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ROTORUA LAKES CYANOBACTERIAL DATA ANALYSIS – 2022

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Prepared for Bay of Plenty Regional Council

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EXECUTIVE SUMMARY

Cyanobacterial blooms have occurred in some of the Rotorua lakes since at least the 1970s. These blooms create a suite of water quality issues and because some species produce cyanotoxins, they also pose a health risk to recreational users.

As part of the five-yearly Science Review related to Plan Change 10, the Bay of Plenty Regional Council (BOPRC) commissioned the Cawthron Institute to:

- evaluate trends in the abundance and composition of cyanobacteria in four Rotorua lakes (Ōkaro, Rotoehu, Rotoiti, Rotorua) and one site in the Kaituna River
- collate and analyse data on health warnings, measurements of cyanotoxins, and shifts in the presence of potentially toxic species, with a focus on Lake Rotorua
- provide recommendations on further monitoring or analysis that could assist BOPRC with obtaining a robust understanding of future trends in cyanobacterial blooms and their toxins.

Data collected as part of the BOPRC cyanobacterial recreational monitoring programme were provided for twelve sites spread across Lakes Ōkaro, Rotoehu, Rotoiti and Rotorua, and one site in the Kaituna River. While these data are appropriate for use in a recreational monitoring programme, they are not ideal for assessing long-term trends in cyanobacterial abundance and composition at a whole-lake level. This is because the samples are only collected from the shore and only from late spring to early autumn. Additionally, no physicochemical data are collected in parallel. These and other caveats detailed in the report need to be considered when interpreting the results.

Statistical results indicated that cyanobacterial biovolumes had very likely decreased over the last approximately 20 years (data from March 1997 to April 2022, but the time frames vary among lakes) for Lakes Rotoiti and Rotorua and the Kaituna River site. In Lake Ōkaro the total cyanobacterial biovolume had not changed significantly, whereas in Lake Rotoehu it has very likely increased. We also analysed trends in the data before and after two interventions aimed at improving water quality: alum dosing in Lake Rotorua and the construction of the Ōhau Channel diversion wall. The before and after analysis of the application of alum indicates that this may have had a positive impact on Lake Rotorua and the Kaituna River site. However, it is less clear whether the building of the diversion wall has had a positive impact on cyanobacterial abundance in Lake Rotoiti with biovolumes increasing post installation at all sites except Okere Arm.

The long-term trends need to be interpreted with some caution as analysis of the data with generalised additive models highlights variability, with significant periods of increasing and decreasing cyanobacterial biovolume identified. Without corresponding physicochemical data, it is difficult to explore the drivers of these shifts. However, the sinusoidal and almost synchronous fluctuation in total cyanobacterial biovolume prompted us to undertake a very preliminary investigation into whether global climate patterns may explain some of the

observed shifts. Alignment of the cyanobacterial biovolume data with the Southern Oscillation Index indicates that climate patterns could be involved, but further investigation is recommended.

Although cyanobacteria were almost always present in the samples collected from Lake Rotorua, health warnings were only issued for 2% of the samples collected between 2012 to 2022 (the period analysed for health warnings does not span the full dataset). In all instances the health warnings were triggered by exceedance of the potentially toxic biovolume threshold (> 1.8 mm^3/L; Situation 1 in the planktonic cyanobacteria alert-level framework). The recreational cyanobacteria guidelines for Aotearoa New Zealand are currently being revised and if the recommended new 'toxic species only cell concentrations' thresholds are adopted by BOPRC, the number of health warnings issued over this period would decrease to 1.25% of samples.

Cyanotoxin data are extremely limited for Lake Rotorua with data available for 12 samples, two of which contained low levels of the hepatotoxic (affecting the liver) microcystin. The occasional presence of high concentrations of the toxin producers *Microcystis* sp. and *Cuspidothrix issatschenkoi* suggest cyanotoxins could reach levels which are dangerous to human users of the lake.

Based on the analysis undertaken in this study, we recommend:

1. **Improvements to the current recreational monitoring programme.** These should include incorporating new technologies to complement current approaches which could allow lakeside health warnings to be issued. We also suggest participation in an annual interlaboratory validation process to improve quality control of cyanobacterial taxonomy.
2. **Cyanotoxin analysis is incorporated in the recreational monitoring programme**. This should follow a stepwise process, where samples are initially screened for genes involved in cyanotoxin production, followed by chemical analysis of positive samples.
3. **A programme aimed at understanding algal ecology in the Rotorua lakes is developed and instigated.**
4. **Analysis of high frequency data, water quality and other relevant data is undertaken to enhance knowledge on the drivers of changes in cyanobacterial biovolumes**. Where phycocyanin data are available, analysis of the high frequency data from the automatic monitoring platforms should be undertaken. Water quality results, and other information, such as climate data should be included.

Developing robust monitoring approaches for the future, and undertaking detailed analysis of current data, will help in understanding the drivers of cyanobacterial blooms and toxin production in the Rotorua lakes. Climate change predictions indicate that cyanobacterial blooms will become more prevalent in many lakes across Aotearoa New Zealand. A detailed understanding of the variables regulating cyanobacteria in the Rotorua lakes will help in developing management practices to minimise their impact under future pressures.

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1. GENERAL INTRODUCTION

Cyanobacteria, also known as blue-green algae, are photosynthetic prokaryotic organisms found in a diverse range of ecosystems (Whitton 2012). In freshwater ecosystems when environmental conditions are favourable, cyanobacteria cells can multiply and form cyanobacterial blooms. These blooms are aesthetically unpleasant and can cause a suite of water quality issues. Additionally, some cyanobacterial species produce natural toxins collectively known as cyanotoxins. These natural toxins are a threat to humans and animals when consumed or through contact with them. The toxicity mechanisms for cyanotoxins are very diverse, ranging from hepatotoxicity (affecting the liver) and neurotoxicity (affecting the nervous system), to dermatotoxicity (effects on the skin).

Because of the health risks posed by cyanobacteria, routine monitoring of lakes used for recreational activities is often undertaken by regional authorities. The Bay of Plenty Regional Council (BOPRC) set up the Rotorua lakes' cyanobacteria monitoring programme in the early 1990s when local communities became concerned about frequent cyanobacterial blooms in Lakes Rotorua and Rotoehu (Wilding 2000; Burns et al. 2005).

Bay of Plenty Regional Council is required to undertake a five-yearly Science Review as a condition of Plan Change 10 (a policy to manage nutrient losses from land use in the Lake Rotorua catchment). As part of this process, in 2022 an independent peer reviewer identified that additional research was required related to cyanobacteria. They requested that a review of cyanobacterial blooms in the Rotorua lakes be undertaken, specifically:

> Harmful algal blooms (HABs, toxic cyanobacteria/blue-green algae) were identified in the previous report review as a water quality issue requiring attention in the future. If a suitable lead author can be found, a specific report could provide a summary of cyanobacteria data and bloom events from past studies and ongoing monitoring, e.g., public health advisories, presence of toxic species, toxicity texts, data availability for trend analysis in the future.

The Cawthron Institute (Cawthron) was identified as having experts with previous knowledge and experience analysing BOPRC cyanobacterial data (e.g., Wood et al. 2006a; Wood et al. 2008a; Wood et al. 2014; Smith et al. 2016). While the Plan Change 10 Science Review is only applicable to Lake Rotorua and its catchment, BOPRC was also interested in extending the review to other Rotorua lakes with cyanobacterial monitoring data.

This report is divided into three sections to reflect the requests of the BOPRC. Section 2 evaluates trends in the abundance and composition of cyanobacteria in four

of the Rotorua lakes (Rotorua, Rotoiti, Ōkaro, Rotoehu) and one site in the Kaituna River. This section also explores changes in:

- the relative abundance of potentially toxic versus non-toxic cyanobacterial species
- the relative abundance of species capable of nitrogen fixing versus those that do not have this capability
- the dominant genera in each year for individual lakes and the Kaituna River site and changes in species richness.

Section 3 of the report focuses primarily on Lake Rotorua. It includes a more detailed investigation into toxin production and the consequences of that, including:

- health warnings issued by Toi te Ora Public Health
- measurements of cyanobacterial toxins
- the presence of potentially toxic species and how they have changed in Lake Rotorua.

Section 4 of the report provides recommendations on further monitoring and analysis that would provide BOPRC with a robust understanding of future trends and drivers of cyanobacterial blooms and their toxins.

2. ANALYSIS OF CYANOBACTERIAL DATA FOR LAKES ROTORUA, ROTOITI, ROTOEHU AND ŌKARO, AND KAITUNA RIVER

2.1. Introduction – cyanobacterial data analysis

Seasonally recurrent cyanobacterial blooms have been reported in Lakes Ōkaro and Rotoehu since at least the 1970s (Cassie 1978; Dryden & Vincent 1986) and in Rotorua and Rotoiti since the 1990s (Wilding 2000). In response to the blooms, particularly in Rotoiti and Rotorua, the BOPRC established a cyanobacterial monitoring programme in the early 1990s aimed at protecting human health.

When the initial monitoring programme was established, up to 18 sites were monitored across five lakes (Ōkaro, Rotoehu, Rotoiti, Rotorua, and Tarawera). Other sites were sampled occasionally in response to reports from locals (Wilding 2000). Health warnings were originally issued when cell concentrations exceeded 15,000 cells/mL.

Since about 2000, there has been more consistency with the sites and frequency of monitoring. A total of 13 sites (spread across four lakes, Rotorua, Rotoiti, Rotoehu and Ōkaro) and one site in the Kaituna River are now routinely monitored from late spring to early autumn. Lake Tarawera is also sampled occasionally if blooms are reported. As suggested in the ‘New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters’ (MfE and MoH 2009) health warnings for these lakes and the Kaituna River are now issued when set thresholds of cyanobacterial biovolumes are exceeded. The rationale for the switch to biovolume, as opposed to the previously-used cell concentrations, is that biovolume takes into account the variability in size of different species and is considered to be a better indicator of the potential health risk. However, determining biovolumes for each species present in a sample requires time-consuming measurement of individual cells. Rather than undertaking these measurements on each sample, most laboratories use data from species-specific databases and conversions are undertaken post-analysis.

In this section of the report, we evaluated biovolumes and composition of cyanobacteria in Lakes Ōkaro, Rotoehu, Rotoiti and Rotorua and one site in the Kaituna River. Trends and possible causes for any identified trends or step changes are discussed, with particular reference to intervention actions undertaken by BOPRC to improve water quality. This section also explores changes in:

- the relative abundance of potentially toxic versus non-toxic cyanobacteria species
- the relative abundance of species capable of nitrogen fixing versus those that do not have this capability
- the dominant cyanobacterial genera in each year for individual lakes and the Kaituna River site and changes in cyanobacterial species richness.

There are multiple caveats that must be considered when interpreting the results of this study:

1. All data used in this report come from sampling sites that are at the edge of the lakes and river. While this is appropriate for a recreational monitoring programme because these are the areas of greatest human interaction, it is not ideal for assessing long-term trends in cyanobacterial abundance and composition at a whole lake level. Most cyanobacteria are not homogeneously distributed throughout a lake or river. Many cyanobacteria are buoyant and often form wind-accumulated blooms (also called scums) at the downwind side of a lake. Therefore, depending on the wind direction and given that the sites are not evenly distributed around the lake shore, the sampling approach used for the recreational monitoring will likely cause biases. The dataset also has a seasonal bias with most sampling occurring from late spring to early autumn (because this is when cyanobacterial blooms generally occur and is the period of greatest recreational contact).
2. Some of the sampling sites are in relatively enclosed bays, for example Kennedy Bay (Lake Rotoehu) and Ōkawa Bay (Lake Rotoiti). It is likely that cyanobacteria become 'trapped' in these bays. As noted above, while taking samples from these sites for a recreational monitoring programme is sensible, they are probably not representative of cyanobacterial dynamics in the wider lake.
3. Because the samples are taken for recreational health monitoring purposes, no physicochemical data are collected in parallel. This makes it extremely challenging to identify drivers of change. While it might be possible to make some associations with mitigation actions at a very high level, without corresponding physicochemical data these inferences lack scientific robustness.
4. Accurate cyanobacterial taxonomy is challenging, especially when trying to differentiate to species level. It takes years of training to recognise and identify some species. Additionally, picocyanobacteria (those less than 3 µm) are very difficult to identify and are often overlooked unless microscopes with at least 600 to 1,000× magnification are used. Over the time that the dataset used for this study was collected, the taxonomists have changed regularly (often yearly) and a new, more powerful microscope was purchased. In recent years a training programme has been instigated for the new taxonomists. It is likely that these inconsistencies have resulted in discrepancies in the dataset.
5. Species-level identification of some cyanobacteria, using microscopy alone, requires the presence of specialised cells to be present. For example, identification of most *Dolichospermum* species, one of the most common bloom-forming genera in the Rotorua lakes, requires akinetes (a dormant cell that forms when environmental conditions are unfavourable) and heterocytes (specialised cells involved in nitrogen fixation) to be observed. In most instances, the absence of these specialised cells only allows these cyanobacteria to be identified to genus level. This is problematic when using biovolumes, as each species has a specific biovolume and reference databases are used for these conversions (Wood et al.

2008a). If a species is wrongly identified or cannot be identified, the associated biovolume assigned will not be accurate.

6. Not all cyanobacteria produce toxins and within a species known to produce toxins, both toxic and non-toxic strains exist. It is not possible to determine if a species is toxic based on microscopic analysis. Species that produce toxins contain a cluster of genes encoding enzymes that are responsible for producing the toxins. Therefore, either genetic or chemical / biochemical analysis is required to confirm toxin production. A further complication is that species can contain the genes but not be producing toxins at that point in time, therefore, genetic analysis usually only provides information on the potential of a species to produce toxins. Based on several decades of research, Cawthron has a good indication of which species are likely to be toxin producers in the Rotorua lakes, and this information has been used in the analysis undertaken for this report. However, we know that both toxic and non-toxic strains co-exist in the Rotorua lakes, and because of this it is likely that we have overestimated the proportion of toxin producers in this analysis.
7. Some cyanobacterial species can fix atmospheric nitrogen when nitrogen levels are low in the water. This gives them an advantage over other cyanobacteria and phytoplankton when nitrogen limits growth. Nitrogen fixation is generally undertaken in specialised cells known as heterocytes. These thick-walled cells exclude oxygen, allowing nitrogenase enzymes to function. In this analysis we have identified species that are capable of nitrogen fixation; however, this does not necessarily mean that they are undertaking this process in the study lakes at the time of sampling. Nitrogen fixation is an energetically expensive process and is usually only performed by cyanobacteria when required (i.e., low concentrations of nitrogen sources in the water). The presence of heterocytes within a cyanobacterial filament can provide a strong indication that nitrogen fixation is occurring; however, these cells are not differentiated in the BOPRC dataset. It is therefore important to note that our analysis compares the biovolume of cells with nitrogen fixation capability, to those that are not known to have this ability.

These caveats need to be considered when interpreting the results presented. In the discussion of this section (Section 2.4) and in Section 4 we provide some further suggestions for establishing a robust monitoring programme which aims to overcome some of these limitations and assist in understanding the composition of cyanobacteria and toxins at the whole lake level, and provide insights into the drivers of change.

2.2. Methods – cyanobacterial data analysis

2.2.1. Data preparation

Cyanobacterial data from Lakes Ōkaro, Rotoehu, Rotoiti, Rotorua and Kaituna River were provided by BOPRC on 7 April 2022. The data were provided in a spreadsheet and included information on the:

- sampling location (the lake or river that the sample was collected from)
- sampling site (the specific place where the sample was collected from at the lake or river)
- date the sample was collected
- cyanobacterial taxa identified in each sample (either defined to species or genera level)
- biovolume of each cyanobacterial taxa identified in each sample.

