then the 'Action Level' is triggered (see section 3.3.3 for example calculations). When a health alert has been issued using the cell concentration thresholds for Action Level (red mode) – Situation 1, but subsequent analysis shows that toxin concentrations are lower than the Situation 4 thresholds, the mode may revert to Alert Level (amber mode) if the total cyanobacterial biovolume is < 10 mm^3/L.

j) Testing for toxin-production genes provides information on the potential for cyanotoxin production and the types of cyanotoxins that might be present. When a health alert has been issued using the cell concentration thresholds for Action Level (red mode) – Situation 1, but subsequent analysis is negative for toxin-production genes, the mode may revert to Alert Level (amber mode) if the total cyanobacterial biovolume is < 10 mm^3/L.

Action Box notes

Action Box 1

1. Increase sampling frequency to at least weekly because blooms can change rapidly in some water bodies.
2. If possible, multiple sites should be inspected and sampled.
3. Notify public health staff.
4. Consider erecting information signs. To avoid desensitisation and unnecessary avoidance of water bodies that do not pose a health risk, information signs should not contain the 'danger' signals featured in the warning signs (see section 5.5 for more information).

Action Box 2

1. Continue monitoring as for Alert Level (amber mode; see Points 1 and 2 from Action Box 1).
2. Notify the public of a potential risk to human health (see section 5.5 for more information).
3. Samples should be tested for toxin-production genes or cyanotoxins to continue growing our knowledge on toxin-producing cyanobacteria in Aotearoa New Zealand.

3.4 Integrating new technologies into lake monitoring programmes

A range of new and emerging technologies are likely to become useful additions to the monitoring toolbox by enabling *in situ* estimation of cyanobacterial biovolumes, early detection, greater spatial coverage, and additional information such as the genetic potential for toxin production to aid risk assessment (Table 3). Here, we outline some key emerging technologies, and how they might be integrated into a lake cyanobacteria monitoring programme, to complement the best-practice approach described in Decision Chart 1 and the considerations needed when exploring these technologies.

Table 3: Summary of lake cyanobacteria monitoring strategies that might be utilised at different stages in a public health response

Information	Tools
Lake selection Identify lakes suffering from cyanobacterial blooms	 Satellite imagery can help with selection of lakes that suffer from cyanobacterial blooms but are not part of existing monitoring networks. At present, this is limited by lake size, image resolution and the impact of cloud cover. See section 3.4.1 for more information.
Sample site selection Identify high-risk sampling sites at a lake	 Satellite and drone imagery can be used both qualitatively (visual image assessment) and quantitatively (hyperspectral imagery and models) to identify the extent and locations of blooms within monitored lakes. This could be used to select high-risk sites where blooms are present or when setting up new monitoring locations for the first time.
Site visit Rapid on-site estimation of cyanobacterial density Sample collection for laboratory-based measures Assess extent of bloom (if present)	 CyanoFluor™ can be used to rapidly estimate biovolume on-site (see section 3.4.3 for details) and escalate alert-levels if lake-specific biovolume-phycocyanin relationships are available. Corresponding samples can be taken and processed using microscopy to identify potentially toxic taxa. Consider using satellite or drone imagery to delineate bloom extent and identify any further high-risk sites for sampling.
Amber / red mode Gather other information to augment risk assessment Additional site visits and tests as required	 Consider sending samples for toxin gene analysis or toxin quantification to aid in the assessment of the risk posed by the bloom. Continue sampling using the suite of available tools to maintain an up-to-date assessment of the risk.

3.4.1 Satellite imagery

Satellite images may enable more frequent and extensive monitoring of planktonic cyanobacteria in lakes than traditional in-field sampling methods do, due to the large areas and remote locations that can be assessed. This can be particularly helpful in lakes with patchy bloom distributions and where access to lakes for sampling is difficult.

Remote sensing using satellite imagery may allow broadening of the scale and, in some cases, the frequency of cyanobacterial monitoring[42-44]. This technique relies on linking the reflectance spectra of water bodies and the abundance of cyanobacteria[45]. It typically uses the spectral signal of phycocyanin (a cyanobacteria-specific pigment) to infer cyanobacterial abundance or density[46]. It has been used internationally in large lakes such as Lake Erie (USA), Harsha Lake (Ohio, USA) and Lake Taihu (China)[47-49]. Models using species-specific reference spectra are in development and early field-testing; for example, the work of Legleiter et al (2022)[50], where reference spectra generated for a range of cyanobacteria were used to discriminate the dominant cyanobacterial taxa throughout a lake. A limitation of these approaches is that cyanobacterial pigment composition and concentrations can change depending on their growth stage and the growing conditions (for example, irradiance and nutrient status[51]), which can impact the ability to robustly discriminate spectra.

Research into generic models to estimate cyanobacterial blooms from satellite data is ongoing. The most advanced cases are currently in certain lakes where the bloom-forming species are known, and lake-specific algorithms linking spectral reflectance signals to cyanobacterial biovolumes can be developed using field data. For lakes where field data and satellite imagery are available, models or calibrations can be developed that approximate cyanobacterial biovolumes in the lake. This could be used as an early warning system to trigger targeted on-site sampling, if thresholds are exceeded, that indicate a bloom may be present. Alternatively, satellite images may be visually assessed for the presence of cyanobacterial blooms in lakes known to experience highly coloured surface blooms, which could either trigger additional sampling or provide information about bloom extent to inform a response. Because satellite imagery cannot penetrate deep into lake water, some cyanobacterial species that do not form surface blooms may be missed.

The choice of imaging platform depends on the site and application of monitoring. Satellite imagery can provide more frequent data with minimal effort from those undertaking the monitoring, as the images are collected automatically when satellites pass overhead. These images, however, require significant processing to generate usable input data due to atmospheric distortion and cloud cover that need to be excluded from the images. Frequent passes are likely to be required to garner useful images without cloud cover. Image resolution from satellites can limit the lake size. If the resolution is not sufficiently detailed, the pixels can contain elements from the surrounding land (for example, trees and grass) that interfere with predictions of cyanobacteria. This means that many small lakes cannot yet be assessed using these methods, but as satellite technology improves and becomes cheaper, this will become less of a limitation.

3.4.2 Drone imagery

Drone-based imagery can be used to collect similar data as satellite imagery, and so overcomes several issues mentioned above. As the cameras are closer to the lake surface, the spatial resolution is higher, and this approach can be used in lakes that are too small for current satellite imagery to assess. Drones fitted with hyperspectral cameras (rather than a

conventional RGB camera) also have the potential to discriminate between cyanobacteria and other microalgae based on spectral reflectance, as described above, for satellite imagery.

Because the use of drones requires lakes to be visited in person, this will limit sampling frequency; however, using them could be a valuable addition to the assessment toolbox to determine the spatial extent or localisation of blooms in lakes as part of routine monitoring. Many drones can now also be equipped to collect samples, and this may be useful if offshore sampling is required.

3.4.3 On-site fluorometric assessment of total cyanobacterial biovolume

Handheld chlorophyll and phycocyanin fluorometers designed to measure the concentration of cyanobacterial pigments using their fluorescence signal (for example, the CyanoFluor™) can be used to infer cyanobacterial biomass[52]. Although submersible probe fluorometers have been previously evaluated for the same purpose, results were not sensitive enough to distinguish the Alert Level (amber mode) – Situation 2 threshold[53, 54].

For in-field use of the CyanoFluor™ (or similar handheld chlorophyll and phycocyanin fluorometers), a small subsample of water (two to three millilitres) is taken, placed into a cuvette and read in the fluorometer. The fluorometric device then provides measurements of chlorophyll and phycocyanin RFUs (raw fluorescence units), with phycocyanin fluorescence being used as a proxy for cyanobacterial abundance. Consistency among samples is improved by lysing the cells using a sonicator, which breaks open the cells and distributes the pigments more evenly throughout the liquid. If a field sonicator isn't available, multiple replicates (for example, three replicate subsamples) should be evaluated, and the results averaged to reduce the impact of analytical variability.

Several steps need to be taken to integrate the use of handheld fluorometry into an already established biovolume monitoring programme for cyanobacteria. Firstly, there can be inter-device variability in fluorometric output data among different handheld fluorometers (including different CyanoFluor machines from the same manufacturer). To overcome this, each device needs to have its unique conversion factor to convert the output phycocyanin RFU to a standardised phycocyanin concentration (that is, µg/L). To calculate this conversion factor, the concentration of a phycocyanin extract sample (for example, from spirulina) is determined by spectrophotometry. The same phycocyanin sample is then diluted to obtain a standard curve (ideally ranging from 0.01 µg/L to 1,000 µg/L). Our recommendation is to measure three separate standard curves, with each phycocyanin concentration measured in triplicate, to obtain an average phycocyanin RFU for each phycocyanin concentration. Linear regression analysis is used to examine the relationship between phycocyanin RFU and phycocyanin concentration. The conversion factor is determined by calculating the gradient of the line, with the intercept set to zero. An example of this is provided in Thomson-Laing et al (2020)[52].

The use of phycocyanin concentration, as measured using a handheld fluorometer, to assess cyanobacterial biovolume in water samples requires robust field validation. Phycocyanin measurements using a handheld fluorometer should be made in parallel with conventional microscopy and biovolume measurements on lake water samples throughout regular monitoring seasons. It is recommended that this parallel measurement approach be implemented for individual lakes, and across a seasonal timeline, that encompasses changes in cyanobacterial abundance and community composition as pigment concentrations in cyanobacteria can be species- and condition-specific[52-54]. Ideally, this timeline should cover at least two repeated monitoring seasons. The approaches need to be as consistent and

repeatable as possible, with in-field sonication recommended as part of the fluorometry protocol.

Linear regression analysis should then be used to examine the relationship between phycocyanin and cyanobacterial biovolumes. Ideally, this is done separately for each lake due to inter-lake variability in cyanobacterial abundance and community composition. When assessing the performance of these linear models, there should be a significant correlation ($p < 0.05$) between phycocyanin and biovolume, and an r^2 value > 0.7 is recommended. A lower r^2 value signifies increased variability in the data around the linear relationship and can lead to more misassigned samples to the correct alert-level thresholds when using phycocyanin as a proxy.

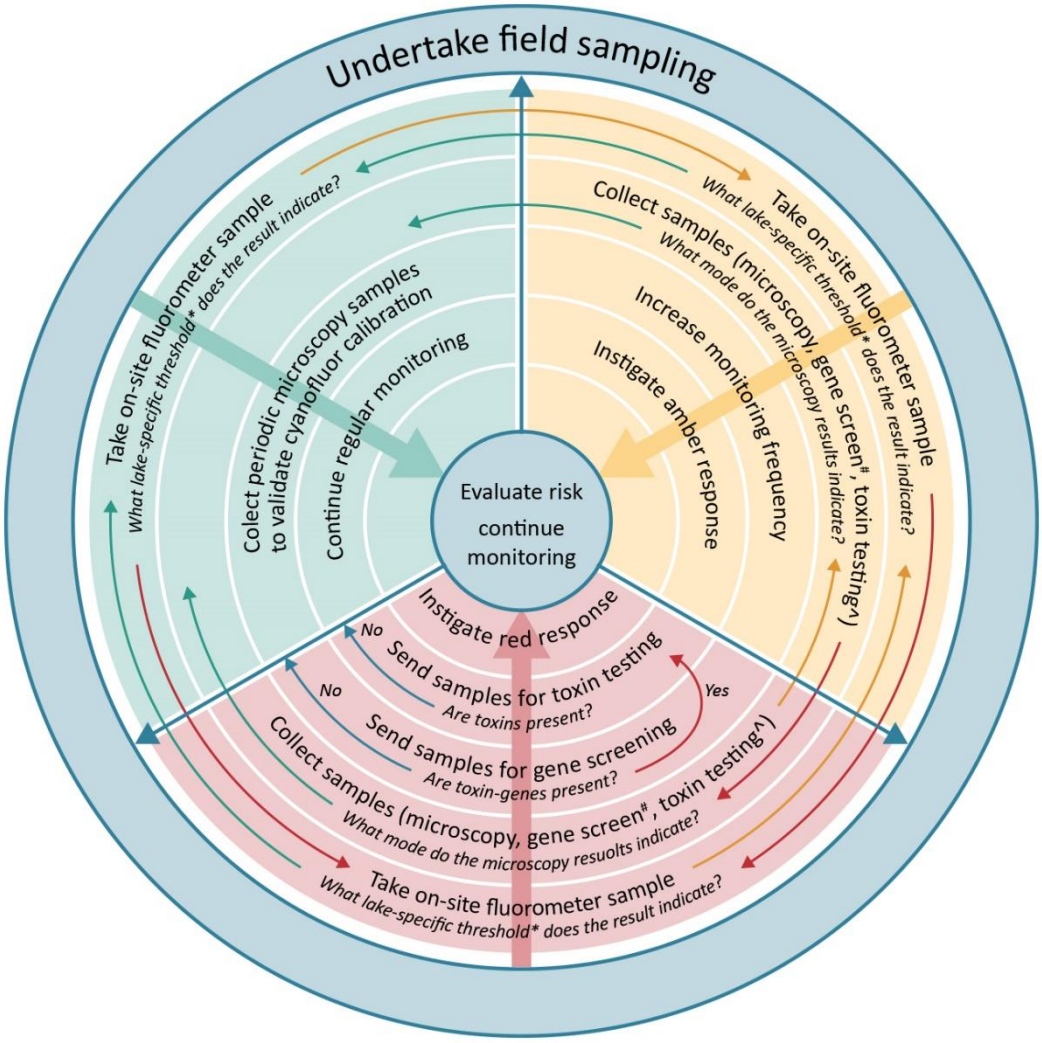
If the linear relationship is performing well, it can be used to develop lake-specific phycocyanin thresholds, equivalent to the biovolume thresholds for planktonic cyanobacteria (those described in Situation 2 of Decision Chart 1). Thomson-Laing et al (2020)[52] demonstrated that a model encompassing multiple lakes led to 74 per cent of samples being assigned to the correct Alert Level (amber mode) but recommended that lake-specific phycocyanin-biovolume thresholds should be developed because of clear differences in phycocyanin response between lakes.

On-site fluorometers have several advantages over traditional microscopy-based approaches; in particular, results are available immediately. If combined with pre-defined phycocyanin RFU thresholds for each lake, this enables point-of-sampling results and identifies immediate action that need to be taken if thresholds are exceeded. There are, however, some limitations to the data available using an on-site fluorometer. As no taxonomic analysis is undertaken, the results only reflect the total cyanobacterial community, irrespective of whether or not they are potential toxin producers. Although this limits its use in triggering the Situation 1 thresholds, the on-site measurements could be used to trigger the alert-level thresholds through Situation 2 (total cyanobacterial biovolume), allowing warning signs to be installed without waiting for samples to be processed by a lab. The on-site fluorometer measurements would also be useful for determining when a lake is still in Surveillance Level (green mode), and microscopy samples are not necessarily required.

Figure 1 provides an example of how the on-site fluorometer might be integrated into the alert-level framework for planktonic cyanobacteria.

Samples for microscopy should still be collected and analysed in Alert Level (amber mode) to determine if toxin-producing cyanobacteria are present at levels that reach the Action Level (red mode) and trigger this threshold through Situation 1. Periodically collecting and analysing microscopy samples will also allow the calibration of the phycocyanin RFU model for the lake to be maintained over time.

Figure 1: Example of integrating on-site fluorometer measurements (for example, using the CyanoFluor™) into a cyanobacteria monitoring programme for recreational public health



The diagram is read from outside to the inside within an alert mode, unless a result indicates a shift between alert modes (indicated by the small arrows). In all modes, the emphasis is on using the information collected to evaluate the human health risk and respond accordingly.

* = See Thomson-Laing et al (2020)[52] for more information on the calibration of lake-specific biovolume thresholds.

^ and # = sections 4.5.2 and 4.5.3 (respectively) for details on sample collection for toxin testing and gene screening.

Part B: Benthic cyanobacteria

3.5 Benthic cyanobacteria: Introduction

These guidelines are designed to manage risks to *recreational users*. They have been designed to protect users from the risks associated with the ingestion of water and mats. The levels given in these guidelines are not relevant for addressing risks to dogs that actively seek out and consume cyanobacterial mats. Appendix 6 provides further explanation on how the values are derived.

Benthic mat-forming cyanobacteria are widespread throughout rivers in Aotearoa and are found in a wide range of water-quality conditions, including oligotrophic waters[12, 55]. The most common mat-forming benthic cyanobacteria genus in Aotearoa is *Microcoleus* (previously *Phormidium*). During stable flow conditions, *Microcoleus* mats can proliferate, at times forming expansive black-brown leathery mats across large expanses of river substrate (see appendix 11). Flow conditions, substrate, water chemistry and species composition can influence the macroscopic appearance of benthic cyanobacterial mats (see appendix 11), and at times they may easily be confused with other algal groups (for example, diatoms or green algae). Microscopic confirmation of the dominant organisms in benthic mats should be undertaken by either competent regional council staff or by a laboratory with microalgae identification expertise (see appendix 12).

Dog deaths associated with the consumption of benthic cyanobacteria have become increasingly common around Aotearoa[13-15, 37]. In most instances, these deaths have been associated with the presence of anatoxins[13], which often results in the rapid death of the animal. The production of microcystins by benthic cyanobacteria in Aotearoa (*Nostoc* sp and *Planktothrix* sp) has also been observed[16, 21], and in at least one instance a dog death was caused by microcystins[16]. Saxitoxin production by benthic cyanobacteria (*Scytonema* spp) has been reported in Aotearoa lakes[34, 56]. The production of nodularin by unidentified benthic cyanobacteria in Lake Tikitapu | Blue Lake (Rotorua) has also been reported[57, 58]. In other parts of the world, benthic species are also known to produce cylindrospermopsins[59].

Recent research suggests that the presence of cytotoxic (toxic to cells) compounds are affecting mammalian cells from multiple *Microcoleus* species collected around Aotearoa. Therefore, health warnings should not rely solely on the presence of known toxins. In-depth studies of the spatial and temporal distribution of *Microcoleus* mats in rivers around Aotearoa have shown that toxin concentrations within mats can vary markedly among sampling sites and over short timeframes (for example, a week[15, 60-63]). It has also been demonstrated that the presence and concentrations of anatoxins within the mat are not related to the abundance of the *Microcoleus* mats[15, 17]. 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 the edges of waterbodies (see appendix 11). During these detachment events, the risk to human health is higher, due to the accessibility of the cyanobacterial mats to water users and the increased likelihood of ingesting floating mat particles. The highest risk to water users is through ingestion of the cyanobacterial mats, as the toxin concentrations in the mats can be very high[12, 62]. The likelihood of this occurring increases during detachment events, as the mats are accessible on the edge of the water body. Although it is not expected that an adult would

intentionally consume the cyanobacterial material, there is a real possibility that a child playing at the water's edge might do so. Although not well understood, direct contact with cyanobacterial mats (for example, touching and handling without gloves) should be avoided as a precaution. The risk associated with detached *Microcoleus* mats and their associated toxins floating into estuaries and coastal environments is also not well understood.

Research on the quantity of anatoxins released from *Microcoleus* mats into the surrounding river water indicates that concentrations in the water are unlikely to reach levels that pose a human health risk during recreational activities[62]. Traditional water column sampling (taking a grab sample) only provides a snapshot 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)[64] has been applied to assess anatoxin released into overlying water in river environments[62]. While SPATT samplers do not provide a quantitative measure of the concentration of anatoxins present in the water, they are a useful and economical tool for early warnings of the presence of extracellular toxins in rivers.

Although not specifically covered in these guidelines, benthic cyanobacteria do occur in lakes and ponds, where they have caused animal fatalities[18, 65]. As observed in rivers, benthic cyanobacteria mats in lakes can also detach and accumulate on shorelines (see appendix 13, figure A13.2B). Where this occurs in recreational areas, it is recommended that samples be collected for microscopic identification, gene screen and cyanotoxin analysis, and that the percentage of affected shoreline is estimated. Further information on toxin-producing benthic cyanobacteria in lakes around Aotearoa is provided in appendix 13.

3.6 Alert-level framework: Benthic Microcoleus in rivers

This section of the guidelines specifically covers benthic *Microcoleus* in rivers and streams. As noted in table 2 (section 3.2) and table A5.1 (appendix 5), other toxic benthic cyanobacteria have been detected in rivers and lakes in Aotearoa and elsewhere in the world (see Appendices 13 and 14 for more information). Limited data currently prevent guidelines being developed for other benthic toxin-producing cyanobacterial taxa (such as *Nostoc* spp, *Planktothrix* spp and *Scytonema* spp) or for the occurrence of benthic toxin-producing cyanobacteria in lakes and ponds. If such problems are encountered in recreational freshwaters, access expert advice on how to evaluate and manage the situation, and health risks, through the National Public Health Service.

Decision Chart 2: Alert-level framework for benthic Microcoleus in rivers (see section 2.4 for the recommended framework for roles and responsibilities relating to actions, and the information provided in section 3.7 for advice on interpreting the guidance in this table).

Alert-Level[a]	Actions
Surveillance level (green mode) **Situation 1:** Up to 20% coverage[b] of *Microcoleus* attached to the river 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 level (amber mode) **Situation 1:** 20–50% coverage[b] of *Microcoleus* attached to the river substrate.	Increase monitoring frequency to at least weekly.[c] Notify public health staff. Consider erecting a sign that provides the public with information on the appearance of mats and the potential risks. Consider increasing the number of survey sites to enable risks to recreational users to be more accurately assessed. Consider testing samples for cyanotoxins or toxin-production genes.[d]
Action level (red mode) **Situation 1:** Greater than 50% coverage[b] of *Microcoleus* attached to the river substrate; or **Situation 2:** Up to 50% coverage,[b] but *Microcoleus* mats are visibly detaching from the substrate, accumulating as scums along the river's edge or becoming exposed on the river's edge as the water level decreases.	Continue monitoring as for Alert Level (amber mode).[c] Immediately notify public health staff. Notify the public of the potential risk to health. Consider testing samples for cyanotoxins or toxin-production genes.[d]

a. The alert-level framework is based on an assessment of the percentage of riverbed that Microcoleus mats cover at each site. However, local knowledge of other factors that indicate an increased risk of toxic cyanobacteria (for example, human health effects, animal illnesses and prolonged low flows) should be used when assessing a site status and may, in some cases, lead to an elevation of site status (for example, from Surveillance Level to Action Level), irrespective of mat coverage.

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

c. Benthic Microcoleus proliferations can grow rapidly in some water bodies, hence the recommended weekly sampling regime.

d. Cyanotoxin and toxin-production gene testing is useful to provide further confidence on potential health risks when a health alert is being considered.

3.7 Details of the framework: Benthic Microcoleus in rivers

3.7.1 Surveillance level (green mode)

Surveillance Level (green mode) is the lowest alert-level. Benthic cyanobacterial mat coverage of < 20 per cent will be common in many rivers and is unlikely to pose human health risks when mats are not actively detaching. Site surveys should be conducted as described in section 4.4. Microscopic identification may need to be undertaken on samples to confirm the presence of *Microcoleus*. Weekly surveys should be performed at representative locations along the river from spring to autumn and during peak recreational use periods. A single site 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 3.8) might be used to trigger the Alert Level (amber mode).

3.7.2 Alert level (amber mode)

The Alert Level (amber mode) is triggered when there is 20 per cent to 50 per cent coverage of *Microcoleus* mats attached to the substrate. The Alert Level (amber mode) requires notification and consultation with public health staff for ongoing assessment of the status of the cyanobacterial proliferation (see section 2.4 for guidance on roles and responsibilities). 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 but high cyanotoxin concentrations are detected, the risk level might be increased to Action Level (red mode).

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 at different recreational sites.

The Alert Level (amber mode) is also a good time to raise public awareness of the potential risk to water users. Section 5 provides more information on communicating with the public about the risk from toxic cyanobacteria in recreational freshwaters. Installing information signs that provide the public with information on the appearance of mats and the potential risk should be considered (see appendix 15 for some examples).

3.7.3 Action level (red mode)

The Action Level (red mode) is triggered when representative site surveys and sampling reveal either greater than 50 per cent coverage of *Microcoleus* mats attached to the substrate; or where up to 50 per cent of the available substrate is covered by *Microcoleus* mats and these are visibly detaching from the substrate, accumulating as scums along the river's edge or becoming exposed on the river's edge as river levels drop.

At Action Level (red mode), public health staff should warn the public of the existence of potential health risks. In some regions, local and regional councils might play a role in

communicating the risk with the public (see section 2.4 for suggestions on the role and responsibilities of each agency). This should be done (although not exclusively) through media releases, notifications through the communications channels of the responsible agencies in the region, and requesting territorial authorities to erect signs at affected water bodies. Appendix 17 provides an example of information to include in a media release, and appendix 18 provides a warning sign template for benthic *Microcoleus* in rivers. 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, as it is a notifiable disease under the Health Act – Chemical poisoning arising from contamination of the environment. Section 5 provides more information on communicating with the public about toxic algae.

Based on data from planktonic cyanobacteria (for example[38–40]), there is an increased likelihood of gastrointestinal, respiratory, eye-irritation and allergic symptoms from exposure to large amounts of cyanobacterial material, irrespective of toxicity or of the presence of known toxins. This background forms the rationale for Action Level (red mode) Situation 1. As benthic cyanobacterial mats detach, they can accumulate along a river's edge. Because of the increased availability of these mats (since they can end up on the edge of the waterway), and the increased likelihood of accidental ingestion, this is a period of high risk regardless of the percentage coverage in a water body (see Action Level – Situation 2, Decision Chart 2).

3.7.4 Changes in alert-levels over time

It is recommended that the Action Level (red mode) is not changed from a higher to a lower level (for example, from Action Level to Alert Level) until the percentage cover falls below the Action Level threshold on two successive surveying occasions (collected at weekly intervals).

