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2022 REVISIONS TO THE ALERT-LEVEL FRAMEWORK FOR PLANKTONIC CYANOBACTERIA IN THE 'NEW ZEALAND GUIDELINES FOR CYANOBACTERIA IN RECREATIONAL FRESH WATERS'

2022 REVISIONS TO THE ALERT-LEVEL FRAMEWORK FOR PLANKTONIC CYANOBACTERIA IN THE 'NEW ZEALAND GUIDELINES FOR CYANOBACTERIA IN RECREATIONAL FRESH WATERS'

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EXECUTIVE SUMMARY

The 'Interim New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters' were released by the Ministry for the Environment | Manatū Mō Te Taiao (MfE) and the Ministry of Health | Manatū Hauora (MoH) in 2009. In 2018, the Recreational Cyanobacteria Guidelines were reviewed, with one of the recommendations being improvements to the alert-level framework (ALF) for planktonic cyanobacteria. The aim of the current project was to revise the ALF for managing planktonic cyanobacteria in recreational waterbodies in-line with the end-user recommendations from the 2018 review. This involved:

1. determining toxin quotas for selected toxin-producing planktonic cyanobacteria observed in New Zealand and collating toxin quotas from literature
2. developing taxa-specific cell concentration thresholds for toxin-producing planktonic cyanobacteria observed in New Zealand to trigger the Action level /Red mode of the ALF
3. consulting with end-users on the revised ALF and incorporating final changes.

Taxa-specific thresholds for confirmed toxin-producing planktonic cyanobacteria observed in New Zealand were developed using the toxin quota dataset described above and the World Health Organization (WHO) guideline values for cyanotoxins in recreational waters. The taxa-specific thresholds were integrated into the ALF for planktonic cyanobacteria in recreational freshwaters and feedback was sought from end-users. The end-user feedback indicated that the taxa-specific thresholds were an improvement on the previous ALF for planktonic cyanobacteria and identified other opportunities to improve the planktonic cyanobacteria ALF and the guidance associated with it. Some of the suggestions received were outside of the scope of the current project but were strongly aligned with feedback received during the 2018 review of the Recreational Cyanobacteria Guidelines. Feedback was also sought on whether to align the naming of the alert levels in the Recreational Cyanobacteria Guidelines (where a traffic light system is used; green, amber, red) with the naming found in the WHO Guidelines and the New Zealand Drinking-Water Cyanobacteria Guidelines (where a number system is used; Vigilance, Alert Level 1, Alert Level 2, etc.). The majority of end-users who participated in the survey did not support the name change and we recommend that it remains as is (a traffic light system).

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GLOSSARY

ALF:	Alert-level framework
ATX:	Anatoxin-a
ATXs:	Anatoxins
CYNs:	Cylindrospermopsins
dhATX:	Dihydroanatoxin-a
dhHTX:	Dihydrohomoanatoxin-a
DHB:	District Health Board
ESR:	Institute of Environmental Science and Research
FOV:	Field of view
HTX:	Homoanatoxin-a
LAWA:	Land, Air, Water Aotearoa
LC-MS/MS:	Liquid chromatography-tandem mass spectrometry
Max:	Maximum
Med:	Median
MCs:	Microcystins
Min:	Minimum
MfE:	Ministry for the Environment | Manatū Mō Te Taiao
MoH:	Ministry of Health | Manatū Hauora
NIWA:	National Institute of Water and Atmospheric Research
NOF:	National objectives framework
NPS-FM:	National Policy Statement for Freshwater Management
NODs:	Nodularins
NOAEL:	No-observed-adverse-effect level
TA:	Territorial authority
WHO:	World Health Organization

1. BACKGROUND INFORMATION

Cyanobacteria are photosynthetic prokaryotic organisms that are an integral part of many terrestrial and aquatic ecosystems. Under favorable conditions, cyanobacteria cells can multiply and form blooms in freshwater environments. Because many cyanobacteria species have toxin-producing strains, high levels of cyanobacteria in lakes and rivers can pose a health risk to humans and animals.

The 'Interim New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters' were released by the Ministry for the Environment | Manatū Mō Te Taiao (MfE) and the Ministry of Health | Manatū Hauora (MoH) in 2009 (MfE and MoH 2009). In 2018, the interim guidelines were reviewed and a key recommendation was that the alert-level framework (ALF) for planktonic cyanobacteria be revised to include thresholds for toxin-producing cyanobacteria observed in New Zealand (Wood et al. 2018a). The rationale for this recommendation was that a decade's worth of research since the interim guidelines were released had identified four species/genera of toxin-producing planktonic cyanobacteria in New Zealand. Therefore, the Action / Red mode thresholds could be tailored to avoid unnecessary escalations in alert level when the risk to recreational water users was low. Whilst benthic cyanobacteria also occur in lakes in New Zealand (Wood et al. 2015), development of an ALF for managing both benthic and planktonic cyanobacteria in lakes was outside of the scope of the current project.

Developing taxa-specific thresholds requires knowledge on safe levels of cyanotoxins in a waterbody being used for intermittent recreational use (guideline values) and knowledge on the amount of cyanotoxins produced by cyanobacteria (the toxin quota). The World Health Organization (WHO) recently developed recreational guideline values for the four commonly-observed classes of cyanotoxin: anatoxins (World Health Organization 2020a), cylindrospermopsins (World Health Organization 2020b), microcystins (World Health Organization 2020c) and saxitoxins (World Health Organization 2020d). Toxin quotas for microcystin-producing *Microcystis* spp. Reported in New Zealand were collated during the 2018 review of the Recreational Cyanobacteria Guidelines (Wood et al. 2018a). Toxin quotas for anatoxin-, cylindrospermopsin- and saxitoxin-producing planktonic cyanobacteria reported in New Zealand and other countries were collated during the 2020 review of the Drinking-Water Guidelines for Cyanobacteria (Puddick et al. 2020). Toxin analyses of additional cyanobacterial strains was required to supplement the toxin quota data available from literature.