Other information was also provided in the spreadsheet but was not used for this analysis (e.g., the cell concentration for each cyanobacterial taxa, whether the sample was part of routine sampling or responsive sampling, a classification of whether the cyanobacterial taxa was a potential toxin producer, details of the microscopy assessment).

Not all sampling dates and sampling sites contained in the data packet were retained for the time series analysis. Sampling date selection was based on having regular sampling data for that location (the lake / river) and evidence that the cyanobacteria present had been accurately identified. These criteria resulted in data being removed for Lakes Ōkaro, Rotoehu, Rotorua, and Kaituna River (Table 1). All data from Lake Rotoiti were used.

Sampling site selection was based on having regular sampling data from the initiation of consistent sampling (see above and Table 1) through to 2022. Some sampling sites had a substantial amount of data (e.g., Te Pōhue Bay in Lake Rotoehu and Gisborne Point in Lake Rotoiti), but it could not be used for the time series analysis, and the associated trend analysis, because sampling ceased for long periods of time (e.g., between 2007–2015 for Te Pōhue Bay) or well before 2022 (e.g., in 2012 for Gisborne Point). The sampling sites retained for the time series analysis are listed in Table 1.

Table 1. Information on the sites and time frames of data used for the time series analysis.

Lake / River	Sites Included	Date Range Included
Lake Ōkaro	Boat Ramp	18/12/2003 to 04/04/2022
Lake Rotoehu	Kennedy Bay	16/04/1998 to 04/04/2022
	Ōtautū	09/03/1998 to 04/04/2022
Lake Rotoiti	Hinehopu	10/03/1997 to 04/04/2022
	Ōkawa Bay	10/03/1997 to 04/04/2022
	Okere Arm	10/03/1997 to 04/04/2022
	Ōtaramarae	17/02/1999 to 04/04/2022
	Te Weta	29/01/1999 to 04/04/2022
Lake Rotorua	Hamurana	16/04/1998 to 04/04/2022
	Holdens Bay	03/02/1999 to 04/04/2022
	Ngongotahā	16/11/1998 to 04/04/2022
	Ōhau Channel	01/12/1998 to 04/04/2022
Kaituna River	Trout Pool	22/03/2004 to 04/04/2022

Eight time points were removed (Table 2) as the cell concentrations and corresponding biovolumes for at least one of the species present in that sample were excessively high and deemed to be either erroneous or outliers. In all cases these samples were collected prior to 2006 (Table 2).

Table 2. Species with high biovolumes that resulted in the corresponding time point been removed from the analysis.

Lake	Site	Date	Cyanobacterial Taxa	Cell Conc. (cells/mL)	Biovolume (mm^3/L)
Rotoehu	Kennedy Bay	25/11/1999	*Dolichospermum spiroides*	3,781,200	983
Rotoehu	Ōtautū	08/01/2001	*Microcystis aeruginosa*	85,195,517	7,412
Rotoehu	Ōtautū	07/02/2001	*Microcystis aeruginosa*	15,743,075	1,370
Rotoehu	Ōtautū	11/04/2001	*Microcystis aeruginosa*	20,780,640	1,808
Rotoehu	Ōtautū	09/01/2006	*Dolichospermum circinale*	2,387,941	1,375
Rotoiti	Okere Arm	26/05/1999	*Dolichospermum spiroides*	14,466,427	3,761
Rotoiti	Te Weta	27/05/1999	*Dolichospermum spiroides*	9,051,204	2,353
Rotorua	Ōhau Channel	10/05/2004	*Microcystis aeruginosa*	38,569,675	3,356

2.2.2. Suggested combining of species and taxonomic changes

As molecular taxonomy continues to be implemented internationally, ongoing changes in the nomenclature of cyanobacteria are occurring. Many of the names used in the BOPRC dataset are now out of date or both the old and new names have been used. Suggestions for changes to the nomenclature used in the BOPRC cyanobacteria database, to bring it in line with current international practice, are provided in Table 3. The use of old nomenclature is unlikely to have caused significant issues in this analysis.

We believe it is likely that some species have been wrongly identified, e.g., to our knowledge *Dolichospermum flos-aquae* has never been identified in these lakes and it should be corrected to *Dolichospermum lemmermannii* (Table 3). In some instances, we suggest that it would be very challenging to speciate some cyanobacterial taxa using the microscopes available and that it would be better to maintain the resolution at genus level; e.g., *Aphanocapsa* (Table 3). Because of the caveats given in the introduction of this section, all the analysis performed in this report was undertaken at the genus level. Therefore, the suggestions provided in Table 3 mostly do not impact the results presented. The only exception is the calculation of species richness where these suggestions were implemented (Section 2.3.5 of the Results). However, it is important to note that each species, even if we thought it was potentially wrongly identified, was given the biovolume associated with it provided in the BOPRC database. This likely introduces some errors. We did not attempt to correct this because there was no way for us to retrospectively determine if a misidentification had occurred.

Table 3. Suggestions for combining of cyanobacterial species and nomenclature changes.

Species	Suggested Misidentifications and Required Nomenclature Updates
Anabaena affinis	Change to *Dolichospermum planctonicum* - Most likely a misidentification
Anabaena circinalis	Change to *Dolichospermum circinale*
Anabaena flos-aquae	Change to *Dolichospermum lemmermannii* - Most likely a misidentification
Anabaena holsatica	Change to *Dolichospermum* sp.
Anabaena inaequalis	Change to *Dolichospermum planctonicum* - Most likely a misidentification
Anabaena lemmermannii	Change to *Dolichospermum lemmermannii*
Anabaena planktonica	Change to *Dolichospermum planctonicum*
Anabaena smithii	Change to *Dolichospermum planctonicum* - Most likely a misidentification
Anabaena solitaria	Change to *Dolichospermum planctonicum* - Most likely a misidentification
Anabaena sp.	Change to *Dolichospermum* sp.
Anabaena spiroides	Change to *Dolichospermum spiroides*
Anabaena spiroides var minima	Suggest that this is species combined with *Dolichospermum spiroides*
Anabaena torulosa	Change to *Dolichospermum planctonicum* – Most likely a misidentification
Aphanizomenon flos-aquae	Combine with *Aphanizomenon gracile* – Most likely a misidentification
Aphanizomenon issatschenkoi	Change to *Cuspidothrix issatschenkoi*
Aphanocapsa delicatissima	Combine and use *Aphanocapsa* sp. – Very hard to distinguish at a species level
Aphanocapsa elachista	Combine and use *Aphanocapsa* sp. – Very hard to distinguish at a species level
Aphanocapsa holsatica	Combine and use *Aphanocapsa* sp. – Very hard to distinguish at a species level
Aphanocapsa incerta	Combine and use *Aphanocapsa* sp. – Very hard to distinguish at a species level
Aphanocapsa nubilum	Combine and use *Aphanocapsa* sp. – Very hard to distinguish at a species level
Aphanocapsa sp.	Combine and use *Aphanocapsa* sp. – Very hard to distinguish at a species level
Aphanothece alascense	Combine and use *Aphanothece* sp. – Very hard to distinguish at a species level
Aphanothece clathrata	Combine and use *Aphanothece* sp. – Very hard to distinguish at a species level
Aphanothece stagnina	Combine and use *Aphanothece* sp. – Very hard to distinguish at a species level
Microcystis botrys	Combine with *Microcystis aeruginosa* – Genetic work suggests all one species
Microcystis flos-aquae	Combine with *Microcystis aeruginosa* – Genetic work suggests all one species
Microcystis panniformis	Combine with *Microcystis aeruginosa* – Genetic work suggests all one species
Microcystis sp.	Combine with *Microcystis aeruginosa* – Genetic work suggests all one species
Microcystis sp. (large)	Combine with *Microcystis aeruginosa* – Genetic work suggests all one species
Microcystis sp. (small)	Combine with *Microcystis aeruginosa* – Genetic work suggests all one species

2.2.3. Classification of cyanobacterial traits

Toxin-producing cyanobacterial taxa

We did not use the classifications of potential toxin-producing cyanobacteria provided in the BOPRC database. The rationale for this was that the BOPRC data come from a list of toxin producers known internationally and provided in the recreational cyanobacteria guidelines (MfE and MoH 2009) and other studies since this publication. Based on several decades of research on toxic cyanobacteria in Aotearoa New Zealand, there is now a good understanding of the toxin-producing species that

occur here (Table 4). This list is more accurate and was used in this analysis. We suggest this should be adopted by BOPRC in the future. The revised guidelines for managing cyanobacteria in recreational freshwaters, which are yet to be released, will also follow this approach (Puddick et al. 2022).

While *Dolichospermum* has previously been considered as a 'potential toxin producer' by water managers in Aotearoa New Zealand, there is insubstantial evidence that species / strains of this genus produce toxins in this country. Wood (2004) reported anatoxin-a production in a single environmental sample dominated by *Dolichospermum lemmermannii*. Kouzminov et al. (2007) reported saxitoxin production in *Dolichospermum planctonicum* from the Waikato River, but this was based on low level positives from environmental samples using an enzyme-linked immunosorbent assay (ELISA; which can be subject to false positives). To date, no isolated cultures of *Dolichospermum* from Aotearoa New Zealand have tested positive for cyanotoxins.

Table 4. Confirmed toxin-producing cyanobacteria in Aotearoa New Zealand that were observed in the Rotorua lakes dataset.

Cyanobacterial Genus / Species	**Growth Strategy**	**Cyanotoxin**	**Reference/s**
Cuspidothrix issatschenkoi	Planktonic	Anatoxin-a	Wood et al. (2007a)
Raphidiopsis raciborskii	Planktonic	Cylindrospermopsins*	Wood and Stirling (2003)
Microcystis spp.	Planktonic	Microcystins	Wood et al. (2008b)
Planktothrix sp.	Bi-phasic	Microcystins	Wood et al. (2010)
Nostoc sp.	Benthic	Microcystins	Puddick et al. (2019)
Oscillatoria sp.	Benthic	Anatoxins*, microcystins*	Hamill (2001)
Microcoleus autumnalis	Benthic	Anatoxins	Wood et al. (2007b) Heath and Wood (2010)

* = Observations were made using environmental material rather than cultured cyanobacterial strains.

Microcystin-producing *Planktothrix* has been identified in benthic mats from a river in the Canterbury region (Wood et al. 2010), however, the strains are bi-phasic in culture (i.e., they will use both benthic and planktonic growth strategies at different times). For this reason, and because of a lack of knowledge on *Planktothrix* in Aotearoa New Zealand we have assigned this as a toxin producer for this analysis.

At times, confirmed toxin producing cyanobacterial taxa generally considered as benthic were observed in samples (e.g., *Nostoc* and *Phormidium*, now renamed to *Microcoleus*), these were also classified as toxin producers in this analysis.

Microcystis wesenbergii was not included as a toxin-producing taxon since it can be distinguished microscopically from other *Microcystis* species and research to date has not found toxin-producing *Microcystis wesenbergii* in Aotearoa New Zealand. Other species of *Microcystis* were classified as toxin-producing.

A list of the cyanobacterial taxa (present in the dataset) that were classified as toxin producers for the purpose of this analysis is provided in Appendix 1.

Nitrogen-fixing cyanobacterial taxa

Cyanobacterial taxa in the samples were classified by their ability to fix atmospheric nitrogen under certain environmental conditions (i.e., whether they can produce heterocytes). This included species from the genera *Aphanizomenon*, *Dolichospermum*, *Gloeotrichia*, *Nostoc*, *Raphidiopsis*, *Tolypothrix* and *Trichodesmium*. A list of the cyanobacterial taxa (present in the dataset) classified as capable of nitrogen-fixing for the purpose of this analysis is provided in Appendix 2.

2.2.4. Assessing the effect of water quality interventions

Although multiple mitigation actions have been undertaken in the Rotorua lakes to improve water quality, based on our initial analysis of the cyanobacterial data, we determined it was appropriate to assess only two main interventions.

Targeted before and after sampling involving the collection of a suite of physicochemical parameters is required to accurately investigate the effects of mitigation actions. As noted previously the data analysed in this study was collected for the purpose of protecting human health and the samples are taken from sites on the shorelines of the lakes or river without any corresponding physicochemical data.

The two interventions included in our analysis are:

1. The construction of Ōhau Channel diversion wall—this was completed in July 2008 (Hamilton et al. 2010). The purpose of the wall is to divert water from the outlet of Lake Rotorua directly into the Kaituna River, thereby preventing it entering the main basin of Lake Rotoiti. The effect of this intervention on total cyanobacterial biovolume in Lake Rotoiti was investigated.
2. The dosing of alum to the inflows of Lake Rotorua—initial dosing of alum in the Utuhina Stream began in 2006, and continuous dosing started in this stream in 2007 and in Puarenga Stream in 2010 (McIntosh 2012). The purpose of the alum dosing is to lower the dissolved reactive phosphorus concentration in the inflows and lake water of Lake Rotorua. We used an initiation date of January 2007 in our analysis (aligning with the commencement of continuous dosing) and investigated

the effect on total cyanobacterial biovolumes in Lake Rotorua and the Kaituna River site.

2.2.5. Data analysis

All data analysis and plots were performed with the R and RStudio software (R Development Core Team 2022).

For all analyses using total biovolume, the biovolume for each taxa was summed for each time point at each site. The analysis was undertaken using hydrological years, where any samples collected after 1 July are labelled with the following year. For example, August 2011 would appear in the 2012 year.

Assessment of long-term trends in total cyanobacterial biovolume — Kendall's tau test

A Kendall's tau test was undertaken for each site and for each lake / river (data for all sites at each time point combined) to assess the long-term trend in total cyanobacterial biovolume. This analysis approach is also used by LAWA (Land Air Water Aotearoa) to calculate trends in water quality data (see LAWA 2022).

A Kendall's tau test is a 'non-parametric' trend analysis, meaning that it does not assume a straight-line trend, an exponential growth or decay, or any other specific curve. It makes all pairwise comparisons exhaustively from the available data and tallies the number of pairs where the later sample was higher than the earlier (and vice versa). In random data it should be close to 50:50; a more consistent increase or decrease would result in a bias from 50:50. The test generates a Cd value. This is the confidence in a decreasing trend, which returns a value of 0 to 1 (as the probabilities of an increasing and decreasing trend must sum to 1). Values closer to 0 indicate an increasing trend, while values closer to 1 indicate a decreasing trend. We have used the same approach as LAWA to interpret the Cd value (Snelder et al. 2021). A Cd value < 0.1 is categorised as 'highly likely increasing', while a value < 0.33 is categorised as 'likely increasing'. A Cd value > 0.9 is categorised as 'highly likely decreasing', while a value < 0.67 is categorised as 'likely decreasing'.

To explore the possible effect of several major intervention actions undertaken by BOPRC for Lakes Rotoiti and Rotorua and the Kaituna River site we also undertook this analysis before and after the following:

- Rotoiti—before and after July 2008 when the diversion wall was installed.
- Rotorua and Kaituna River—before and after January 2007 when continuous alum dosing began.

Assessment of long-term trends in total cyanobacterial biovolume – generalised additive model

To identify periods of statistically significant change in total cyanobacterial biovolume, generalised additive models (GAMs) were fitted to the data using the '`->gam`' function from package mgcv v1.8-36 (Wood 2017; Simpson 2018). Generalised additive models are a powerful method of modelling the relationships between individual predictor and dependent variables that do not assume linear relationships. The technique is an additive modelling approach where smoothing functions (which can be non-linear) capture the impact of the predictive variables, allowing non-linear patterns to be modelled without the risk of overfitting that occurs with high order polynomial fitting approaches.