3.8 Benthic Microcoleus and river flows

A correlation between benthic *Microcoleus* mat abundance, water temperature and a lack of 'flushing flow' conditions has been observed in some rivers[13, 15, 66]. In some instances, the length of time since a flushing flow event can be used as an early warning of elevated risk of benthic *Microcoleus* proliferations. However, the flow velocity required to shift *Microcoleus* mats from the riverbed will vary depending on factors such as the riverbed substrate type and size. For example, a river with a sandy substrate will require a markedly smaller flow to flush benthic *Microcoleus*, compared to a river with a large cobble substrate. In addition, the length of time required for *Microcoleus* to proliferate following a flushing flow event will vary. If these data are available, it might be possible to develop site-specific models which can assist with guiding monitoring and management requirements (for example References 61 and 67).

3.9 Integrating new technologies into river monitoring programmes

3.9.1 Satellite imagery

Because the resolution of satellite imagery is currently coarse, this precludes its use in assessing or monitoring benthic cyanobacteria in rivers, although this may change in the future as technologies improve.

3.9.2 Drone imagery

Drone-based aerial imagery has great potential to be integrated into monitoring workflows because it can be obtained when visiting a site for other measurements and because drones are relatively low cost. Although the use of drones and automated image-assessment tools is promising, there are limitations inherent with the technology, and considerable research is needed to establish the relationships between cyanobacterial cover over entire reaches and the risk to human health[68]. While these relationships are being established, drone imagery might be used to assess a much larger area of riverbed than visual surveys conducted with bathyscopes, which may augment visual survey data by extending them to areas not typically monitored. The images and video collected using drones might also be useful for public communication and public education initiatives, and to increase public engagement through warnings posted on social media.

There are limitations to images obtained using drones, meaning they may not be appropriate in all circumstances. These include sites where the river is turbid or highly coloured, the water is too deep, and where significant overhanging vegetation impedes riverbed views. Time of day can also be an important consideration due to reflections from the water surface. A major limitation for some sites is the presence of controlled airspace and the need for trained drone pilots. Despite these limitations, drones can provide useful information on the extent of blooms and a visual estimate, even if quantitative analysis of the images is not used. This will help in understanding which areas of the river are subject to cyanobacterial proliferations.

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 waders) 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. Organisations should develop standard operating procedures to mitigate any potential risks.

4.2 Biosecurity

The procedures detailed in this section involve entering water bodies that may contain aquatic pests or invasive species such as didymo (*Didymosphenia geminata*), *Lagarosiphon*, alligator weed (*Alternanthera philoxeroides*), lake snow (*Lindavia*), *Egeria* or hornwort *(Ceratophyllum demersum)*. When someone leaves a water body, all equipment and clothing should be decontaminated to restrict the spread of these organisms. In addition to aquatic pests, other biosecurity considerations may be required when crossing private land to access water bodies; for example, the bacterium *Mycoplasma bovis* is found on farms. Although most recreational waterways will be entered across public land, consider regular engagement with private landowners if this is not the case. For up-to-date information on invasive species and biosecurity threats in Aotearoa, as well as decontamination protocols, field samplers should check the Ministry for Primary Industries website.

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 are 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 while other species may excrete toxins into the surrounding 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 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 help focus the monitoring programme on critical periods and locations in the water body of interest. The sampling protocols outlined below will 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 (2020)[2], and protocols for sampling for ecological and other studies are provided by Pridmore (1987)[69], Codd et al (1999)[70] and Hötzel and Croome (1999)[71].

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 consistent data through time, and can be 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.

- 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 reveal sites most likely to harbour scums or 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 (for example, 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-centimetre depth-integrated column (hosepipe) samples collected relatively randomly along a 20–30 -metre transect (parallel to the lake shore) and mixed into a single container (for example, a bucket). From this composite sample, subsamples are taken for the cell counts, phycocyanin fluorometry measurements, gene screening and toxin analysis or both.

The rationale for this sampling is that:

- the 50-centimetre 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-centimetre zone also covers the accumulation of buoyant cyanobacteria near the surface under calm conditions

- the recommendation for five pooled samples accounts for spatial variability within a single site.

The volume of each subsample required will vary, and care needs to be taken to mix the water in the container well before taking each one (so that they all contain the same cell density). When sampling eutrophic lakes, 100 millilitres is usually sufficient for cyanobacterial identification and 500 millilitres for cyanotoxin analysis. In oligotrophic lakes, two 200-millilitre samples are required: one for identification and one for cyanotoxins (see sections 4.5.1 and 4.5.2). For cyanotoxin gene screening, a 500-millilitre sample collected into a sterile sampling container is usually sufficient (see section 4.5.3). Samples for microscopy should be preserved with Lugol's iodine (see appendix 22), whereas samples for cyanotoxin or gene screen analysis should not be preserved (see section 4.5). Measurement of phycocyanin concentrations using handheld fluorometers usually requires only two to three millilitres of water and can be conducted on-site.

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

When wading, or when boat access is not available, the alternative is to collect a pooled surface-grab (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
- photos of cyanobacteria or algae observed can also be useful.

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.

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

(surface, depth or integrated depth) or the place or time of collection. An example of a typical field sheet is provided in appendix 20.

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 per cent) 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 (completion of the survey form and sample collection, appendix 21).

4.4.1 Site selection and collecting background site information

Refer to sections 2.3.3 and 4.3.1 for key factors that should be considered when selecting sampling sites. *Microcoleus* mats (the most prevalent type of benthic cyanobacteria observed in Aotearoa) tend to proliferate initially in riffles (sections of river where rocks create water turbulence at the surface) then runs (wadable sections of river without riffles), so priority should be given to examining these habitat types.

On the first visit to the site, choose a 40–60 -metre 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 (bed substrate type – such as cobbles, gravels, sand-silt)
- water velocity
- amount of shade at each survey reach
- bank vegetation (descriptive – this could be captured photographically)
- hydrology (such as the time since the last flood, 2× and 3× median flow).

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 viewers or bathyscopes (Figure 2) are commercially available. 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 21): forms should preferably be printed on waterproof paper.
- Sampling containers and permanent marker pen or equivalent (for labelling).

Figure 2: Using an underwater viewer (or bathyscope) to visually assess periphyton growth

Photos: SA Wood (Cawthron).

For health and safety and logistical reasons, the survey should be undertaken in teams of two: one observer and one scribe.

All monitoring should be undertaken under similar flow conditions (for example, 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.

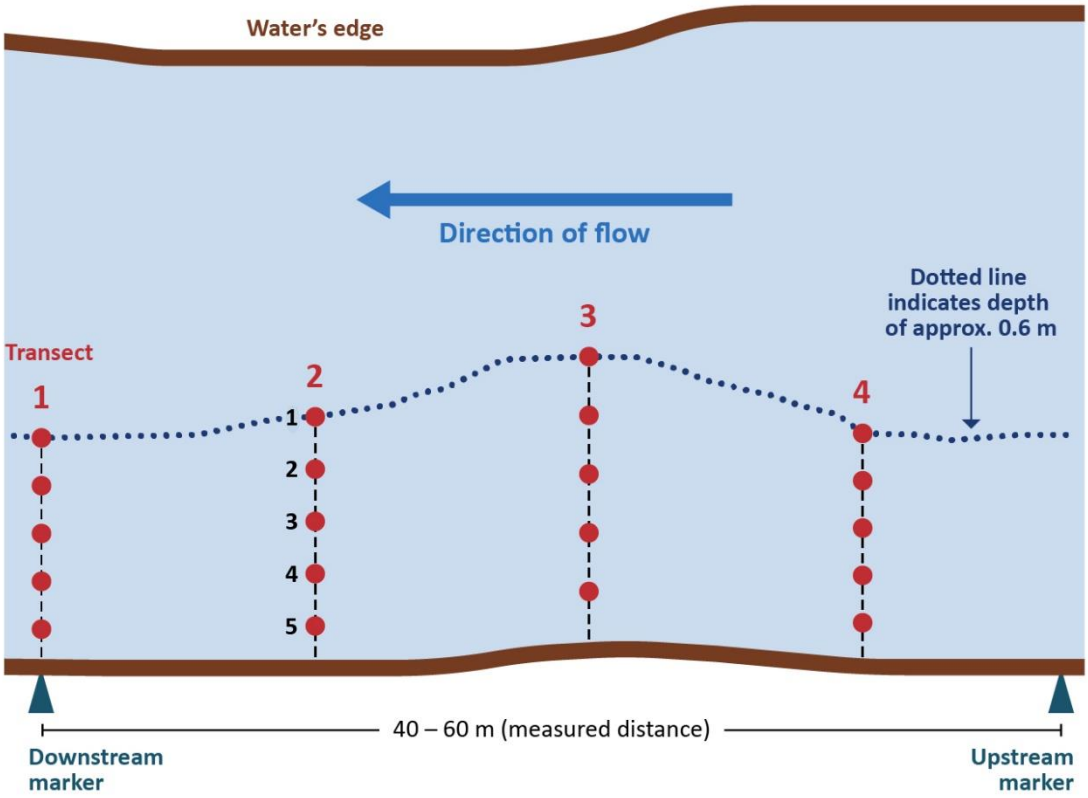
4.4.3 Monitoring procedure

1. After arriving at a survey area, spend approximately 5 minutes looking along a 30−60 -metre section of the riverbed for 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 metres apart.
2. Complete the first section of the monitoring form (appendix 21) with site, date, time and other relevant details, and note the general presence or absence of cyanobacterial mats 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 metres (see figure 3 and figure 4) and A standard maximum depth of 0.6 metres 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.
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 centimetres 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 5. Cyanobacterial mats are usually dark black, dark brown or dark green in colour, are leathery, and have an earthy, musty odour. Refer to appendix 11 for a photographic guide to benthic *Microcoleus* and other benthic algae commonly observed in rivers around Aotearoa. Coverage should only be recorded if mats are greater than one-millimetre thick, although it is useful to record the presence of thin mats.
6. If there is any doubt about the identity of the mat cover (that is, whether it is *Microcoleus*) at any sampling point, take a sample for microscopic identification. Samples should be collected by scraping an egg-sized clump of the mat into a sampling pottle. Samples for microscopy should be preserved with Lugol's iodine (see appendix 22), whereas samples for cyanotoxin or gene screen analysis **should not** be preserved (see section 4.5). Because toxin content can vary markedly between rocks within a site[17, 62, 63], take 10 samples from separate rocks or stones (where possible) and pool these for cyanotoxin and gene screen analysis or both. If the amount of toxin within an area needs to be estimated, the sample should be taken from a known area of the rock (for example, 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 (for example, point one is always the deepest into the water and point five is always closest to the water's edge; (see figure 3 and figure 4). Record at which sites samples were taken (if any). Record any notes regarding other algal cover (for example, green filaments overgrowing cyanobacterial mats).
8. Space the points evenly along the transect to a depth of 0.1–0.15 metres nearest to the water's edge, although this depth will vary according to the type of river. For example, if the riverbank is incised (channelled), the closest survey point will be deeper.
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 3.6) and the appropriate actions taken.

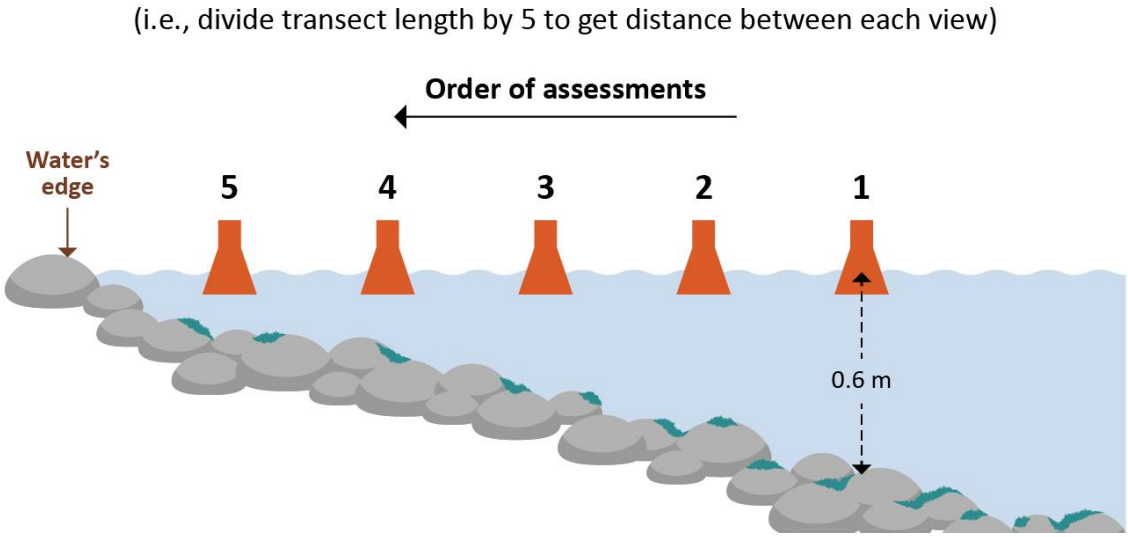
The frequency of samples collected at any one location is dictated by the alert-level framework (section 3.6).

Figure 3: **Schematic layout of transects (numbered in red) and survey areas (red circles, numbered in black) at a site (not to scale)**



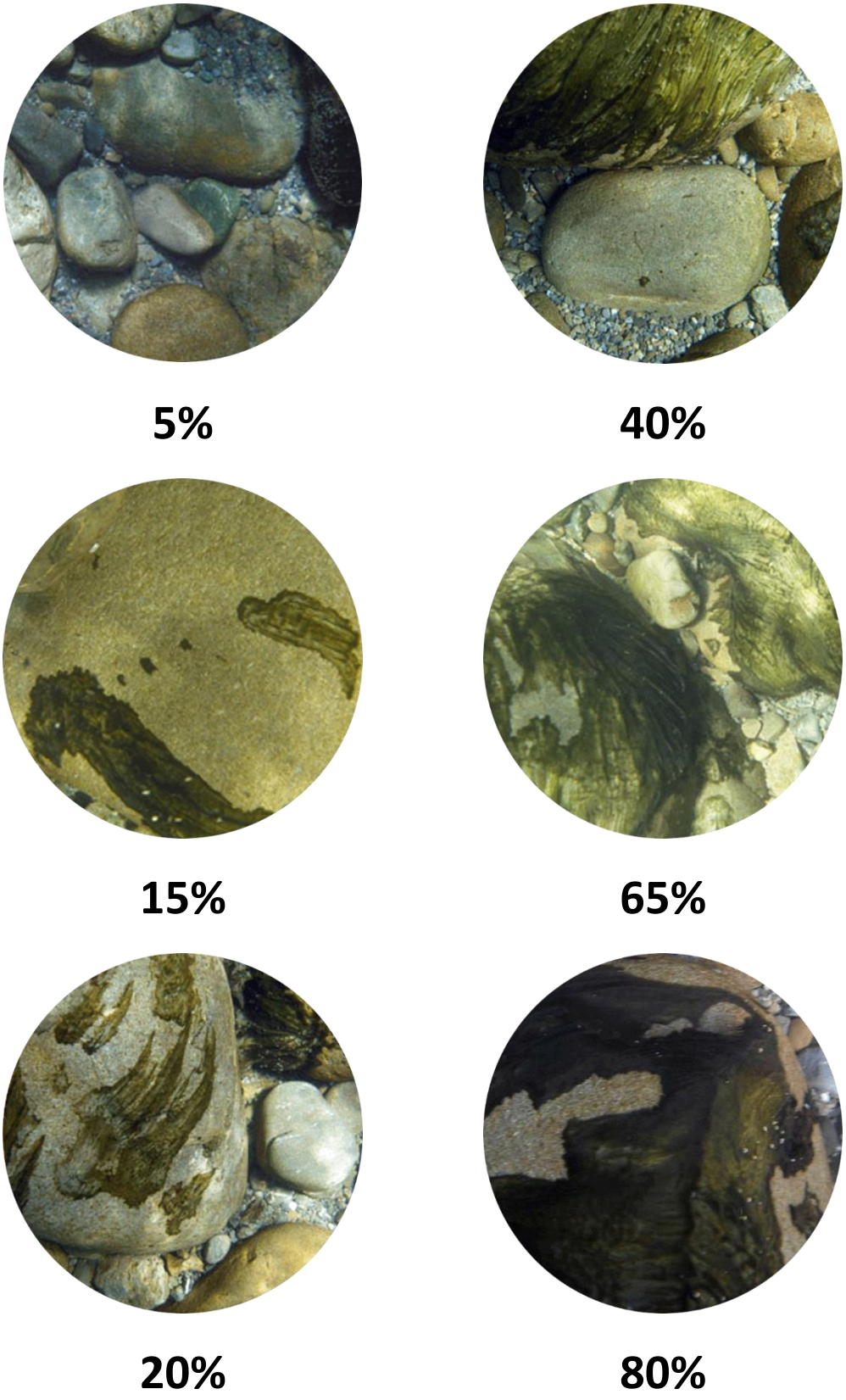
Notes: The numbering indicates the order in which assessments are made and corresponds to the numbers on the monitoring form (appendix 21). The transects are spaced evenly along the survey reach. It may not always be possible to have five viewer results (for example, in steep-sided rivers). In these circumstances, take as many views as practical per transect. If the river does not exceed 0.6 metres in depth, the transect should span its entire width. Reproduced from originals by C Kilroy (NIWA).

Figure 4: **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. **Reproduced from originals by** C Kilroy (NIWA).

Figure 5: Examples of different levels of cyanobacterial cover viewed through an underwater viewer



Photos: M Heath (Victoria University).

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 12) well before sample collection.

4.5.1 Cyanobacterial identification and enumeration

Subsamples should be preserved as soon as possible after collection by the addition of approximately one per cent Lugol's iodine preservative (appendix 22). Lugol's is added drop by drop until the sample is the colour of beer or weak tea (approximately 4 drops per 100 millilitres in water). Dense samples (for example, scum material or benthic mats) will absorb Lugol's and may require additional Lugol's if long-term storage is required. In that case, check samples at approximately monthly intervals and add more Lugol's if the colour fades.

Samples should be stored in the dark. Some plastic bottles (polyethylene) tend to absorb iodine very quickly into the plastic, so take care 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 a note as to whether Lugol's has been added.

4.5.2 Sampling for cyanotoxin testing

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 millilitres of water should be collected. Benthic samples should be collected as described in section 4.4.3 (Point 6).

Cyanotoxins can degrade, both photochemically (in light) and microbially. Samples should be transported in dark, cold conditions, and kept refrigerated prior to analysis. If samples for toxin analysis will not reach the analytical laboratory within 24 hours, they can be stored frozen. Note, however, that freezing releases cyanotoxins from the cells, so only the total amount of toxins in a sample can be determined. If information on dissolved toxin is required, filter samples before freezing.

4.5.3 Sampling for cyanotoxin gene screen testing

Samples for gene screen analysis should be stored in sterile sample bottles. If non-sterile bottles are used, these should be sterilised using a two per cent bleach solution for a minimum of 10 minutes, then rinsed thoroughly with water and dried. The volume of sample required depends on the type of analysis. For planktonic samples, at least 500 millilitres of water should be collected. Benthic samples should be collected as described in section 4.4.3 (Point 6).

DNA can degrade with exposure to high temperatures, ultraviolet light and microbial activity. Samples should, therefore, be transported in dark, cold conditions and kept refrigerated prior to analysis. If samples for gene screen analysis won't reach the analytical laboratory within 24 hours, they can be stored frozen.

Section 5. Communications

5.1 Background

Since the *Interim Guidelines for Cyanobacteria in Recreational Freshwaters* were introduced in 2009, the way that councils and public health staff communicate with the public about toxic cyanobacteria has evolved. Originally, warning signs and press releases were the main communication mechanisms, but nowadays a range of communication methods have been added: digital platforms, social media and public engagement.

A successful communication programme on the risk posed by toxic cyanobacteria in recreational freshwaters relies on two interlinked facets:

- continual education of communities on the potential risk from toxic cyanobacteria, what to look out for and how to stay safe, and
- responsive communication of warnings and the associated escalation of health risks for water users.

The information provided below, and in the cited appendices, is intended to act as a guide to developing a communications plan about the risk of toxic algae in recreational freshwaters, and to document successful approaches that have been undertaken in Aotearoa to date. Because every region in Aotearoa faces different issues, and has different communities to reach, each operates using a slightly different response framework and has different resourcing available – there is no one-size-fits-all approach.

It may be that only certain approaches described here are adopted in your region, however, we do encourage you to think about:

- Who do you want to reach? – *your target audiences*
- What do you want to let them know? – *your key messages*
- How are you going to get the message to them most effectively? – *the communication mechanisms you adopt.*

As there is already much good content out there in Aotearoa, we suggest using the examples provided here, and contacting colleagues from other regions, as a basis for developing content for your region. At times, you will need to tailor the material and content to the specific issues you see in your region, or to make it relevant to audiences in your region, but starting from a working example will you help do this more efficiently.

5.2 Communications plan

A communications plan is a great way to get everyone to work together and to make day-to-day communications activities easier. Because health risks associated with toxic algae are predominant in the summer months, a communications plan provides you with a good starting point and minimises the impacts associated with staff turnover during the winter months.

A communications plan should document your goals, key messages, target audiences and how you plan to communicate with your target audiences (your communication mechanisms), as well as information on how the plan will be actioned (by whom and when). A well-developed

communications plan should also tailor messages and communications mechanisms to different target audiences. Some examples include:

- targeting dog owners using dog-registration information or providing information to local vet clinics
- targeting water users through water recreation clubs, community groups and recreation forums (for example, Facebook groups)
- targeting landowners who have access to the waterway through direct communication (especially when the waterway is not easily accessed by the public).

The communications plan for your region would, ideally, be collaboratively developed by the responsible agencies in your region, and involve communications staff as well as water-quality scientists and public health professionals (see section 5.3 below for more information). The communications plan can be revised and expanded each year, and lessons from previous years should be incorporated.

5.2.1 Example key messages

Below are examples of key messages consolidated from those currently used by Land, Air, Water Aotearoa (LAWA), councils, and HNZ. Although these will provide a solid basis to develop key messaging for a toxic algae communications plan, they should be adapted to be more relevant to your region.

- Focus on the issues encountered in your region; for instance, if benthic cyanobacteria in rivers is the main issue in your region, then concentrate your messaging on this.
- Draw on regionally specific information; for instance, direct people to your monitoring data rather than using a generic description.
- Provide people with useful local information; for instance, direct people to a safe alternative swimming location rather than just saying they can't go swimming.

We have used 'toxic algae' throughout this section and in the examples of key messages below; however, some regions in Aotearoa also use the term 'potentially toxic algae'. This is to avoid confusion for the public between messaging on bacterial contamination of waterways (such as *E. coli*) and cyanobacteria in waterways – especially in rivers, where the key times to avoid swimming are different for each hazardous organism. (Avoid swimming after periods of rain because of bacterial contamination, and avoid swimming after periods of no rain because of toxic algae levels.) In areas where *Microcoleus* (benthic cyanobacteria) in rivers is an issue, care should be taken with the use of 'blue-green algae' as the public can be confused that the colour mentioned doesn't match that of the black-brown mats. Regardless of the term chosen, use it consistently in your communications to avoid confusing the public with multiple terms which refer to the same thing.

General messaging on toxic algae:

- Toxic algae can produce toxins that are harmful to humans, dogs, livestock and wildlife when ingested.
- Toxic algae are naturally occurring and live in our lakes and rivers.
- Toxic algae are more common in the warmth of summer – posing increased health risks to humans and animals during this time – but can also occur at other times of the year.

- Play it safe – if you see toxic algal blooms in rivers or lakes, avoid contact and choose another site to swim at, don't drink the water, do not collect food (kai) from the waterway, and wash hands before consuming food.
- If you think you are experiencing a serious reaction to toxic algae, seek urgent medical attention and advise the doctor of the potential exposure to toxic algae; also ask them to notify the local Public Health Service.
- If your dog shows signs of toxic algae poisoning – including lethargy, muscle tremors, fast breathing, twitching, paralysis and convulsions – treat it as an emergency and contact your vet immediately. You or your vet can report any animal illness resulting from contact with toxic algae to your local council.
- The best thing you can do is to stay informed. Look for the latest monitoring results of toxic algae, check for any warnings or alerts before you head to a river or a lake, and learn what to look out for.

Benthic cyanobacteria in rivers:

- Toxic algae in rivers grow as dark brown or black mats attached to the rocks and stones on the riverbed (also called benthic).
- Toxic algae can occur in low-nutrient 'clean' rivers.
- Stable river flows and warm temperatures encourage the growth of toxic algae in rivers.
- At times, the toxic algae mats can become detached and wash up on the riverbank where they are easily accessible to children and dogs.
- Toxic algae mats have a strong musty odour that can attract dogs to eat them.
- If there has been an alert or warning issued, or you have seen toxic algae, keep your dog on a lead and away from the water's edge to ensure it does not eat any algal mats.
- Benthic toxic algae that grow in rivers differ from harmless bright green algae, which often form long filaments.

Planktonic cyanobacteria in lakes:

- In lakes and slow-flowing waters, toxic algae grow in a free-floating form (also called planktonic) which can cause the water to become murky or cloudy.
- Planktonic cyanobacterial blooms are generally greenish in colour and can give lakes a 'pea soup' appearance.
- They can also form visible green films or 'scums' on the water's surface, especially at the water's edge.
- If there has been an alert or warning issued, or if the lake water looks green, cloudy or discoloured, it is better to be cautious and wait until the water clears before swimming in the lake.

5.2.2 Example communication mechanisms

Communication mechanisms have changed rapidly over the past decade and will continue to change heading into the future. Social media has allowed engagement with a wide array of people at a low cost, while also helping retain ownership of the content and messaging. Although public engagement through traditional media is on the decline, it still reaches many people and shouldn't be overlooked – and don't ignore the value of information and warning signs on toxic algae (as they reach audiences who are directly interacting with an at-risk water body). Public engagement with different communication mechanisms can be regionally specific, so collecting data on engagement levels is important for understanding where to prioritise communications efforts in your region. For example:

- information and warning signs (see Figure 6)
- social media posts
- direct communication – for example, emails, letters, phone calls and text messages
- media interviews on radio and television, or in newspapers to discuss toxic algae warnings or providing general information in the lead-up to summer
- website content – general information on toxic algae, factsheets, FAQs, and information supplied in the LAWA 'Can I swim here?' module
- pamphlets
- information videos – for example, Cawthron's Toxic Algae in our Rivers video
- seminars, either for the wider public or targeted to specific audiences
- information sessions – for example, a stall at a local science day, on a popular dog-walking route or at a water recreation event
- advertisements to push content and messages including on radio, newspapers, magazines, online advertising, social media and billboards

Linking content through different communication mechanisms is a powerful way to engage with a wide array of audiences and get your message across. This might involve:

- directing people to a central source for information such as LAWA or the council website through (for example) social media posts, and media articles
- including information videos in media articles through an embedded link
- QR codes on information or warning signs to push people to web content or reporting apps (for example, iNaturalist).