Revising the ALF for planktonic cyanobacteria in-line with the recommendations of the 2018 review of the Recreational Cyanobacteria Guidelines involved three stages:

1. determining toxin quotas for planktonic cyanobacteria observed in New Zealand and collating available toxin quota information from scientific literature

2. developing taxa-specific Action / Red mode thresholds for toxin-producing planktonic cyanobacteria observed in New Zealand
3. collecting end-user feedback on the revised ALF and incorporating suggestions into the final output.

This report summarises the work undertaken to develop the revised ALF for planktonic cyanobacteria in recreational freshwaters, collates feedback from end-users on the revised ALF and provides recommendations for future work in this area.

2. DETERMINATION AND COLLATION OF TOXIN QUOTAS

The 2018 review of the Recreational Cyanobacteria Guidelines (Wood et al. 2018a) identified that only four toxin-producing planktonic cyanobacteria have been confirmed in New Zealand: *Cuspidothrix issatschenkoi* (formerly *Aphanizomenon issatschenkoi*), *Microcystis* spp., *Nodularia spumigena* and *Raphidiopsis raciborskii* (formerly *Cylindrospermopsis raciborskii*). This presents an opportunity to develop taxa-specific cell concentration thresholds to trigger the Action / Red mode threshold in the ALF for planktonic cyanobacteria. These new thresholds would reduce unnecessary alert-level escalations in situations where the risk to recreational water users is low.

Microcystin-producing *Microcystis* spp. Have been observed in a range of New Zealand lakes (Wood et al. 2017b; Puddick et al. 2019), and nodularin-producing *N. spumigena* has been reported in brackish lakes / lagoons (Dolamore et al. 2017; Wood et al. 2017b). Anatoxin-producing *C. issatschenkoi* has been observed in a small number of lakes, primarily in the central North Island (Wood et al. 2007; Wood et al. 2009). While *R. raciborskii* is commonly found in lakes in the Waikato region, to our knowledge cylindrospermopsin has only been detected on one occasion in 2003 from Lake Waahi (Wood & Stirling 2003).

Although very low levels of saxitoxin have been detected in New Zealand freshwater bodies, this has not been confirmed using a reliable detection method (Kouzminov et al. 2007). Additionally, no planktonic saxitoxin-producing cyanobacteria have been isolated to-date and, therefore, collated saxitoxin quotas are not presented here. If required, more information on saxitoxin quotas in planktonic cyanobacteria can be found in Puddick et al. (2020).

Guanitoxins / anatoxin-a(S) (Fiore et al. 2020) was not included in the revised ALF for planktonic cyanobacteria in recreational freshwaters because guanitoxin-producing cyanobacteria have not been identified in New Zealand to-date.

As described in Section 1, the development of taxa-specific thresholds requires toxin quotas for the cyanobacteria of interest. A review of microcystin quotas from New Zealand *Microcystis* compared to microcystin quotas reported internationally indicated that there were regional differences and that *Microcystis* from New Zealand produced higher levels of microcystins compared to strains elsewhere in the world (Puddick et al. 2019). These observations demonstrate the importance of using local toxin quota data when possible. A review of reported cyanotoxin quotas was undertaken during revisions of New Zealand's drinking-water guidelines for cyanobacteria management and concluded that there was sufficient regional information for microcystin-producing *Microcystis*, but that international data would need to be drawn upon for anatoxin- and cylindrospermopsin-producing cyanobacteria (Puddick et al. 2020). The review also identified that there was a lack of nodularin quota information available, with only a single nodularin quota found in the literature (Wood et al. 2008). To fill this knowledge

gap, nine nodularin-producing *N. spumigena* strains were isolated from New Zealand lakes and their toxin quotas were determined. Cyanotoxin quotas were also determined for existing strains of *C. issatschenkoi* (two), *Microcystis* sp. (six) and *N. spumigena* (one) maintained in the Cawthron Institute Culture Collection of Microalgae (Rhodes et al. 2016).

2.1. Determination of toxin quota values

Cyanotoxin cell quotas were determined in actively growing cyanobacterial cultures as described in Appendix 1. Anatoxin quotas for the two *C. issatschenkoi* strains were similar to each other (0.03 and 0.06 pg/cell; Table 1), but were lower than several anatoxin quotas reported in the literature for this species (see Appendix 2). Microcystin quotas for *Microcystis* strains varied from 0.13 to 2.9 pg/cell (Table 1). These levels were consistent with those previously reported in the literature (see Appendix 3). Nodularin quotas for *N. spumigena* strains varied from 0.26 to 3.96 pg/cell (Table 1). To the best of our knowledge, only one nodularin quota has been reported in the literature (0.35 pg/cell from *N. spumigena* CAWBG-020 / CYN-43) (Wood et al. 2008). The nodularin quotas measured here bracket the previously reported value, but the new nodularin quotas were mostly higher (see Appendix 4). Because of the lack of reported nodularin quotas, the addition of ten more nodularin quotas from *N. spumigena* represents a significant increase in our knowledge base.

Table 1. Toxin quotas measured in New Zealand planktonic cyanobacteria.

Cyanobacteria Species	Strain ID[a]	Toxin Type	Toxin Conc. (ng/mL)[b]	Cell Conc. (cells/mL)[c]	Toxin Quota (pg/cell)[d]
Cuspidothrix issatschenkoi	CAWBG-002	ATXs	110	3,450,000	0.03
Cuspidothrix issatschenkoi	CAWBG-031	ATXs	51	830,000	0.06
Microcystis sp.	CAWBG-011	MCs	68	103,000	0.66
Microcystis sp.	CAWBG-563	MCs	100	234,000	0.44
Microcystis sp.	CAWBG-570	MCs	30	143,000	0.21
Microcystis sp.	CAWBG-617	MCs	1,100	1,380,000	0.78
Microcystis sp.	CAWBG-624	MCs	290	102,000	2.90
Microcystis sp.	CAWBG-706	MCs	52	404,000	0.13
Nodularia spumigena	CAWBG-021	NODs	44	167,000	0.26
Nodularia spumigena	CAWBG-703	NODs	110	219,000	0.50
Nodularia spumigena	CAWBG-704	NODs	100	55,700	1.86
Nodularia spumigena	CAWBG-709	NODs	16	3,910	3.96
Nodularia spumigena	CAWBG-710	NODs	21	8,690	2.43
Nodularia spumigena	CAWBG-711	NODs	39	25,800	1.50
Nodularia spumigena	CAWBG-712	NODs	12	4,300	2.80
Nodularia spumigena	CAWBG-713	NODs	28	9,890	2.79
Nodularia spumigena	CAWBG-714	NODs	20	23,300	0.85
Nodularia spumigena	CAWBG-715	NODs	12	7,680	1.50

ATXs = Anatoxins, MCs = Microcystins, NODs = Nodularins. [a] Strain identifier from the Cawthron Institute Culture Collection of Microalgae (https://cultures.cawthron.org.nz/). [b] Values are rounded to two significant figures. [c] Values are rounded to three significant figures. [d] Toxin quotas were calculated using non-rounded toxin- and cell-concentrations.