Each generalised additive model was fitted with two smooth terms: day-of-year and days-since-start. The day-of-year smoother was used to remove regular seasonal fluctuations, to allow for investigation of long-term trends. The significance of the slope (first derivative) of the long-term trend was determined and shown as orange (significant increasing) or blue (significant decreasing) on the fitted smoothing line.

To undertake a preliminary exploration of the possibility that global climate patterns might be influencing total cyanobacterial biovolume, the 6-month rolling average Southern Oscillation Index (SOI; accessed from Stats NZ 2020) was plotted and visualised in parallel to the total cyanobacterial biovolume data. El Niño and La Niña are opposite phases of a naturally occurring global climate cycle known as the El Niño Southern Oscillation, or ENSO. ENSO influences rainfall, temperature, and wind patterns around the world. The SOI that was plotted in this report measures the difference in pressure between Tahiti and Darwin (Australia). Over a period of three months or more, values below -1 correspond to El Niño conditions while values above 1 correspond to La Niña conditions. Two smoothing curves were also plotted. The first was a kernel smoothing function with a bandwidth of 20 and number of points contributing to the fit of 80, which shows the finer changes in the ENSO data. The second smoothing curve was fitted using the lowess function, with an f of 0.08 and five iterations. The second curve smooths some of the larger peaks to display the trends over longer timescales.

Assessment of long-term trends – relative abundance of potential toxin producers versus non-producers

This analysis was only undertaken at the whole lake level; i.e., all sites combined, and the Kaituna River site. Assignment of toxin producers is described in Section 2.2.3. Trends in the relative abundance of toxic cyanobacterial taxa were assessed using the Kendall's tau test and GAMs as described above.

Assessment of long-term trends – relative abundance of taxa capable of nitrogen fixing versus those with no nitrogen fixing capacity

This analysis was only undertaken at the whole lake level and the Kaituna River site. Assignment of taxa capable of nitrogen fixation is described in Section 2.2.3. Trends in the relative abundance of taxa with the ability to fix nitrogen were assessed using the Kendall's tau test and GAMs as described above.

Shifts in dominant cyanobacteria genera

Species were grouped at the genus level and the top three were plotted for each hydrological year for each lake (all sites combined) and the Kaituna River site.

Changes in overall diversity

To explore whether there have been changes in species richness (either due to environmental shifts or changes in microscopy analysts), we calculated species richness in each lake and the Kaituna River site for each hydrological year. For this analysis, the merging of species and nomenclature changes in Table 3 were used.

2.3. Results - cyanobacterial data analysis

2.3.1. Assessment of long-term trends in total cyanobacterial biovolume

Kendall's tau test

There were no clear long-term changes in cyanobacterial biovolume in Lake Ōkaro (Table 5). When data across all sites were combined, the Kendall tau results indicated that it was highly likely that cyanobacterial biovolume in Lake Rotoehu was increasing, although the analysis was indeterminate for the Ōtautū site (Table 5).

When data across all sites were combined, the Kendall's tau results indicated that it was highly likely that cyanobacterial biovolume is decreasing in Lake Rotoiti, although this pattern is not clear when the data are analysed pre- and post-July 2008 (installation of the diversion wall). Pre-2008, the combined data analysis suggests there was little change in cyanobacterial biovolume over the study period. Post-2008, the analysis of the combined data indicated that total cyanobacterial biovolume was increasing, with an increasing trend also observed at three of the five sites (Table 5).

The Kendall's tau test results show that cyanobacterial biovolume was very likely decreasing over the study period in Lake Rotorua, both in the combined and individual site data (Table 5). When the data were analysed pre- and post-2007 (when alum dosing was initiated) there was a clear pattern of a highly likely increasing biovolume pre-2007, followed by highly likely decreasing biovolume post-2007 (Table 5). The analysis indicated that total cyanobacterial biovolume was decreasing in the Kaituna River (Trout Pool site) across the study period. It also indicated that cyanobacteria biovolume was decreasing before and after alum dosing began in 2007 (Table 5).

Table 5. Results of the Kendall's tau test for total cyanobacterial biovolume for each site and the entire lake (all sites combined). For Lake Rotoiti, the trend in cyanobacterial biovolume (before and after July 2008 when the diversion wall was installed) was analysed and for Lake Rotorua and the Kaituna River the analysis was undertaken before and after January 2007 when continuous alum dosing began. The green shading denotes an increasing trend, blue is indeterminate and red, an increasing trend. The Cd value is the confidence in a decreasing trend, which returns a value of 0 to 1 (as the probabilities of an increasing and decreasing trend must sum to 1). Values closer to 0 indicate an increasing trend, values closer to 1 indicate a decreasing trend and values close to 0.5 indicate no trend.

Site	Interpretation	Cd Value
Lake Ōkaro		
Boat Ramp	Indeterminate	0.64
Lake Rotoehu		
All sites	Likely increasing	0.11
Kennedy Bay	Likely increasing	0.11
Ōtautū	Indeterminate	0.36
Lake Rotoiti		
All sites	Highly likely decreasing	1.00
Hinehopu	Highly likely decreasing	1.00
Ōkawa Bay	Highly likely decreasing	1.00
Okere Arm	Highly likely decreasing	1.00
Ōtaramarae	Highly likely decreasing	0.98
Te Weta	Highly likely decreasing	1.00
Lake Rotoiti - pre July 2008		
All sites	Indeterminate	0.52
Hinehopu	Indeterminate	0.50
Ōkawa Bay	Highly likely decreasing	0.93
Okere Arm	Likely increasing	0.29
Ōtaramarae	Likely decreasing	0.84
Te Weta	Highly likely decreasing	1.00
Lake Rotoiti - post July 2008		
All sites	Highly likely increasing	0.09
Hinehopu	Highly likely increasing	0.08
Ōkawa Bay	Highly likely increasing	0.01
Okere Arm	Highly likely decreasing	0.93
Ōtaramarae	Highly likely increasing	0.04
Te Weta	Highly likely increasing	0.00
Lake Rotorua		
All sites	Highly likely decreasing	1.00
Hamurana	Highly likely decreasing	1.00
Holdens Bay	Highly likely decreasing	1.00
Ngongotahā	Highly likely decreasing	1.00
Ōhau Channel	Highly likely decreasing	1.00

Table 5, continued

Site	Interpretation	Cd Value
Lake Rotorua – pre January 2007		
All sites	Highly likely increasing	0.02
Hamurana	Highly likely increasing	0.00
Holdens Bay	Likely increasing	0.23
Ngongotahā	Highly likely increasing	0.08
Ōhau Channel	Highly likely increasing	0.03
Lake Rotorua - post January 2007		
All sites	Highly likely decreasing	0.99
Hamurana	Highly likely decreasing	0.99
Holdens Bay	Highly likely decreasing	0.99
Ngongotahā	Highly likely decreasing	0.96
Ōhau Channel	Highly likely decreasing	1.00
Kaituna River		
Trout Pool	Highly likely decreasing	1.00
Kaituna River - pre January 2007		
Trout Pool	Highly likely decreasing	0.94
Kaituna River - post January 2007		
Trout Pool	Highly likely decreasing	0.96

Generalised additive models

The GAMs analysis shows periods of significant increasing and decreasing biovolumes in all lakes and at the Kaituna River site over the study period (Figure 1). In general, the oscillations in cyanobacterial biovolume in Lakes Rotoiti, Rotorua, Rotoehu and the Kaituna River follow the same timing. Of note were the significant increases in cyanobacterial biovolume beginning about 2000, followed by a period of decreasing biovolume beginning in 2005 in all except Lake Rotoehu where this decline started earlier. A further significant increase occurred across all of these lakes in 2013 and in most lakes again in 2020. In all of the study lakes except Lake Rotoehu, the five- and one-year trend shows increasing biovolume. In Rotoehu, the one-year trend was indeterminate whereas the five-year trend suggests decreasing biovolumes (Figure 1).

Although Lake Ōkaro shows a similar pattern of variable periods of increasing and decreasing cyanobacterial biovolume, the timing of these changes is slightly different from the other lakes and the Kaituna River site. Significant increases in cyanobacterial biovolume begin at c. 2010, 2016 and 2020 (a five-year cycle; Figure 1). The one- and five-year trends at this lake show increasing cyanobacterial biovolume.

The GAMS plots for the individual sites in Lakes Rotoiti, Rotorua and Rotoehu are provided in Appendix 3. Most of the individual sites followed the same patterns as observed when all the site data were combined. There were a few exceptions among

the Lake Rotoiti sites, most notably in Ōkawa Bay where the GAMs indicated no change in cyanobacterial biovolumes at this site in the last one or five years (Appendix 3).

A visual assessment of the GAMs plots for each lake and the SOI index suggests some broad alignment. In general, when the SOI index value was positive, cyanobacterial biovolumes tended to be lower (Figure 1). Conversely when the SOI index was negative, cyanobacterial biovolumes tended to be higher.

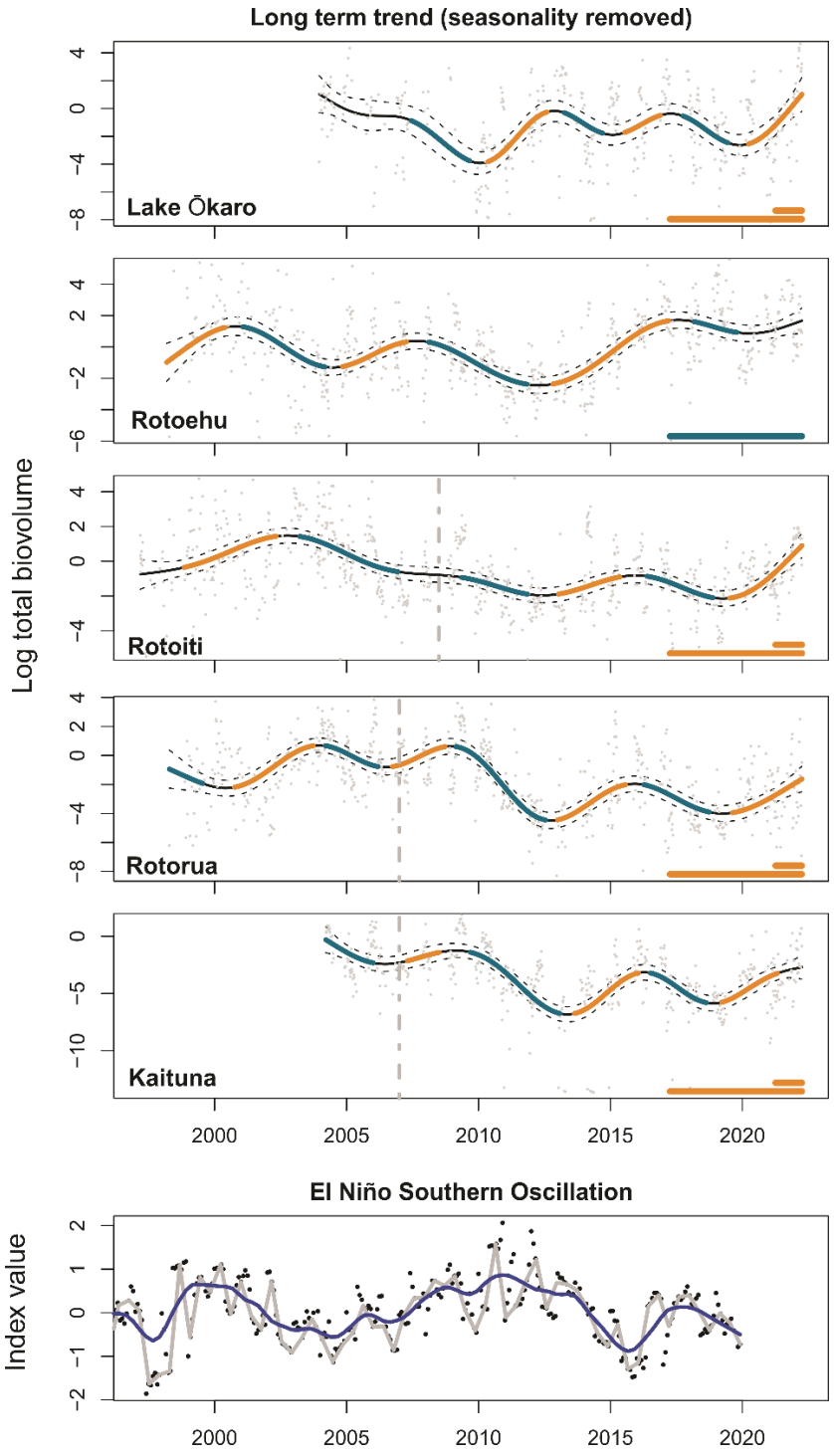

Figure 1. Results of generalised additive models for total cyanobacterial biovolume with all sites combined for each lake. Plots show raw data in grey and fitted generalised additive mixed models (black line) with 95% confidence intervals shown by the dashed black line. Dashed vertical lines show interventions—alum dosing (Rotorua and Kaituna) and diversion wall (Rotoiti). Significant changes are denoted by blue (decreasing) and orange (increasing) line segments. Horizontal orange or blue bars at the right-hand end of the x-axis summarise whether the cyanobacterial biovolume in the last year (short bar) or five years (longer bar) has been overall increasing (orange) or decreasing (blue). The El Niño Southern Oscillation index is plotted below, with kernel smoothing (grey) and lowess smoothing (blue) overlaying the raw data.

2.3.2. Assessment of long-term trends – relative abundance of potential toxin producers versus non-producers

Kendall's tau test

The results of the Kendall's tau test indicate that the portion of the cyanobacteria community that are potentially toxin producers was likely or highly likely increasing in Lakes Ōkaro, Rotoiti and Rotorua and highly likely decreasing in Lake Rotoehu and the Kaituna River (Table 6).

Table 6. Results of the Kendall's tau test for the portion of the cyanobacterial biovolume that contains potential toxin producers for each lake / river (all sites combined). Green shading denotes an increasing trend and red an increasing trend. The Cd value is the confidence in a decreasing trend, which returns a value of 0 to 1 (as the probabilities of an increasing and decreasing trend must sum to 1). Values closer to 0 indicate an increasing trend, while values closer to 1 indicate a decreasing trend.

Lake / River	Interpretation	Cd Value
Lake Ōkaro	Likely increasing	0.13
Lake Rotoehu	Highly likely decreasing	1.00
Lake Rotoiti	Highly likely increasing	0.00
Lake Rotorua	Highly likely increasing	0.00
Kaituna River	Highly likely decreasing	0.99

Generalised additive models

The lakes with the highest portion of their cyanobacteria community comprising potentially toxin-producing taxa were Ōkaro and Rotoehu, albeit high proportions were also recorded in all lakes at specific time points (Figure 2). Although there were periods of increasing and decreasing trends, there were no consistent patterns across lakes. Of note was the decrease in the five- and one-year trends in Lakes Rotoiti and Rotorua and the Kaituna River site (Figure 2). The opposite pattern was apparent in Ōkaro and Rotoehu which was largely due to an increase in *Microcystis* (Section 2.3.4).