Figure 6: Information (left) and warning (right) signs used in the Nelson region

5.2.3 Example target audiences

Regions may differ when it comes to which target audiences are the highest priority, depending on their waterways and cyanobacteria issues. New target audiences may be identified with time and experience, for instance:

- recreational water users
- parents
- tangata whenua
- interest groups
- dog owners
- dog care professionals (for example, vets, groomers or the SPCA)
- children (who may be excellent communicators to the adults in their lives)
- internal staff (enabling them to spread the right message).

As discussed above in section 5.2.1, tailoring key messaging and communication mechanisms towards a high-priority target audience is the gold standard for communications; however, general messaging and communication mechanisms will still reach and help inform a range of audiences.

5.3 Collaboration

As discussed in section 2.4, a successful programme that monitors cyanobacteria and provides a public health response requires collaboration between the different agencies in different regions. This is also very important for a successful toxic algae communications programme. An inter-agency collaboration to develop, review and revise a toxic algae communications plan for your region means that everyone can work together and agree to the messaging adopted. This makes it much more likely that consistent messaging and content will be disseminated in your region, avoiding confusion among your communities.

Another advantage of taking a collaborative approach is that different agencies in a region will have better relationships and connections with certain parts of the community – and so a particular agency might take the lead on connecting with key stakeholders (referred to as 'target audiences' earlier in this section). For example, territorial authorities in a region might have access to the contact information for registered dog owners in their districts and could directly communicate information with them, whereas public health staff might have good connections with iwi or hapū associated with a certain water body. A collaborative approach may also be helped by the sharing of resourcing and specialist skillsets (for example, expertise in communications, videography, content development, social media and photography).

In the area of toxic algae communications, another important collaboration is between the subject experts (water-quality scientists and public health staff) and the communications staff at their agencies. The expertise of communications personnel will not only be valuable during the development of a communications plan (and its related content), but early engagement will also make implementation of the adopted approach easier. Involving communications staff in inter-agency planning meetings will help them to run an effective communications programme.

5.4 Continual community education

Councils cannot monitor every water body in Aotearoa, and the potential exists for high variability in cyanobacteria density or coverage within waterways. Therefore, educating communities on the risk posed by toxic algae in their waterways helps them to make decisions independent of public health warnings. Education also helps reduce apprehension about potential risks that are not always present and so encourages people to interact with their waterways when they are safe to use. Because of the positive benefits associated with recreation, water users should not be discouraged from using recreational water bodies when there is a low risk or no risk.

An exemplar case study on toxic algae community education from the Wellington region is provided at the end of this section (box 2). Greater Wellington and the responsible agencies from the Wellington region used a combination of written information, signage, video, social media, traditional media, public seminars, digital communication platforms and advertising to communicate information on toxic algae to their communities. It is not expected that all regions would use the same approach, but the case study might provide inspiration for strategies that could be adopted in your region.

As a minimum, a council website should provide information on toxic algae. This could include a factsheet with information on the risk from toxic algae, what it looks like and what to do if someone encounters it (see appendix 18 for an example). The same information might also be provided as a physical pamphlet to provide at local information centres, council receptions, and veterinarian practices in the region (see appendix 19 for an example).

Frequently asked questions (or FAQs) can be a means of supplying more detailed information on specific elements of the topic. Appendix 23 is a collation of FAQs on toxic algae and the associated responses, which may provide a starting point for website content. FAQs are also very useful for frontline staff (at call centres or reception) who may get queries from the public about toxic algae.

Responsible agencies might also take a more active approach to educate the public by using social media, public seminars, advertising and videos. When developing this communications content, remember to link back to the key messages in your communications plan, and think about who your high-priority audiences are and how to best reach them.

5.5 Responsive communication of health warnings

As described in sections 3.3 and 3.7, the communication of public health warnings occurs when the Action Level (red mode) threshold has been breached. Warnings about the potential health risk should be communicated to water users through a combination of warning signs, media releases, and other useful communication mechanisms at the disposal of the responsible agencies, such as social media, website information (including LAWA) and direct communication.

At this point, primary messaging should focus on the potential health risk, what to do to stay safe, and what to do if people think that they (or others in their care) have been exposed to cyanotoxins; for example:

- Tests carried out by [name of agency] have found potentially dangerous levels of toxic algae (cyanobacteria) at [name of water body].
- Humans and animals should avoid contact with [name of water body] until health warnings are removed.
- Toxic algae produce toxins that are harmful to humans and animals if swallowed and, in some cases, through contact with skin when swimming or undertaking activities such as [insert likely activities for the affected water body].
- Exposure to water containing toxic algae has been reported to cause symptoms such as skin rashes, nausea, tummy upsets, and tingling or numbness around the mouth or fingertips.
- If you have had contact with contaminated water and experience health symptoms, visit a doctor immediately and contact [name of agency].
- Boiling water does not remove the toxins, and drinking contaminated water should be avoided.
- Fish and shellfish can concentrate toxins, so their consumption should be avoided. If fish are eaten, remove the gut and liver and wash in clean water.
- For further information, visit [website address] or contact [contact information].

To increase awareness that toxic algae could also be a problem in other unmonitored sites, you might also include broader information on toxic algae and their occurrence in our waterways as secondary messaging. This might include messaging described in section 5.2.1

on, for example, how toxic algae and cyanobacteria are naturally occurring, why they are more prominent in warmer periods of the year, and what to look out for. As mentioned earlier, tailor key messages so they are relevant to the issues experienced in your region.

Warning signs erected in response to a health warning should have clear ‘danger’ signals (bright contrasting colours, exclamation points and clear warning graphics) and inform the public of what they should do to stay safe (see Appendices 10 and 16 for some examples). Links to further information, and images of what to look out for, are also useful. To avoid ‘message de-sensitisation’, remove the warning signs when the health warning is no longer in effect. To educate water users on toxic algae, some regions will have permanent information signs at sites where blooms are frequently observed (see appendix 15 for some examples). These should not contain the ‘danger’ signals featured in the warning signs, but have a more neutral aesthetic.

Box 2: Case study on toxic algae communications practices in the Wellington region

Case Study

Greater Wellington's Evolution in Toxic Algae Communications

In November 2005, a dog that had been swimming in Te Awa Kairangi / Hutt River suffered a sudden and unexpected death. This was followed by four more dog deaths in the Hutt Valley during the summer period. These dog deaths were the catalyst of Greater Wellington's toxic algae communications journey that has evolved over the course of the last 18 years.

The Power of Collaboration

These dog deaths shocked the local community and led to the formation of an inter-agency group to respond to cyanobacteria poisoning cases in the region, and better manage the risk to human and animal health. The inter-agency group included Greater Wellington, Upper Hutt City Council, Hutt City Council, Masterton District Council, Kāpiti Coast District Council and the Public Health Service of Te Whatu Ora. In 2007, the group established a traffic light framework and a monitoring programme – which was also later adopted in the interim 'recreational cyanobacteria guidelines'.

During the early stages of the response, the communications component relied predominantly on warning signs at rivers, press releases and traditional media (newspapers and radio; although media coverage of warnings was minimal). However, dogs continued to die in the region and the public were uneasy about the situation. Feedback from the Wellington public included that:

- People weren't aware of health warnings for the river – they didn't hear about them in the media or they didn't see the warning signs.
- The current warning signs weren't effective – people ignored them because they were up all the time.
- People were avoiding using the river – even when it was safe to swim and walk dogs.
- In general, people had little understanding about toxic algae and what to look for.

To address this feeling of unease, protect the community and encourage safe association with rivers in the region, the inter-agency group developed a targeted Toxic Algae Strategy and Communications Plan which continued to evolve over the following decade. The Strategy and

Communications Plan aimed to:

1. Educate the Wellington community about how to stay safe when accessing rivers in the region.
2. Connect with the Wellington community through a wider range of communication mechanisms.

A critical factor in the Wellington region's successful work on toxic algae communications is the collaboration between the various responsible agencies from the Wellington region. Throughout the past decade, the inter-agency approach has provided clarity on who is doing what, enabled sharing of resources and expertise, and has allowed consistent messaging throughout the Wellington region. It's proved more efficient than each district doing their own thing and potentially confusing the public with mixed messages.

Communication Evolution

Warning Signs

The public feedback recieved in the Wellington region indicated that warning signs that stayed up all year led to de-sensitisation of their messaging. To fix this, permanant signage for the Wellington region was re-developed to have a less intimidating look and be educational in nature - i.e., it provided information on toxic algae and what to look for. The messaging on the new signage included:

- That toxic algae could be present.
- Showed people what toxic algae looked like.
- Told people where to go for more information.
- Later versions of the signage also told people what to do if a person or dog swallowed toxic algae.

Information (left) and warning (right) signs for toxic algae in rivers around the Wellington region. Information signs are permanently installed at sites where toxic algae is commonly observed and warning signs are erected when toxic algae reaches potentially dangerous levels.

Greater Wellington embraced digital platforms to educate their community on the risks from toxic algae and to communicate river monitoring data and toxic algae warnings in their region.

Only when the "Action" threshold (50% cover) was breached, were the traditional "danger" warning signs utilised (see image overleaf) to warn the public about dangerous levels of toxic algae in the river, and care was taken to ensure these signs were removed after the toxic algae bloom had disappeared (usually after a decent rainfall event had flushed the river clean).

Public Education

In 2013/2014, the Wellington region undertook a series of public education seminars to inform their communities on the risks from toxic algae, what to look out for and how to stay safe. Whilst these were well recieved and well attended (60-80 people attending each session), with one million people visiting Te Awa Kairangi / Hutt River each year, the reach was too limited. To broaden its reach, the information from the public seminar was compiled into a factsheet that was freely-available online on the Greater Wellington website.

To increase engagement with the information on toxic algae, a 2-min web-based video was developed. This provided clear and concise messaging on the risks and what to look out for. The video was complimented by the more comprehensive information available on the website and through FAQs.

Going Digital

In 2014, 'Is it Safe to Swim?' was launched in the Wellington region. This was a 'one-stop-shop' for recreational water quality information including an interactive map displaying monitoring data and the current warnings in effect for each monitoring site in the region. This allowed for traffic to be directed to one location instead of multiple pages of warnings and water quality data. So that the community knew this information existed, active promotion of this site was undertaken through traditional channels, social media and even back of bus advertising .

Greater Wellington captured drone footage of cyanobacterial blooms in Te Awa Kairangi / Hutt River to demonstrate the extent of cyanobacterial blooms via social media.

Like many other regions, the Wellington region have now centralised toxic algae warnings and data on the LAWA 'Can I swim here?' module (alongside other recreational water quality warnings/data). Web traffic is directed here as a single source for information.

Social Media

In 2015/2016, Greater Wellington began using social media (Facebook, Twitter and Neighbourly) to share recreational water quality information, especially warnings about toxic algae in the region. These platforms were used to communicate information on any recreational water quality issues in the region, including where toxic algae is present and algae levels. They were also used as an opportunity to reinforce the key messages (i.e., it can't hurt you if you don't eat it, dogs are most at risk, and know what to look for). These posts were usually put out towards the end of the week to inform people ahead of the weekend.

During the past seven years of communicating toxic algae information on social media, Greater Wellington has experimented with different approaches to captivate and engage audiences. One successful campaign developed a persona around a Pug called Tank. Tank provided weekly advice to other dogs (and their owners) in a 'first person' perspective. This allowed Greater Wellington to communicate more informally with people, contributing to record engagement levels, along with the associated sharing and tagging of content amongst social media users. Another benefit of the Tank content was that the interactions and comments were very positive and appreciative. Whilst toxic algae is a serious issue and not to be trivialised, there are very real advantages to taking an "infotainment" approach in a world where people are saturated with information and will quickly move on if their attention is not captured.

The use of drone footage capturing the large cyanobacterial blooms in Te Awa Kairangi / Hutt River (see image above) has also had great engagement on Greater Wellington's social media channels. One of these posts has had 66,000 views and 1,200 shares, generating a 'reach' of several hundred thousand people. But the visuals from the drone footage have also done a great job in demonstrating to the public that whilst a monitoring site might have no toxic algae present, upstream could be inundated – reinforcing the need for people in the community to know what it looks like and not to rely on site warnings being relevant to the entire length of the river.

Some learnings from Greater Wellington's work communicating with the wider public on social media included:

- that social media encourages conversations, so you need to be prepared to continue the conversation and respond to people's messages/comments;
- most comments will be positive and inquisitive (especially when they are more informal or take an "infotainment" approach), but a small proportion won't and you need to have a strategy for how to respond to these;
- video and picture content are viewed far more than text alone, which plays very well with toxic algae education where you want people to know what it looks like;
- people want advice not just information and respond well to information on alternatives rather than soley focussing on restrictions (waterways that are safe/free of toxic algae they can access in the region);
- that you need to keep putting up new content to maintain engagement with social media users (and you can't just recycle old content); and
- that entertaining your social media audience whilst educating them leads to better. engagement with the content – they interact with it, digest all the information and share it with others.

For Greater Wellington, social media provided a very effective way to communicate with the wider public about toxic algae and recreational water quality. It costs less than conventional advertising campaigns, but reaches far more people – whilst thousands of people might be reached using their earlier conventional toxic algae advertising campaigns, tens-of-thousands to hundreds-of-thousands were reached using social media. They were also able to magnify their reach through posting engaging and entertaining content. Paid promotion of content on social media platforms has also proved a very cost-effective means of extending content reach, especially when targeting certain geographic areas.

Key Messaging

Whilst key messages might vary between regions (see Section 5 of the Recreational Cyanobacteria Guidelines) and the key messages used in the Wellington region have also developed during the past ten years, the key messages initally adopted were:

1. Know what toxic algae looks like and avoid it.
2. Toxic algae can't hurt you if you don't eat it.
3. Dogs are most at risk because they love the smell and will try to eat it.

Because Greater Wellington can't monitor for toxic algae in every waterway in their region all of the time, they wanted their community to be educated on how to protect themselves, their children and their pets, and to not be overly fearful of the potential risk from visiting rivers in their region.

We have presented these intial key messages and goals here because they are still relevant and will be useful for regions that are just beginning to develop their own toxic algae communications plan. We anticipate that many regions will have region-specific messaging that they would include. Their messaging will also evolve with increased awareness amongst their community and additional resources being developed.

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Glossary

Term	Meaning
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 cyanobacteria	Grow attached to the substrate of lakes, ponds, rivers and streams.
Cyanobacteria	A phylum of bacteria (also called blue-green algae) 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.
ELISA	Enzyme-linked immunosorbent assay – an antibody-based method used to measure cyanotoxins.
Eutrophication	Degradation of water quality due to enrichment by the nutrients 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 (for example, 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, particularly water.
LC-MS	Liquid chromatography-mass spectrometry – an analytical method used to measure cyanotoxins.
Macroscopic	Large enough to be seen by the unaided eye.
Microcystin	A hepatotoxic cyanotoxin produced by a range of cyanobacteria.
Monomictic	A lake that mixes through its entire depth once a year.
Neurotoxic	Toxic to nerves or nerve tissue.
Nodularin	A hepatotoxic cyanotoxin produced by the planktonic cyanobacterium *Nodularia spumigena.*
Oligotrophic	A water body with low primary 'productivity'. These water bodies are typically clear and have high water quality due to low algal biomass from low nutrient concentrations.
Periphyton	The mixture of algae, cyanobacteria, heterotrophic microbes and detritus found attached to submerged surfaces in most aquatic ecosystems.
Planktonic cyanobacteria	Grow free-floating (drifting) in the water body.

Polymictic	Lakes in which the water column undergoes frequent periods of stratification and remixing (common in shallow lakes).
Pool	A deep, slow-moving region of a river, usually with fine substrate, often containing eddies.
Prokaryote	An organism where the nucleus is not clearly defined (bacteria and cyanobacteria, but not microalgae, plants, fungi or animals).
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 known as paralytic shellfish poison.
SPATT	Solid-phase absorption toxin tracking – a passive sampling technique used to detect low levels of toxins or to integrate multiple pulses of toxins released into water.
Stratification	The formation of separate layers (of temperature, plant or animal life) in a water body. Each layer has similar characteristics (for example, all water in the layer has the same temperature).
Toxigenic	Produces toxin/s.

Appendix 1
Cyanotoxin accumulation in aquatic organisms

Cylindrospermopsins, microcystins, nodularin and saxitoxins can accumulate in a variety of freshwater and marine organisms[72-75]. When this occurs, warnings to avoid consuming aquatic foodstuffs or mahinga kai should be included in media releases and on warning signs (Appendices 9, 10, 16 and 17), and made in collaboration with the Ministry for Primary Industries (MPI), which is responsible for food-related illnesses.

In Aotearoa, microcystin accumulation has been noted in rainbow trout and kākahi (freshwater mussels)[76]. The accumulation of nodularin has been found in kōura (freshwater crayfish)[58] and tuna (eels)[28]. Feeding studies have also shown microcystin accumulation in kākahi and kōura[77]. In local and international research, microcystin accumulation has been recorded in aquatic plants[78, 79] and in land-based crops irrigated with microcystin-contaminated water[78, 80-83]. As with microcystins, the accumulation of nodularin has also been found in plants[84]. Results in Aotearoa are similar to international studies of the accumulation of nodularins and microcystins in aquatic foodstuffs[85]; that is, toxin concentrations in kōura, trout and tuna were highest in the hepatopancreas and the liver, but toxin was also found in the flesh.

Although there is no official recommendation on a safe level of cyanotoxins in aquatic foodstuffs in Aotearoa, Mulvenna et al (2012)[86] proposed 'safe levels' for human consumption. Based on these values, the toxin levels observed in most samples analysed from trout and tuna flesh in Aotearoa[28, 76] are unlikely to result in adverse health effects when eaten as part of a regular balanced diet. However, when cyanobacterial blooms are more intense, toxin levels will be higher. In one instance during the Wairewa study of nodularins in tuna[28], toxin levels in the flesh exceeded the 'safe level' suggested by Mulvenna et al (2012)[86].

As a precaution, fish, tuna and kōura collected from water bodies experiencing toxic cyanobacterial blooms should be gutted and thoroughly washed in clean tap water before eating. The organs of fish and tuna should not be eaten because of the higher levels of toxins that accumulate in the liver. Shellfish should not be collected from lakes where there are toxic cyanobacteria blooms, or the estuaries and rivers that they flow into, because toxins concentrate in the edible portions.

The downstream effects of water bodies containing cyanobacterial blooms should also be considered. For example, Lake Ōmāpere (Northland) experiences blooms containing microcystins, and flows into the Hokianga Harbour where microcystins have been found in shellfish. Cultural harvest of mahinga kai in the Kaituna River and Maketu estuary (Bay of Plenty) has previously been affected by cyanobacterial blooms in Lakes Rotorua and Rotoiti.

For further advice on appropriate levels of cyanotoxins in aquatic organisms, contact New Zealand Food Safety (part of the Ministry for Primary Industries) and refer to its website information.

Appendix 2
Effect of climate change on cyanobacterial blooms

Scientific forecasts predict that rivers, lakes and other water bodies will experience increased water temperature, thermal stratification and water column stability, with more extreme flooding and longer droughts. These changes may increase the risk of cyanobacterial blooms, through the following effects:

- Climate change is likely to cause general warming of freshwater lakes and rivers, leading to increased extremes in water temperature in summer[87]. Higher water temperatures may favour bloom-forming cyanobacteria whose growth rates increase faster than other phytoplankton[88]. Warmer surface waters will increase vertical stratification and the chance for buoyant cyanobacteria to accumulate at the surface[89]. Buoyant cyanobacteria can form dense surface blooms, enhancing their ability to shade out and dominate other phytoplankton.
- Rising temperatures and more stratified waters are expected to increase the availability of sediment-bound nutrients as well as decreasing bottom-water oxygen, thereby enriching the water column with nutrients. Increased numbers of extreme storms can cause greater nutrient and sediment runoff into lakes and rivers[90]. Both scenarios will result in nutrient enrichment, further promoting cyanobacterial growth and bloom formation[91] in conditions that may also promote toxic over non-toxic strains of cyanobacterial species (see appendix 5). More deposits of sediment in rivers associated with more storms may also promote benthic cyanobacterial blooms[92].
- Climate change is predicted to increase the intensity of the El Niño–Southern Oscillation (ENSO) cycle. An increase in its typical westerly winds is likely to lead to more rainfall in western regions and drought conditions in eastern regions of Aotearoa[93]. Extended droughts may result in longer periods of stable flows, increasing the probability of benthic cyanobacterial blooms in rivers for example,[15, 60].

For more information on the predicted effects of climate change on toxic freshwater cyanobacteria in Aotearoa, see Puddick et al (2022)[94].

Appendix 3
Management of freshwater cyanobacterial blooms

Managing cyanobacteria is usually part of a wider strategy that extends across whole catchments to manage nutrient loads to receiving water bodies[95]. Diverse actions can contribute to a comprehensive management programme of lakes, streams and rivers. Recent review articles on lake restoration and management[96, 97], and 12 papers on restoration in a special issue of the *New Zealand Journal of Marine and Freshwater Research*[98], provide guidance on some of the options for freshwater aquatic systems that could reduce the frequency and magnitude of cyanobacterial blooms.

Monitoring of cyanobacteria is often part of a broader assessment that may be required for state of the environment reporting, detection of environmental trends, and to evaluate the effects of management actions. Monitoring can also provide input data for models shown to be useful for allowing different management scenarios to be simulated (for example,[99]). These are sometimes as a prerequisite for understanding the high costs of investing in specific management actions[100].

Most catchment-based actions are long-term investments in the health of receiving waters in order to reduce nutrient loads and thus mitigate occurrences of cyanobacterial blooms[101-104]. They often take some time to show positive results[105]. Catchment-based actions can be complemented with in-lake options when there is a pressing and immediate problem with lake health or water quality[105]. Such management approaches have been successful for cyanobacteria management in Lake Rotorua (Bay of Plenty), balancing the time scales for achieving long-term reductions in external (catchment) nutrient loads with short-term actions that can alleviate severe cyanobacteria blooms[106]. Trying to treat acute symptoms of lake health degradation with in-lake treatments (sometimes known as lake geoengineering) usually has high levels of risk and high costs; it also puts managers in a difficult position of trying to evaluate operations or products that can have a strong commercial imperative but limited scientific assessment. Where these interventions are successful, the problem returns unless external nutrient loads are also sufficiently reduced.

What follows is a brief review of scientific literature and a commentary on in-lake options to manage cyanobacterial blooms. The options are those that attempt to deal with symptoms and directly remove cyanobacteria (such as flushing, ultrasound, hydrogen peroxide and booms) and those that attempt to deal with the primary causal factors that interfere with the environmental conditions that trigger blooms (for example, by removing nutrients or promoting mixing).

Flushing

Flushing is only effective when water residence times can be reduced to a point where phytoplankton are flushed from the lake at a rate that exceeds the generation time for new biomass production[96]. Not achieving a sufficiently low water residence time (< 20 days) to prevent bloom formation may actually increase phytoplankton biomass because it enhances the flux of ‘new’ (external) nutrients to a lake. A high proportion of these nutrients are likely to

be in bioavailable forms compared with the existing proportions in lake water. In hydro lakes, flushing can be a protective mechanism against cyanobacteria blooms, except where the lakes have poorly flushed side-arms[107]. In most natural lakes, there is limited capacity (water availability) to increase flushing rates to a level that directly controls cyanobacterial blooms.

Inflow diversions

The best-known case of a major inflow diversion is the Ōhau Channel wall, in Lake Rotoiti, Bay of Plenty. It takes water from the Ōhau Channel before it enters Lake Rotoiti and diverts it towards the Kaituna River outflow, bypassing most of the lake. This water originates from Lake Rotorua, and the nutrients and cyanobacteria that it carries were considered to have had a major role in the severe cyanobacterial blooms in Lake Rotoiti in the early 2000s[97]. Another inflow diversion case is the Lake Ngarotoiti (Waikato) inflow diversion from Lake Ngaroto to the lake outlet. Modelling suggests that the diversion has had a modest impact on the water quality of Lake Ngaroto[108].

Phosphorus inflow locking

'Phosphorus locking' involves dosing water with a chemical such as alum [$KAl(SO_4)_2 \cdot 12(H_2O)$] to bind with (flocculate) and precipitate phosphorus. It has been used in the Utuhina Stream inflow to Lake Rotorua (Bay of Plenty) since 2006 and in the Puarenga Stream inflow to Lake Rotorua since 2010[106]. This method is an interim measure while 'excess' catchment nutrient loads are addressed. The operation has involved 'overdosing', so there is enough aluminium to fully precipitate with phosphate (PO_4^{3-}) and any unspent aluminium will remain active in locking phosphorus within the lake itself. Consideration needs to be given to a range of anions and cations that may interfere with the phosphorus flocculation process by alum, the potential to acidify poorly buffered stream and lake water, and localised in-lake accumulations from sedimentation of flocs around the inflows that are dosed with flocculant[109].

Ultrasound

The use of ultrasound is being promoted for cyanobacteria control by some distributors of ultrasound equipment. There are no reviews of ultrasound treatments specific to Aotearoa, but research and reviews have been carried out overseas[110, 111]. Ultrasound devices are purported to collapse the gas vesicles that allow cyanobacteria to float to the surface of a lake. The loss of gas vesicles would reduce light capture and inhibit photosynthesis, while breaking down cyanobacteria cells. The most authoritative and scientifically based research on ultrasound indicates, however, that it is highly unlikely to have any control effect of cyanobacteria in natural systems unless the cyanobacteria cells are very small and extremely high-intensity ultrasound is applied.