2.2. Collation of toxin quota values

As part of a review of the ALF for planktonic cyanobacteria in drinking-water reservoirs for the New Zealand Drinking-Water Guidelines (MoH 2020), literature was searched for published studies reporting toxin quotas for planktonic cyanobacteria. The results from this collation of toxin quota values (Puddick et al. 2020) were supplemented with several new toxin quotas identified in literature and the toxin quotas measured in New Zealand cyanobacteria strains (see Section 2.1). For new toxin quotas from the literature, the same principles described in (Puddick et al. 2020) were applied; only the maximum toxin quota value was included for culturing studies with multiple time-points and environmental studies that were conducted over a short period of time (e.g., multiple measurements of a single surface scum), but multiple toxin quota values were included for environmental studies that were conducted over a longer period of time (e.g., measurements made at fortnightly intervals). This approach was used because it provided a conservative measure of toxin quotas and

didn't bias the dataset with multiple measurements of the same cyanobacterial strain under similar conditions.

Not all of the toxin quota data identified during the New Zealand Drinking-Water Guidelines review were retained for the development of the ALF for planktonic cyanobacteria in recreational freshwaters. The international data identified for anatoxin- and cylindrospermopsin-producing cyanobacteria were limited to the cyanobacterial species observed in New Zealand (*C. issatschenkoi* for anatoxins and *R. raciborskii* for cylindrospermopsins). As described earlier in Section 2, Action / Red mode thresholds for saxitoxin-producing planktonic cyanobacteria were not developed, as planktonic saxitoxin-producing cyanobacteria have not been identified in New Zealand to date. Therefore, saxitoxin quotas were not evaluated here.

The resulting dataset for anatoxins was compiled mostly from New Zealand data with one measurement from a German *C. issatschenkoi* strain (Appendix 2). The dataset for cylindrospermopsins was based on studies from Australia and Saudi Arabia (Appendix 5) as these were the only countries to have reported cylindrospermopsin-producing *R. raciborskii*. The microcystin and nodularin toxin quota datasets (Appendices 3 and 4) were both compiled entirely from New Zealand studies. No international data for nodularin toxin quotas was identified. As described at the beginning of Section 2, the microcystin toxin quota database was sufficiently large that international data were not required; but we acknowledge that there is an abundance of international data available for microcystin-producing *Microcystis* spp. More information on the individual toxin quota values (e.g., country of origin, sample type and reference information) can be found in Appendices 2 to 5.

The minimum, maximum, median and mean toxin quotas observed for each toxin type were similar (i.e., within an order of magnitude; Table 2). The highest median toxin quota was observed in nodularin-producing cyanobacteria (Table 2); however, this is potentially due to the limited dataset available for this toxin type and strains were isolated from only two lakes (i.e., lower toxin quotas might be observed with wider investigation). For microcystin-producing cyanobacteria, there was a wide range of toxin production capacities observed, but the majority of the data were low (< 10% of the maximum value; Figure 1). However, there were multiple observations of microcystin quotas at the high end of the range, indicating that it is probable that cyanobacteria with high toxin quotas will be encountered in New Zealand lakes.

Table 2. Summary of toxin quota data from a literature review of studies on New Zealand and international planktonic cyanobacteria.

Toxin	n	Toxin Quota (pg/cell)				
		Min	Max	Median	Mean	95th Percentile
Anatoxins[a]	6	0.03	0.41*	0.10	0.18	- [e]
Cylindrospermopsins[b]	33	0.004	14.60	0.03	1.15*	6.72
Microcystins[c]	50	0.006	5.95	0.17	0.77*	3.68
Nodularins[d]	11	0.26	3.96	1.50	1.71*	- [e]

Min = Minimum, Max = Maximum.
[a] A mixture of New Zealand and international data were used.
[b] Because no New Zealand data were available, international data were used.
[c] Because sufficient data were available, only New Zealand studies were used.
[d] Because no international data were available, only New Zealand data was used.
[e] Unable to calculate a 95th percentile value due to insufficient data.
* These toxin quota values were used for formulating cell concentration thresholds in the alert levels framework.
N = number of datapoints in dataset.