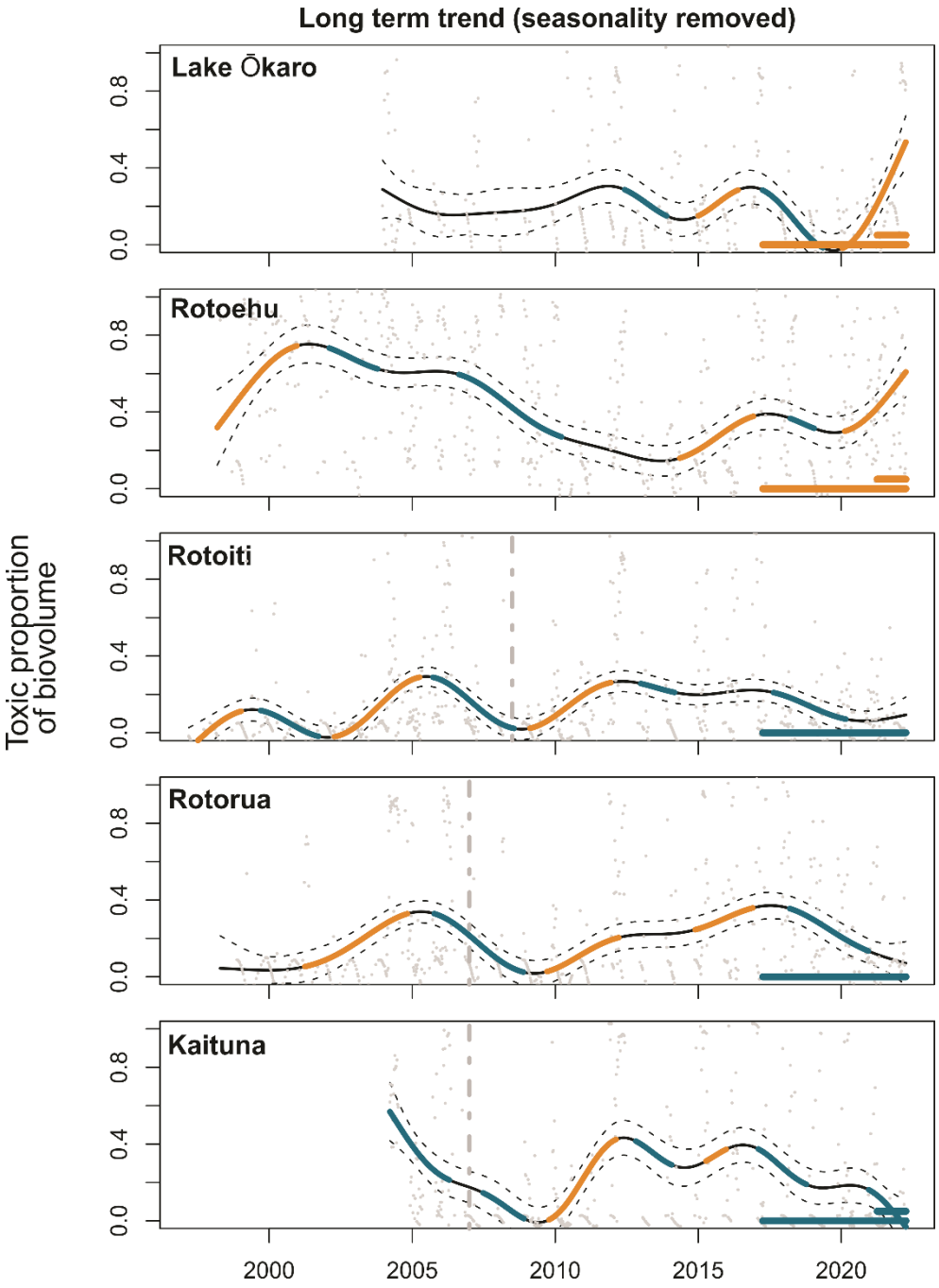

Figure 2. Results of generalised additive models showing changes in the portion of the cyanobacterial community that are potential toxin producers with all sites combined for each lake. Plots show raw data in grey and fitted generalised additive mixed models (black line) with 95% confidence intervals shown by the dashed black line. Dashed vertical lines show interventions—alum dosing (Rotorua and Kaituna) and diversion wall (Rotoiti). Significant changes are denoted by blue (decreasing) and orange (increasing) line segments. Horizontal orange or blue bars at the right-hand end of the x-axis summarise whether the cyanobacterial biovolume in the last year (short bar) or five years (longer bar) has been overall increasing (orange) or decreasing (blue).

2.3.3. Assessment of long-term trends - relative abundance of taxa capable of nitrogen fixing versus those with no nitrogen fixing capacity

Kendall's tau test

Analysis using the Kendall's tau test of the long-term trend in the proportion of the cyanobacterial community that was comprised of species capable of nitrogen fixation demonstrated that there has highly likely been a decrease in their relative abundance in Lakes Ōkaro, Rotoiti and Rotorua and no change in either Lake Rotoehu or the Kaituna River site (Table 7).

Table 7. Results of the Kendall's tau test for total potential nitrogen fixing taxa (expressed as biovolume) for each lake / river (all sites combined). The green shading denotes an increasing trend and blue indeterminate. The Cd value is the confidence in a decreasing trend, which returns a value of 0 to 1 (as the probabilities of an increasing and decreasing trend must sum to 1). Values closer to 0 indicate an increasing trend, whilst values closer to 1 indicate a decreasing trend.

Lake / River	Interpretation	Cd Value
Lake Ōkaro	Highly likely decreasing	0.97
Lake Rotoehu	Indeterminate	0.65
Lake Rotoiti	Highly likely decreasing	1.00
Lake Rotorua	Highly likely decreasing	1.00
Kaituna River	Indeterminate	0.45

Generalised additive models

All the study lakes and the Kaituna River site have at times had a high proportion of their cyanobacterial community having the ability to fix atmospheric nitrogen (Figure 3). Although there were significant periods of increasing and decreasing trends, there were no consistent patterns across lakes or the Kaituna River site (Figure 3). A general pattern of decreases in cyanobacteria with the ability to fix atmospheric nitrogen between the start of monitoring and c. 2012 was apparent in Lakes Rotoehu, Rotoiti and Rotorua, although this has been more variable over the last ten years. The one- and five-year trends in Ōkaro, Rotoehu and Rotoiti suggest the proportion of cyanobacterial taxa with the ability to fix nitrogen is declining, whereas the five-year trend is increasing in Rotorua and the Kaituna River site (Figure 3).

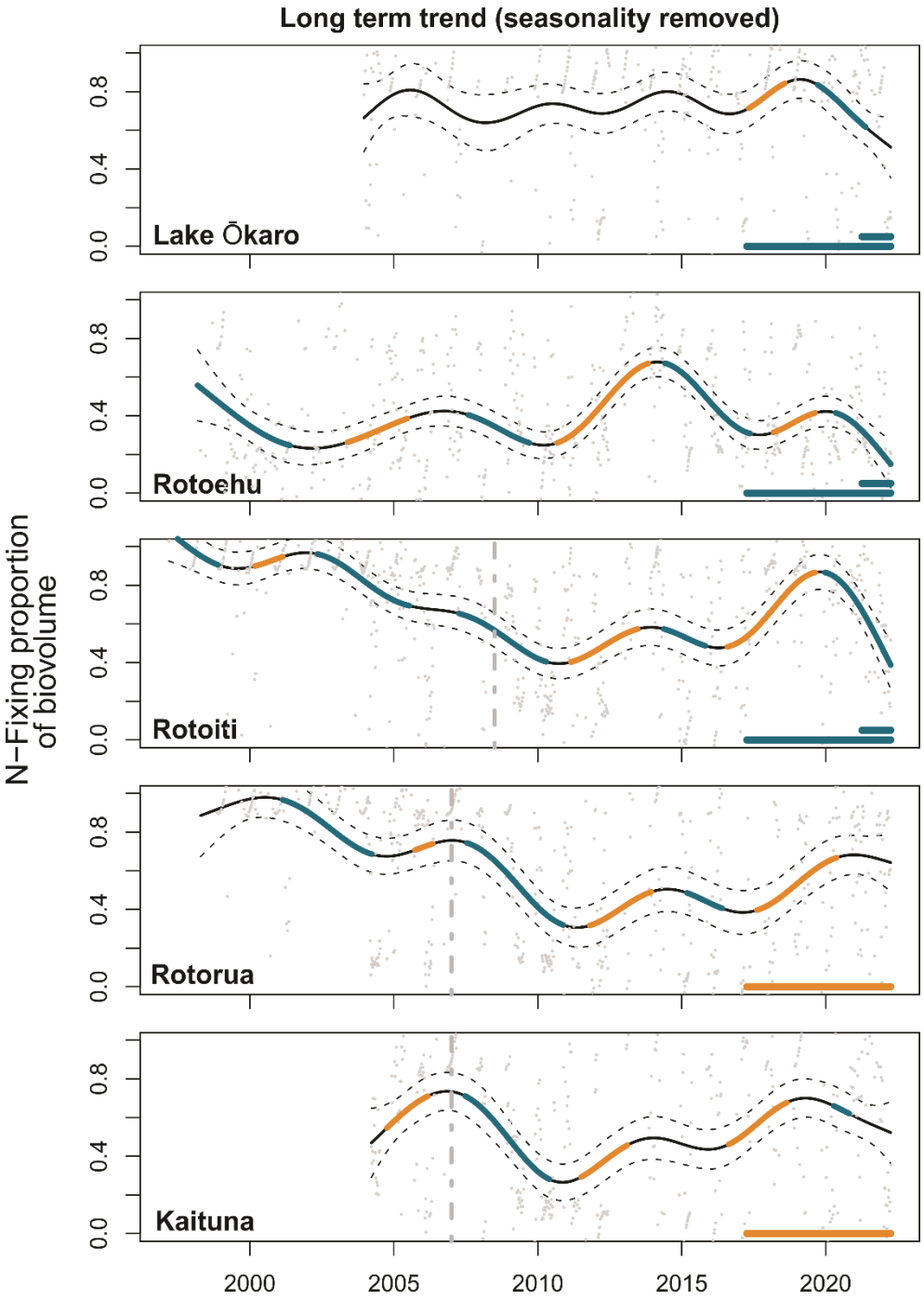

Figure 3. Results of generalised additive models showing changes in the portion of the cyanobacterial community that are capable of nitrogen fixation with all sites combined for each lake. Plots show raw data in grey and fitted generalised additive mixed models (black line) with 95% confidence intervals shown by the dashed black line. Dashed vertical lines show interventions—alum dosing (Rotorua and Kaituna) and diversion wall (Rotoiti). Significant changes are denoted by blue (decreasing) and orange (increasing) line segments. Horizontal orange or blue bars at the right-hand end of the x-axis summarise whether the cyanobacterial biovolume in the last year (short bar) or five years (longer bar) has been overall increasing (orange) or decreasing (blue).

2.3.4. Shifts in dominant cyanobacteria genera

The two most dominant genera across all lakes and the Kaituna River site were *Dolichospermum* and *Microcystis* (Figure 4). There were occasional exceptions to this pattern. In Lake Ōkaro, *Dolichospermum* was dominant in all but 2022 (where *Microcystis* was dominant). In Lake Rotoehu, *Dolichospermum* and *Microcystis* switched in their dominance among years, with the only exception being 2003 when *Aphanizomenon* was most abundant. A similar pattern was observed in Lake Rotoiti with oscillating *Dolichospermum* and *Microcystis* dominance except in 2012 when *Planktothrix* was most abundant, in 2015 when *Oscillatoria* was the dominant genus and 2022 when *Chroococcus* dominated. In Lake Rotorua, *Pseudanabaena* was most abundant in 2012 and 2013, whilst *Cuspidothrix* was most abundant in 2015, *Synechococcus* in 2017, *Planktothrix* in 2018, with all other years dominated by either *Dolichospermum* or *Microcystis* (Figure 4). At the trout pool site in the Kaituna River there were six years where *Dolichospermum* and *Microcystis* were not the most abundant genera: 2012 (*Planktothrix*), 2015 (*Cuspidothrix*), 2016 (*Synechococcus*), 2018 (*Planktothrix*), 2019 (*Oscillatoria*) and 2022 (*Chroococcus*).

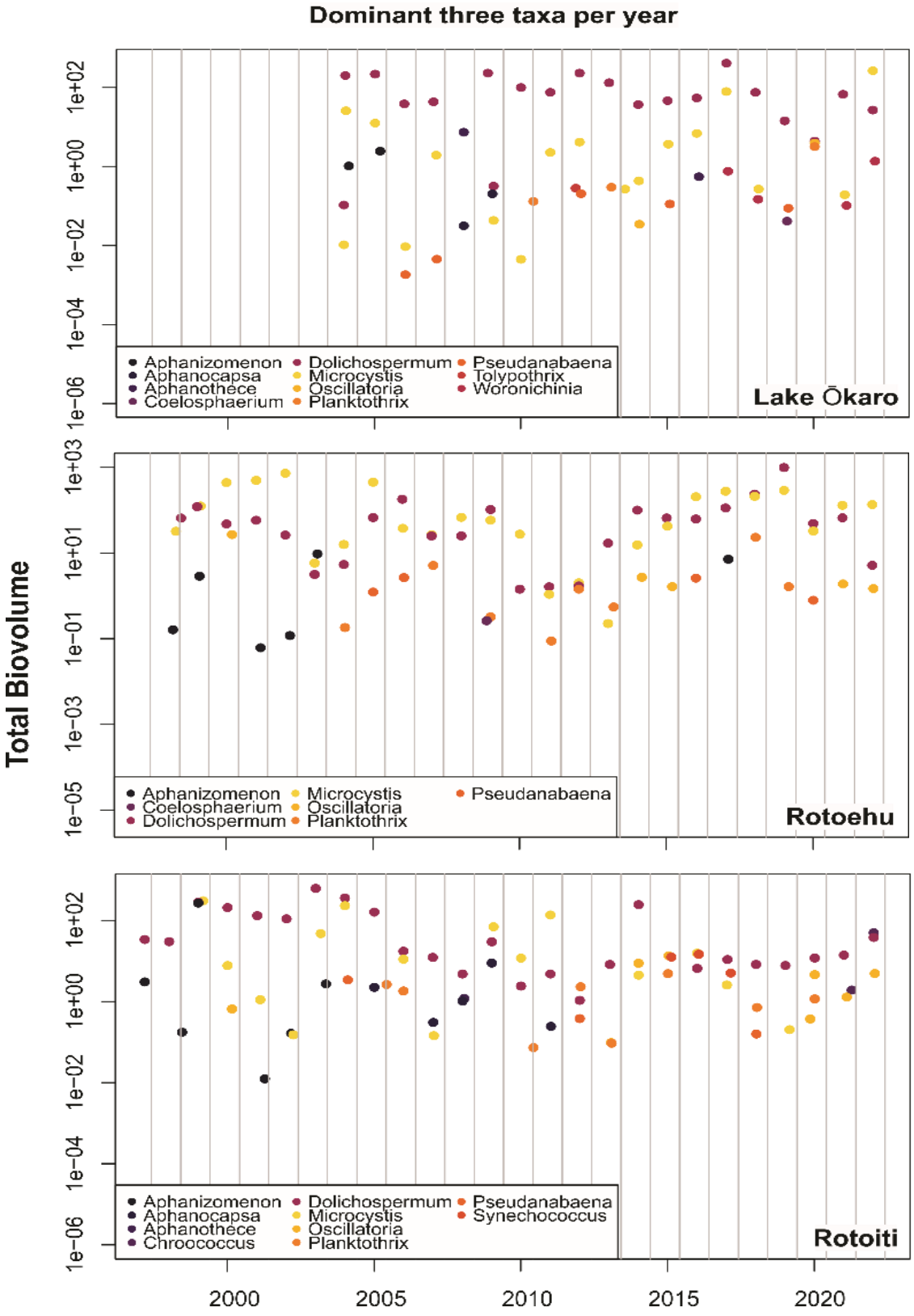

Figure 4. The top three dominant taxa in each hydrological year for each lake / river (all sites combined).