Hydrogen peroxide

As with ultrasound, there are no working examples in Aotearoa of hydrogen peroxide used for cyanobacteria control. Internationally, it has been reported that hydrogen peroxide can selectively target removal of cyanobacteria over other algae[112]. Hydrogen peroxide may also oxidise and degrade cyanobacteria toxins; for example, microcystins[113]. A major advantage of hydrogen peroxide is its rapid breakdown to harmless by-products (water and oxygen) so that

there is no chemical legacy from dosing with this compound. Effective dose-response relationships vary from approximately 2–100 mg L^{-1} of hydrogen peroxide. It is important to recognise that hydrogen peroxide deals with the symptoms of degraded water-quality (blooms) rather than the causal factors (nutrients).

Booms

We are not aware of booms being used for the purpose of concentrating and removing cyanobacteria. This technique exploits the buoyancy of many cyanobacteria as a potential control measure. Booms were used as an emergency measure for a *Microcystis* bloom in the Swan River in Western Australia in 2000, but their efficacy was not quantitatively evaluated[114]. The algal scum was discharged to the sewer system.

Harvesting

In natural systems, very high filtration rates are required to achieve control of cyanobacteria. As with flushing, harvesting is only effective if the relevant water mass can be filtered within time periods of less than 20 days. The Bay of Plenty Regional Council ran a trial to filter water from the Ōhau Channel outlet of Lake Rotorua to remove algae and nutrients. The conclusions from this trial were that filtration rates were inadequate to achieve the desired removal rates of algae and nutrients[115].

Surface mixers, aerators, artificial destratification and oxygenation

Artificial mixing of lake water can have multiple benefits for reducing cyanobacterial blooms. By preventing seasonal temperature stratification, the entire water column can remain oxygenated, reducing the release of phosphate from the bottom sediments that would otherwise be released when dissolved oxygen is depleted from bottom waters. Higher rates of mixing also tend to favour algal growth (usually diatoms) that are tolerant of high turbulence. Calm conditions will favour cyanobacteria that can control their buoyancy, whereas high turbulence increases the time they spend below a critical mixing depth, where they stop growing due to light limitation. The most common mixing techniques include mechanical mixers and bubble plume destratifiers. As part of the planning stage, undertake proper evaluations of whether artificial mixing will be successful in reducing cyanobacteria in a lake; for example, if nutrient levels are sufficiently high enough, the cyanobacterial biomass may persist. See Burch et al (2021)[116] for more details.

Mechanical mixers mounted at the water's surface pump water from the surface towards the bottom of a lake, ideally creating sufficient flow to stop cyanobacteria from accumulating at the surface and forming blooms[117]. Mechanical mixers also deepen the surface mixed layer, creating lower levels of light exposure for phytoplankton circulating in this layer, thus reducing growth rates. Surface mixers generally require considerable energy to be effective and achieve basin-wide circulation. Solar-powered devices (for example, SolarBee®) operate in a different way; they float on the lake surface and draw deep water up through a draft tube before discharging the water to the surface of the lake. We are aware of SolarBee® devices that have operated on Virginia Lake (Whanganui)[118] and Pegasus Lake (North Canterbury), but their impact has not been well quantified.

Bubble plume destratifiers use compressed air pumped through a sparge line near the bottom of the water column to generate a bubble curtain that entrains and lifts deep water to the surface. The entrained water is lifted towards the surface, then separates to form multiple circulation cells as it moves towards the lake surface. These circulation cells entrain low-oxygen water from the bottom and mix it with the oxygenated waters higher in the water column while sending oxygenated water to the bottom (see figure A3.1 for installation of a destratification draft tube designed to constrain bubbles and direct water from the bottom to the surface water). The destratifier shown in figure A3.1 was found to have limited horizontal extent in the relatively large but shallow Lake Rotoehu (8.1 km^2), Bay of Plenty[119]. Bubble curtain destratifiers have been successfully used for more than 10 years for water-quality control in water supply dams managed by Auckland Council[120]; in this situation, it decreased the occurrence of cyanobacteria proliferations developing in the reservoirs as well as reducing levels of iron and manganese in the water supplied to the city.

Destratification should not be confused with oxygenation, which involves directly providing oxygen (or occasionally air) to the bottom waters of lakes without interfering with the seasonal temperature stratification. Very small air bubbles (sometimes referred to as micro-bubbles or nanobubbles, although the prefix is not indicative of actual bubble size) or liquid oxygen can be used to increase dissolution of oxygen in bottom waters. Nanobubble techniques have successfully managed cyanobacteria proliferations in trials in Japan but there is little published information about the technique and its limitations in larger lakes. Hawke's Bay Regional Council has been trialling methods of oxygenating or destratifying Lake Waikopiro as a pilot-scale study to consider oxygenating or destratifying adjacent Lake Tūtira. An attempt was made in the 1970s to destratify Lake Tūtira, but the operation was quickly abandoned. The timing of mechanical destratification is critical as, once stratification has formed, reduced metals and nutrients will be redistributed from surface to bottom waters, and it can be difficult to remove the existing stratification.

Figure A3.1: Commissioning of a destratification device for Lake Rotoehu (Bay of Plenty), which was designed to fully mix the water column using air-lift

Photo: David Hamilton.

Floating wetlands

Installing floating wetlands (figure A3.2) in lakes allows nutrient uptake and control. This occurs through nutrient uptake by plants, also providing a substrate to promote denitrification, by converting nitrate (a plant nutrient) to nitrogen gas. Ideally, plants are harvested from a floating wetland to remove nutrients. Considering the scale of investment of installing floating wetlands in lakes, there is scant evidence for their benefit as a nutrient-control method. Nutrient removal rates appear to be modest[121] and may be compromised by birds that use the new 'terrestrial' habitat.

Figure A3.2: Floating wetlands have been used as an in-lake nutrient control option (Lake Rotoehu, Bay of Plenty)

Photo: David Hamilton.

Hypolimnetic siphoning

Gravitational siphoning of water from the bottom of the lake to an outlet at a lower level can preferentially remove nutrient-enriched bottom waters[122]. This technique has been used successfully in stratified lakes and reservoirs internationally, but there are some limitations to its application that should be evaluated in the planning stages[116]. We are not aware of this technique being used in Aotearoa, although it has been considered among a range of potential management options for Lake Ōkareka, Bay of Plenty[123].

Biological treatments

We have yet to find good scientific documentation on bacterial treatments that have produced significant changes in lake water quality. This does not include heavily organically loaded systems: for instance, oxidation ponds, where breakdown of organic material may be constrained in some way by the availability of suitable microbes and their ability to rapidly generate biomass. Diatomix may be classified as another type of biological treatment. It contains micronutrients (for example, magnesium and molybdenum) that are bound to nano-particles of silica so that they are only available to, and promote the growth of, algae (particularly diatoms) which require silica as a macronutrient. The additional silica would give diatoms a competitive advantage over other members of the algal assemblage only if there was silica limitation of their growth. To date, rigorous confirmation of the effectiveness of this approach is lacking. A recent trial of Diatomix in one of the University of Waikato campus lakes (an area of 0.7 hectares) found no improvement in water quality post-treatment (personal communication with Brendan Hicks, University of Waikato).

Dredging

This option appears to be an obvious and straightforward method to deal with accumulated nutrients in the bottom sediments of a lake, especially when the sediments are exposed to anoxic overlying waters, which increases resupply of nutrients to the water column. It can also remove akinetes (dormant cells; for example, of *Dolichospermum* or *Raphidiopsis*) or overwintering vegetative cells (*Microcystis* spp). Dredged sediment must be disposed of according to local regulations, and options may be restricted according to how the 'spoil' is classified and compliance with environmental safeguards. Costs for small lakes may be of the order of $100,000 per hectare[96]. The efficacy of dredging varies with several factors including the dredging depth; the composition of underlying, now exposed sediments (as dredging is based on the premise that deeper sediments have lower levels of nutrients and organic matter); the scale of disturbance from the operation; the disruption of benthic biota; and the ability to accurately target the deeper, organic-rich sediments[124, 125]. Also consider the effective timespan of the intervention, as lakes that still have a high sediment load will eventually have the same problems again.

Sediment capping with clean sand or gravel may achieve a similar outcome to dredging, effectively resetting the bottom sediments to substantially reduce negative impacts of the sediments on the overlying water quality. As with dredging, careful evaluation of the cost and longevity of capping would be required.

Lake geoengineering

Lake geoengineering refers to the deliberate manipulation of lake processes – using natural and engineered processes to achieve a desired chemical or ecological outcome. Flocculants (sometimes referred to as phosphorus inactivation agents) are a type of geoengineering material used in different situations to deal with eutrophication and to reduce the incidence of cyanobacteria blooms. Their mode of action is to floc with dissolved sediment and particulate material in the water column, but they can also act to chemically precipitate phosphorus. They are not completely risk-free. Under alkaline conditions, a dissociation product of alum [$KAl(SO_4)_2 \cdot 12(H_2O)$] is $Al(OH)_4^-$ (poly-aluminium chloride or PAC) can be toxic to biota[126]. This is one reason why research is exploring alternate materials, some of which have been applied in Aotearoa. Such materials include Phoslock™ – a patented material that uses the rare earth lanthanum (La^{3+}) instead of aluminium (Al^{3+}) to bind phosphorus – and Aqual-P™, which uses a zeolite carrier but has aluminium as the active phosphorus-locking compound. Some of these compounds have been tested for potential eco-toxicological effects[127].

General principles for the use of flocculants have been developed through case studies undertaken in Aotearoa[122, 128], primarily because of Bay of Plenty Regional Council experience in the Rotorua–Te Arawa lakes. Table A3.1 provides additional information on some of the flocculants that have been used for experimental testing purposes or for whole-lake treatments. Longevity of a one-off treatment appears to be variable, depending on a number of lake-specific variables including morphology, phosphorus loading and the presence of benthic organisms that cause bioturbation, which may reactivate phosphorus from the bottom sediments[129].

Table A3.1: Flocculants used as phosphorus inactivation agents in lake treatments

Flocculant	Active compound	Carrier	Requirements	Approx. cost	Potential side effects / toxicity	Application difficulty
Alum, PAC	Al^{3+}	None	Mostly used with a buffer; careful checks required to avoid acidification	Low	Free Al ion toxicity to biota	Low-medium
Phoslock®	La^{3+}	Bentonite	-	Medium-high	Low-alkalinity waters could lead to greater susceptibility of biota to side effects from La^{3+}	Medium
Chitosan[130]	Crustacean shell fibre	Has been used in association with flocculants for sinking (ballast) purposes	Check for contaminants released by flocculant (if used)	High	Benign: toxicity to higher organisms highly unlikely but appears to act as an algaecide to cyanobacteria	High
Oxygen nanobubble-modified natural particles[131]	-	A locally mined soil is often used, impregnated with oxygen nanobubbles	Has not been scaled up; still experimental (laboratory-scale)	Likely to be high	Benign unless the modified soil releases contaminants	Medium
Aqual-P™	Al^{3+}	Zeolite	-	Medium	Evidence to date indicates Aqual-P™ is relatively benign	Medium

Appendix 4
The impact of toxic freshwater cyanobacteria on marine environments

International research has shown that cyanotoxins – specifically microcystin and nodularin – can be present in estuarine environments in both the water column and surface sediments[132-135]. Despite experiencing optimal growth in fresh water, toxin-producing cyanobacteria including *Microcystis* sp, *Dolichospermum* sp, and *Anabaenopsis* sp can tolerate the higher salinity (greater than 10 ppt) of estuarine waters[136-138]. When salinities are higher than 10 ppt, cell lysis / death will probably take place; however, extracellular microcystins can persist in the water column for up to three weeks for example[132, 138].

Cyanotoxins from freshwater sources can accumulate in marine organisms, posing a subsequent health risk to humans and marine organisms that consume them for example[132, 134]. Microcystins have been found in marine or estuarine fish, mussels, oysters and other shellfish species[134, 139], with toxins detectable in organisms for considerable periods; for example, up to eight weeks post-exposure in mussels[134]. In California, microcystin and nodularin were, respectively, detected in the tissue of dead sea otters and bottlenose dolphins, and were determined to be the cause of death in the sea otters[132, 133]. The authors proposed that the mammals are exposed to the toxins after ingesting filter-feeding organisms that have accumulated the toxins.

Increased risk of cyanotoxins in estuaries has been linked to the following conditions:

- Severe toxic cyanobacterial blooms in freshwater sources; that is, lakes that flow into the sea[132]. Toxin concentrations in the estuaries can be seasonal (highest during autumn and spring), consistent with the seasonal patterns of freshwater cyanobacterial blooms[132, 140].
- Where severe bloom events in coastal freshwater catchments coincide with high-flow events. For example, Miller et al (2010)[132] found that, with minimal freshwater runoff, samples collected in estuaries tested negative for microcystin but, in the rainy season, microcystins were detected in many samples. Robson and Hamilton (2003)[141] described a storm event that resulted in a toxic *Microcystis* bloom being forced downstream into an estuary.

Monitoring of toxic cyanobacteria is undertaken primarily in freshwater environments, but many freshwater systems that contain cyanobacteria flow into estuaries or sheltered coastal environments. Examples from Aotearoa include the Kaituna River (outflow of Lakes Rotorua and Rotoiti) that drains into the Maketu Estuary, and the Utakura River (outflow of Lake Ōmāpere) that flows into the Hokianga Harbour. Lake Ōmāpere (Northland) has experienced blooms containing microcystins – and microcystins have been found in shellfish in the Hokianga Harbour, although the analytical results were inconclusive[142]. Further research is required to identify whether there is a significant risk in Aotearoa from freshwater toxin transfer to marine environments.

Appendix 5
Cyanotoxins in Aotearoa

Cyanotoxins are a diverse assemblage of natural toxins with several toxicity mechanisms; for example, hepatotoxicity (toxic to the liver) and neurotoxicity (toxic to nerves or nerve tissue). Some cyanotoxins have also been shown to promote liver-tumour growth when ingested in low doses over extended periods[143, 144]. Based on their chemical structure, cyanotoxins can be divided into the following groups: cyclic peptides (microcystins and nodularins) and alkaloids (cylindrospermopsins, anatoxins and saxitoxins). Exposure to cyanobacteria has also been linked to dermatotoxicity (affecting the skin); however, this has not been linked to any of the cyanotoxins described in this section.

Cyanotoxin-producing cyanobacteria globally

Internationally, many cyanobacteria have been shown to produce cyanotoxins. Table A5.1 provides a list of toxin-producing cyanobacteria identified internationally; however, new research is continuously being conducted in this area and all cyanobacteria blooms should be treated with some caution until proven otherwise. Not all strains of a toxin-producing species will be able to produce toxins, and toxic strains cannot be identified microscopically. Therefore, cyanotoxin testing and molecular assessment of toxin-production genes should be used to determine the inherent risk when blooms of potentially toxic cyanobacteria are observed in waterways.

Table A5.1: Summary of known cyanotoxin-producing cyanobacteria identified internationally by isolation of cultured strains. Species in bold type are known to produce the associated toxin (also in bold type) in Aotearoa

Cyanobacteria genus / species	Cyanotoxin(s)
Anabaena sp	Microcystins
Anabaenopsis millenii	Microcystins
Annamia toxica	Microcystins
Aphanizomenon flos-aquae	Anatoxin-a, Cylindrospermopsins, Saxitoxins
Aphanizomenon gracile	Anatoxin-a, Cylindrospermopsins, Saxitoxins
Aphanizomenon ovalisporum	Cylindrospermopsins
Aphanizomenon sp	Anatoxin-a, Cylindrospermopsins, Saxitoxins
Aphanocapsa cumulus	Microcystins
Arthrospira fusiformis	Anatoxin-a
Chrysosporum ovalisporum	Cylindrospermopsins
Coelosphaerium kuetzingianum	Microcystins
Cuspidothrix issatschenkoi	**Anatoxin-a**
Cylindrospermum stagnale	Saxitoxins

Cylindrospermum sp	Anatoxin-a, Saxitoxins
Dolichospermum circinale	Anatoxin-a, Saxitoxins
Dolichospermum flos-aquae	Anatoxin-a, Guanitoxin / Anatoxin-a(S), Microcystins
Dolichospermum lapponica	Cylindrospermopsins
Dolichospermum lemmermannii	Anatoxin-a, Guanitoxin / Anatoxin-a(S), Microcystins
Dolichospermum sp	Anatoxin-a, Cylindrospermopsins, Guanitoxin / Anatoxin-a(S), Microcystins
Dolichospermum spiroides	Guanitoxin / Anatoxin-a(S)
Dolichospermum subcylindrica	Microcystins
Dolichospermum ucrainicum	Microcystins
Dolichospermum variabilis	Microcystins
Fischerella sp	Microcystins
Geitlerinema amphibium	Saxitoxins
Geitlerinema carotinum	Anatoxin-a, Microcystins
Geitlerinema lemmermannii	Saxitoxins
Geitlerinema splendidum	Anatoxin-a, Microcystins
Gloeotrichia natans	Microcystins
Hapalosiphon hibernicus	Microcystins
Heteroleiblenia kuetzingii	Microcystins
Iningainema pulvinus	Nodularin
Leptolyngbya sp	Microcystins
Limnothrix mirabilis	Microcystins
Microcoleus autumnalis (previously Phormidium autumnale)	**Anatoxins**
Microcoleus sp	**Anatoxins**, Microcystins,
Microcystis aeruginosa	**Microcystins**, Saxitoxins
Microcystis botrys	Microcystins
Microcystis flos-aquae	Microcystins
Microcystis ichthyoblabe	Microcystins
Microcystis novacekii	Microcystins
Microcystis panniformis	Microcystins
Microcystis sp	Anatoxin-a, **Microcystins**, Saxitoxins
Microcystis viridis	Microcystins
Microcystis wesenbergii	Microcystins

Microseira wollei	Cylindrospermopsins, Saxitoxins
Nodularia spumigena	**Nodularin**
Nodularia sphaerocarpa	Nodularin
Nostoc commune	Microcystins
Nostoc linckia	Microcystins
Nostoc muscorum	Microcystins
Nostoc sp	**Microcystins**, Nodularin
Nostoc spongiiforme	Microcystins
Oscillatoria agardhii	Microcystins
Oscillatoria formosa	Anatoxins
Oscillatoria limnetica	Anatoxin-a
Oscillatoria limosa	Microcystins
Oscillatoria margaritifera	Microcystins
Oscillatoria sp	Anatoxin-a, Cylindrospermopsins, Microcystins
Phormidium ambiguum	Cylindrospermopsins
Phormidium corium	Microcystins
Phormidium favosum	Anatoxin-a
Phormidium sp	Anatoxin-a, Microcystins
Phormidium splendidum (Syn. Geitlerinema splendidum)	Microcystins
Phormidium uncinatum	Anatoxin-a, Microcystins, Saxitoxins
Planktothrix agardhii	Microcystins
Planktothrix rubescens	Microcystins
Planktothrix sp	Anatoxins, **Microcystins**, Saxitoxins
Plectonema boryanum	Microcystins
Pseudanabaena frigida	Microcystins
Pseudocapsa dubia	Microcystins
Radiocystis fernandoi	Microcystins
Raphidiopsis brookii	Saxitoxins
Raphidiopsis curvata	Cylindrospermopsins
Raphidiopsis mediterranea	Anatoxins, Cylindrospermopsins
Raphidiopsis raciborskii	**Cylindrospermopsins**, Saxitoxins, Microcystins
Rivularia biasolettiana	Microcystins
Rivularia haematites	Microcystins

Schizothrix rivulariarum	Microcystins
Scytonema cf. crispum	**Saxitoxins**
Scytonema drilosiphon	Microcystins
Snowella sp	Microcystins
Sphaerospermopsis torques-reginae	Guanitoxin / Anatoxin-a(S)
Synechococcus lividus	Microcystins
Synechocystis sp	Microcystins
Tolypothrix distorta	Microcystins
Tychonema bourrellyi	Anatoxins
Umezakia natans	Cylindrospermopsins
Woronichinia sp	Microcystins

Notes: This table is a compilation of worldwide information but, because new toxin-producing species are always being identified, it should not be regarded as comprehensive. The toxin-producing species listed here are only those where toxin production has been confirmed in an isolated strain (not in environmental samples).

Variability among cyanobacterial strains

Research continues to highlight the intraspecific or between-strain variability in growth rates, biovolumes, toxin production and responses to environmental conditions in cyanobacterial species. For example, from a single lake surface bloom sample of *Raphidiopsis raciborskii* (previously *Cylindrospermopsis raciborskii*) from Australia, 24 individual trichomes (unbranched chains) were isolated with distinct differences in morphology (straight or coiled), cell biovolume, growth rates and toxin quotas[145]. In Aotearoa, 30 Microcoleus autumnalis (previously *Phormidium autumnale*) strains were isolated from 1 cm^2 sections of *Microcoleus autumnalis*-dominated mats. Of these strains, 60 per cent were anatoxin producers; within the toxin-producing strains, there was a 100-fold variation in toxin content[146]. Different saxitoxin profiles of two strains of *Scytonema* cf. *crispum* can arise from differences in the biosynthetic gene cluster associated with tailoring enzymes that influence the composition of the saxitoxin congeners[147].

Several environmental conditions have been found to promote the dominance of toxic over non-toxic strains of cyanobacteria, although results from different studies often provide confounding data.

- Nutrient enrichment, specifically with nitrogen, can promote the dominance of toxic strains over non-toxic strains in cyanobacterial genera including *Raphidiopsis raciborskii*[148], *Microcystis*[149-153] and *Planktothrix*[153]. The type of nutrient can differentially impact strains. For example, one study found that the growth of a toxic *Microcystis* strain can be stimulated by inorganic nitrogen rather than organic nitrogen, whereas the opposite was observed in a non-toxic strain of *Microcystis*[151].
- Both higher[150, 152] and lower[154] water temperatures can promote the dominance of toxic strains of *Microcystis*. Heath et al (2011)[15] suggested that temperatures over 13.4°C favour

toxic over non-toxic *Microcoleus* strains, although subsequent research has not verified this in environmental mats[60, 155].

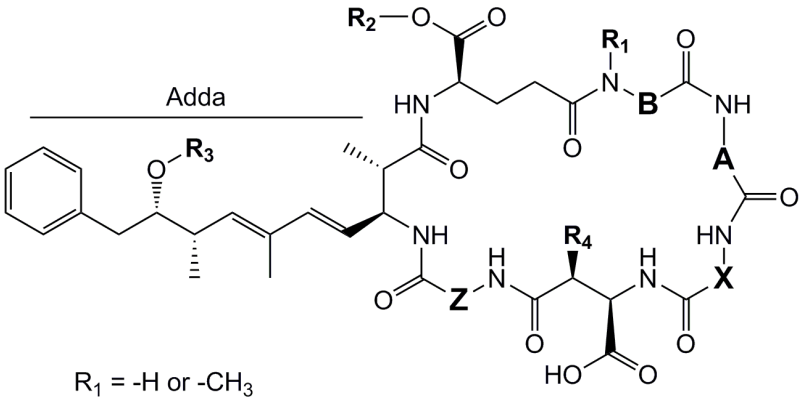
- Low pH can increase the ratio of toxic to non-toxic Microcystis populations[154].
- Higher light intensity was a promoting factor for toxic strains of *Microcystis aeruginosa* and *Dolichospermum circinale* (previously *Anabaena circinalis*) over non-toxic strains[156]. Furthermore, Xiao et al (2017)[157] found that intraspecific variation in this species, in response to light and temperature fluctuations, was greater than the interspecific variation in strains of *Microcystis aeruginosa* and *Raphidiopsis raciborskii*.
- Seasonality is also important; for example, seasonal bloom succession in a Dutch lake resulted in a switch from a toxic-genotype-dominated population of *Microcystis* at the onset of a bloom, to a non-toxic-genotype-dominated population at the end of the bloom[158].

This knowledge highlights the complexity of attributing risk to cyanobacterial blooms. Given the high variability in cell biovolumes and toxin production, caution is required when using this metric when defining thresholds for toxic species. As toxic and non-toxic strains of the same cyanobacteria species cannot be distinguished through microscopy, it is recommended that toxin gene screens, in concert with toxin testing, should be used to refine the human health risk posed by the bloom. Although the alert-level frameworks in section 3 (Decision Charts 1 and 2) provide a conservative mechanism to manage recreational risks to human health from cyanobacteria – based on cyanobacterial biomass (cell counts, biovolumes and benthic mat coverage) – information on toxin concentrations and toxin production potential (via the toxin gene screen) will allow more nuanced management that could limit unnecessary restrictions to recreational water bodies.

Microcystins

Globally, microcystins (MCs) are the most frequently detected cyanotoxin[159]. More than 250 microcystin congeners have been identified to date[160]. Each variant differs structurally with respect to the level of methylation and acetylation, and the amino acids incorporated into the peptide ring (see figure A5.1). The amino acids in the X and Z positions are highly variable, but variation is also observed in several of the other amino acids (see figure A5.1). These structural changes result in pronounced differences in toxicity among the variants, according to their ability to inhibit protein phosphatase enzymes and their uptake by cells[161, 162]. The amino acid Adda (see figure A5.1) is unique to microcystins and nodularins, and is required for their biological activity[159]. The large number of microcystin congeners identified also creates challenges for their detection and quantification.