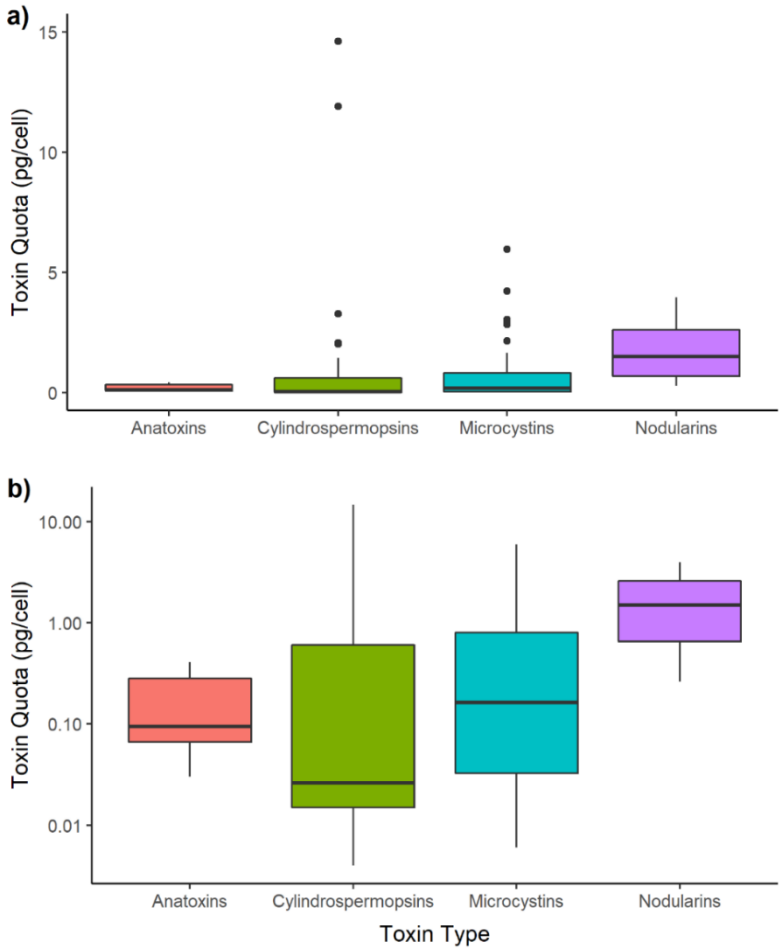

Figure 1. Box plots of toxin cell quotas for anatoxins, cylindrospermopsins, microcystins and nodularins from planktonic cyanobacteria that have been observed in New Zealand using; **a)** a natural scale, and **b)** using a $\log_{10}$ scale. Toxin quotas for microcystins and nodularins are based solely on New Zealand data, toxin quotas for cylindrospermopsins are based solely on international data and toxin quotas for anatoxins are a mixture of New Zealand and international data. Boxes indicate the upper and lower quartiles, the horizontal line in the centre of the box indicates the median, the whiskers stretch to the minimum and maximum values within 1.5-times the inter-quartile range and dots are values beyond this range.

Adverse health effects underpinning the WHO guideline values for cylindrospermopsin and microcystin exposure are based on toxicological studies that have allowed a clear 'no observed adverse effect level' (NOAEL) to be defined for the chronic effects observed from these toxins (Chorus & Welker 2021). Due to the similarity in chemical structure and mode of action, toxicity data for microcystin have been applied to nodularin. People exposed to these cyanotoxins through recreational activity will encounter cyanobacteria with a range of toxin production capacities during their lifetime and, therefore, their habitual exposure will be best represented by the mean toxin quota. For calculating cell concentration thresholds (see Section 3.1), the mean toxin quota was used for determining thresholds for cylindrospermopsin-, microcystin- and nodularin-producing cyanobacteria.

Adverse health effects due to anatoxin exposure are less well studied and there are insufficient toxicology data to define a NOAEL (World Health Organization 2020a). Anatoxins lead to acute effects (death by asphyxiation due to effects on the nervous system) rather than chronic effects through repeated low-level exposure (e.g., promotion of cytotoxicity and liver toxicity as is observed for cylindrospermopsins, microcystins and nodularins). As adverse effects from anatoxin exposure would occur as the result of a single exposure, the maximum toxin quota was used for determining thresholds for anatoxin-producing cyanobacteria. Maximum values are not ideal for guideline setting, as they potentially represent errors in sampling or analysis. For this reason, a high percentile of the distribution of values (90^{th}, 95^{th} or 97.5^{th}) is generally used. However, because it was not possible to determine a 95^{th} percentile toxin quota, due to the limited data available, the maximum value was used as a conservative approach.

Adverse effects from recreational exposure to cyanotoxins are tracked (in New Zealand) through the Hazardous Substances Disease and Injury Reporting Tool. This information is collated by Massey University and the Environmental Health Intelligence New Zealand system to identify trends in exposure to hazardous substances.

3. PROPOSED REVISIONS TO THE ALERT-LEVEL FRAMEWORK FOR PLANKTONIC CYANOBACTERIA

As described in Wood et al. (2018a), our current knowledge on toxin-producing cyanobacteria in New Zealand indicates that taxa-specific thresholds could be introduced to the ALF for planktonic cyanobacteria in the Recreational Cyanobacteria Guidelines. This approach would reduce escalations in alert-levels for situations where non-toxic cyanobacteria are dominant in a waterbody. This modification to the ALF would address feedback received from end-users during a workshop held as part of the 2018 recreational cyanobacteria guidelines review project (Wood et al. 2018b). Adapting the ALF would also allow for the recent updates in WHO cyanotoxin guidance (World Health Organization 2020d, 2020a, 2020b, 2020c) to be incorporated into New Zealand's cyanobacteria risk management framework.

3.1. Development of taxa-specific alert-level thresholds

The following principles were used when developing the revised ALF:

- That the WHO guideline values for cyanotoxins in recreational waters (World Health Organization 2020a, 2020b, 2020c) would be used to develop taxa-specific cell concentration thresholds for the ALF.
- The WHO guidelines do not include a guideline value for nodularins in recreational waters. Due to similarities in structure, mode of action and toxicity; the New Zealand recreational guideline value adopted for nodularins would match the WHO recreational guideline value for microcystins (World Health Organization 2020c).
- To determine taxa-specific cell concentration thresholds; the mean toxin quota would be used for cylindrospermopsins, microcystins and nodularins and the maximum toxin quota would be used for anatoxins (see Section 2.2).
- The cell concentration threshold in the Surveillance / Green mode would equate to a toxin concentration ≤ 10% of the WHO recreational guideline value for each toxin, and a consistent total cyanobacterial cell concentration would be adopted.
- The biovolume option for the Surveillance / Green mode threshold would be retained. This is for lakes where picocyanobacteria are present in high cell concentrations, but in low biomass because of their small cell size.
- The Action / Red mode thresholds would be set to 100% of the WHO recreational guideline value (for that cyanotoxin).
- The total cyanobacterial biovolume Action / Red mode threshold would be retained (at the existing value of 10 mm^3/L) as it protects human health from the risks associated with irritant compounds produced by cyanobacteria (Pilotto et al. 2004; Stewart et al. 2006; Chorus & Welker 2021).