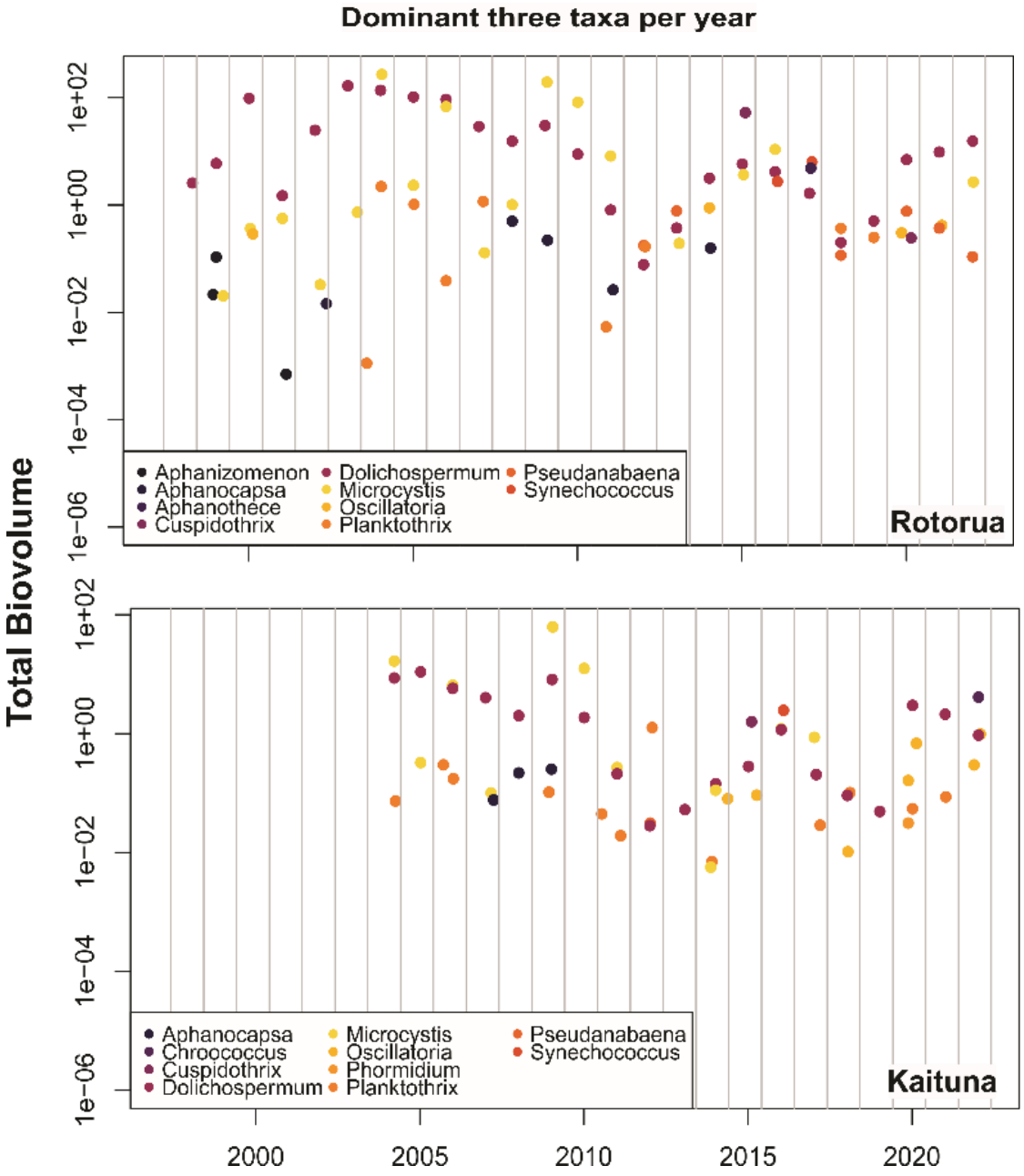

Figure 4, continued.

2.3.5. Cyanobacterial species richness

Species richness is the number of different species (or genera when they can't be identified to species level) that are identified in a sample. There was a general pattern of increasing cyanobacterial species richness across all lakes over the study period. There were notable increases in cyanobacterial species richness starting in 2003 and 2013 (Figure 5).

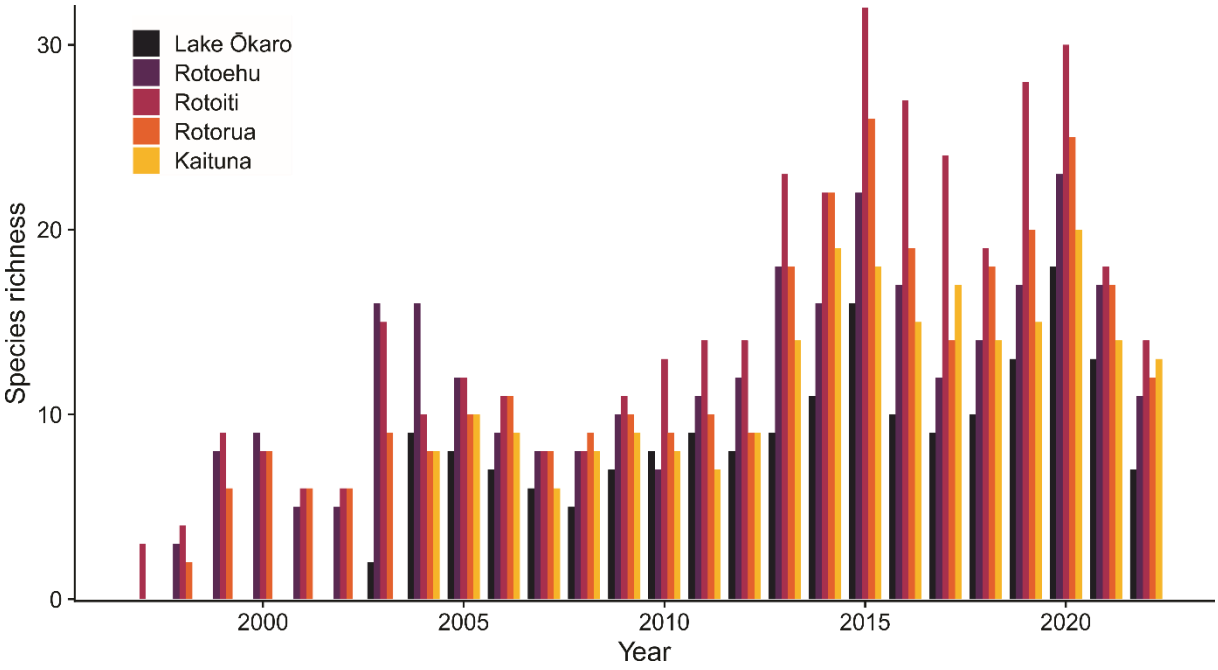

Figure 5. Cyanobacterial species richness per lake or river (all site data combined) per hydrological year.

2.4. Discussion - cyanobacterial data analysis

The caveats provided in Section 2.1 need to be considered when interpreting these data. Probably the most important of these caveats is that the data used in this study were collected for the purpose of protecting human health, not for measuring changes in abundance and composition of cyanobacteria in these lakes and the Kaituna River. The samples are collected from the shore, they are largely taken during summer, and no parallel physiochemical data are collected.

However, because cyanobacterial blooms usually occur in summer, and in Lakes Rotorua and Rotoiti there has been a relatively even spread of sampling sites around the lakes, we believe that there is value in using these data to explore broad changes and trends in the dataset.

A positive outcome of the long-term trend analysis was that for Lakes Rotoiti and Rotorua and the Kaituna River the analysis indicates that cyanobacterial biovolumes are highly likely decreasing over the c. 20-year period (although see below regarding Lake Rotoiti). However, the analysis suggests that in Lake Ōkaro the total cyanobacterial biovolume has not changed significantly and in Lake Rotoehu it is increasing. This is despite the mitigation actions that have been implemented including alum dosing, artificial wetland construction and weed harvesting (e.g., Quinn et al. 2004; Hudson & Nagels 2011; Tempero 2015).

The before and after analysis of the application of alum to the inflows of Lake Rotorua indicate that this may have had a positive impact on this lake and the Kaituna River site, with the trend switching in Lake Rotorua from one of increasing total cyanobacterial biovolume prior to dosing, followed by a decreasing trend post dosing. However, it is less clear whether the building of the Ōhau channel diversion wall has had a positive impact on total cyanobacterial biovolumes in Lake Rotoiti, with biovolumes increasing post installation at all sites except for the Okere Arm site.

The long-term trend analysis needs to be interpreted with some caution as the GAMs analysis highlights variability in the data, with significant periods of increasing and decreasing biovolume in all lakes and the Kaituna River site. These changes do not necessarily align with inventions: for example, immediately following the initiation of alum dosing there is an increase in total cyanobacterial biovolume in Lake Rotorua and the Kaituna River site. In contrast, following the construction of the diversion wall there is a decrease in cyanobacterial biovolume as might be expected but this is then followed by an increase about five years later. We acknowledge that the effects of mitigation actions are likely to have an impact over multiple years and we wouldn't necessarily expect to see immediate impacts, but the natural cyclic variation in the cyanobacterial data demonstrates why long-term data are required to understand the impacts of mitigation actions. Without corresponding physicochemical data and a carefully planned sampling strategy (e.g., not biased by seasonality and shoreline sampling) we do not believe it is appropriate to draw any further conclusions of the impact of these interventions of cyanobacterial biovolumes.

The sinusoidal and almost synchronous fluctuation in total cyanobacterial biovolume prompted us to undertake a very preliminary investigation into whether global climate patterns could be driving some of the observed changes in total cyanobacterial biovolume. Alignment of the SOI suggests that climate patterns could be involved and that these warrant further investigation. Because climate patterns have varying regional effects, and probably lake-specific effects (i.e., some bays may be more sheltered or exposed to the prevailing wind direction), we recommend further investigation using local climate data. Given the caveats with the cyanobacteria dataset it might be more appropriate to undertake this analysis using data from the automatic high-frequency monitoring platforms which have collected continuous high-frequency temperature, chlorophyll-*a*, dissolved oxygen, turbidity and, in some

cases, phycocyanin data for more than 10 years at some of these lakes. As part of this analysis, extreme climate events (droughts and storms) should be considered as it is highly likely that this also plays a major role in regulating cyanobacterial abundance. It would also be valuable to include water quality data in this analysis. Based on the patterns observed in these datasets, we strongly believe that climate and related variables are a major driver of fluctuations in cyanobacterial biovolume in these lakes. It is extremely important to improve knowledge on climate and water quality factors; without this information it is challenging to assess the impact of mitigation actions on cyanobacterial biovolumes.

The analysis of the potential toxin producers in the Rotorua lakes showed no obvious patterns, except that their presence was highly variable. It also highlighted that the likelihood of detecting cyanotoxins is higher in Lakes Ōkaro and Rotorua.

Similarly, the change in cyanobacterial taxa with the ability to fix atmospheric nitrogen was somewhat inconclusive. Of most significance was a general decline in potential nitrogen fixers in Lakes Rotoiti and Rotorua between 1997–1998 and c. 2012. A similar pattern was noted by Smith et al. (2016), who also showed a general pattern of decreasing total nitrogen over this period in Lake Rotorua. Further investigation should be undertaken to determine if this, and other, shifts in taxa can be related to changes in water quality parameters.

The analysis of cyanobacterial species richness highlighted two years where there appeared to be a step-increase in the number of species identified. While it is possible that this could be related to environmental factors, we suggest this is most likely due to analytical inconsistencies (e.g., changes in the microscope used and the implementation of more robust training procedures). We did not investigate this further but highlight this as a caveat of this dataset. Given that the most abundant taxa in many samples were *Dolichospermum* and *Microcystis* (which have been identified consistently across the dataset) and that many of the additional taxa were only in low abundance, it is likely that these inconsistencies have had minimal effect on the assessment of trends in total cyanobacterial biovolume undertaken in this study.

3. REVIEW OF HEALTH WARNINGS, CYANOTOXINS AND TOXIC CYANOBACTERIAL SPECIES IN LAKE ROTORUA

3.1. Introduction – health warnings and cyanotoxins

This section focuses primarily on Lake Rotorua. It includes a review of health warnings issued by Toi te Ora Public Health, data on cyanotoxins and shifts in potentially toxic cyanobacterial species. No systematic analysis of cyanotoxins has been undertaken on Lake Rotorua, and the available data come from several national surveys. A targeted study was also undertaken on Lakes Rotoehu and Rotoiti as part of a PhD study (Wood 2004), and a summary of these findings are included as it provides some context for the likelihood of cyanotoxins in Lake Rotorua.

3.2. Methods

3.2.1. Data – health warnings

The data packet provided by BOPRC included information on whether each site in Lake Rotorua was in green (surveillance mode), amber (alert mode) or red (action mode) level according to the alert-level framework (ALF) for planktonic cyanobacteria in recreational freshwaters (see Table 8; MfE and MoH 2009). These data covered the period from 20 November 2012 to 04 April 2022. Health warnings were issued when a site was in the red level / action mode. The potentially toxic cyanobacteria used in these data are as defined by the Bay of Plenty Regional Council based on international data and differ from the data classification used in Section 2 of this report (outlined in Section 2.2.3).

Table 8. Biovolume alert-level thresholds used by Bay of Plenty Regional Council and Toi te Ora to issue health warnings. Heath warnings are issued when thresholds in the red level / action mode are exceeded. Note that the Situation 1 green level / surveillance mode threshold is not included because it is based on cell concentrations rather than biovolumes.

Cyanobacterial Alert-Level Thresholds
Green level (surveillance mode) **Situation 2:** Biovolume equivalent of ≤ 0.5 mm^3/L for the combined total of all cyanobacteria
Amber level (alert mode) **Situation 1:** Biovolume equivalent of > 0.5 to < 1.8 mm^3/L of potentially toxic cyanobacteria; **or** **Situation 2:** > 0.5 to < 10 mm^3/L total biovolume of all cyanobacterial material (where known cyanotoxin producers are not present).
Red level (action mode) **Situation 1:** biovolume equivalent of ≥ 1.8 mm^3/L of potentially toxic cyanobacteria; **or** **Situation 2:** ≥ 10 mm^3/L total biovolume of all cyanobacterial material (where known cyanotoxin producers are not present).

These data were analysed to determine how many times a site breached the alert level / action mode thresholds, and in each instance whether this was caused by the biovolume of potentially-toxic cyanobacteria (Situation 1 in the ALF; see Table 8) or the total cyanobacterial biovolume (Situation 2 in the ALF; see Table 8).

3.2.2. Data – cyanotoxin data

Two national surveys have been undertaken in Aotearoa New Zealand that have measured cyanotoxins in lakes:

- The first was undertaken between 1 January 2001 and 20 January 2004 (Wood et al. 2006b). A total of 61 samples were analysed from eight lakes in the Rotorua region. Of these, nine were from Lake Rotorua. The samples were analysed using enzyme-linked immunosorbent assay (ELISA) for two cyanotoxins; microcystins and saxitoxins.
- The second survey was undertaken between 11 December 2012 and 27 April 2013 (Wood et al. 2017). A total of 18 samples were analysed from eight lakes in the Rotorua region, including three samples from Lake Rotorua. In this study, samples were initially screened for genes involved in cyanotoxin production and positive samples were then analysed by liquid chromatography-mass spectrometry (LC-MS).