Figure A5.1: The general structure of microcystin and some of its structural modifications



R_1 = -H or -CH_3
R_2 = -H, -CH_3 or -C_3H_6OH
R_3 = -H, -CH_3 or -$COCH_3$
R_4 = -H or -CH_3
A = D-Alanine, D-Leucine, D-Serine or Glycine
B = Dehydroalanine, Dehydrobutyrine, Serine or Lanthionine
X = Variable L-amino acids
Z = Variable L-amino acids

Microcystins are hepatotoxins that block protein phosphatase 1 and 2A in affected organisms[163]. This binding is inhibitory, highly specific and irreversible. The chief pathway into cells for microcystins is the bile acid carrier, which is found in high levels in liver cells and, to a lesser extent, in intestinal epithelia[164, 165]. Most microcystins are highly toxic, with intraperitoneal (ip) mouse toxicities ranging between 50 and 300 µg/kg body weight[159]. 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)[166] published evidence for disruption of nasal tissues by the common hydrophilic variant microcystin-LR. Toxicity by oral uptake is generally an order of magnitude lower than toxicity by ip injection. However, intra-nasal application in these experiments was as toxic as ip injection, and membrane damage by microcystin enhanced the toxicity of anatoxin-a. This uptake route may be relevant for water sports such as water skiing that leads to inhalation of spray and droplets.

Fitzgeorge et al (1994)[166] demonstrated that that the damage from microcystins can be cumulative if exposure is repeated. 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[167, 168]. Sub-acute liver injury is likely to go unnoticed for two reasons: liver injury shows externally noticeable symptoms only when it is severe; and 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[169, 170], Serbia[171, 172] and the United States[173] has linked the continual consumption of low doses of

microcystins in drinking water to primary liver cancer. The International Agency for Research on Cancer has classified microcystin-LR as a group 2B carcinogen[174]. Group 2B compounds are also considered possible carcinogens to humans.

Numerous incidents of animal deaths and some human poisonings have been attributed to microcystins[175]. 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[176]. In a further case from Brazil, the deaths of 88 people, mostly children, were associated with drinking water from a newly constructed reservoir[177]. Although no data for microcystins are available, the heavy bloom of *Microcystis* spp in the reservoir was the suspected cause.

Microcystins in Aotearoa

Microcystins are the most commonly observed cyanotoxin in planktonic cyanobacteria in Aotearoa, and they have been identified in many water bodies around the country[20, 21, 26, 32, 37, 76, 90, 178-181]. Some of the highest concentrations of microcystins reported include cyanobacterial scum samples from Lake Rotorua (Kaikōura; 2,150 µg/L[182]) and Lake Horowhenua (Levin; 36,500 µg/L[21]).

Microcystis sp is the only confirmed planktonic producer of microcystins in Aotearoa[27] although, internationally, many different cyanobacterial genera have been confirmed as microcystin producers (see table A5.1). The production of microcystins by *Dolichospermum* (previously *Anabaena*) and planktonic species of *Planktothrix* in Aotearoa is suspected due to the detection of microcystins in environmental samples dominated by these cyanobacteria species[21, 26, 178]. This has, however, not been confirmed through culturing studies.

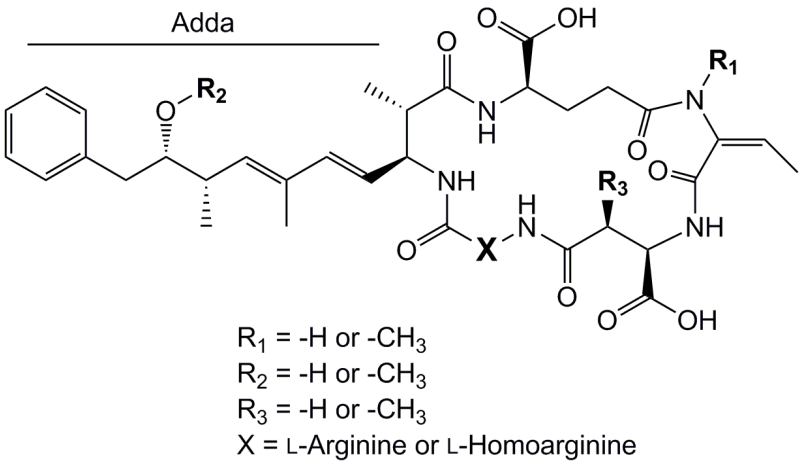
Analysis of environmental and cultured samples indicated that microcystins are also produced by benthic species[27, 37] including a *Planktothrix* species[16]. In March 2003, the eastern shore of Lake Taupō was lined with thick gelatinous mats of *Nostoc* sp (appendix 13) that contained high levels of microcystins (708 mg/kg). Gelatinous colonies accumulated along the shoreline following a storm that likely dislodged the *Nostoc* sp from underwater rocks. A water sample collected close to the shoreline at Lake Taupō also contained microcystins[21]. Small sponge-like balls of *Nostoc* sp collected from the Hakataramea River (Canterbury region) have also been shown to produce microcystins[27].

A range of microcystin congeners (structural analogues) have been found in freshwater environments throughout Aotearoa[27]. The most frequently encountered microcystin congener profiles in cyanobacteria from Aotearoa are due to a *Microcystis* that produces mainly [Dha^7] MC-LR with lower levels of [Asp^3,Dha^7] MC-LR and MC-LR, and a *Microcystis* that produces a wide array of toxin congeners (> 27 congeners including MC-RR, MC-YR, MC-LR, MC-FR, MC-WR, MC-RA, MC-RAba, MC-LA, MC-FA, MC-WA, MC-LAba, MC-FAba, MC-WAba;[32]). Labs involved in toxin testing should be aware of the wide array of microcystin congeners found in this country, especially when conducting analysis by liquid chromatography-mass spectrometry (LC-MS), due to the high degree of specificity of this technique and the potential for specific congeners to be overlooked.

Nodularins

Nodularins have a very similar structure to microcystins but contain only five amino acids (figure A5.2). Nodularins are generally produced by *Nodularia spumigena*, primarily a brackish-water species; however, nodularin production has recently been documented in *Iningainema pulvinus* from Australia[183]. Like microcystins, nodularin is also a potent inhibitor of protein phosphatases 1 and 2A[168, 184] and has an ip LD_{50} of 60 µg/kg body weight (mouse)[33]. Much like microcystins, nodularins have also been demonstrated to promote liver tumours[185]. Because of the toxicological and chemical similarities of nodularins and microcystins, they are often considered as a single class of compound for health-risk assessments.

Figure A5.2: The general nodularin structure and some of its structural modifications



Nodularins in Aotearoa

Nodularin has been identified from planktonic blooms of *Nodularia spumigena* in Te Waihora | Lake Ellesmere[186], Te Roto o Wairewa | Lake Forsyth[26, 28] and Whakakī Lake[11]. A long history of stock deaths is associated with nodularin intoxication from *Nodularia spumigena* blooms[186-188]. *Nodularia* sp is known to occur in other lakes around Aotearoa such as Lake Clearwater, Lake Ōkataina, Lake Rotoiti (Rotorua region), Lake Rotomahana, Lake Rotorua (Rotorua region) and Lake Taharoa[189], although the occurrence of nodularin in these lakes has not been investigated. Nodularin has also been found in benthic mats from Lake Tikitapu | Blue Lake, although the producing organism was not identified[57].

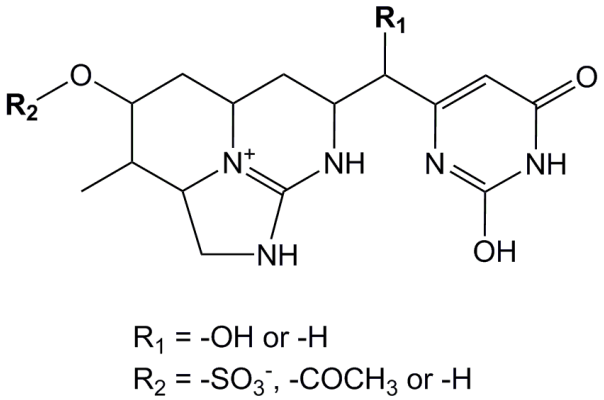
There are few reports on nodularin toxin quotas (the amount of toxin per cell), but the nodularin quotas for 10 *Nodularia spumigena* cultures isolated from Aotearoa were evaluated during the development of the revised alert-level framework for planktonic cyanobacteria included in these guidelines[11]. This research found that nodularin quotas from nodularin-producing *Nodularia spumigena* (0.3–4 pg/cell) in Aotearoa were in a similar range as microcystin quotas from microcystin-producing *Microcystis* spp (0.006–6 pg/cell) in Aotearoa.

Cylindrospermopsins

Cylindrospermopsin (CYN) causes extensive damage to the liver and kidney, and is a potent inhibitor of protein synthesis[190-192]. Clinical symptoms may appear several days after exposure, so it is often difficult to find a cause-effect relationship. Falconer and Humpage (2001)[193]

suggest that cylindrospermopsin may also act directly as a tumour initiator, which has implications for both short- and long-term exposure. Crude extracts of *Raphidiopsis raciborskii* (a common cylindrospermopsin producer, previously called *Cylindrospermopsis raciborskii*) injected or given orally to mice also induce pathological symptoms in the kidneys, spleen, thymus and heart[194]. Other structural variants of cylindrospermopsin include 7-epicylindrospermopsin (7-epi-CYN), a toxic minor metabolite of the cyanobacterium *Aphanizomenon ovalisporum*[195] and deoxycylindrospermopsin[196], which is thought to be as toxic as cylindrospermopsin (see figure A5.3). Purified CYN / 7-epi-CYN exhibited an ip LD_{50} of 2.1 mg/kg body weight (mouse) at 24 hours post-exposure and 0.2 mg/kg after 5 to 6 days had passed[197].

Figure A5.3: The general cylindrospermopsin structure and some of its structural modifications



Raphidiopsis 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 gastroenteritis after a local water supply on Palm Island (Australia) was dosed with copper sulphate to control a dense algal bloom[198, 199]. The copper sulphate caused the cells to break apart (lyse) and resulted in the release of cyanotoxins into the water supply[200]. In addition, cattle deaths in Queensland (Australia) have been attributed to cylindrospermopsin[201].

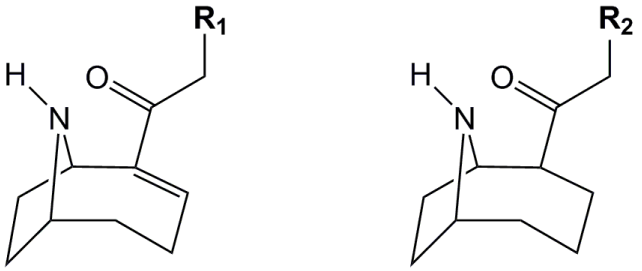
Cylindrospermopsins in Aotearoa

Cylindrospermopsin was first identified in Aotearoa in Lake Waitawa (Otaki) in 1999, although the species responsible for its production was not confirmed[20]. *Raphidiopsis raciborskii* (previously *Cylindrospermopsis raciborskii*) was identified for the first time in a bloom in Lake Waahi (in March 2003), and LC-MS confirmed the presence of cylindrospermopsin and deoxycylindrospermopsin[29]. These two incidents are the only records of cylindrospermopsin in Aotearoa. Multiple samples with high concentrations of *Raphidiopsis raciborskii* have been analysed, but no cylindrospermopsin was detected[202], indicating that not all strains of *Raphidiopsis raciborskii* in Aotearoa produce this toxin.

Anatoxins

Anatoxins are neurotoxic poisons which are powerful depolarising neuromuscular blocking agents, acting through the nicotinic acetylcholine receptor[203]. Four anatoxin congeners are commonly produced by cyanobacteria: anatoxin-a (ATX), homoanatoxin-a (HTX), dihydro-anatoxin-a (dhATX) and dihydro-homoanatoxin-a (dhHTX; see figure A5.4 below).

Figure A5.4: The general anatoxin structure and its common structural modifications



ATX R_1 = -H
HTX R_1 = -CH_3

dhATX R_2 = -H
dhHTX R_2 = -CH_3

To date, most toxicology work has been conducted on ATX and some work has been conducted using HTX. There are very few studies of the toxicity of the dihydro-anatoxin congeners (dhATX and dhHTX); however, the presence of high levels of these congeners during incidents of dog deaths[18] has led researchers to believe that the dihydro-anatoxins also pose a health risk. Recent toxicology testing has shown that the oral toxicity of dhATX is considerably greater than previously assumed[204]. Although it was originally thought that the dihydro-anatoxin congeners (dhATX and dhHTX) were breakdown products of ATX and HTX (respectively), it has now been established that they are produced by cyanobacteria[205, 206].

Because of their small size, anatoxins are rapidly absorbed when ingested orally. In affected animals, these toxins can cause convulsions, coma, rigours, cyanosis, limb twitching, hypersalivation and death. Anatoxin-a is often linked with animal and wildfowl poisonings[175], but there have been no reported human fatalities from ATX. Anatoxin-a and HTX have an ip LD_{50} of 200−250 µg/kg body weight (mouse)[207, 208], while dhATX has an ip LD_{50} of 730 µg/kg body weight (mouse). The LD_{50} value for dhHTX has not been established. In contrast, dhATX is more toxic than ATX via oral admission (both gavage and feeding in mice): dhATX has an LD_{50} of 2.5 mg/kg (gavage) and 8 mg/kg (feeding) and ATX has an LD_{50} of 10.6 mg/kg (gavage) and 25 mg/kg (feeding)[204]. These results highlight the potential risk of less well-known congeners and the need for toxicological data beyond ip injection (through realistic exposure scenarios) to enable accurate risk assessments.

Wood et al (2018)[62] determined that anatoxins are found in the water close to *Microcoleus* mats. Although the concentrations of anatoxins in the water were lower than levels that posed a risk to recreational users, they were detectable and quantifiable. The higher concentrations of anatoxins in *Microcoleus* mat material compared to those in the overlaying river water (at one site, 0.9 µg/L vs. 500 mg/kg dry weight[62]) poses a greater risk to water users.

Anatoxins in Aotearoa

Following the rapid deaths of dogs near the Waikanae River (Lower North Island) in 1998, the toxicity of a benthic mat of *Oscillatoria* sp was investigated using a mouse bioassay and high-performance liquid chromatography with fluorescence detection (HPLC-FLD). Anatoxin-a 'degradation products' were reported, which were likely to be the dihydro-anatoxin congeners now shown to be naturally-produced structural congeners[37]. 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[37]. Wood et al (2006)[21] identified ATX in three planktonic samples collected from Lake Rotoehu (Rotorua), Lake Henley

(Masterton), and Lower Karori Reservoir (Wellington); all three samples were dominated by *Dolichospermum* sp (previously *Anabaena*).

Cuspidothrix issatschenkoi (previously *Aphanizomenon issatschenkoi*) was identified for the first time in Aotearoa in 2003, and LC-MS analysis of a strain isolated from Lake Hakanoa (Waikato) confirmed it was producing ATX[22]. Interestingly, despite the absence of cylindrospermopsin production, genes implicated in the biosynthesis of cylindrospermopsin were successfully amplified from the *Cuspidothrix issatschenkoi* strain.

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 the presence of anatoxin congeners ATX, HTX, dhATX and dhHTX[13]. The causative species was identified as *Microcoleus autumnalis* (previously *Phormidium autumnale*)[13]. Since this incident, anatoxin-producing *Microcoleus*-dominated mats have been detected in many regions of Aotearoa, including the Bay of Plenty[17], Canterbury[60, 146], Kaikōura[18], Southland[209], and Wellington[13, 15, 17, 146]. A review from 2016 documented the rivers in Aotearoa where *Microcoleus* had been reported, and provided a summary of the available anatoxin data to that time[12]. Over the past decade, *Microcoleus* blooms in rivers have been linked to dog-poisoning events around Aotearoa (for example, Bay of Plenty, Canterbury, the Nelson-Tasman area, and Wellington) and in a farm pond in Kaikōura[18]. The presence of high amounts of 'algal material' in the stomach contents of the dead dogs suggests that they ingested the cyanobacterial mats containing high levels of toxins, rather than through direct exposure by drinking water with lower levels of toxins than the mats. It is unknown whether dogs are more susceptible to anatoxin poisoning than other organisms.

Research into *Microcoleus*-dominated blooms has demonstrated that *Microcoleus*-dominated mats generally contain a mixture of *Microcoleus*-dominated strains[14, 210]. These studies, along with another[146], demonstrated that the different *Microcoleus* strains isolated from the same mat can have vastly different anatoxin-production potential, making it difficult to predict the expected toxin level in a sample. Furthermore, when the spatial variability of *Microcoleus*-dominated blooms was assessed, vastly different anatoxin levels were observed in mats sampled from different sites in the same river[15] and from mats in very close proximity (< 1 m^2)[17, 62]. Overall, these findings show that, without assessing for toxins or toxin-production genes, *Microcoleus*-dominated blooms should be assumed to be toxic – and when sampling blooms for toxin analysis, a representative sample from multiple rocks should be collected and homogenised. The study by Wood et al (2010)[17] indicated that pooling 10 samples from different rocks, and analysing the composite sample, provided confidence in detecting anatoxins at a site if the detection limit of the analytical method was suitably low. An assessment of rivers containing *Microcoleus*-dominated mats found that anatoxins were present in the river water. The level of anatoxins detected in the water was related to the severity of the bloom (for example, 0.9 µg/L of anatoxins was detected in the water of a river with 51 per cent *Microcoleus* mat coverage and an average anatoxin concentration of 500 mg/kg dry weight, while 0.05 µg/L was detected in the water of a river with 23 per cent mat coverage and 40 mg/kg dry weight anatoxins[62]).

Flow, nutrient availability, sediment inputs and substrate stability have been further investigated as drivers of *Microcoleus* proliferations. McAllister et al (2018)[60] identified that both dissolved reactive phosphorus and dissolved inorganic nitrogen concentrations were important factors in the accrual of *Microcoleus* mats. Subsequent work has further emphasised that nutrient concentrations, along with flow rates, are very important in the establishment of blooms[211-214]. Following mat accrual, *Microcoleus* can use organic nutrient sources to assimilate nutrients for growth, even when inorganic nutrient concentrations in the overlying water column are low[215, 216].

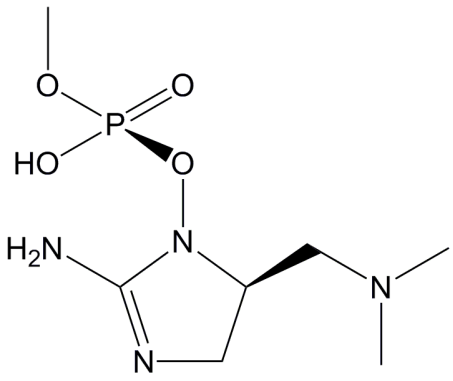
Genomic differences between toxic and non-toxic strains of *Microcoleus* indicate that the nutrient-acquisition strategies differ among strains – and suggest that non-toxic strains have the ability to use a wider range of compounds than toxic strains[216-218]. Thomson-Laing et al (2021)[219] investigated *Microcoleus* mat expansion, finding that in addition to previously established drivers, metal availability may influence mat accrual and growth, even though the relative importance of the various drivers differs among sites. Substrate stability was implicated as an important variable in previous habitat-suitability work, and this was studied by Neverman (2018)[220]. Subsequently, Haddadchi et al (2020)[221] investigated the relationships between the flows required for periphyton removal in relation to sediment entrainment, and developed a national model that estimates the flows required to remove periphyton based on substrate mobility. These models indicated that the general approach of three times the median flow is overly conservative in some regions (particularly more mountainous areas), while underestimating the flows required to flush out periphyton in lowland areas.

Several studies have used molecular approaches to investigate the bacterial communities associated with *Microcoleus* proliferations among rivers and between countries[222, 223]. Both studies found differences in the bacterial communities among locations, which suggests that *Microcoleus* is not reliant on, nor does it lead to, a particular bacterial community structure.

Guanitoxin / anatoxin-a(S)

Guanitoxin, previously known as Anatoxin-a(S), is structurally different from anatoxin-a (see figure A5.5 vs. figure A5.4) and was recently renamed guanitoxin in order to avoid confusion[224]. It is a cholinesterase inhibitor that causes hypersalivation, diarrhoea, shaking, and nasal mucus discharge in mammals[225, 226]. Guanitoxin was originally identified in *Dolichospermum lemmermannii*[227] and *Dolichospermum flos-aquae*[226] (previously both *Anabaena*), but has recently also been identified in *Sphaerospermopsis torques-reginae*[228-230]. It has an ip LD_{50} of 20 µg/kg body weight (mouse)[225]. Detection and measurement of guanitoxin) is frequently conducted using acetylcholine esterase inhibition assays, although it should be noted that these assays are susceptible to interference by organophosphate-based pesticides.

Figure A5.5: Structure of guanitoxin / anatoxin-a(S)



Guanitoxin / anatoxin-a(S) in Aotearoa

To the best of our knowledge, there have been no studies specifically analysing for guanitoxin in Aotearoa. The primary reason is that no commercial standards are available for the toxin,

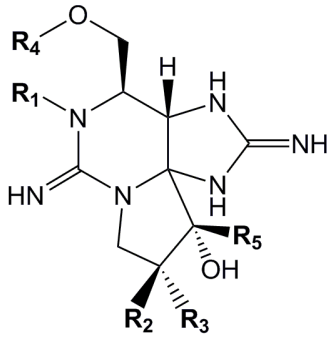
limiting the ability to set up robust detection methods. The recent identification of guanitoxin in *Sphaerospermopsis torques-reginae*, and the associated characterisation of the biosynthetic pathway for the toxin[230], may lead to future opportunities to understand the prevalence and distribution of this under-researched cyanotoxin. The reliable production of guanitoxin from *Sphaerospermopsis torques-reginae*[228, 229] may also lead to standards for the toxin becoming available, allowing detection by analytical methodologies.

Saxitoxins

Saxitoxins are fast-acting neurotoxins that inhibit nerve conduction by blocking sodium channels[231]. Saxitoxins are also produced by various marine dinoflagellates under the name of paralytic shellfish poisons (PSPs), and the health effects on humans caused by saxitoxins are well described by numerous reports of toxicity associated with eating shellfish containing relatively high concentrations of PSPs. No PSP-like illnesses have been reported in humans from the consumption of drinking water or from contact with recreational water-containing saxitoxins[23]. More than 30 saxitoxin variants have been isolated and characterised (figure A5.6). Saxitoxin has an ip LD_{50} of 10 µg/kg body weight (mouse). Other analogues are mostly less toxic than saxitoxin.

Saxitoxins have caused sheep mortalities in Australia[232] and were identified in an extensive bloom of *Dolichospermum circinale* (previously *Anabaena circinalis*) in 1990 on the Murray Darling River (Australia), which resulted in the death of over 1,600 stock[233].

Figure A5.6: Structure of saxitoxins and some of its common structural modifications



R_1 = -H or -OH
R_2 = -H or $-SO_3^-$
R_3 = -H or $-SO_3^-$
R_4 = -H, $-COCH_3$, $-CONH_2$,
$-CONHSO_3^-$ or $-COC_6H_4OH$
R_5 = -H or -OH

Saxitoxins in Aotearoa

A cyanobacterial bloom (predominantly *Dolichospermum planctonicum*, previously *Anabaena planctonica*) 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[36], but levels were well below the provisional maximum acceptable values set out in the *Drinking-water Standards for New Zealand 2005*[234]. The saxitoxin-producing organism(s) were not identified.

The production of saxitoxin has been identified in *Scytonema* cf. *crispum* from the Canterbury region[34, 56]. Using an ELISA and neuroblastoma assay, Wood et al (2006)[21] detected low levels of saxitoxins in 38 different water bodies. Although only low levels of saxitoxins were detected, the results imply that saxitoxin-producing cyanobacteria may be present in water bodies from Aotearoa.

β-N-methylamino-L-alanine

β-N-methylamino-L-alanine (BMAA) is a non-protein amino acid that has been observed in some cyanobacteria (for example, certain *Nostoc* species). BMAA has been proposed as a possible cause of the amyotrophic lateral sclerosis / Parkinsonism–dementia complex (ALS-PDC) 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;[235]). A possible link between cyanobacteria and neurodegenerative disorders has also been proposed based on epidemiological studies that have identified higher rates of neurodegenerative disorders in people who live close to lakes or associate with lakes more frequently than others[236-240]. However, a strong toxicological link between BMAA and neurodegenerative disorders has not yet been established[241], and WHO guidelines for this cyanotoxin have not been developed.

There is debate on the occurrence of BMAA in other cyanobacterial genera (for example[242, 243]) as different analytical methods give different results. The original derivatisation method gives higher concentrations than the more modern hydrophilic interaction liquid chromatography (HILIC) methods.

β-N-methylamino-L-alanine in Aotearoa

Recent work investigated the prevalence of BMMA in cyanobacteria from Aotearoa[244, 245]. A HILIC mass spectrometry method was used to analyse for 'total BMAA' in 34 cyanobacteria samples. The samples included 20 cyanobacterial genera from at least 23 species and covered a range of morphologies (for example, single-celled, colonial and filamentous) and growth strategies (benthic and planktonic). BMAA was only detected in a benthic species of *Planktothrix* sp., which is not prevalent in lakes and rivers around Aotearoa (see appendix 14).

Cyanotoxin and toxicity testing

For health assessments in water bodies used for recreation, it is recommended that total toxin content (that is, combined intracellular and extracellular toxins) is measured. A range of methods have been developed to detect, identify and measure cyanotoxins in a range of

samples (see Lawton et al (2021)[246] for an in-depth review). In Aotearoa, four methods are currently commercially available for cyanotoxin analysis (appendix 12).

Liquid chromatography-mass spectrometry (LC-MS)

Liquid chromatography-mass spectrometry methods are commercially available for microcystins, nodularin, anatoxins, cylindrospermopsins and saxitoxins in Aotearoa. 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 250 structural congeners exist, each varying in its toxicity. Routine LC-MS screens may miss unusual microcystin and saxitoxin variants, and therefore underestimate the total toxin load. Because the ionisation efficiency of different toxin congeners varies, a direct standard will provide the most accurate results. Diverting a portion of the column outflow to a photodiode array (PDA) detector, or using a system with a PDA and a mass spectrometer in sequence, can improve quantification accuracy, as the PDA response is likely to be more consistent between congeners than the mass spectrometry response.

Enzyme-linked immunosorbent assay (ELISA)

An ELISA is available for detecting total Adda-containing microcystins and nodularin[247]. It uses antibodies raised against Adda (an amino acid unique to microcystins and nodularin; see figures A5.1 and A5.2) 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. Nodularin is, however, predominantly produced by *Nodularia spumigena,* a brackish-water species, and so this is unlikely to be problematic. Research in Aotearoa has shown a high correlation between the Adda-ELISA and LC-MS for microcystin detection when working with water samples[248, 249], but differences commonly occur when working with more complex matrices (for example, fish or shellfish tissue[142] or algal mats[249]).