- Toxin concentration thresholds would also be included in the Action level / Red mode threshold and would be the WHO recreational guideline values.

Using these principles, the cyanobacterial cell concentrations that equated to 10% and 100% of the WHO recreational cyanotoxin guideline values were calculated (Table 3). The cyanobacterial cell concentrations at 10% of the cyanotoxin guideline values equated to between 520 and 14,600 cells/mL (dependent on toxin type). For ease of use, one cell concentration threshold for total cyanobacteria was the most desirable option for the Surveillance / Green mode threshold (and this would also be consistent with the current ALF). The currently-adopted total cyanobacterial cell concentration threshold of 500 cells/mL used for the Surveillance / Green mode threshold was appropriate for anatoxin-, cylindrospermopsin-, microcystin- and nodularin-producing planktonic cyanobacteria as cell concentrations at 10% of the cyanotoxin guideline values were all > 500 cells/mL (Table 3).

The cyanobacterial cell concentrations at 100% of the recreational guideline values (Table 3) varied by over an order of magnitude with a threshold of 5,200 cells/mL for cylindrospermopsin-producing cyanobacteria and 146,300 cells/mL for anatoxin-producing cyanobacteria. Due to the low cell concentration thresholds for some toxin types, but not for others, taxa-specific thresholds were adopted for the Action / Red mode to avoid unnecessary escalations through the ALF when less potent cyanobacteria were present. Because of the low sample numbers in the toxin quota datasets for anatoxin- and nodularin-producing planktonic cyanobacteria (Table 2) heavier rounding of the cell concentrations was undertaken for the adopted thresholds in order to provide an additional level of protection.

Table 3. Calculation of cyanobacterial cell concentration thresholds for each toxin type using the mean or maximum toxin quota values.

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

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

The adopted taxa-specific Action / Red mode thresholds were:

- 100,000 cells/mL for *C. issatschenkoi* (anatoxins),
- 5,000 cells/mL for *R. raciborskii* (cylindrospermopsins),
- 30,000 cells/mL for *Microcystis* spp. (microcystins),
- 10,000 cells/mL for *N. spumigena* (nodularins).

3.2. Structuring of the alert-level framework for planktonic cyanobacteria

The Surveillance / Green mode and Action / Red mode thresholds developed in Section 3.1 were applied to the existing three-tier ALF for planktonic cyanobacteria (Table 4). The Situation 1 'triggers' (the use of cell concentrations) were adapted to incorporate the taxa-specific thresholds developed for confirmed toxin-producing cyanobacteria observed in New Zealand. The Situation 2 'triggers' (the use of biovolumes) were retained because of the irritant effects on eyes, skin and respiratory tract caused by high levels of any type of cyanobacteria (not just toxin-producing cyanobacteria) (Pilotto et al. 2004; Stewart et al. 2006; Chorus & Welker 2021). The biovolume thresholds for the Surveillance / Green mode (0.5 mm^3/L) and Action / Red mode (10 mm^3/L) were not modified from the existing ALF for planktonic cyanobacteria.

Situation 3 from the existing alert levels framework; 'cyanobacterial scums consistently present', was removed because councils commonly use microscopy measurements to make decisions rather than visual observations, and the 10 mm^3/L biovolume threshold (Action / Red mode – Situation 2) is below the cyanobacteria levels that would be observed in a cyanobacterial scum.

Table 4. Proposed revised alert-level framework for planktonic cyanobacteria in recreational freshwaters.

Decision Chart 1: Alert-level framework for planktonic cyanobacteria	
Alert Level	**Action** (See Section X for the recommended framework for roles and responsibilities relating to actions, and the text box at the beginning of Section X for advice on interpreting the guidance in this table.)
Surveillance (green mode) **Situation 1:** The cell concentration of total cyanobacteria is **< 500 cells/mL** or **Situation 2:** The biovolume equivalent for the combined total of all cyanobacteria **< 0.5 mm^3/L**[a]	• Undertake weekly or fortnightly visual inspections[b] and sampling of water bodies where cyanobacteria are known to proliferate between spring and autumn.
Alert (amber mode) **Situation 1:** The cell concentration of total cyanobacteria is **> 500 cells/mL** and the cell concentrations for toxin-producing cyanobacteria (observed in NZ) are;[c] *Cuspidothrix issatschenkoi* **< 100,000 cells/mL** *Raphidiopsis raciborskii* **< 5,000 cells/mL** *Microcystis* spp. **< 30,000 cells/mL** *Nodularia spumigena* **< 10,000 cells/mL**, or **Situation 2:** **0.5 to < 10 mm^3/L** total biovolume of all cyanobacteria[d]	• Increase sampling frequency to at least weekly.[e] • Notify the public health unit. • Multiple sites should be inspected and sampled.
Action (red mode) **Situation 1:** Cell concentration thresholds for toxin-producing cyanobacteria (observed in NZ);[c] *Cuspidothrix issatschenkoi* **≥ 100,000 cells/mL** *Raphidiopsis raciborskii* **≥ 5,000 cells/mL** *Microcystis* spp. **≥ 30,000 cells/mL** *Nodularia spumigena* **≥ 10,000 cells/mL**, or **Situation 2:** **≥ 10 mm^3/L** total biovolume of all cyanobacteria,[d] or **Situation 3:** Cyanotoxin concentration thresholds;[f] Anatoxins **≥ 60 µg/L** Cylindrospermopsins **≥ 6 µg/L** Microcystins / Nodularins **≥ 24 µg/L** Saxitoxins **≥ 30 µg/L**	• Continue monitoring as for alert (amber mode).[e] • If potentially toxic taxa are present (see Table X and Table AX.X), then consider testing samples for cyanotoxins.[f] • Notify the public of a potential risk to health.
a) Biovolumes are useful when high concentrations of picocyanobacteria are present in a waterbody (described in more detail in Section X) and Situation 2 applies when 'non-toxigenic' cyanobacteria taxa are abundant in samples.	