Wood et al. (2006a) undertook a study of microcystins in Lakes Rotoiti and Rotoehu as part of a larger study to explore the potential for these toxins to accumulate in rainbow trout and kākahi. Water samples were collected approximately weekly from 14 November 2003 to 2 May 2004 from three sites in Lake Rotoiti (Ōkawa Bay, Te Weta Bay and Hinehopu) and three sites in Lake Rotoehu (Kennedy Bay, Te Pōhue and Ōtautū). These samples were tested for microcystins using ELISA. As part of this study, microcystins were also measured intensively in Te Weta Bay (Lake Rotoiti) between 7 March 2004 and 27 March 2004. Surface water samples were taken twice daily (at ca. 10 am and 3 pm). Samples were also taken at 1, 2 and 3 m depths at the 3 pm sampling.

Wood et al. (2012a) studied the abundance, taxonomy, and toxin content of benthic mats in Lakes Tikitapu, Ōkāreka and Rotoiti. Samples were initially screened for genes involved in cyanotoxin production and positive samples were then analysed by LC-MS. Although this study focused on benthic not planktonic cyanobacteria, we include a summary of the results to allow a complete inventory of cyanotoxin data in the Rotorua lakes to be assembled.

No other toxin data were available for analysis and reporting. We believe that some sporadic analysis was likely undertaken in the early 2000s as part of the Ministry of Health / ESR public health advice programme that provided testing and advice to Public Health Officers, however, we were unable to source these data.

3.2.3. Shifts in the relative abundance of potentially toxin producing taxa

The methods and results describing shifts in the relative abundance of potentially toxic genera in Lake Rotorua are given in Sections 2.2.5 and 2.3.2, but are further explored in the discussion of this section (Section 3.4).

3.3. Results – health warnings and cyanotoxins

3.3.1. Health warnings

At the Holdens Bay site there were a total of five health warnings issued between 2012–2022. In all instances these were because of the potentially toxic biovolume threshold being breached (Situation 1 in the planktonic cyanobacteria ALF; Figure 6). At the Ngongotahā site there were three health warnings issued and all were via the potentially toxic biovolume threshold (Situation 1). There were two red level / action mode breaches due to exceedance of the potentially toxic threshold at Hamurana (Situation 1). Ōhau Channel had the highest number of action mode breaches, with the red level / action mode threshold being exceeded on nine occasions. All of these were due to the potentially toxic threshold being exceeded (Situation 1), albeit on one occasion the total biovolume threshold (Situation 2) was also exceeded on the same date (Figure 6).

The cyanobacterial taxa (and their respective biovolumes) observed in Lake Rotorua samples when the red level / action mode threshold was breached between 2012–2022 are listed in Table 9. The cyanobacterial taxa responsible for the threshold breaches were *Cuspidothrix issatschenkoi*, *Dolichospermum* spp., *Microcystis aeruginosa* and *Microcystis wesenbergii* (Table 9).

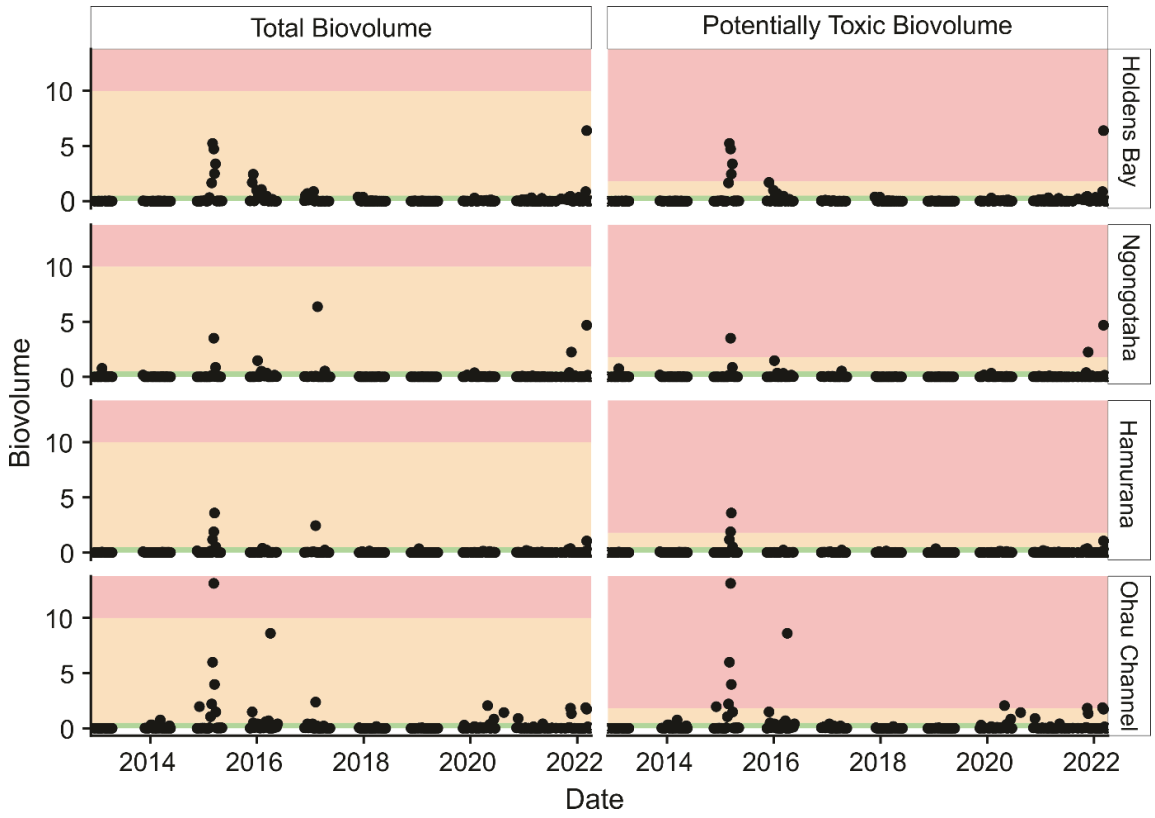

Figure 6. Total cyanobacterial biovolume (mm^3/L) for the four monitoring sites in Lake Rotorua. Shading shows the three biovolume thresholds as given in the alert-level framework for planktonic cyanobacteria in recreational freshwaters (MfE and MoH 2009). The left column shows the thresholds for total cyanobacterial biovolumes (green / surveillance ≤ 0.5 mm^3/L; orange / alert > 0.5 mm^3/L to < 10 mm^3/L; red / action ≥ 10 mm^3/L; Situation 2). The right column shows the biovolume for potentially toxic cyanobacteria (green / surveillance ≤ 0.5 mm^3/L; orange / alert > 0.5 mm^3/L to < 1.8 mm^3/L; red / action ≥ 1.8 mm^3/L; Situation 1). Note that potentially toxic cyanobacteria in these data are as defined by Bay of Plenty Regional Council and differ from the data classification as outlined in Section 2.2.3. A health warning is issued when the action level threshold is breached by either the total cyanobacterial biovolume or the biovolume of potentially toxic cyanobacteria.

Table 9. Cyanobacterial taxa and biovolumes observed in Lake Rotorua samples when the red level / action mode threshold was breached between 2012–2022.

Site	Date	Cyanobacterial Taxa	Biovolume (mm^3/L)
Holdens Bay	04/03/2015	*Cuspidothrix issatschenkoi*	5.99
	12/03/2015	*Cuspidothrix issatschenkoi*	13.13
	17/03/2015	*Cuspidothrix issatschenkoi*	3.98
	24/03/2015	*Cuspidothrix issatschenkoi*	3.38
	07/03/2022	*Dolichospermum planctonicum*	1.26
		Dolichospermum circinale	0.10
		Microcystis aeruginosa	0.37
Ngongotahā	12/03/2015	*Cuspidothrix issatschenkoi*	3.50
	22/11/2021	*Dolichospermum planctonicum*	2.25
	07/03/2022	*Dolichospermum planctonicum*	2.53
		Dolichospermum circinale	1.70
		Microcystis aeruginosa	0.45
Hamurana	12/03/2015	*Cuspidothrix issatschenkoi*	1.88
	17/03/2015	*Microcystis aeruginosa*	3.41
		Cuspidothrix issatschenkoi	0.17
Ōhau Channel	04/12/2014	*Dolichospermum circinale*	1.95
		Chroococcus limneticus	0.01
		Dolichospermum planctonicum	0.002
		Microcystis wesenbergii	0.002
		Microcystis aeruginosa	0.003
	26/02/2015	*Cuspidothrix issatschenkoi*	2.11
	04/03/2015	*Cuspidothrix issatschenkoi*	5.99
	12/03/2015	*Cuspidothrix issatschenkoi*	13.13
	17/03/2015	*Cuspidothrix issatschenkoi*	3.98
	03/04/2016	*Microcystis wesenbergii*	8.61
	28/04/2020	*Dolichospermum circinale*	2.05
	16/11/2021	*Dolichospermum planctonicum*	1.82
	28/02/2022	*Dolichospermum circinale*	0.90
		Dolichospermum planctonicum	0.84
		Pseudanabaena limnetica	0.002
		Microcystis aeruginosa	0.12

3.3.2. Cyanotoxins

In the study of Wood et al. (2006b), very low levels of microcystins were detected in two samples and trace levels of saxitoxin in two samples (Table 10). All positive samples were collected when the lake was experiencing a cyanobacterial bloom.

Table 10. Lake Rotorua cyanotoxin results from the study of Wood et al. (2006b). Microcystins and saxitoxins were determined using ELISA (ND = not detected, NT = not tested).

Date	Bloom present	Dominant species	Microcystins (µg/L)	Saxitoxin (µg/L)
28/01/2002	No	*Planktothrix* sp.	ND	NT
24/02/2003	Yes	*Microcystis aeruginosa, Dolichospermum planctonicum, Dolichospermum crassa*	ND	Trace
24/02/2003	Yes	*Microcystis aeruginosa, Dolichospermum planctonicum, Dolichospermum crassa*	0.30	Trace
24/02/2003	Yes	*Microcystis aeruginosa, Dolichospermum planctonicum, Dolichospermum crassa*	ND	ND
12/03/2003	No	*Dolichospermum planctonicum, Dolichospermum* sp.	ND	ND
24/02/2003	Yes	*Microcystis aeruginosa, Dolichospermum planctonicum, Dolichospermum crassa*	0.20	ND

Microcystins were detected in 27 samples from five other lakes in the Rotorua region as part of Wood et al. (2006b) (Appendix 4). The highest value measured was 350 µg/L in Lake Rotoiti (7 April 2003). Anatoxins were detected in one sample from Lake Rotoehu (6 November 2001) and trace levels of saxitoxins were detected in 21 samples from seven lakes (Appendix 4).

Three samples from Lake Rotorua were tested for cyanotoxin production genes as part of the Wood et al. (2017) study, but no cyanotoxin production genes were detected in these samples. A gene involved in microcystin production was detected in four samples from Lake Rotoiti, and in one sample each from Lakes Ōkataina, Rotoehu and Tarawera (Appendix 5). Subsequent LC-MS analysis only detected trace levels of microcystins in one of the samples from Lake Rotoiti (Appendix 5).

Microcystins were detected in 39% of samples collected from Lake Rotoiti in the Wood et al. (2006a) study, but only 7% from Lake Rotoehu (Appendix 6). The highest concentrations detected in each lake were 410 µg/L for Rotoiti (23 February 2004) and 24 µg/L for Rotoehu (2 May 2004; Appendix 6). Microcystins were detected in

92% of the samples from the intensive Te Weta Bay study. The highest concentration measured was 38 µg/L (26 March 2003; Appendix 6). In general, surface water samples contained more microcystins than those taken at depth (Appendix 6).

The *ndaF* gene (involved in the production of nodularin, a cyanobacterial hepatotoxin) was present in all five cyanobacterial mats collected from Lake Tikitapu in the study of Wood et al. (2012a). LC-MS was then used to confirm the concentration of nodularin in these samples (0.14 to 0.61 mg/kg). Attempts to identify the causative species were unsuccessful. Analysis of water samples using a passive sampling technique showed that very low levels of nodularin were also present in the lake water.

3.4. Discussion – health warnings and cyanotoxins

Although cyanobacteria were almost always present in the samples collected from Lake Rotorua between 2012–2022, health warnings were only issued for 2% of the samples collected during this time (note the period analysed for health warnings does not span the full dataset). During this period the health warnings issued for Lake Rotorua were all triggered by exceedance of the potentially toxic biovolume threshold (≥ 1.8 mm^3/L; Situation 1 in the ALF for planktonic cyanobacteria in recreational freshwaters).

The recreational cyanobacteria guidelines for Aotearoa New Zealand are currently being revised. A key change in the revised cyanobacteria guidelines will be that the concentration of confirmed toxin-producing cyanobacteria in Aotearoa New Zealand will be used to trigger the Situation 1 threshold in the ALF for planktonic cyanobacteria, rather than the biovolume of potentially-toxic cyanobacteria based on international data (as is currently used in the guidelines; Puddick et al. 2022). Following nearly two decades of research on toxic cyanobacteria, there is consensus that there is a good understanding of which cyanobacterial species are toxin producers in Aotearoa New Zealand. The proposed revised ALF for planktonic cyanobacteria will be based on cell concentration thresholds for known producers in Aotearoa New Zealand, of which two occur in Lake Rotorua, *Microcystis* spp. and *Cuspidothrix issatschenkoi. Microcystis* spp. produces the hepatoxic microcystins (Puddick et al. 2019) and *Cuspidothrix issatschenkoi* produces neurotoxic anatoxins (Wood et al. 2007a). At times, both taxa have reached very high concentrations in Lake Rotorua. When this occurs, caution should be taken, and health warnings should be issued due to the likelihood of cyanotoxins being present in the lake.

As noted in Section 2.2.3, no *Dolichospermum* species isolated from lakes in Aotearoa New Zealand have been confirmed as cyanotoxin producers. Biovolumes of this genus have caused health warnings to be issued on seven occasions between 2012 and 2022. When the revised cyanobacteria guidelines are finalised, we recommend adopting the new thresholds based on confirmed toxin-producing

cyanobacterial taxa observed in Aotearoa New Zealand, which will likely lessen the number of health warnings issued. In the interim, we recommend undertaking toxin testing on bloom samples when *Dolichospermum* is present to build up more knowledge on toxic cyanobacteria in the Rotorua Lakes (see Section 4.2 for more information).

Although the revised cyanobacteria guidelines will include cell concentration thresholds for confirmed toxin producers (Situation 1 in the ALF), the total cyanobacterial biovolume red level / action mode threshold of 10 mm^3/L (Situation 2 in the ALF) will still apply as high densities of cyanobacteria can cause skin irritation and respiratory issues regardless of whether they produce cyanotoxins.

Cyanotoxin data for Lake Rotorua are extremely limited and comes from several national studies that took only single time point samples and the most recent was undertaken over nine years ago. The data from one of these studies found low levels of microcystins, likely due to *Microcystis* sp. observed in the lake. Because *Cuspidothrix issatschenkoi* is present in Lake Rotorua, we suggest it is highly likely that there are also anatoxins in the lake. Because the cyanotoxin data are so limited, it is not possible to make any conclusive statements on cyanotoxins in Lake Rotorua. We recommend that a more robust process should be implemented to obtain a better understanding of cyanotoxins in Lake Rotorua. In Section 4 we provide recommendations for generating robust data on the occurrence of cyanotoxins to allow the assessment of long-term patterns and to help refine risk assessments related to potentially toxic cyanobacteria.