ELISA kits to measure anatoxin, BMAA, cylindrospermopsin and saxitoxins are also available commercially. However, there is limited knowledge about the cross-reactivity of the assays for different structural congeners of the toxins and about the specificity of the assays to detect only the target toxin.

Dip-stick tests based on the same technology are available to assess microcystins, nodularin, anatoxin and cylindrospermopsin. While these do not provide quantitative results, they can provide quick presence / absence results in the field to help with water management decisions. The performance of the dip-stick tests has not currently been assessed in Aotearoa.

Scotia rapid test / Jellett test

The Scotia rapid test (sometimes referred to as the Jellett test) is an antibody-based technique that detects saxitoxin variants to varying degrees. The test determines the presence or absence of saxitoxins. It is not truly quantitative and does not provide information on which saxitoxin congeners are present.

Acetylcholinesterase assay

The biochemical activity of guanitoxin / 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. However, this assay is not routinely available in Aotearoa.

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 Aotearoa, 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. A positive mouse test, however, 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. More information on toxicity testing and bioassays can be found in Lawton et al (2021)[246].

Appendix 6
Derivation of guideline values

Planktonic cyanobacteria

The Action Level (red mode) – Situation 4 cyanotoxin concentration thresholds (see Decision Chart 1 in section 3 of the guidelines) are based on the 2020 WHO recreational guideline values for anatoxins (60 µg/L)[7], cylindrospermopsins (6 µg/L)[8], microcystins (24 µg/L)[9] and saxitoxins (30 µg/L)[10]. These WHO guideline documents review toxicological information on each class of cyanotoxin and the calculations used to derive each guideline value. These calculations are also provided in boxes 3–6 below. Thresholds for nodularins (described in these guidelines for Aotearoa) are based on the guideline value for microcystins (due to the similar structure, toxicity and mode of action shared by the two toxin classes).

Box 3: Calculation of guideline value for anatoxin-a in recreational waters

This calculation is for the 2020 WHO provisional recreational water health-based reference value for anatoxin-a (section 8.1 of the WHO background document for anatoxin-a and analogues; p15[7]):

$$\mathrm{GV} = \frac{\mathrm{NOAEL} \times \mathrm{bw}}{\mathrm{UF} \times \mathrm{C}} = \frac{98 \times 15}{100 \times 0.25} = 58.8\ \mu\mathrm{g/L} \approx 60\ \mu\mathrm{g/L}$$

Where:

GV	=	guideline value for recreational waters
NOAEL	=	no-observed-adverse-effect level (98 µg/kg bw/day; based on neurotoxicity in the study of Fawell et al 1999[250])
bw	=	body weight (15 kg for a child)
UF	=	uncertainty factor (100 = 10 for interspecies variation × 10 for intraspecies variation)
C	=	daily incidental water consumption (0.25 L for a child)

The calculation is based on toxicology data for anatoxin-a, which are very limited. Dihydroanatoxin-a has been demonstrated to have higher oral toxicity than anatoxin-a[204]; therefore, during a review of New Zealand's maximum acceptable values for cyanotoxins in drinking water, it was recommended that a toxicity equivalence factor of three is used for dihydroanatoxin-a[251]. Because no robust toxicology data were available for homoanatoxin-a and dihydrohomoanatoxin-a, a toxicity equivalence factor of one was suggested for these anatoxin congeners[251]. Testing providers may be able to provide more up-to-date information on toxicity equivalence factors for anatoxins.

Box 4: Calculation of guideline value for cylindrospermopsin in recreational waters

This calculation is for the 2020 WHO provisional recreational water guideline value for cylindrospermopsin (section 8.1 of the WHO background document for cylindrospermopsins; p 21–22[8]):

$$GV = \frac{NOAEL \times bw}{UF \times C} = \frac{30 \times 15}{300 \times 0.25} = 6\ \mu g/L$$

Where:

GV	=	guideline value for recreational waters
NOAEL	=	no-observed-adverse-effect level (30 µg/kg bw/day; based on cytotoxicity in the study of Humpage and Falconer, 2003[252])
bw	=	body weight (15 kg for a child)
UF	=	uncertainty factor (300 = 10 for interspecies variation × 10 for intraspecies variation × 3 for database deficiencies)
C	=	daily incidental water consumption (0.25 L for a child)

The calculation is based on toxicology data for cylindrospermopsin. Due to similar toxicity observed in cylindrospermopsin congeners (based on limited evidence), WHO recommends that total cylindrospermopsins are assessed as molar equivalents (pg 22 of the WHO cylindrospermopsins guideline document[8]). Testing providers may be able to provide more up-to-date information on toxicity equivalence factors for cylindrospermopsins.

Box 5: Calculation of guideline value for microcystin-LR in recreational waters

This calculation is for the 2020 WHO provisional recreational water guideline value for microcystin-LR (section 8.1 of the WHO background document for microcystins; pg 40[9]):

$$\mathrm{GV} = \frac{\mathrm{NOAEL} \times \mathrm{bw}}{\mathrm{UF} \times \mathrm{C}} = \frac{40 \times 15}{100 \times 0.25} = 24\ \mu\mathrm{g/L}$$

Where:

GV = guideline value for recreational waters

NOAEL = no-observed-adverse-effect level (40 µg/kg bw/day; based on liver toxicity in the study of Fawell, et al 1999[253])

bw = body weight (15 kg for a child)

UF = uncertainty factor (100 = 10 for interspecies variation × 10 for intraspecies variation)

C = daily incidental water consumption (0.25 L for a child)

The calculation is based on toxicology data for microcystin-LR. In the absence of oral toxicity data for other microcystin congeners, WHO recommends that total microcystins are assessed as gravimetric or molar equivalents (p 40 of the WHO microcystins guideline document[9]). Although not explicitly stated in the WHO guidance, nodularins should also be assessed in the same manner. A toxicity equivalence factor of one should be used for all microcystin and nodularin congeners unless new oral toxicity information becomes available. Testing providers may be able to provide more up-to-date information on toxicity equivalence factors for microcystins.

Box 6: Calculation of guideline value for saxitoxins in recreational waters

This calculation is for the 2020 WHO recreational water guideline value for saxitoxins (section 8.1 of the WHO background document for saxitoxins; p 18[10]):

$$GV = \frac{LOAEL \times bw}{UF \times C} = \frac{1.5 \times 15}{3 \times 0.25} = 30\ \mu g/L$$

Where:

GV	=	guideline value for recreational waters
LOAEL	=	lowest-observed-adverse-effect level (1.5 µg STX-eq/kg bw/day; based on neurotoxicity in the 2009 EFSA study[254])
bw	=	body weight (15 kg for a child)
UF	=	uncertainty factor (3 for use of a LOAEL rather than a NOAEL)
C	=	daily incidental water consumption (0.25 L for a child)

The calculation is based on human poisoning data for saxitoxins reported as STX-equivalents. Saxitoxin measurements in recreational freshwaters should also be assessed as STX-equivalents. Toxicity equivalence factors for saxitoxin congeners, assessed in the regulatory monitoring for saxitoxins in bivalve molluscan shellfish in Aotearoa, can be found in Cawthron Report 3219[255]. This includes updates recommended in the 2016 FAO / WHO technical paper[256], as well as toxicity equivalence factors adopted for other saxitoxin congeners (not included in the 2016 FAO / WHO technical paper) in our country's regulatory monitoring for saxitoxins in bivalve molluscan shellfish. In the absence of a saxitoxin toxicity equivalence factor, and with no oral toxicity data to base it on, a toxicity equivalence factor of one should be used. This aligns with the advice provided by WHO; to either evaluate total saxitoxins as gravimetric or molar equivalents, or as toxicity equivalents relative to saxitoxin (p 18–19 of the WHO saxitoxins guideline document[10]). Testing providers may be able to provide more up-to-date information on toxicity equivalence factors for saxitoxins.

Box 7: Rationale for the Situation 1 alert-level thresholds

For each of the cyanotoxins produced by planktonic cyanobacteria in Aotearoa (anatoxin-producing *Cuspidothrix issatschenkoi*, cylindrospermopsin-producing *Raphidiopsis raciborskii*, microcystin-producing *Microcystis* spp and nodularin-producing *Nodularia spumigena*), cell concentration thresholds were developed using toxin quota data. Depending on the data available, the toxin quota datasets were either based entirely on data from Aotearoa (microcystins and nodularins), based entirely on international data (cylindrospermopsins) or based on a mixture of data from national and international data (anatoxins). The summary statistics for this toxin quota data can be found in box 8 and more information can be found in Cawthron Report 3726[11].

The Action Level (red mode) – Situation 1 cell concentration thresholds for cyanotoxin-producing planktonic cyanobacteria observed in Aotearoa (see Decision Chart 1 in section 3 of the guidelines) were derived using the 2020 WHO cyanotoxin guideline values and the toxin quota data. For cylindrospermopsins, microcystins and nodularins, the mean toxin quota was used and, for anatoxins, the maximum toxin quota was used. The threshold derivation can be found in box 9 and more information can be found in Cawthron Report 3726[11].

The Surveillance Level (green mode) – Situation 1 cell concentration threshold, which triggers Alert Level (amber mode), was set at 500 cells/mL for each of the cyanotoxin-producing planktonic cyanobacteria observed in Aotearoa (*Cuspidothrix issatschenkoi*, *Raphidiopsis raciborskii*, *Microcystis* spp and *Nodularia spumigena*). Calculations indicated that this would provide safety, as cell concentrations for each cyanotoxin class were > 500 cells/mL at 10 per cent of the recreational guideline values (box 9). Note, that this is slightly different from the approach suggested in Cawthron Report 3726[11], where the total cyanobacteria cell concentration was used, but still provides advanced warning of a potential risk to human health.

Note that an Action Level (red mode) – Situation 1 cell concentration threshold was not developed for saxitoxin-producing cyanobacteria because no **planktonic** saxitoxin-producing cyanobacteria have been recorded in Aotearoa to date. The saxitoxin Action Level (red mode) – Situation 4 cyanotoxin concentration threshold has been retained for the eventuality that saxitoxin-producing planktonic cyanobacteria are observed in Aotearoa in the future and for situations where saxitoxin-producing benthic cyanobacteria need to be managed (see appendix 5 – Saxitoxins in Aotearoa).

Box 8: Summary of toxin quota data from a literature review of studies on planktonic cyanobacteria

		Toxin Quota (pg/cell)[a]				
Toxin	**n**	**Min**	**Max**	**Median**	**Mean**	**95th Percentile**
Anatoxins[b]	6	0.03	**0.41***	0.10	0.18	-[f]
Cylindrospermopsins[c]	33	0.004	14.6	0.03	**1.15***	6.72
Microcystins[d]	50	0.006	5.95	0.17	**0.77***	3.68
Nodularins[e]	11	0.26	3.96	1.50	**1.71***	-[f]

a More information on the toxin quota data evaluated can be found in Cawthron Report 3726[11]. b A mixture of data from Aotearoa and international data was used. c Because no data from Aotearoa were available, international data were used. d Because sufficient data were available, only studies from Aotearoa were used. e Because no international data were available, only data from Aotearoa were used. f Unable to calculate a 95th percentile value due to insufficient data. * These toxin quota values were used for formulating cell concentration thresholds in the alert levels framework.

Box 9: Calculation of cyanobacteria cell concentration thresholds for each toxin type using the mean or maximum toxin quota values

Calculation Component	**ATXs**	**CYNs**	**MCs**	**NODs[a]**
Toxin quota value (pg/cell)	0.41[b]	1.15[c]	0.77[c]	1.71[c]
Recreational guideline values (µg/L)	60	6	24	24
10% recreational guideline value (µg/L)	6	0.6	2.4	2.4
Cell concentration threshold (cells/mL)	14,600	520	3,100	1,400
Adopted Surveillance Level threshold (cells/mL)	500	500	500	500
100% recreational guideline value (µg/L)	60	6	24	24
Cell concentration threshold (cells/mL)	146,300	5,200	31,000	14,000
Adopted Action Level threshold (cells/mL)	100,000	5,000	30,000	10,000

ATXs = Anatoxins, CYNs = Cylindrospermopsins, MCs = Microcystins, NODs = Nodularins, WHO = World Health Organization. a WHO does not have a defined guideline value for nodularins, but the microcystin guideline value is used here due to the similar toxicity and mode of action for these cyanotoxins. b The maximum toxin quota has been used. c The mean toxin quota has been used.

Box 10: Rationale for the Situation 2 and 3 alert-level thresholds

A second guideline (Action Level [red mode] − Situation 2 in Decision Chart 1; see section 3 of the guidelines) is required for circumstances where high cell densities of cyanobacteria are present; that is, where the cyanobacterial population is not expected to contain known cyanotoxins (anatoxins, cylindrospermopsins, microcystins, nodularins or saxitoxins). Even when toxin-producing taxa are not present, it is appropriate to issue warnings if the total biovolume of all cyanobacterial material exceeds 10 mm3/L (Situation 2) or cyanobacterial scums are present (Situation 3).

This guideline recommendation is based on the work of Stewart et al (2006)[39], showing an increased 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)[39], where a total surface area of 12 mm2/mL is given as being equivalent to a total biovolume of approximately 12.5 mm3/L. This value has been rounded down to a more conservative value of 10 mm3/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 by the consistent presence of scums (that is, 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[257]. This also allows water managers to respond to the presence of high levels of cyanobacteria in a water body without needing to wait for cell enumeration results (which might take a week or longer to be processed).

Box 11: Rationale for the Situation 4 alert-level threshold

The Action Level (red mode) − Situation 4 guideline provides a mechanism for water managers to respond to the human health risks posed by new toxin-producing cyanobacteria that could be identified in Aotearoa in the future (but are not currently included in the Situation 1 thresholds). Cyanotoxin testing can also be used de-escalate from Action Level (red mode) when it has been triggered through Situation 1 (cell concentrations for confirmed toxin-producing cyanobacteria observed in Aotearoa) but where cyanobacteria biovolumes do not breach the threshold for Situation 2 (≥ 10 mm^3/L total biovolume of all cyanobacteria; see section 3.3.3).

Benthic Microcoleus in rivers

The *Microcoleus* alert-level framework (Decision Chart 2 in section 3 of the guidelines) is based on three tiers of alert-levels, with benthic mat abundance and detachment of mats as the triggers for changes in thresholds. Methods for conducting a site survey are found in section 4.4. 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 Aotearoa 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 coverage (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, so recreational water users should be more vigilant.

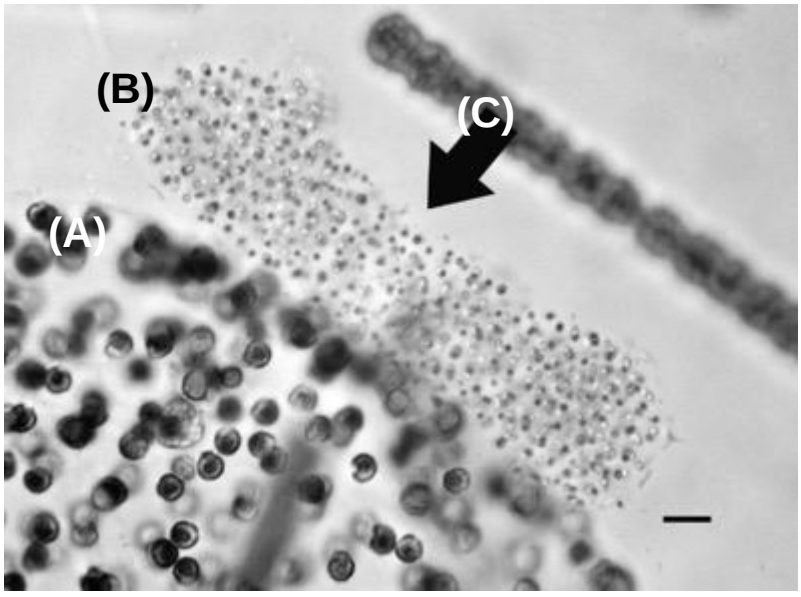
The *Microcoleus* alert-level framework is based on preliminary research and observations, and it is anticipated that these will require further refining as knowledge and monitoring tools improve. Note that the alert-level framework is designed to manage risks to **recreational water users**. The levels given in the framework are not relevant for addressing risks to dogs that actively seek out and eat *Microcoleus*-dominated cyanobacterial mats. Raising public awareness through information and warning signs (see Appendices 15 and 16), media releases (see appendix 17), and information pamphlets and factsheets (see Appendices 18 and 19) are recommended to reduce human exposure and dog poisonings. Refer to section 5 of these guidelines for comprehensive information on toxic algae communications.

Toxin testing can be used to help further define the health risk at sampling sites. However, quantitatively measuring toxin levels (even if samples are collected quantitatively) in *Microcoleus*-dominated cyanobacterial mats can be problematic. This arises from 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. Estimating the human health risk of toxin-containing cyanobacterial mats, and determining acceptable thresholds, is also difficult and requires more work by risk-modelling experts. For these reasons, no toxin concentrations have been recommended within the framework. Future research may enable the inclusion of toxin thresholds, as well as the potential refinement of the percentage coverage thresholds currently used in the alert-level framework for benthic *Microcoleus* in rivers.

Appendix 7
Biovolumes explained

Cell concentration measurements do not account for variability in the size of different cyanobacterial taxa (see figure A7.1). This is particularly relevant when there are high concentrations of picocyanobacteria (cyanobacteria that are very small, < 2 µm; for example, *Aphanothece* sp and *Aphanocapsa* sp), which have been reported with increasing frequency in recent years. Using biovolumes to evaluate the total abundance of planktonic cyanobacteria in a water body (the Situation 2 thresholds in the alert-level framework for planktonic cyanobacteria; see Decision Chart 1 in section 3 of the guidelines) avoids health warnings being needlessly issued in water bodies dominated by picocyanobacteria.

Figure A7.1: Light photomicrographs demonstrating the difference in cell size among A) *Microcystis* sp; B) *Aphanocapsa holsatica*; and C) *Dolichospermum planctonicum* (Arrow points towards *Ap. holsatica)*. Note: Scale bar = 10 µm



It is time-consuming to measure and calculate mean cell volumes for every taxon identified in routine counting, so it is recommended that standardised species lists with fixed biovolumes are used. Where possible, these should be specific to the water bodies being monitored and updated periodically through measuring the volumes of 10–20 cells of a certain cyanobacterial species when they appear in samples. In conjunction with the development of these guidelines, biovolumes for 22 of the most problematic species in Aotearoa lakes have been established (table A7.1).

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

1. In taxa that contain specialised cells such as akinetes and heterocysts or 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)[258] showed that preserving samples with Lugol's iodine (a preservative commonly used in Aotearoa) causes shrinkage rates of up to 40 per cent, depending on the 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 iodine-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 (μm^3)

1×10^6 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 *Dolichospermum planctonicum* and 5,600 cells/mL of *Microcystis wesenbergii* is calculated as follows:

BV (*D. planctonicum*) = $(10{,}200 \times 399^*) / 1 \times 10^6$

= $4.07\ mm^3/L$

BV (M. *wesenbergii*) = $5600 \times 182^* / 1 \times 10^6$

= $1.02\ mm^3/L$

Total BV = 4.07 + 1.02

= **$5.09\ mm^3/L$**

* Using values from table A7.1.

Table A7.1: Biovolumes of common cyanobacteria in Aotearoa New Zealand

Cyanobacterial taxa	Average volume (mm^3)	Length (mm)	Width (mm)	Diameter (mm)	Source	Shape	Count (n)
Aphanocapsa holsatica	1.7			1.4 (0.8, 2.3)	Kaituna River, Lakes Ōkareka, Okaro, Rotoiti, Waahi	OVOR	48
Aphanizomenon gracile	32	4.4 (2, 11.3)	2.8 (1, 5)		Lakes Rotoiti, Tarawera, Waikare	CYL	50
Anathece clathrata (previously *Aphanothece clathrate)*	2.1	2.3 (1.8, 3.2)	1 (0.7, 1.4)		Lakes Ōkareka, Okaro, Tarawera	CYL	30
Chroococcus cf. minutus	35	2.7 (2.1, 3.4)	4.9 (3.6, 6.1)		Lake Waahi	OVO	30
Coelosphaerium kuetzingianum	8.9	2.0 (1.2, 2.9)	2.7 (2, 4)		Kaituna River, Lake Rotoiti	OVO	32
Cuspidothrix issatschenkoi (previously *Aphanizomenon issatschenkoi)*	89	10.7 (6.5, 264.1)	3.2 (1.6, 4.7)		Lake Kainui	CYL	30
Dolichospermum circinale (previously *Anabaena circinalis)*	208	5.9 (4, 8.2)	8.2 (6.1, 10.9)		Lakes Kainui, Maraetai	OVO	39
Dolichospermum lemmermannii (previously *Anabaena lemmermannii)*	116	5.5 (3.1, 8.5)	6.3 (4, 8.5)		Lakes Karapiro, Ōkareka, Rotoehu, Rotoiti, Rotorua, Tarawera	OVO	50
Dolichospermum planctonicum (previously *Anabaena planctonica)*	399	6.8 (3.9, 10.2)	10.5 (7.3, 13.3)		Kaituna River, Lakes Karapiro, Ngaroto, Okaro, Rotoiti, Rotorua, Tarawera	OVO	75
Leptolyngbya cf. subtilis	8.6	3.2 (2, 5)	1.8 (1.2, 2.6)		Lake Kainui	CYL	30
Merismopedia punctata	6.4	2.8 (2, 3.8)	2 (1.5, 2.8)		Lake Forsyth	OVO	30
Microcystis sp. (small)	19			3.2 (2.4, 4.2)	Lake Ngaroto, Ōkareka, Okaro, Rotoehu, Rotoiti, Tarawera	OVOR	60
Microcystis sp. (large)	93			5.5 (4.1, 7.4)	Kaituna River, Lakes Rotoehu, Rotoiti, Rotorua, Tarawera	OVOR	54

Cyanobacterial taxa	Average volume (mm^3)	Length (mm)	Width (mm)	Diameter (mm)	Source	Shape	Count (n)
Microcystis wesenbergii	182			6.9 (4.6, 9)	Lakes 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)		Lake Waikare	CYL	30
Planktolyngbya limnetica (previously *Planktolyngbya subtilis)*	3	3 (2, 4.5)	1.1 (0.8, 1.6)		Lakes Waahi, Waikare	CYL	42
Planktothrix cf. agardhii	28	3 (2, 4.8)	3.4 (2.9, 4.1)		Oxidation pond (Horowhenua)	CYL	30
Pseudanabaena limnetica	8.3	3.7 (1.9, 6.8)	1.6 (1.1, 2.2)		Lakes Karapiro, Ōkareka, Rotoehu, Rotoiti	CYL	30
Raphidiopsis raciborskii (previously *Cylindrospermopsis raciborskii)*	15	6.5 (3.6, 9.9)	1.7 (1, 3.9)		Lake Whangapehe	CYL	30
Snowella lacustris	99	5 (3.5, 7.7)	6.0 (4.2, 8.6)		Lakes Rotoiti, Rotorua	OVO	48
Trichodesmium iwanoffianum	102	4.3 (3.4, 5.3)	5.4 (4.4, 7.1)		Lakes Ōkareka, Okaro, Rotoiti, Tarawera	CYL	30

Note: Equations given by the United States Environmental Protection Agency (2007)[259] 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 Aotearoa New Zealand.

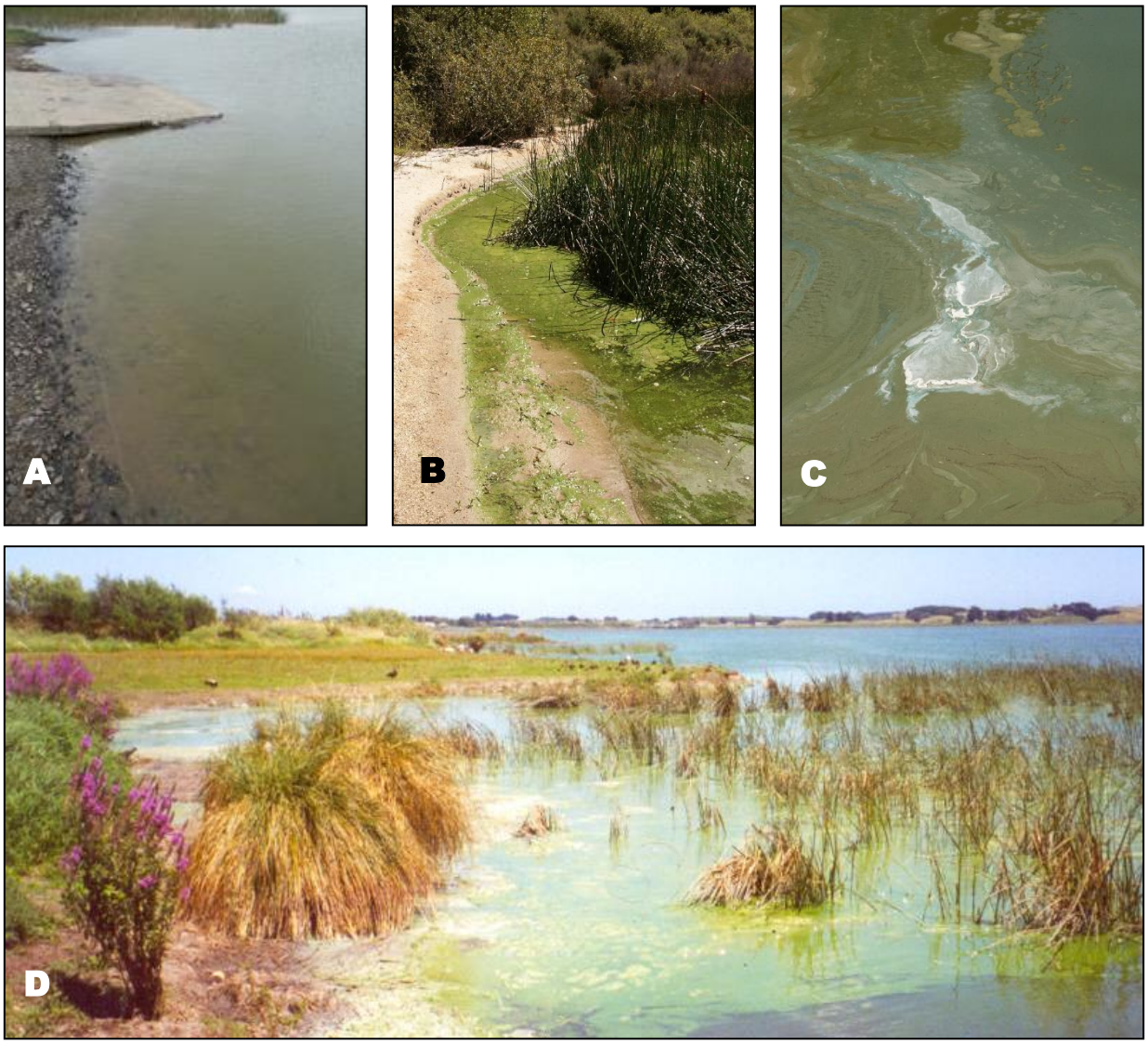
Table A7.2: Volume equations for common cyanobacteria cell shapes

Cell shape	Formula
Ovoid (round)	$V = ((4 / 3) \times p \times (\text{diameter} / 2)^3)$
Ovoid	$V = (4 / 3) \times p \times (\text{width} / 2)^2 \times (\text{length} / 2)$
Cylinder	$V = (p \times (\text{width} / 2)^2 \times (\text{length}))$

Source: United States Environmental Protection Agency (2007)[259].
p = pi (π; 3.142)

Appendix 8
Photographs of planktonic blooms

This appendix shows examples of blooms and cyanobacterial scums of different planktonic cyanobacterial species.