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

c) Cell concentration thresholds for planktonic toxin-producing cyanobacteria observed in New Zealand were developed using toxin quotas and the 2020 World Health Organisation guideline values for cyanotoxins in recreational waters (anatoxins, cylindrospermopsins and microcystins). When multiple toxin-producing cyanobacteria are present in a waterbody at the same time, the cumulative effects should be accounted for using the ratio of each cell concentration to the relevant 'Action' level thresholds and summing the ratios. Should this sum exceed 1, then the 'Action' level is triggered. Example calculations are provided in Section X.

d) Situation 2 applies where high cell concentrations of 'non-toxigenic' cyanobacteria taxa are present and the 10 mm^3/L threshold is to protects human health from the risks associated with irritants produced by cyanobacteria.

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

f) Cyanotoxin testing is useful to provide further confidence on potential health risks when a health alert is being considered and to show that residual cyanotoxins are not present when a toxic cyanobacteria bloom subsides. Toxin concentration thresholds are based on the 2020 World Health Organisation guideline values for cyanotoxins in recreational waters (anatoxins, cylindrospermopsins, microcystins and saxitoxins).

Cyanotoxin concentrations (based on the WHO recreational guideline values) were also included as a 'trigger' for the Action / Red mode. These would likely be used to deescalate the alert level to Alert / Amber mode in circumstances where Situation 1 has been used to trigger Action / Red mode and toxin testing has been undertaken to evaluate the inherent risk. Its inclusion will also provide a mechanism for water managers to respond to the human health risks posed by new toxin-producing cyanobacteria that could be identified in New Zealand in the future (but are not currently included in the Situation 1 taxa-specific thresholds). To account for the possibility that saxitoxin-producing planktonic cyanobacteria might be observed in New Zealand in the future (e.g., species from other countries might be introduced), saxitoxins were included in the cyanotoxin concentration thresholds (Situation 3 in the revised ALF).

The names for the alert levels in the ALFs from the Recreational Cyanobacteria Guidelines (both planktonic and benthic cyanobacteria) currently differ from those adopted in the WHO recreational guidelines for cyanobacteria (World Health Organization 2021) and the cyanobacteria section of the New Zealand Drinking-Water Guidelines (MoH 2020). In the New Zealand Recreational Cyanobacteria Guidelines, the different alert-levels are named 'Surveillance / Green mode', 'Alert / Amber mode' and 'Action / Red mode'. In the WHO recreational cyanobacteria guidelines and the New Zealand drinking-water guidelines the alert levels are named 'Vigilance', 'Alert Level 1' and 'Alert Level 2'. Adopting the same naming convention in the New Zealand Recreational Cyanobacteria would provide better continuity. However, the authors of this report felt that this decision should be made by the end-users and was included as a question during the end-user survey (see Section 4).

To account for the presence of multiple cyanotoxins (e.g., microcystins and anatoxins) or co-occurring toxin-producing taxa (e.g., *Microcystis* spp. And *C. issatschenkoi*) in a lake, the use of a calculation to account for cumulative effects was added to the ALF (under 'note c'). As end-users of the guidelines may not be familiar with these types of calculations, several hypothetical examples were included in Section 3.3 of the Recreational Cyanobacteria Guidelines (and also in Appendix 6 of this report).

Other sections of the Recreational Cyanobacteria Guidelines that were updated included:

- The 'Change from current practice' section (Section 2.3.2 in the recreational cyanobacteria guidelines) was updated to describe the revised ALF for planktonic cyanobacteria.
- The 'Details of the framework: planktonic cyanobacteria' section (Section 3.3 in the recreational cyanobacteria guidelines) was adapted to describe how the new alert-level thresholds should be applied and provide additional guidance on dealing with cyanobacteria that have been observed to produce toxins overseas (where confirmed producers have not been observed in New Zealand).
- The 'Derivation of guideline values' appendix (Appendix 5 in the recreational cyanobacteria guidelines) was updated with the calculations for the revised Action / Red mode thresholds (these are also provided in Appendix 7 of this report).

4. END-USER REVIEW OF PROPOSED REVISIONS TO THE ALERT LEVEL FRAMEWORK

An information packet and online survey were prepared and distributed to regional councils and public health officers. The information packet included links to the Interim Cyanobacteria Guidelines (MfE and MoH 2009), the report on the 2018 guidelines review (Wood et al. 2018a), and a revised version of the planktonic cyanobacteria section of the guidelines (Section 3 Part A of the Recreational Cyanobacteria Guidelines; including the revised ALF, see Section 3 above). The online survey comprised seven questions. The first four questions gauged general feelings on the revisions made to the ALF for planktonic cyanobacteria and the accompanying guidance, and allowed end-users to supply comments. Two questions gathered feedback on specific queries relating to the guidelines (ease of use and naming of the thresholds). The final question inquired about any additional suggestions to improve the ALF for planktonic cyanobacteria.

4.1. Survey Questions

Q1. The revised alert levels framework for planktonic cyanobacteria is an improvement from that currently included in the Interim Guidelines.

Five options from 'Strongly Agree' to 'Strongly Disagree' (with an opportunity to comment).

Q2. The revised alert levels framework for planktonic cyanobacteria will likely lead to less unnecessary alert level escalations in my region.

Five options from 'Strongly Agree' to 'Strongly Disagree' (with an opportunity to comment).

Q3. The additional guidance on using the revised alert levels framework for planktonic cyanobacteria was informative.

Five options from 'Strongly Agree' to 'Strongly Disagree' (with an opportunity to comment).

Q4. The 'actions' in the revised alert levels framework for planktonic cyanobacteria are reasonable and useful.

Five options from 'Strongly Agree' to 'Strongly Disagree' (with an opportunity to comment).

Q5. Taking into account the alert levels framework (Decision Chart 1) and the additional guidance provided around the framework (the associated text), do you feel that you could navigate more complex situations that might arise (e.g., multiple toxin-producing cyanobacteria present, the presence of potential toxin-producing cyanobacteria)?

Yes/No (with an opportunity to comment).

Q6. Do you support renaming the alert levels in the NZ recreational cyanobacteria guidelines to align with the WHO guidelines (i.e., Vigilance Level, Alert Level 1, Alert Level 2)?