The presence of cyanotoxins in other lakes in the Rotorua region, particularly the high concentrations of microcystins measured in Lake Rotoiti, demonstrates that cyanotoxins are present and, at times, pose a health risk to recreational users in this region. These toxins can also accumulate in aquatic organisms, and Wood et al. (2006a) showed this occurs in rainbow trout and kākahi in Lakes Rotoiti and Rotoehu. Cyanotoxins can also have a detrimental effect on the health of native species, as demonstrated by Clearwater et al. (2014) for kākahi and kōura. The presence of cyanotoxins in benthic mats from Lake Tikitapu (Wood et al. 2012a) and the accumulation of these in kōura (Wood et al. 2012b) highlights another source of cyanotoxins in these lakes, and further investigation should be undertaken to assess the distribution and health risks posed by benthic cyanobacteria in the Rotorua lakes.

The analysis of shifts in toxin-producing cyanobacteria in Lake Rotorua undertaken in Section 2.3.2 showed a fluctuating pattern with no clear trends. Shifts in the proportion of toxic cyanobacteria were largely driven by fluctuations in the abundance of *Microcystis* sp. Between 2009 to c. 2012, *Microcystis wesenbergii* was the dominant species of *Microcystis* present in this lake. Because this species is not known to produce toxins in Aotearoa New Zealand, it was not assigned as a toxin producer in our analysis. Without undertaking a detailed analysis of environmental drivers over the

corresponding period, it is impossible to make inferences about what causes the shifts in cyanobacterial species composition in Lake Rotorua. It is also likely that both toxic and non-toxic strains occur in this lake. Genetic and / or chemical methods should be used in the future to aid in understanding shifts in toxic and non-toxic strains.

4. RECOMMENDATIONS FOR FUTURE MONITORING

4.1. Improvements to the current recreational cyanobacterial monitoring programme

The data analysed in this report were generated as part of a cyanobacterial recreational monitoring programme that is aimed at protecting human health. The results of this study demonstrate that it is imperative that this monitoring programme continues. High cyanobacterial biovolumes were detected at all sites included in this study on multiple occasions. Currently there are no mechanisms to predict when cyanobacterial blooms might occur in these study lakes (or the Kaituna River). Regular monitoring is an important part of managing the risk posed by cyanobacteria to human health in the Rotorua lakes.

There is a wider need across Aotearoa New Zealand to modernise how cyanobacteria monitoring is undertaken. Cyanobacterial blooms are highly changeable and often by the time results are generated using microscopy the concentration of cells at a site has changed. Consideration should be given to incorporating technologies such as field-portable fluorometers that could be used on the lakeshore to measure phycocyanin (which is then converted to a biovolume equivalent using a lake-specific model; Thomson-Laing et al. 2020). This would enable health warnings to be issued onsite when set thresholds are exceeded. We also recommend that samples continue to be taken and analysed using microscopy, at least at some sites, to maintain knowledge on species composition. Another promising technique for cyanobacterial monitoring is remote sensing using satellites. A recently completed MBIE Smart Idea research programme (Eye on lakes, UOWX1802) has developed some valuable approaches that, with further customisation, could be included in a multi-tiered approach to cyanobacterial bloom monitoring.

An issue highlighted in the present study was the step-change observed in species richness at two time points, which we suggest is likely due to inconsistencies in the approach used in the microscopy analysis. We are aware that a training procedure has now been instigated and we strongly advocate for this to continue. We also suggest that BOPRC includes an inter-laboratory comparison as part of their annual quality control process. This should involve a selection of samples, ideally from different lakes taken across the sampling season being sent to an algal laboratory, accredited by International Accreditation New Zealand (IANZ), for cross-laboratory comparison of taxonomic assignment.

4.2. Incorporate cyanotoxin analysis into the recreational cyanobacterial monitoring programme

We recommend that cyanotoxin gene and chemical analyses are incorporated into BOPRC's cyanobacterial recreational monitoring programme. This will help to improve knowledge on cyanotoxins and toxin-producing species in the Rotorua lakes and allow long-term trends in cyanotoxin abundance to be assessed.

We recommend that samples are screened using a polymerase chain reaction-based approach for genes involved in cyanotoxin production when a potentially-toxic cyanobacteria species is present at biovolumes > 0.5 mm^3/L (i.e., those known to produce toxins either in Aotearoa New Zealand or overseas, see Appendix 7). Some cyanobacteria can contain the gene for toxin production but may not produce toxins. Therefore, if the results of the gene screen are positive, the sample should be analysed for the appropriate cyanotoxin (established based on the gene detected) using a chemical method such as LC-MS. The result of the chemical analysis also provides quantitative data on the concentration of toxin present in the sample. To be able to undertake this analysis, a non-preserved sample will need to be collected in parallel to the samples used for microscopy analysis. If desired, we can provide further advice on collecting and storing samples for the analysis described above.

From a public health protection standpoint and in the context of the Rotorua lakes, samples containing *Dolichospermum* sp., *Aphanizomenon*, *Microcystis wesenbergii* and *Planktothrix* sp. would be sensible candidates for prioritising in the analysis described above (if funding is restricted). This is because of their high abundance in the Rotorua lakes at certain points in time, their potential to produce cyanotoxins and a need for more information (both nationally and locally for the Rotorua lakes). Whilst no toxin-producing strains of *Dolichospermum* sp. and *Microcystis wesenbergii* have been identified in Aotearoa New Zealand to date, these cyanobacterial taxa have been reported to produce cyanotoxins overseas. As described in Section 2.2.3, microcystin-producing *Planktothrix* sp. has been observed in Aotearoa New Zealand, but we have relatively little information on the prevalence and distribution of toxin-producing *Planktothrix* around the country and in the Rotorua lakes. If funding is available, then information on the toxin production capacity of a wider range of 'potentially toxic cyanobacteria' will prove beneficial from a risk management perspective and long-term data on toxin concentrations in the Rotorua lakes will allow for trends and drivers to be explored in the future.

When cyanotoxins are detected in a sample, determining the cyanobacterial species responsible for toxin production is an additional useful step as most samples will contain a mixture of different taxa. This is especially pertinent if there are no species in the sample known to produce the detected cyanotoxin in Aotearoa New Zealand. To do this, we recommend that the positive gene fragment is amplified and sequenced. The sequence of genes involved in toxin production are usually species-

specific and this can be checked against international genetic databases to ascertain the most likely producer. Further confirmation can be obtained by isolating and culturing the species from the positive sample. This is a time-consuming and lengthy process and using the gene sequencing to guide which species to isolate is recommended. Because of the investment required for isolating and culturing, we recommend that this is undertaken only when there is strong evidence that a species is present that has not already been confirmed as a toxin-producer in Aotearoa New Zealand.

4.3. Develop a robust algal monitoring programme

Algal communities are sensitive to changes in their environment, and thus, the amount and types of algae present in a lake provide valuable information on the state and drivers of water quality. For example, Paul et al. (2012) showed that phytoplankton composition in eleven lakes from the Rotorua region was related to land use in their catchments.

We recommend that an algal monitoring programme is developed and instigated for a selection of lakes in the Rotorua region. This programme would allow long-term patterns in all algae to be assessed, not just cyanobacteria. If the sampling sites are carefully selected, the data would be much more informative on whole lake changes than the shoreline samples analysed in this study. Algal monitoring programmes have previously been undertaken in multiple Rotorua lakes, although the timing and duration of these has varied (Wilding 2000). Ideally, monitoring should be undertaken at least monthly, and samples should be collected in parallel to water chemistry sampling.

4.4. Analyse high frequency data and explore drivers of change

Because of the caveats described in Section 2.1 we did not explore whether there were environmental or other parameters that might explain the observed shifts in cyanobacterial biovolumes during the current study. We also only undertook a very high-level exploration of whether two mitigation approaches (alum dosing and the Ōhau Channel diversion wall) had an impact on cyanobacterial biovolume.

Automatic high-frequency monitoring platforms which collect continuous high-frequency temperature, chlorophyll-*a*, dissolved oxygen, turbidity and in some cases phycocyanin data are now installed in nine Rotorua lakes. In some lakes, these have been recording data for more than 10 years. To gain further insights into cyanobacterial dynamics in these lakes, where phycocyanin data are available, we recommend that an in-depth analysis of the high-frequency data is undertaken. Where feasible water quality data, and other information, such as alum dosing records,

should be included following a similar approach to that used in Smith et al. (2016). Climate data and information on extreme events (droughts and storms) should be included as it is highly likely that they also play a major role in regulating cyanobacterial biovolumes in these lakes.

Climate change predictions for Aotearoa New Zealand (MfE 2017) indicate that extreme climate events will increase in frequency and severity and this, in concert with other effects of climate change on lakes (e.g., more intense and longer periods of stratification), will likely result in an increase in the frequency and severity of cyanobacterial blooms in the Rotorua lakes. Robust knowledge on the drivers of cyanobacterial blooms will assist in developing management practices that help reduce their severity and frequency in the future.

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7. APPENDICES

Appendix 1. Cyanobacterial taxa in the dataset classified as toxin-producing for the current analysis.

- *Cuspidothrix issatschenkoi*
- *Microcystis aeruginosa*
- *Nostoc linckia*
- *Phormidium* sp.
- *Planktothrix agardhii*
- *Planktothrix* cf. *planktonica*
- *Planktothrix raciborskii*
- *Planktothrix* sp.
- *Raphidiopsis* cf. *mediterranea*

Appendix 2. Cyanobacterial taxa in the dataset classified as nitrogen-fixing for the current analysis.

- *Aphanizomenon gracile*
- *Aphanizomenon* sp.
- *Dolichospermum aphanizomenoides*
- *Dolichospermum circinale*
- *Dolichospermum lemmermannii*
- *Dolichospermum oscillarioides*
- *Dolichospermum planctonicum*
- *Dolichospermum* sp.
- *Dolichospermum spiroides*
- *Gloeotrichia* sp.
- *Nostoc linckia*
- *Raphidiopsis* cf. *mediterranea*
- *Tolypothrix* sp.
- *Trichodesmium iwanoffianum*

Appendix 3. Results of generalised additive models (GAMs) for individual sites in Lakes Rotoiti, Rotorua and Rotoehu.

Plots show raw data in grey and fitted generalised additive mixed models (black line) with 95% confidence intervals shown by the dashed black line. Dashed vertical lines show interventions—alum dosing (Rotorua and Kaituna) and diversion wall (Rotoiti). Significant changes are denoted by blue (decreasing) and orange (increasing) line segments. Horizontal orange or blue bars at the right-hand end of the x-axis summarise whether the cyanobacterial biovolume in the last year (short bar) or five years (longer bar) has been overall increasing (orange) or decreasing (blue).

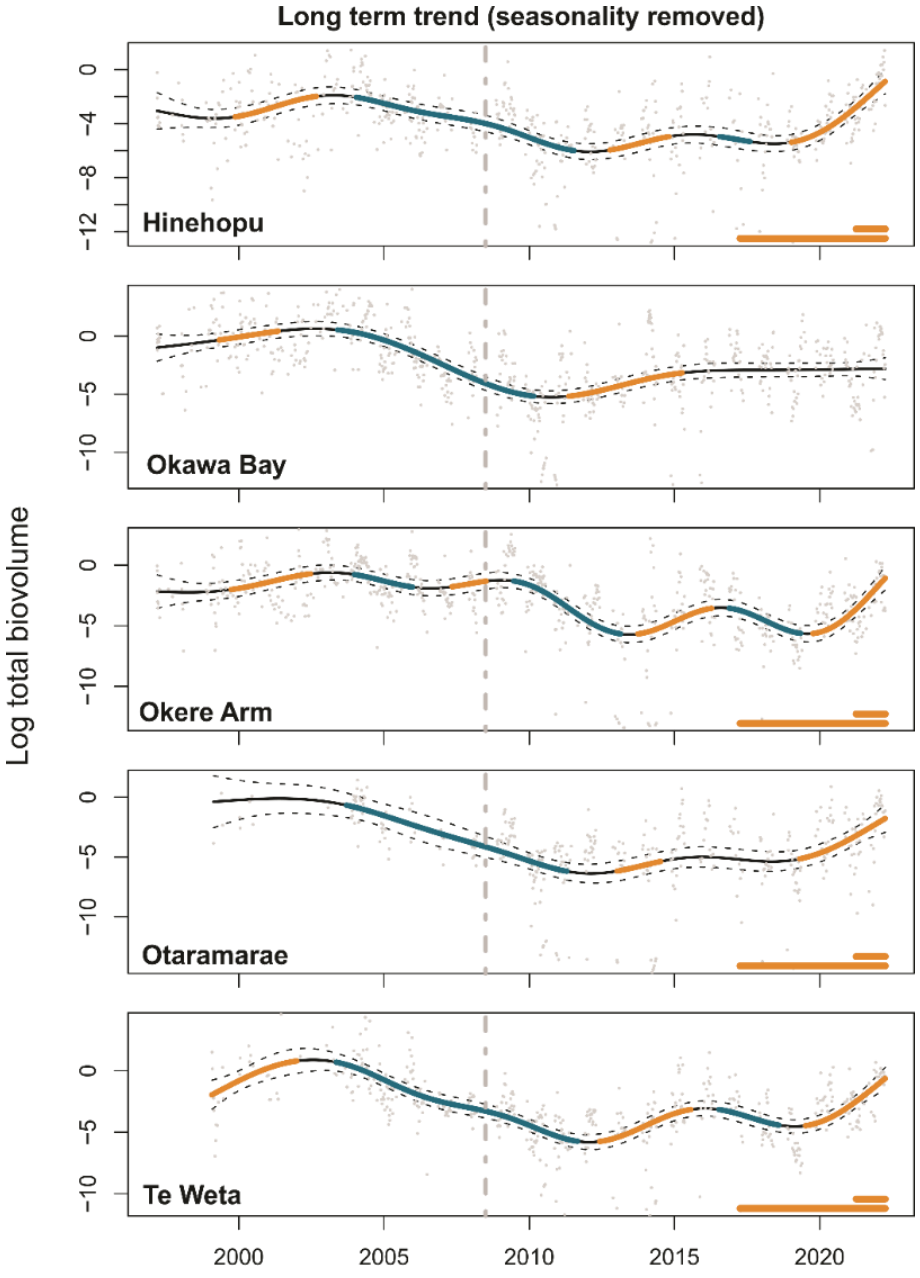

Figure A3.1. Results of generalised additive models for total cyanobacterial biovolume for separate sampling sites in Lake Rotoiti.

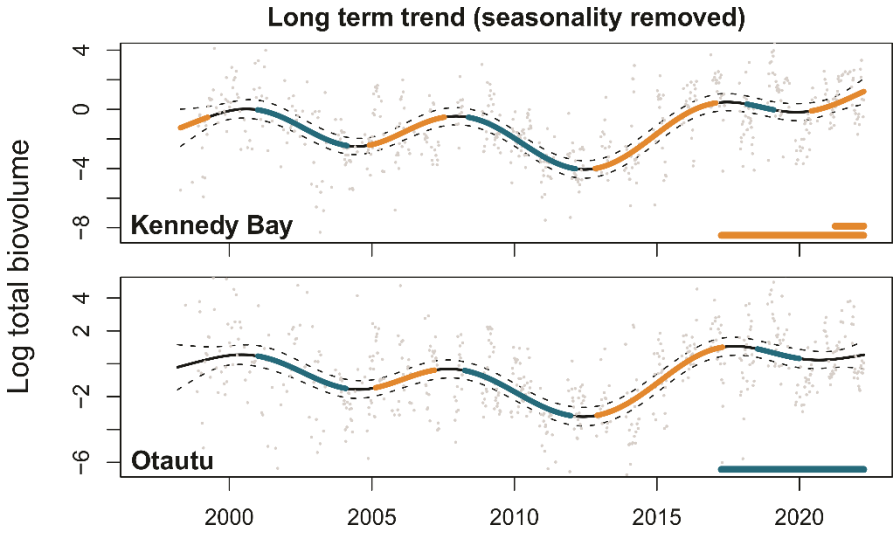

Figure A3.2. Results of generalised additive models for total cyanobacterial biovolume for separate sampling sites in Lake Rotoehu.