(a) Toxic bloom of *Raphidiopsis raciborskii* (Lake Waahi, Waikato) of approximately 200,000 cells/mL. Cells were not visible to the eye. The water had a slight brown tinge but was not markedly different from the usual colour of this peat lake.

(b) Toxic bloom of *Microcystis panniformis* (Lake Rotoehu, Rotorua). Colonies were visible in the water and scum had formed along the shoreline.

(c) A non-toxic bloom of *Dolichospermum circinale* and *Dolichospermum 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 as blooms decline.

(d) Toxic bloom of *Microcystis* spp. (Lake Horowhenua, Levin). A thick scum had accumulated along the downwind shoreline.

Photos: SA Wood (Lincoln University).

(e) A non-toxic bloom of *Dolichospermum planctonicum* (Lower Karori Reservoir, Wellington). This species rarely forms scums.

(f) Shoreline scum of toxic *Microcystis* spp. (Lake Wiritoa, Levin).

(g) Shoreline scum of toxic *Microcystis panniformis* (Lake Rotoehu, Rotorua).

Photos: SA Wood (Lincoln University).

(h) Bloom of *Dolichospermum lemmermannii* (Lake Waihola, Dunedin).

(i) Bloom of *Dolichospermum lemmermannii* (Lake Waihola, Dunedin).

Photos: J Milne (Wellington Regional Council).

Appendix 9
Sample media release – planktonic cyanobacteria in lakes

The following is sample text for inclusion in media releases relating to recreational water bodies affected by levels of planktonic cyanobacteria that may cause adverse effects to human health.

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 (sometimes called 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 during recreational activities. Accidentally or deliberately swallowing water containing cyanobacteria may cause symptoms such as nausea, tummy upset, breathing difficulties, and tingling or numbness around the mouth or the tips of fingers. Some people can also experience rashes through contact with the skin. 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 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. Cyanobacterial concentrations can change quickly with changing environmental conditions (for example, 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 10
Example health warning signs for planktonic cyanobacteria in lakes.

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>

WARNING

Toxic algae may be present.

Until further notice:

Do not swim in areas of scum

Do not drink lake water

Keep pets and livestock away

Clean fish well and discard guts

Avoid areas of scum when boating

Call your doctor or veterinarian if you or your animals have sudden or unexplained sickness or signs of poisoning

Report toxic algae to the Pollution Hotline: 0800 800 033

Otago Regional Council · Queenstown Lakes District Council · Southern District Health Board

Check out ORC's latest recreational water quality results for waterways across Otago at: **www.lawa.org.nz/swim**

LAWA LAND AIR WATER AOTEAROA

Appendix 11
Photos of benthic *Microcoleus* and other benthic algae

(a) *Microcoleus*-dominated mat (Hokitika River, West Coast).

(b) *Microcoleus*-dominated mat (Hokitika River, West Coast).

(c) *Microcoleus*-dominated mats drying out on the river's edge. (Hokitika River, West Coast).

(d) Detached *Microcoleus*-dominated mat drying out on the river's edge.

Photo: K Shearer (Cawthron).

(e) Dark brown *Microcoleus*-dominated mats (Hutt River, Wellington). Tyre tracks run through the centre of the mats

Photo: M Heath (Victoria University).

(f) Detached *Microcoleus*-dominated mat drying on the river's edge.

Photo: SA Wood (Lincoln University).

(g) Detached *Microcoleus*-dominated mat (Hutt River, Wellington) on the river's edge.

Photo: SA Wood (Lincoln University).

(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 *Microcoleus*-dominated mat growing on a rock taken from the Makarewa River (Southland).

Photo: K Meijer (Environment Southland).

(j) Thick *Microcoleus*-dominated mat growing on a rock taken from the Whakatane River (Whakatane).

Photo: SA Wood (Lincoln University).

(k) *Microcoleus*-dominated mat attached to a large boulder, Hutt River, Wellington.

Photo: M Heath (Victoria University).

(l) *Microcoleus*-dominated mats in the Wakapuaka River (Nelson).

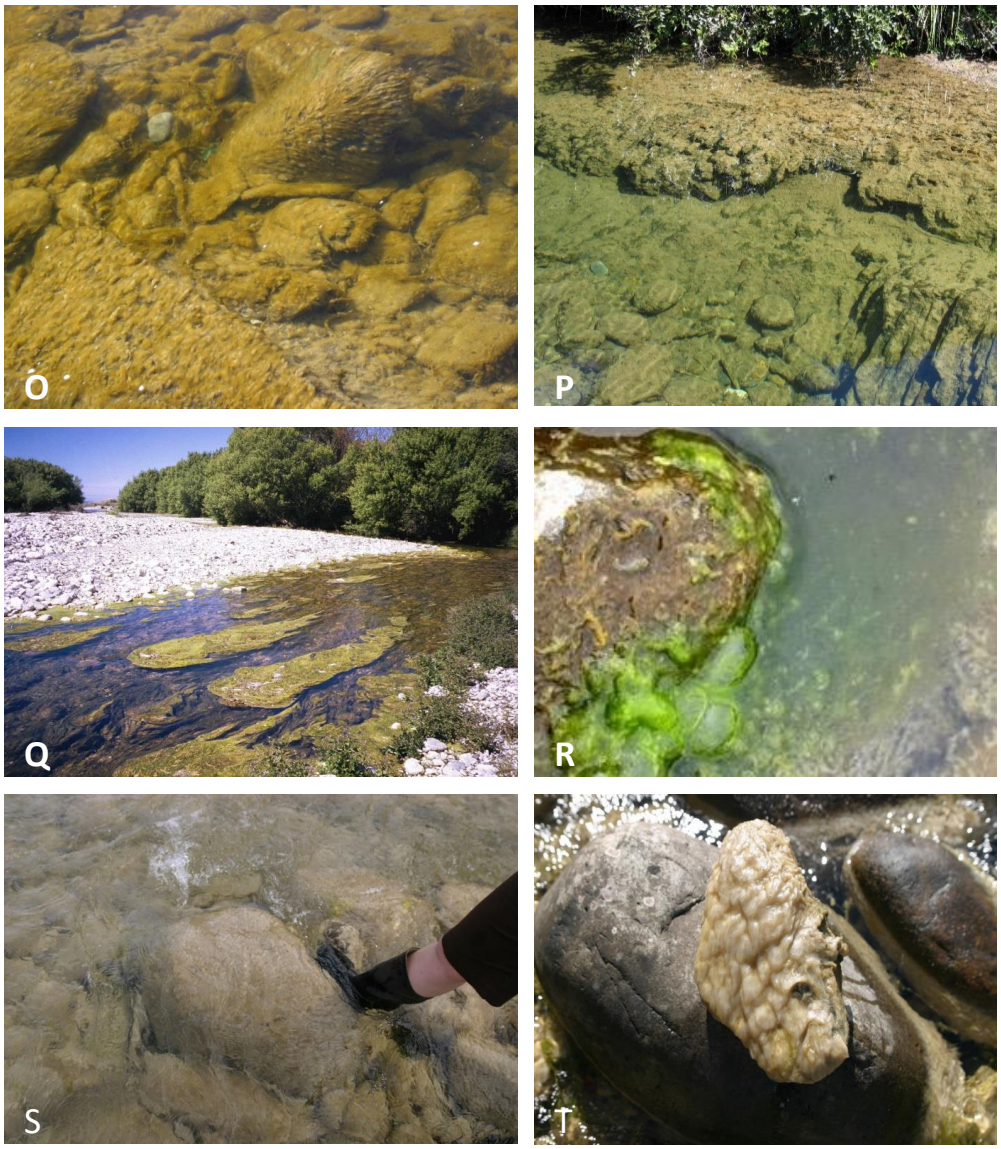
Photo: A Crowe (Cawthron).

(m) *Microcoleus*-dominated mats at the Silverstream Bridge in the Hutt River (Lower Hutt, Wellington).

(n) *Microcoleus*-dominated mats at the Silverstream Bridge in the Hutt River (Lower Hutt, Wellington).

Photos: J Milne (Wellington Regional Council).

Non-cyanobacterial benthic mats



(o) Native diatom mats.

Photo: A Crowe (Cawthron).

(p) Native diatom mats.

Photo: A Crowe (Cawthron).

(q) Filamentous green algae.

Photo: A Crowe (Cawthron).

(r) Filamentous green algae.

Photo: A Crowe (Cawthron).

(s) Mats of the invasive diatom didymo.

Photo: SA Wood (Lincoln University).

(t) Mat of the invasive diatom didymo.

Photo: SA Wood (Lincoln University).

Appendix 12
Cyanobacteria and cyanotoxin analysis capabilities in Aotearoa

Table A12.1: Freshwater microalgae / cyanobacterial analysis capabilities in Aotearoa

Organisation	IANZ accred.*	Location	Contact
Cawthron	Yes	Nelson	Phytoplankton Testing Laboratory Ph: 03 548 2839 Email: NaturalToxinsSection@cawthron.org.nz Web: cawthron.org.nz/what-we-do/cawthron-laboratories
Manaaki Whenua / Landcare Research	No	Lincoln	Allan Herbarium Plant Identification Service Ph: 03 321 9797 Mobile: 027 411 0203 Email: Plantinfo@landcareresearch.co.nz Web: landcareresearch.co.nz/tools-and-resources/collections/allan-herbarium/services/
NIWA	Yes	Hamilton	Algal Monitoring Service Ph: 07 856 7026 Email: algalservices@niwa.co.nz Web: niwa.co.nz/our-science/freshwater/our-services/specialist-analytical-services/algal-monitoring-service
SLR Consulting	No	Dunedin	Ben Ludgate Ph: 03 390 8500 Email: ben.ludgate@slrconsulting.com Web: www.slrconsulting.com
Watercare Laboratory Services	Yes	Auckland	Ph: 0800 522 365 Email: labsales@water.co.nz Web: watercarelabs.co.nz/specialties/water-testing/environmental-and-recreational/

While current in February 2023, contact details and testing services offered may have changed since then. * 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. IANZ accreditation is required for water bodies used as drinking-water supplies.

Table A12.2: Cyanotoxin analysis capabilities in Aotearoa

Laboratory	Cyanotoxins	Method	IANZ accred.*	Contact
AgResearch Hamilton	Total MC / NOD	Adda-ELISA	No	Jan Sprosen Ph: 07 838 5203 Email: jan.sprosen@agresearch.co.nz
Cawthron	MC / NOD	LC-MS	Yes	Biotoxin Testing Laboratory Ph: 03 548 2839 Email: NaturalToxinsSection@cawthron.org.nz Web: cawthron.org.nz/what-we-do/cawthron-laboratories
	ATXs	LC-MS	Yes	
	CYN	LC-MS	Yes	
	SAX	LC-MS	Yes	
	SPATT	LC-MS	No	
Watercare Laboratory Services	MC / NOD	LC-MS	Yes	Organic Chemistry Services Ph: 0800 522 365 Email: labsales@water.co.nz Web: watercarelabs.co.nz/specialties/water-testing/environmental-and-recreational/
	ATX / HTX	LC-MS	Yes	
	CYN	LC-MS	Yes	

Although they are current in February 2023, contact details and testing services offered may have changed since then.
* International Accreditation New Zealand (IANZ) is the national authority for the accreditation of testing and calibration laboratories. Because of the non-routine nature of some situations, IANZ accreditation is not necessarily required, however, you should have confidence in the testing undertaken and an understanding of the limitations of the results. IANZ accreditation is required for water bodies used as drinking-water supplies when analysing for cyanotoxins specified in the *Drinking-Water Standards for New Zealand*.
[#] Not routinely used but potentially available on request. LC-MS = liquid chromatography-mass spectrometry; ELISA = enzyme-linked immunosorbent assay; MC = microcystin; NOD = nodularin; ATX = anatoxin-a; HTX = homoanatoxin-a; ATXs = anatoxins (ATX, HTX and the dihydro-congeners of each); CYN = cylindrospermopsin; SAX = saxitoxin; SPATT = solid-phase absorption toxin tracking (tests for MC / NOD and ATXs / CYN are available).

Appendix 13
Benthic cyanobacterial mats in lakes

Although commonly associated with rivers in Aotearoa, benthic cyanobacteria can also be found in lakes and ponds; in some instances, these taxa also produce cyanotoxins. Generally, benthic cyanobacteria in lakes either occur as mats that are initially attached to the substrate or as 'rafts' that detach and float on the surface or subsurface, particularly in sheltered bays. Cyanobacteria can also grow at the edge of lakes and ponds among macrophytes, where they can be epiphytic (attached to plants) or form long filaments growing amongst the macrophytes (known as metaphyton).

Below is a summary of known instances of toxic benthic cyanobacteria in lakes and ponds in Aotearoa. This summary highlights their different growth forms and appearances and the common locations where they might occur in lakes and ponds. Research in this field is limited and it is likely that further toxic benthic cyanobacterial taxa will be identified in lakes and ponds around Aotearoa.

Unknown nodularin producer

A survey of periphyton (depth 6–7.5 m) in Lake Tikitapu (Rotorua) revealed widespread thick spongy benthic mats with a mixed assemblage of cyanobacterial species from the orders Oscillatoriales, Nostocales and Chroococcales (figure A13.1[57]). Although all benthic mats tested contained nodularin, attempts to identify which species produced the toxin were unsuccessful.

Figure A13.1: Cyanobacterial-dominated mats in Lake Tikitapu (Rotorua).

Photos: Aleki Taumoepeau (NIWA).

Microcoleus (previously *Phormidium*)

Microcoleus-dominated mats are commonly associated with cobble-bed rivers in Aotearoa; however, they also regularly occur in lakes. For example, extensive mats have been identified on the bottom of lakes in the Rotorua region (those tested to date were non-toxic[57]). In 2014, the ingestion of mats dislodged from the bottom of a small farm pond (figure A13.2) caused the death of a dog. Culturing, molecular and toxin analysis confirmed the causative species was *Microcoleus autumnalis*: the same species that causes problems in rivers around Aotearoa[18].

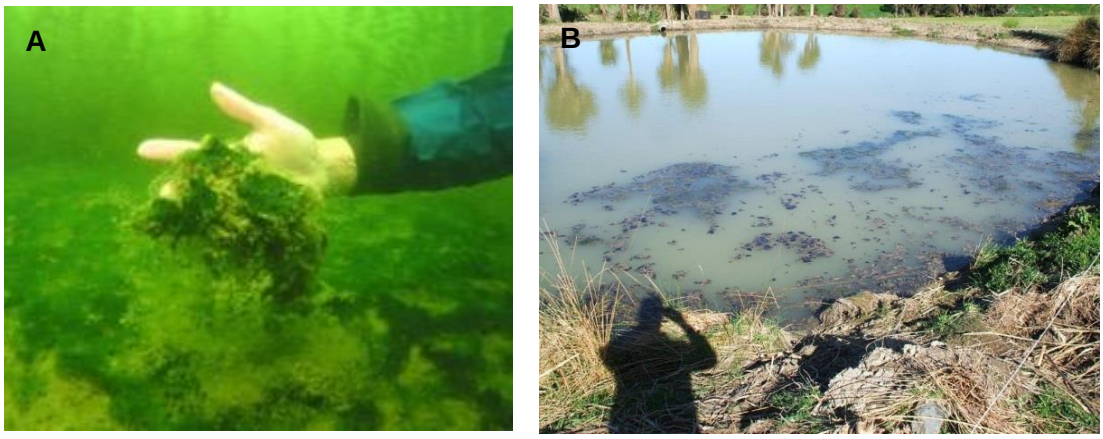


Figure A13.2: A) *Microcoleus*-dominated mats in Lake Rotoiti (Rotorua), and B) detached *Microcoleus autumnalis* mats in a small farm pond (Kaikōura). *Photo A: A Taumoepeau (NIWA).*

Scytonema cf. crispum

Scytonema cf. *crispum*, collected from the metaphyton of a pre-treatment reservoir (Oamaru) for the supply of drinking water; a small eutrophic lake (The Groynes, Christchurch); and a number of recreational lakes in Canterbury tested positive for saxitoxins[34, 56]. *Scytonema* cf. *crispum* forms long, dark-green filaments that grow at the edges of lakes, commonly among macrophytes (figure A13.3). *Scytonema* has been reported in the North Island , where it is common in the littoral zone in Lake Taupō[260], in algal mats in Lake Taharoa and in the Waimangu geothermal lakes[261]. In the South Island it is reported from the benthos of Lake Coleridge[262]. However, toxin testing of mats from these locations has not been undertaken.

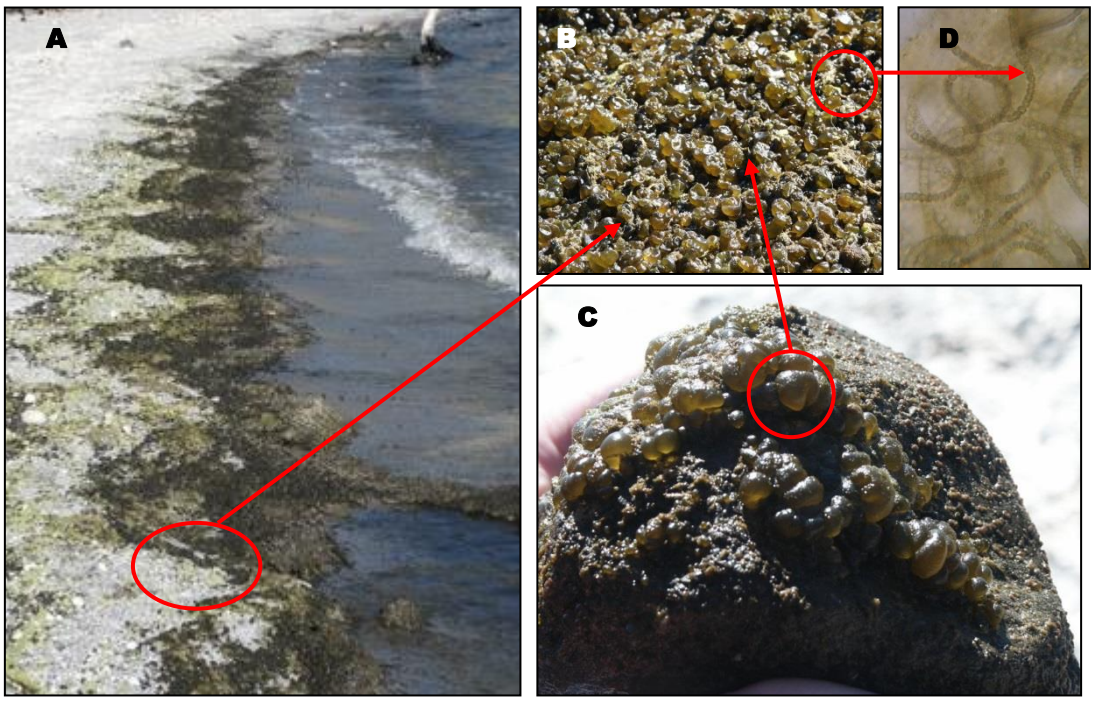
Figure A13.3: A) *Scytonema* cf. crispum growing as metaphyton among aquatic plants, B) dense filamentous mats, and C) light photomicrograph.

Photos: F Harland (University of Canterbury).

Nostoc sp

Thick gelatinous mats of *Nostoc* sp were seen on the eastern shore of Lake Taupō in 2003 (figure A13.4[21]). These mats had accumulated along the shoreline following a storm that dislodged colonies from rocks. The mats contained high levels of microcystins. *Nostoc* is common in many lakes and rivers in Aotearoa.

Figure A13.4: *Nostoc* sp. mats along the shores of Lake Taupō.
A-C) low lake levels and high winds caused *Nostoc* sp. colonies to be dislodged from rocks in the lake and these accumulated along the shoreline;
D) *N. commune* filaments under the microscope.



Photos: S Wood (Lincoln University).

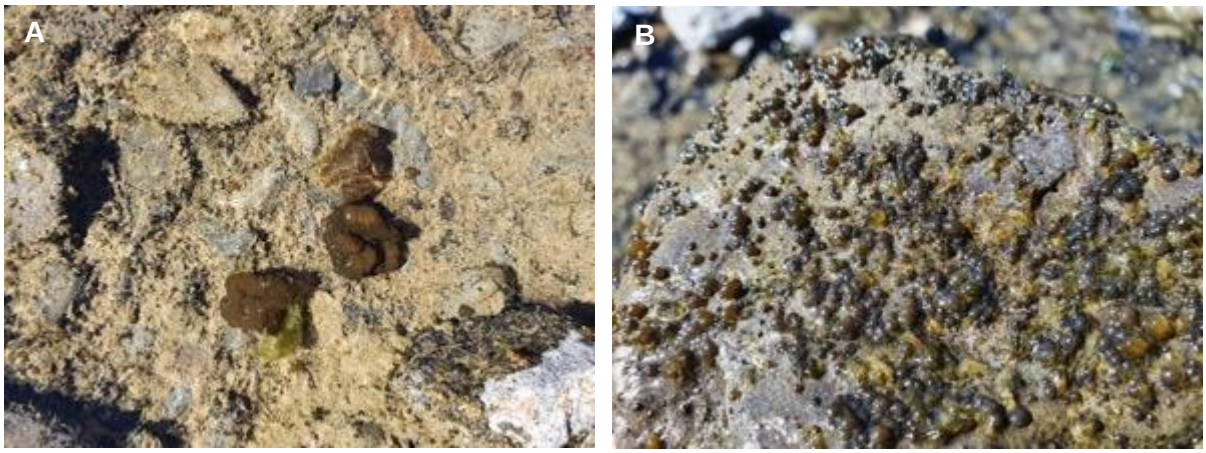
Appendix 14
Other toxin-producing benthic cyanobacteria in rivers

Although *Microcoleus* is the most prevalent benthic cyanobacteria observed in rivers around Aotearoa[263], other toxin-producing benthic cyanobacteria are also found in rivers. If potentially toxic benthic cyanobacteria are encountered in rivers, a good first step is to collect samples for microscopic identification and cyanotoxin testing (see section 4.4.3 – Step 6 for more information). If the high levels of mats or colonies are present at a recreational site, prevalent along a water body or have caused animal poisonings, access expert advice on how to evaluate and manage the situation, and the public health risk, through the National Public Health Service.

Microcystin-producing Nostoc spp

Microcystin-producing benthic *Nostoc* spp have been seen in rivers in Aotearoa[27]; however, their prevalence in river environments and toxin production capacity isn't well-established. Different species with differing morphologies have been found in rivers around Aotearoa to date, from globular morphotypes (figure A14.1A), to smaller jelly-like balls (figure A14.1B), to small sponge-like balls (no image available).

Figure A14.1: *Nostoc* spp found in a tributary of the Waiau Uwha River.



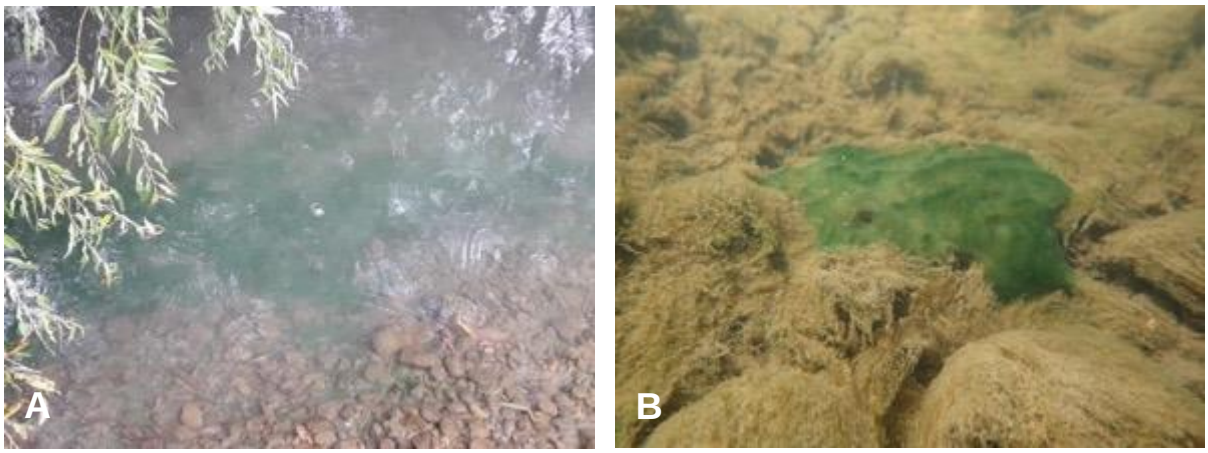
Photos: L Kelly (Cawthron).