Yes/No (no opportunity to comment).

Q7. Do you have any additional suggestions for improving the alert levels framework for planktonic cyanobacteria in the NZ recreational cyanobacteria guidelines?

Open query for participants to supply comments.

The survey was sent to 15 regional councils where cyanobacteria monitoring in lakes is undertaken and responses were received from seven. Because of the COVID-19 public health orders in place at the time the end-user consultation was undertaken (December 2021 to February 2022), we were unable to send the survey directly to public health officers. Instead, the Ministry of Health sent the survey to public health unit managers to pass on to the appropriate staff (if the resourcing capacity was available in their region). One response was received from a public health officer, but via email rather than through the online survey.

Survey responses for the multiple-choice questions to gauge general feelings on the revised ALF (Q1–Q4) were coded using a scale of 0-4:

Strongly Disagree	=	0
Disagree	=	1
Neutral	=	2
Agree	=	3
Strongly Agree	=	4
No Comment	=	Not included in the analysis.

The coded responses are plotted in Figure 2 and summary statistics are provided in Appendix 8. There was general agreement that the revised ALF for planktonic cyanobacteria was an improvement from that currently included in the Interim Guidelines (Q1; Median response = ‘Agree’ or 3 using the number code). There was a wider range of responses on whether the revised ALF for planktonic cyanobacteria would likely lead to fewer unnecessary alert level escalations (Q2; Minimum response = ‘Neutral’ or 2 using the number code, Maximum response = ‘Strongly Agree’ or 4 using the number code). The median response to Question 2 was ‘Neutral’ (2 using the number code). The comments provided suggest that the range of responses received for Question 2 is due to the different species of cyanobacteria observed in different regions or the levels of cyanobacteria commonly observed (see Appendix 8 for verbatim comments). There was general agreement from end-users that the

additional guidance on using the revised alert level framework for planktonic cyanobacteria was informative. (Q3; Median response = 'Agree' or 3 using the number code). There was a wide range of responses on whether the 'actions' in the revised ALF for planktonic cyanobacteria were reasonable and useful (Q4; Minimum response = 'Disagree' or 1 using the number code, Maximum response = 'Strongly Agree' or 4 using the number code). The median response to Question 4 was 'Agree' (3 using the number code). The comments provided for Question 4 provide some insight into this and are explored further below (see Appendix 8 for verbatim comments). In general, the end-users that responded to the survey were supportive of the changes made to the ALF for planktonic cyanobacteria in recreational freshwaters.

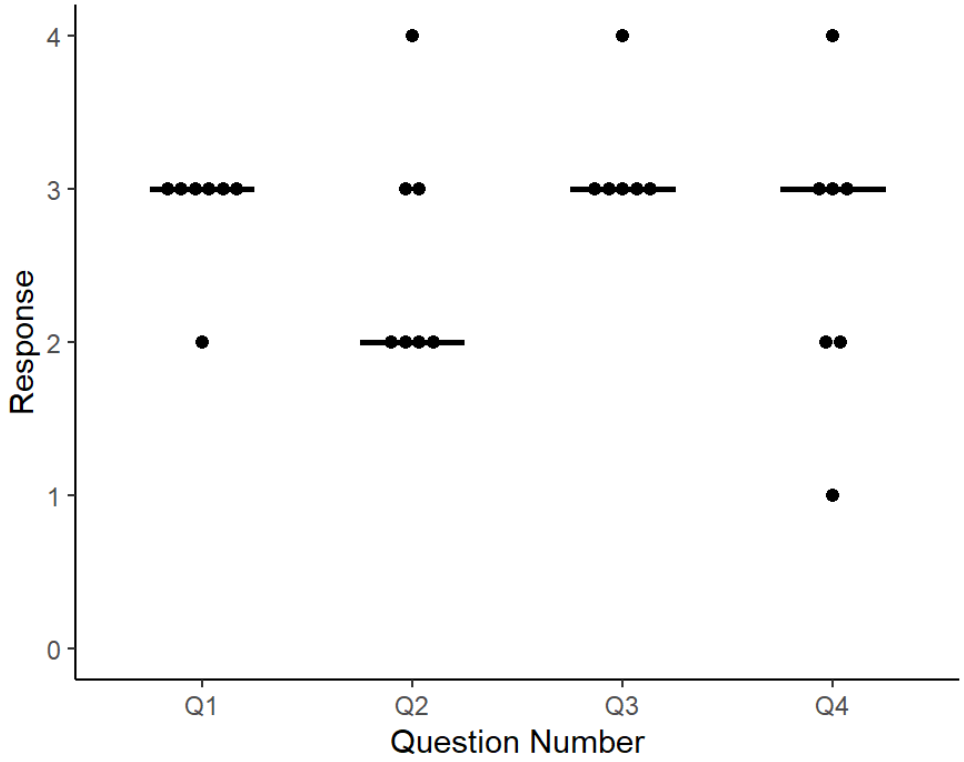

Figure 2. Dot plot of survey response for Questions 1-4 gauging general feelings on the revisions made to the alert-level framework for planktonic cyanobacteria and the accompanying guidance. Responses were assigned a numerical code ranging from 0 = Strongly Disagree to 4 = Strongly Agree. Each dot represents an individual response and the horizontal line indicates the median. Question text can be found above and in Appendix 8.

Question 5 aimed to understand whether the guidance associated with the ALF for planktonic cyanobacteria was comprehensive enough for end-users to apply it. All survey participants responded 'Yes' to Question 5. Comments provided on this question indicated that result summaries from testing providers aligned with the revised thresholds would further improve to usability of the revised ALF (i.e., highlighting the cell concentration for each toxin-producing genera present in a

sample; *C. issatschenkoi*, *R. raciborskii*, *Microcystis* spp. And *N. spumigena*). Another comment indicated that guidance on testing for toxin production genes was not prominent enough. See Appendix 8 for the verbatim comments.