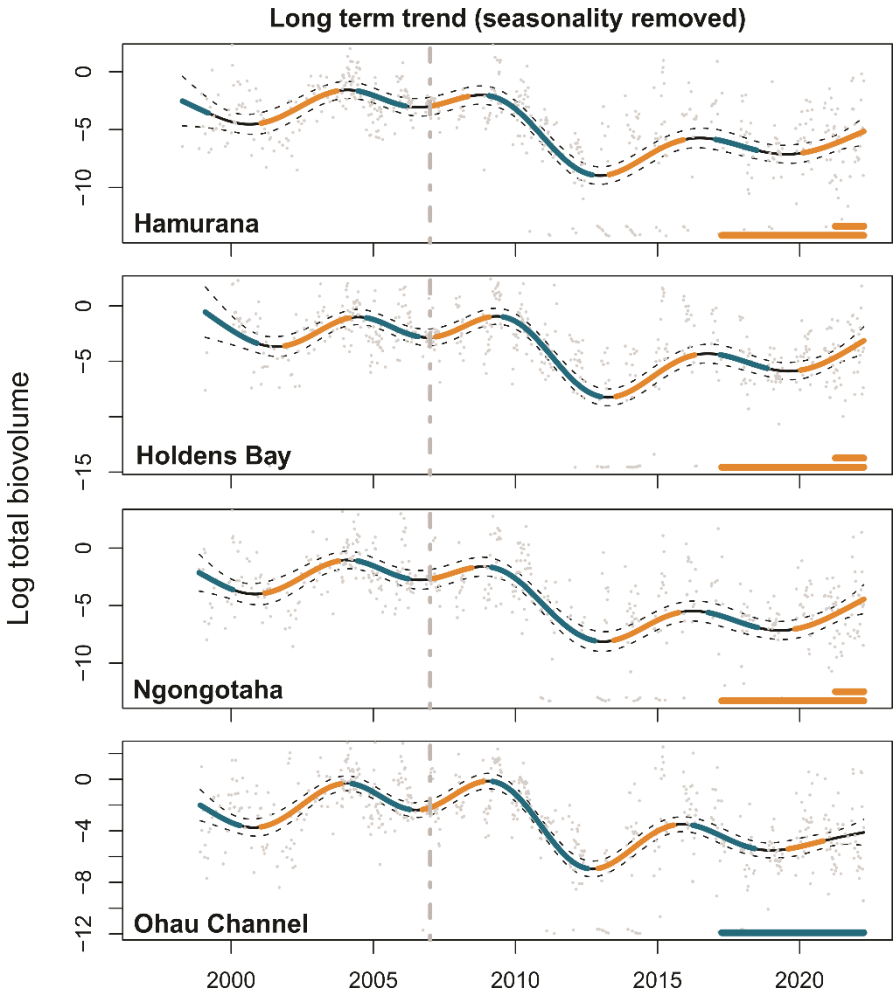

Figure A3.3. Results of generalised additive models for total cyanobacterial biovolume for separate sampling sites in Lake Rotorua.

Appendix 4. Cyanotoxin values for lakes in the Rotorua region from Wood et al. (2006b).

Cyanotoxin results from lakes in the Rotorua region from the study of Wood et al. (2006b). Microcystins and saxitoxins were determined using ELISA and anatoxin was determined using high-performance liquid chromatography. ND = not detected, NT = not tested.

Sample location	Date	Bloom / scum present	Microcystins (µg/L)	Anatoxin	Saxitoxin (µg/kg FDW)	Saxitoxin (µg/L)
Ngahewa	05/01/2002	N	ND	ND	NT	NT
Ngahewa	28/01/2002	Y	0.15	ND	NT	NT
Ngahewa	12/03/2003	Y	ND	NT	NT	ND
Ngahewa	25/04/2003	Y	1.48	ND	NT	ND
Ngapouri	10/12/2001	Y	ND	ND	NT	NT
Ngapouri	12/03/2003	Y	0.38	NT	1.42	ND
Okaro	05/01/2002	N	ND	ND	ND	ND
Okaro	29/01/2002	N	0.15	ND	NT	NT
Okaro	01/12/2002	N	ND	ND	NT	NT
Okaro	01/12/2002	Y	ND	ND	NT	NT
Okaro	12/03/2003	N	ND	NT	NT	ND
Rerewhakaaitu	12/03/2003	N	ND	ND	NT	ND
Rotoehu	26/11/2001	Y	5.00	NT	NT	NT
Rotoehu	05/01/2002	N	ND	ND	NT	ND
Rotoehu	24/02/2003	N	0.20	ND	0.93	0.02
Rotoehu	24/02/2003	N	ND	ND	0.86	0.02
Rotoehu	24/02/2003	N	ND	NT	0.59	0.02
Rotoehu	12/03/2003	N	0.11	NT	NT	ND
Rotoehu	12/03/2003	N	ND	NT	NT	ND
Rotoehu	25/04/2003	Y	43.00	NT	NT	ND
Rotoehu	25/04/2003	Y	16.60	NT	ND	ND
Rotoehu	26/05/2003	Y	5.00	NT	ND	ND
Rotoehu	12/06/2003	Y	4.50	NT	NT	NT
Rotoehu	06/11/2001	Y	ND	Positive	NT	NT
Rotoehu	12/03/2003	Y	ND	NT	ND	ND
Rotoehu	12/03/2003	Y	0.40	NT	0.26	ND
Rotoehu	28/01/2002	Y	25.00	NT	NT	NT
Rotoiti	05/01/2002	N	ND	ND	0.77	0.02
Rotoiti	28/01/2002	N	ND	NT	NT	NT
Rotoiti	28/01/2002	Y	0.60	NT	NT	NT
Rotoiti	28/01/2002	Y	0.25	ND	NT	NT
Rotoiti	28/01/2002	Y	0.25	NT	NT	NT
Rotoiti	28/01/2002	Y	0.27	NT	NT	NT

Sample location	Date	Bloom / scum present	Microcystins (µg/L)	Anatoxin	Saxitoxin (µg/kg FDW)	Saxitoxin (µg/L)
Rotoiti	28/01/2002	Y	0.40	NT	NT	NT
Rotoiti	30/01/2002	Y	0.35	NT	NT	NT
Rotoiti	30/01/2002	Y	0.35	NT	NT	NT
Rotoiti	16/03/2002	N	ND	NT	NT	NT
Rotoiti	24/02/2003	Y	ND	NT	NT	ND
Rotoiti	24/02/2003	Y	ND	NT	NT	ND
Rotoiti	24/02/2003	Y	ND	NT	NT	ND
Rotoiti	24/02/2003	Y	ND	NT	NT	ND
Rotoiti	12/03/2003	N	ND	ND	NT	ND
Rotoiti	12/03/2003	Y	ND	NT	NT	ND
Rotoiti	24/03/2003	Y	0.30	NT	ND	ND
Rotoiti	07/04/2003	Y	44	ND	1.90	NT
Rotoiti	07/04/2003	Y	350	ND	0.43	NT
Rotoiti	07/04/2003	Y	315	ND	1.06	NT
Rotoiti	25/04/2003	Y	ND	NT	0.30	ND
Rotoiti	13/05/2003	Y	2.51	NT	NT	NT
Rotoiti	26/05/2003	Y	ND	NT	NT	NT
Rotoiti	11/06/2003	Y	26.40	NT	NT	NT
Rotoiti	11/06/2003	Y	6.29	NT	NT	NT
Tarawera	01/10/2003	Y	ND	NT	NT	NT
Tarawera	12/03/2003	N	ND	NT	NT	ND
Tarawera	12/03/2003	N	ND	ND	0.76	ND

Appendix 5. Cyanotoxin values for lakes in the Rotorua region from Wood et al. (2017).

Results from Lakes in the Rotorua region of cyanotoxin gene screening followed by subsequent cyanotoxin results from positive samples as described in Wood et al. (2017). Microcystins were determined using liquid chromatography mass spectrometry. ND = not detected, NT = not tested.

Lake	*mcyE* gene	Microcystin (µg/L)
Lake Ōkareka	-	NT
Lake Ōkataina	+	ND
Lake Rotoehu	+	0.01
Lake Rotoehu	+	ND
Lake Rotoiti	-	NT
Lake Rotoiti	-	NT
Lake Rotoiti	-	NT
Lake Rotoiti	-	NT
Lake Rotoiti	+	ND
Lake Rotoiti	+	ND
Lake Rotoiti	+	ND
Lake Rotoiti	+	ND
Lake Rotorua	-	NT
Lake Rotorua	-	NT
Lake Rotorua	-	NT
Lake Tarawera	+	ND
Lake Tikitapu	-	NT
Lake Ōkaro	-	NT

Appendix 6. Cyanotoxin values for Lakes Rotoiti and Rotoehu from Wood et al. (2006a).

Table A6.1 Microcystins concentrations (µg/L) from the approximately weekly surface water sampling undertaken in Lakes Rotoiti and Rotoehu. Microcystins were measured using ELISA. ND = not detected.

Date	Lake Rotoiti			Lake Rotoehu		
	Ōkawa	Te Weta	Hinehopu	Kennedy	Te Pōhue	Ōtautū
14 Nov 2003	ND	ND	ND	ND	ND	ND
19 Nov 2003	ND	ND	ND	ND	ND	ND
25 Nov 2003	ND	ND	ND	ND	ND	ND
01 Dec 2003	ND	ND	ND	ND	ND	ND
08 Dec 2003	ND	ND	ND	ND	ND	ND
15 Dec 2003	ND	ND	ND	ND	ND	ND
18 Dec 2003	ND	ND	ND	ND	ND	ND
05 Jan 2004	ND	ND	ND	5.80	ND	ND
12 Jan 2004	ND	ND	ND	ND	ND	ND
19 Jan 2004	ND	ND	ND	ND	ND	ND
24 Jan 2004	ND	ND	ND	ND	ND	ND
02 Feb 2004	0.01	ND	ND	ND	ND	ND
09 Feb 2004	ND	ND	ND	ND	ND	ND
16 Feb 2004	ND	ND	ND	ND	ND	ND
23 Feb 2004	410.30	38.46	0.07	ND	ND	ND
01 March 2004	7.23	3.02	ND	ND	ND	ND
08 March 2004	6.49	764.20	2.32	ND	ND	ND
15 March 2004	20.23	29.09	3.43	ND	ND	ND
22 March 2004	39.70	7.27	9.33	ND	ND	ND
29 March 2004	4.60	40.35	0.67	ND	ND	ND
05 April 2004	2.06	9.08	2.69	ND	ND	0.12
13 April 2004	0.54	7.14	ND	ND	ND	ND
19 April 2004	0.31	17.58	ND	ND	ND	ND
26 April 2004	7.56	25.70	ND	ND	ND	ND
02 May 2004	1.20	21.10	ND	0.45	23.50	0.20

Table A6.2 Microcystin concentrations (µg/L) in surface and depth samples taken from Te Weta Bay (Lake Rotoiti). Microcystins were measured using ELISA. ND = not detected.

Date	Morning	Afternoon			
	Surface	Surface	1 m	2 m	3 m
07 March 2004	7.98	4.04	ND	ND	9.92
08 March 2004	4.92	6.60	1.81	0.24	0.43
09 March 2004	11.90	1.37	2.19	0.25	10.23
10 March 2004	13.27	17.03	3.62	0.23	22.81
11 March 2004	6.34	4.05	8.46	2.30	5.74
12 March 2004	1.21	3.95	22.28	0.45	7.15
13 March 2004	3.51	28.95	17.50	1.04	ND
14 March 2004	ND	ND	1.17	0.29	6.66
15 March 2004	1.31	2.06	4.99	1.97	1.69
16 March 2004	18.93	21.69	4.01	1.87	6.13
17 March 2004	1.9	5.41	1.89	5.71	2.44
18 March 2004	1.68	21.42	3.3	2.1	2.86
19 March 2004	2.15	5.58	2.54	0.97	1.3
20 March 2004	5.23	8.1	7.64	10.7	13.9
21 March 2004	10.27	2.26	13.1	5.43	13.54
22 March 2004	3.9	2.25	3.41	1.92	4.76
23 March 2004	3.1	2.17	1.52	2.66	3.22
24 March 2004	3.26	1.69	3	3.11	3.92
25 March 2004	2.93	2.45	0.82	6.27	18.35
26 March 2004	7.93	12.5	38.32	11.66	6.72
27 March 2004	8.58	ND	ND	9.38	ND

Appendix 7. Summary of toxin-producing cyanobacteria genera identified internationally (referred to as 'potentially-toxic cyanobacteria' in this report).

Table A7.1 Species in **bold type** are known to produce the associated toxin (also in bold type) in Aotearoa New Zealand. * = Cyanobacterial taxa commonly observed in the Rotorua lakes.

Cyanobacterial Taxa	Cyanotoxin/s
Anabaena sp. *****	Microcystins
Anabaenopsis sp.	Microcystins
Annamia sp.	Microcystins
Aphanizomenon sp. *****	Anatoxin-a, Cylindrospermopsins, Saxitoxins
Aphanocapsa sp. *****	Microcystins
Arthrospira sp.	Anatoxin-a,
Chrysosporum sp.	Cylindrospermopsins
Coelosphaerium sp.	Microcystins
***Cuspidothrix issatschenkoi* ***	**Anatoxin-a**
Cylindrospermum sp.	Anatoxin-a, Anatoxin-a(S), Cylindrospermopsins, Microcystins, Saxitoxins
Dolichospermum sp. *****	Anatoxin-a, Anatoxin-a(S), Cylindrospermopsins, Microcystins
Fischerella sp.	Microcystins
Geitlerinema sp.	Saxitoxins
Gloeotrichia sp.	Microcystins
Hapalosiphon sp.	Microcystins
Heteroleiblenia sp.	Microcystins
Iningainema sp.	Nodularin
Leptolyngbya sp.	Microcystins
Limnothrix sp.	Microcystins
***Microcoleus* sp. ***	**Anatoxins**, Microcystins
***Microcystis* sp. ***	Anatoxin-a, **Microcystins**, Saxitoxins
Microseria sp.	Cylindrospermopsins, Saxitoxins
***Nodularia* sp.**	**Nodularin**
***Nostoc* sp. ***	**Microcystins**, Nodularin
***Oscillatoria* sp.**	**Anatoxins**, **Microcystins**
Phormidium sp.	Anatoxins, Cylindrospermopsins, Microcystins, Saxitoxins
***Planktothrix* sp.**	Anatoxins, **Microcystins**, Saxitoxins
Plectonema sp.	Microcystins

Cyanobacterial Taxa	Cyanotoxin/s
Pseudanabaena sp.	Microcystins
Pseudocapsa sp.	Microcystins
Radiocystis sp.	Microcystins
Raphidiopsis raciborskii	Anatoxins, **Cylindrospermopsins**, Microcystins, Saxitoxins
Rivularia sp.	Microcystins
Schizothrix sp.	Microcystins
***Scytonema* sp.**	Microcystins, **Saxitoxins**
Snowella sp.	Microcystins
Synechocystis sp.	Microcystins
Tolypothrix sp.	Microcystins
Tychonema sp.	Anatoxins
Umezakia sp.	Cylindrospermopsins
Woronichinia sp.	Microcystins

Note: This is a compilation of worldwide information. New toxic species continue to be identified, and all cyanobacteria should be regarded as potentially toxic until evaluated for toxins.