Microcystin-producing Planktothrix sp

Microcystin-producing benthic *Planktothrix* sp occurs in rivers in Aotearoa, although it is not frequently reported[27]. The initial identification of this *Planktothrix* species in Aotearoa was associated with a dog death at the Waitaki River in South Canterbury[16].

Although most *Planktothrix* species seen overseas are planktonic and can form blooms in lakes, the toxin-producing *Planktothrix* observed in Aotearoa to date is benthic, or biphasic when in culture (can exist in benthic and planktonic forms). Rather than the thick benthic *Microcoleus* mats that often grow in the fast-flowing portions of a river, the benthic *Planktothrix* found in Aotearoa forms thin bright-green films on the bottom of the pools found on the sides of rivers and in back-water regions, or in slow-flowing regions of rivers (figure A14.2).

Figure A14.2: Benthic *Planktothrix* sp (the green film) growing A) in a pool to the side of Rakahuri / Ashley River and B) on the base of a river (location unknown).



Photos: J Puddick (A; Cawthron) and SA Wood (B; Lincoln University).

Cryptic cyanobacteria

In California (USA), toxin-producing cyanobacteria have been found growing among other benthic cyanobacteria and algal mats not traditionally considered as dangerous (in this case the green alga *Cladophora glomerata*)[264]. If the toxin-producing cyanobacteria are the sub-dominant taxa in algal mats, they may be difficult to identify by visual observation – and so microscopy or molecular analysis is required.

Although this situation hasn't been recorded in Aotearoa, and would be difficult to manage routinely, the sampling of other periphyton besides cyanobacteria-dominated mats should be considered when investigating suspected cases of cyanobacteria poisoning.

Appendix 15
Example information signs for benthic cyanobacteria in rivers

An effective information sign should tell the public what toxic algae looks like and what to do to stay safe if they come across it in waterways. Photographs on the signs are useful to show people what to look out for. The signs should not be intimidating (for example, use neutral colours and no warning signals) as, otherwise, this can lead to desensitisation to warning signs that are erected when health warnings are issued (see appendix 16 for examples of these). Example information signs used by the Horizons Regional Council and Greater Wellington are given on the following two pages.

IS IT SAFE TO SWIM?

CHECK FOR TOXIC ALGAE

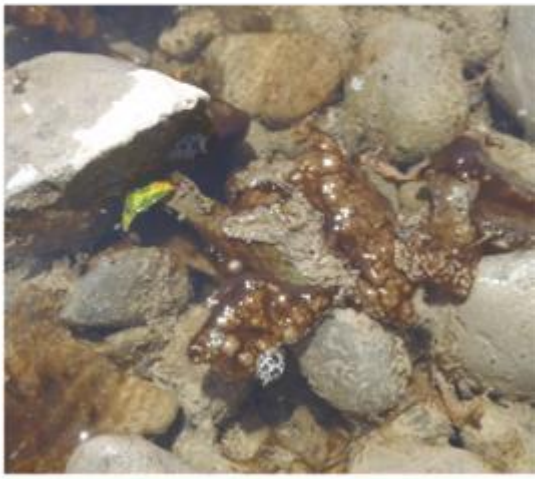
Toxic algae coats rocks in the river and forms floating mats at the water's edge, which can be deadly if swallowed. Please keep an eye on babies and toddlers who like to put things in their mouths.

If a person in your group swallows even a small toxic algae mat (coin sized or less) seek IMMEDIATE medical attention.

DOGS

Toxic algae mats are deadly to dogs, who love the taste of them. When there is a bloom please keep your dog on a leash.

If your dog swallows toxic algae seek IMMEDIATE veterinary attention.



HUTT CITY · UPPER HUTT CITY · Kāpiti Coast DISTRICT COUNCIL · MASTERTON DISTRICT COUNCIL · CARTERTON · SOUTH WAIRARAPA DISTRICT COUNCIL · Absolutely Positively Wellington City Council

Visit the Greater Wellington website for more information on toxic algae.
www.gw.govt.nz/safeswim

greater WELLINGTON
REGIONAL COUNCIL
Te Pane Matua Taiao

Appendix 16
Example health warning signs for benthic cyanobacteria in rivers

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. Warning signs should only be put in place when the Action Level (red mode) threshold is exceeded. Examples of warning signs used by Greater Wellington and Tasman District Council are given on the following two pages.

Toxic cyanobacteria (blue-green algae) health hazard

Warning: potentially toxic cyanobacteria are present in this river or 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 or 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>

DANGER

TOXIC ALGAE IS IN THIS RIVER

IT CAN MAKE PEOPLE SICK AND KILL DOGS

Don't swim here or touch the algae

Don't let dogs near the algae

If you or your dog get sick after being in or near the river, contact your doctor or vet **immediately.**

For more information:
http://www.gw.govt.nz/safeswim/

Greater Wellington Regional Council
(04) 384 5708 or
freephone **0800 496 734**

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Regional Public Health
HAUORA Ā IWI KI TE ŪPOKO O TE IKA A MĀUI
Better health for the greater Wellington region

greater WELLINGTON REGIONAL COUNCIL

Warning!
Kia tupato!

Potentially toxic algae in this part of the river

Toxins produced by blue-green algae (cyanobacteria) have the potential to kill dogs and make humans and other animals sick. Do Not let your dog drink the water.

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 on the Tasman District Council website, www.tasman.govt.nz or call the council on 03 543 8400.

tasman district council

Date: This sign remains current until otherwise advised.

Appendix 17
Sample media release – benthic cyanobacteria in rivers

The following is sample text for inclusion in media releases relating to rivers affected by health-adverse levels of benthic cyanobacteria.

<Day, Month, Year>
<Time>

For Immediate Release:

Tests carried out by **< agency >** have shown high abundance of benthic cyanobacteria (sometimes called 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 during recreational activities. Accidental ingestion of water containing cyanobacteria may also cause symptoms such as nausea, tummy upset, breathing difficulties, and tingling or numbness around the mouth or the tips of fingers. Some people can also experience rashes through contact with the skin. 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. Children playing **< name of water body >** should be supervised so they do not touch or eat the cyanobacterial mats; if you suspect that they have consumed cyanobacteria, take them to a doctor immediately. 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 or 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 18
Example factsheet from Greater Wellington

Staying safe in our waterways

Toxic Algae in the Wellington region

Greater Wellington
Te Pane Matua Taiao

There are lots of different types of algae that can grow in the waterways in our region. It's important to know which algae can be harmful to people and dogs, so that we can enjoy our waterways safely during summer.

Freshwater toxic algae (cyanobacteria) are naturally present in all New Zealand waterways. Usually, toxic algae forms dense blooms when the weather is dry and warm. Algal blooms can be dangerous to aquatic life in our rivers and streams by blocking sunlight and smothering the riverbed.

The bloom will usually last until there is a 'flushing event'. Flushing events happen after heavy rain, washing the algae away. Once the algae reaches the sea, it is no longer harmful as the salt water de-activates the toxins which affect humans and dogs.

River algae

Lake algae (Science Media Centre)

Who is affected?

Anyone who uses waterways in our region can be affected by toxic algae.

Swimmers and recreational water users: Swallowing water containing toxic algae can make people sick, and contact can cause irritation of the skin, eyes, nose and mouth.

Children: Because children are inquisitive, they are more likely to pick up/touch toxic algae and then put fingers in mouths so special care should be taken when swimming with them.

Dogs: Dogs are most at risk as they like the smell and taste of toxic algae. A small amount, the size of a 50 cent piece, can be enough to kill a dog. Dogs are most susceptible when mats wash up along river edges.

Using the waterways safely

In Wellington, we are avid users of our waterways, making the most of their recreational opportunities during summer. It is important to do this safely, especially if you have a dog or child.

The best things you can do to protect yourself, your family and your pets is to know what to look for and check for alerts before you go.

Know what toxic algae looks like

In rivers:

- Look for black, green, brown slime on rocks, or brown or black 'mats' at the river's edge that have a velvety texture and earthy/musty smell.
- If you see them, be cautious and avoid that river site, particularly if you have a dog.
- Check for warning signs before getting in the water or allowing your dog near it.

In lakes: Lakes in the Wellington region are not part of our monitoring program, as river swimming spots are much more popular. However, we encourage you to know what to look for in lakes as well as rivers.

- If the water has a 'pea soup appearance', it could contain toxic algae. Cloudy water with small green blobs suspended in it should be avoided.
- Only high-risk lakes in our region are routinely monitored for cyanobacteria blooms. However, warning signs may be put in place when an issue is detected.

Check for alerts

You can check for any known issues in the waterways in our region by visiting the **Can I swim here** page on the Land Air Water Aotearoa (LAWA) website.

Keeping your dog safe

Check for alerts before you go to a river, look for warning signs, and keep an eye on your dog when you're there.

If there has been an alert issued, or you think you have spotted a toxic algal bloom:

- Keep your dog on a lead
- Keep your dog out of the water
- Ensure it does not eat any algal mats.

Know the signs of poisoning in dogs

If you suspect that your dog has eaten toxic algae, contact your vet **immediately**. In extreme cases, death can occur within 30 minutes after the first signs of illness appear.

Signs a dog has been poisoned by toxic algae include lethargy, muscle tremors, fast breathing, twitching, paralysis and convulsions.

Tell your vet if you suspect your dog has eaten toxic algae, this will ensure that it can be given the best treatment as quickly as possible.

What is being done?

There is no quick or obvious solution to prevent toxic algal blooms, but improving water quality is most likely to help mitigate them.

Greater Wellington works with other councils and Regional Public Health to monitor the safety of our waterways, and issue warnings when blooms occur.

This includes signs at key sites where toxic algae occurs, and updates online. However, people are advised to learn what toxic algae looks like, and swim elsewhere if they see it.

Further Information

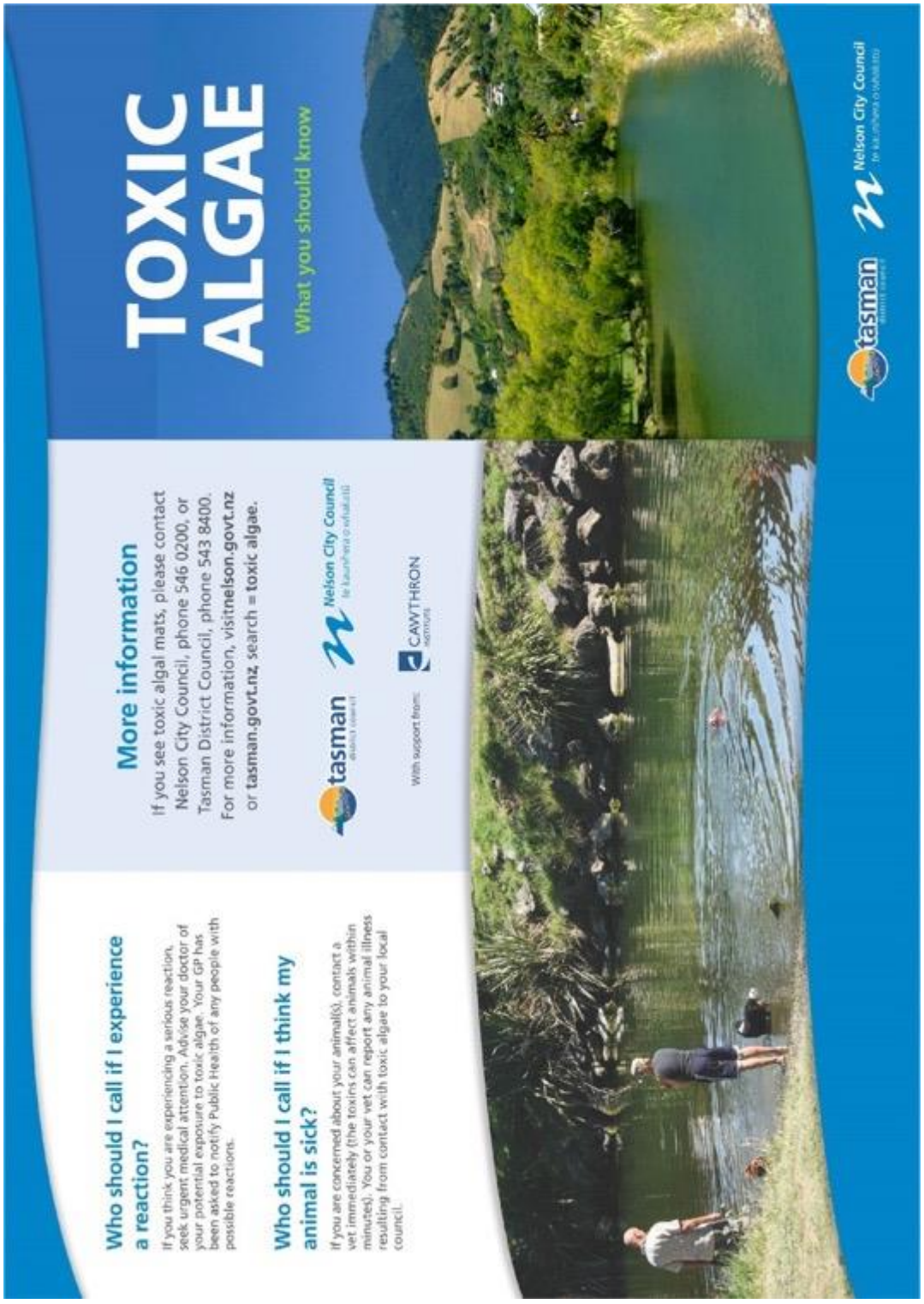
For more information on toxic algae, and where it is safe to swim, head to:
https://www.gw.govt.nz/toxic-algae-faqs/
www.lawa.org.nz/explore-data/swimming/

November 2020

Greater Wellington Regional Council
Ph: 0800 496734

Greater Wellington
Te Pane Matua Taiao

Appendix 19
Example pamphlet from Nelson City / Tasman District Council



What is toxic algae?

Toxic algae (also known as cyanobacteria) are naturally present in many New Zealand waterways. Toxic algae are commonly found in 'clean' rivers and less likely to be present in high nutrient load waters where filamentous algae grow. The growth of toxic algae is encouraged when river flows are stable, and temperatures are warm.

What do the mats look like?

Toxic 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 colour. They also have a strong musty odour, which may attract dogs to eat them.

Dark brown toxic algal mat

Harmless algae that you may see in rivers

What is the problem with toxic algae?

Toxic algae can produce toxins that are harmful to humans, dogs, livestock and wildlife when ingested.

What are the possible health effects?

Swallowing water containing toxic algae can lead to vomiting, diarrhea, 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 toxic algae as they love to scavenge and play near water.

What should I do if I find toxic algae?

1. Avoid contact

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 particularly those that are easily accessible, exposed on river edges or floating in shallow areas of riverbanks or near rocks.

2. Avoid swimming or drinking the water

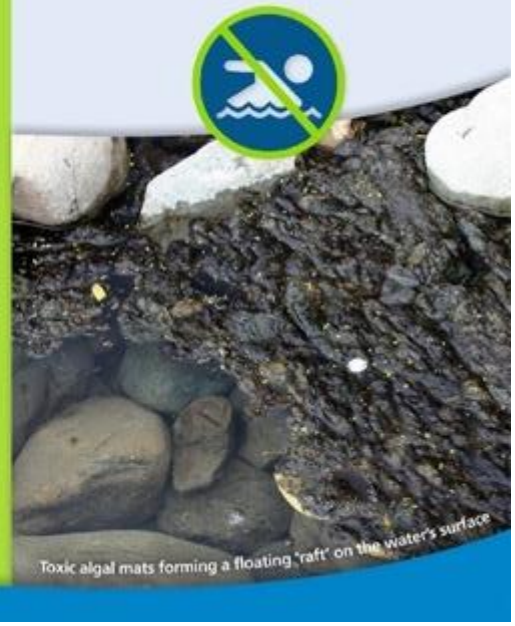
If toxic algal mats are widespread in a river you should presume that the water may be unsafe for bathing or drinking.

What are local councils doing about it?

Nelson City Council will put up 'No Swimming' signs at key sites along the Maitai River when toxic algae levels are high.

Tasman District Council has information signage at popular swimming holes, however people are advised to learn what toxic algae looks like and swim elsewhere if they see it.

Meantime, research is underway by Cawthron scientists and students to find out the key reasons for toxic algal blooms and provide recommendations on how to manage and predict where nuisance blooms may occur. This research will be useful for management of all rivers.



Toxic algal mats forming a floating 'raft' on the water's surface

Appendix 20
Example field sampling sheet for planktonic cyanobacteria

Contact information:

Name of sampler: .....

Ph: .....

Email: .....

Address: .....

Sample information:

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

Sample location (be as detailed as possible; for example, 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; for example, 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? ..... Photos taken? Yes No

Are there any signs of animal / human poisonings (e.g., 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 (e.g., farmland): .....

.....

.....

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

.....

.....

Samples collected for:

□ Cyanobacterial identification (Lugol's preserved)

□ Cyanobacterial identification (unpreserved)

□ Cyanotoxin identification / quantitation (unpreserved)

Additional comments:.....

.....

.....

.....

.....

.....

.....

Appendix 21
Example field sampling sheet for benthic cyanobacteria

Contact information:

Name of sampler: .....

.....

Ph:.....

Email:.....

Address: .....

.....

.....

Sample information:

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

Bank of river: TLB TRB

Sample location (be as detailed as possible and, if possible, provide a sketch on the back of sampling sheet):.....

.....

.....

Benthic cyanobacteria coverage:

Use the method overleaf to determine the percentage cover at the site.

	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?					
Photos taken?					

% cover by benthic cyanobacteria (to nearest 5%)

	Transect 1	Transect 2	Transect 3	Transect 4	Comments
View 1					
View 2					
View 3					
View 4					
View 5					
Mean % cover per transect					Average % cover at site

Method:

- Select an area of riverbed (40–60 metres long) suitable for four transects. It should include areas of riffle and run
- Take each transect across the river, or to a maximum depth of **0.6 metres** for larger, deeper rivers
- Start at the most downstream transect and work upstream to avoid disturbance to areas not yet surveyed
- Divide transect into five 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 percentage cover occupied by benthic cyanobacterial mats at each viewing point. Only record mats if they are greater than one millimetre thick
- Note presence or absence of detached or detaching mats on each transect and exposed mats on the riverbed
- Note bed substrate type (cobbles, gravels, sand-silt, macrophytes)
- If mats are not easily identifiable (for example, if it is unclear if they are *Microcoleus*, or another type of benthic cyanobacteria / periphyton), take photos and collect a sample for microscopic identification.

Appendix 22
Preparation of Lugol's iodine solution

Dissolve :

10 grams of pure iodine crystals,

20 grams of potassium iodide (KI) and

20 grams of glacial acetic acid

in 200 millilitres of distilled water.

Source: Pridmore (1987)[69].

Appendix 23
Example of frequently asked questions

Below is a list of frequently asked questions (FAQs) and an example response collated from council websites and LAWA (Land, Air, Water Aotearoa). You may need to include regionally specific information and to align with communications strategies for your region.

What are toxic algae?

Toxic algae (also known as cyanobacteria or blue-green algae because of the cyan colour of some types) are organisms that have characteristics in common with both bacteria and algae. They live like algae, harvesting energy from the sun to grow, and are naturally present in many waterways around Aotearoa. Benthic toxic algae can grow in 'clean' rivers and are less likely to be present in high-nutrient waters where filamentous green algae will often grow.

In rivers, the growth of toxic algae is encouraged when river flows are low and stable, and growth increases when temperatures are warm.

In lakes, ponds and dams, toxic algae grow faster during the warmer months when sunlight hours are longer (like your lawn during the moist summer months). Wind may cause buoyant cyanobacteria to form a scum or foam along the downwind side of the water body.

What is the problem with toxic algae?

Toxic algae can produce toxins that are harmful to humans, dogs, livestock and wildlife when ingested. To stay safe, you should familiarise yourself with what toxic algae looks like and, if you see it, don't go swimming there and keep your dog on a leash. Check your local council's website for any toxic algae warnings in your region, and look out for warning signs at swimming spots.

What does toxic algae look like?

Benthic toxic algae (cyanobacteria) can be found as mats growing on stones or rocks in the riverbed or on the base of lakes (benthic). These mats are usually dark brown or black in colour. The mats may come loose from the riverbed (or the bottom of the lake) and accumulate along the edge of waterways or lake shoreline. When exposed to the air, the mats may dry out and turn a light brown colour. They also produce a strong musty odour, which can attract dogs to eat the mat.


[Agencies might wish to include locally-acquired image examples – especially if regionally specific cyanobacteria are observed]

In lakes, toxic algae often grown free-floating in the water column (planktonic). This can tint the lake water with colours ranging from bright green to dull brown. Planktonic toxic algae in lakes can sometimes move up and down in the water column, forming dense accumulations (sometimes called scums) on the water's surface that look like green oil slicks.


[Agencies might wish to include locally-acquired image examples – especially if regionally specific cyanobacteria are observed]

What are the possible negative health effects?

Swallowing water containing toxic algae can lead to vomiting, diarrhoea, abdominal pain, cramps, nausea, respiratory problems and other health effects in humans. Skin contact can cause irritation of the skin, eyes, nose and mouth for some people. Exposure to high levels of toxins can result in serious illness or death.

Dogs are particularly susceptible to poisoning from the toxic algae as they love to scavenge and play near water. The musty odour of toxic algae mats that grow in rivers (and some lakes) is very attractive to dogs. If your dog shows signs of lethargy, muscle tremors, fast breathing, paralysis or convulsions, contact your vet immediately. Livestock are also at risk from toxic algae poisoning when they can freely drink from a contaminated water body.

What should I do if I find toxic algae?

River users, particularly those with dogs, should avoid contact with any thick, dark brown-black algal mats, particularly those that are easily reached on river edges or floating in shallow areas of riverbanks or near rocks. If toxic algae mats are widespread in a river, you should presume that the water is unsafe for bathing or drinking.

In lakes, if there is a health warning in place, you should not use the lake for swimming, recreational activities or drinking. If you can't see your toes in calf-deep water, or the lake water looks green and cloudy, it's best to wait until the water clears before taking a dip.

Who should I contact if I experience a reaction to toxic algae?

If you think you are having a serious reaction to toxic algae, seek urgent medical attention. Advise your doctor of your potential exposure to toxic algae. Your doctor has been asked to notify the National Public Health Service of any people with possible reactions.

Who should I contact 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). Let your vet know that you think your animal may have been exposed to toxic algae. You or your vet can report any animal illness resulting from contact with toxic algae to your local council.

Is it safe to drink water containing toxic algae?

No – toxins are not removed by boiling, normal filter systems, Steripen® or UV light or by adding household disinfectant. Arrange alternative drinking water.

Can I eat fish, tuna / eels or shellfish collected from water containing toxic algae?

Eating shellfish from affected water bodies should be avoided as they can concentrate the toxins produced by toxic algae. Shellfish harvested near to the outflow of a lake affected by toxic algae may also be contaminated with the toxin.

If you choose to eat fish and tuna / eels from waters containing toxic algae, you should eat them in moderation. Avoid eating internal organs, such as the liver and kidney, as this is where accumulation of toxins is the greatest. Avoid contact with the water while fishing and wash all fish / tuna in clean water.

Is it safe to boat or canoe in water with toxic algae?

How safe boating and canoeing are depends on the amount of direct contact with the water. If you swallow the water or your skin comes in contact with the water while boating or canoeing, you are at risk from a reaction to toxic algae that may be present.

The higher the concentrations of toxic algae and the longer that people are in contact with the water, the more likely a reaction is to occur.

Wash boats, canoes and life jackets down with clean water after use.

Will wearing a wetsuit protect me from toxic algae?

No – toxic algae may accumulate in the collar and cuff areas of your wetsuit and rub against your skin. This may cause a strong skin reaction in these areas.

If you do choose to wear a wetsuit and go into the water, take care to rinse the wetsuit with clean water.

Can my dog swim in or drink from water containing toxic algae?

No – it is best to keep animals away from water bodies where toxic algae are present. Dogs enjoy scavenging around the water's edge, so it is important to keep dogs on a leash if you are walking by a waterway where toxic algae is present.

If you are visiting waterways where toxic algae frequently occur, it is best to provide an alternative drinking source for your dog.

If you have any concerns about your animal's behaviour or health after contact with toxic algae, contact your local vet immediately.

How is the risk from toxic algae assessed at swimming spots?

The risk from toxic algae (cyanobacteria) present at rivers and lakes is assessed by measuring either the percentage cover of the mat-forming cyanobacteria on rocks in rivers, or the number of cyanobacteria cells suspended in the water of lakes. These are compared to Aotearoa's guidelines for cyanobacteria in recreational freshwaters to determine the risk.

How is toxic algae measured in our waterways?

Toxic algae (cyanobacteria) blooms are much more common in summer than in winter months, and so this is when routine council monitoring usually occurs.

Benthic toxic algae attached to the riverbed are visually assessed with an underwater viewer called a bathyscope. A transect is run across the river and the percentage cover is recorded. This is done multiple times within a river area, and an average percentage cover of potentially toxic algae cover is recorded.

In addition, the river edge is scanned for detached or exposed mats, and scums and foams that may have collected at the river margins. Recreational users and animals have an increased risk of coming in contact with detached mats.

Planktonic toxic algae that grow in lakes are assessed by collecting a sample of water from the surface and counting the number of cells present or the biovolume of the cells (the amount of space within a cell).

Health warnings are issued when the amounts of mat-forming (benthic) and planktonic (free-floating) cyanobacteria are above the levels specified in the Aotearoa New Zealand Guidelines for Cyanobacteria in Recreational Freshwaters.

Where do I get more information?

[Insert relevant contact information and web links for agencies in your region]