Question 6 aimed to understand whether end-users supported renaming the alert levels in the New Zealand recreational cyanobacteria guidelines to align with the WHO guidelines. Five survey participants responded 'No' to this question and two participants responded 'Yes'. A comment on this matter was also received:

> We think that Green amber red or similar is more user friendly for a public conversation. We have spent a long time teaching the public about the traffic light system. The alert level system comes with new learning, bedding in time and confusion with the complexity of its use for other purposes such as COVID-19.

As the majority of the survey participants did not support the change in naming, we recommend that it remains as is.

From the comments provided through Questions 1–4 and in Question 7 (an open opportunity to provide suggestions) several consistent threads were observed:

- There needs to be a clearer distinction between 'potential toxin-producing cyanobacteria' (which is currently used in the planktonic cyanobacteria ALF and encompasses all cyanobacteria species that have been reported to produce toxins anywhere in the world; this includes *Dolichospermum* spp.) and 'confirmed toxin-producing cyanobacteria from New Zealand' (i.e., the taxa-specific thresholds in the revised ALF).
- There needs to be clearer guidance on how to respond to 'potential toxin-producing cyanobacteria' and the toxin testing approach that should be undertaken.
- Sampling multiple sites and dealing with spatial variability are difficult for councils to manage.
- The guidance based on up-to-date science was informative and appreciated.
- Improvements could be made to the wider guidelines, the overall approach taken (mostly due to time delays between taking samples and receiving results), and the integration of real-time technologies into the guidelines. While much of this sits outside the scope of this project focussed on developing taxa-specific thresholds for the planktonic cyanobacteria ALF, there is an opportunity to provide better guidance on how end-users might adapt the risk management framework to incorporate such technologies.

One additional comment asked how:

> this would correspond to NPS-FM (2020) grading since the previous guideline tables are in Table 10 of Appendix 2A, and there has been

> some interpretation around the different languages used in grades A & B (biovolume equivalent for combined total of all cyanobacteria) and C & D (biovolume equivalent of potentially toxic cyanobacteria OR total biovolume of all cyanobacteria).

While we share the concerns of this survey participant that the wording and thresholds used in cyanobacterial NOF (National Objectives Framework) in the NPS-FM (National Policy Statement for Freshwater Management) likely need revising, this is not within the scope of the current project.

Feedback from one public health officer was received via email (rather than through the online survey; see Appendix 8 for verbatim feedback). They felt that guidance on when to undertake toxicity testing needed to be clearer and better integrated into the framework. This was in agreement with comments received via the online survey (see above). We also suggest that they are referring to toxin testing rather than toxicity testing (the effect on an organism; e.g., the mouse bioassay), which is generally not undertaken routinely in New Zealand.

5. UPDATES MADE TO ADDRESS END-USER FEEDBACK

To address the comments described in Section 4, modifications were made to the guidelines text and the ALF for planktonic cyanobacteria.

To improve clarity on the distinction between 'potential toxin-producing cyanobacteria' and 'confirmed toxin-producing cyanobacteria from New Zealand', alterations were made to the wording of sections 3.2 and 3.3.2. More guidance on how to respond to 'potential toxin-producing cyanobacteria' and toxin testing was provided in section 3.3.2 of the guidelines:

> Toxin testing might include testing for the genes involved in toxin production (i.e., cyanotoxin production genes) or for the toxins themselves (e.g., by liquid chromatography-mass spectrometry). When a potentially-toxic cyanobacteria species (i.e., those listed in Appendix 4 – Table A4.1) is present in a waterbody at biovolumes > 0.5 mm^3/L, we recommend undertaking toxin testing as this will help to improve our understanding on toxin-producing cyanobacteria in New Zealand.

It was also specified in the ALF that toxin testing should be considered in Alert / Amber Mode when potentially toxic taxa are present. Testing for toxin production genes was also specified in the ALF (it previously only suggested testing for cyanotoxins).

Several survey participants noted that it was difficult for them to undertake sampling at multiple sites (as suggested in the actions for Alert / Amber Mode). The wording in the ALF was changed to read: 'If possible, multiple sites should be inspected and sampled'. And, some additional guidance was supplied in section 3.3.2 of the guidelines: 'Inspecting multiple sites around the waterbody allows for better understanding on spatial variability. If resourcing is restricted, then monitoring should focus on the areas of greatest risk – places where people commonly access the water and at the downwind end of the lake (on that day).'

To address concerns that real-time technologies were not incorporated into the guidelines, text was added to section 1.4 of the guidelines to further clarify that the risk management approach presented could be modified:

> The management approach described here has been developed to be widely applicable around New Zealand, but that should not limit regional authorities from incorporating new technologies into their management strategies (e.g., drones, phycocyanin fluorometers). When modifications are made, consideration should be given to whether public health is still protected under the revised risk management system. Advice may need to be sought on this.

At the beginning of section 3 of the guidelines there is also a note on ‘interpreting the guidelines framework’ that provides information on how the approach presented in the Recreational Cyanobacteria Guidelines can be modified.

The revised version of the ALF for planktonic cyanobacteria in recreational freshwaters was modified to incorporate the end-user feedback received (Table 5). The actions for Alert / Amber mode were modified as described above. Action / Red mode – Situation 3 (cyanobacterial scums consistently present) was reinstated in the ALF for planktonic cyanobacteria to partially address feedback highlighting problems associated with delays in receiving cell enumeration results to make decisions. This will allow water managers to quickly enact measures to protect human health prior to cyanobacteria enumeration results being returned. Because cyanobacterial scums will likely contain > 10 mm^3/L total cyanobacterial biovolume, this is unlikely to lead to unnecessary escalations in alert level. Other small improvements identified by the authors of this report were also made.

Table 5. Final version of the revised alert-level framework for planktonic cyanobacteria in recreational freshwaters following end-user feedback (additions are noted using yellow highlighting and deletions are noted using strikethrough).

Decision Chart 1: Alert-level framework for planktonic cyanobacteria (See Section X for the recommended framework for roles and responsibilities relating to actions, and the text box at the beginning of Section X for advice on interpreting the guidance in this table).	
Alert Level	**Action** ~~(See Section X for the recommended framework for roles and responsibilities relating to actions, and the text box at the beginning of Section X for advice on interpreting the guidance in this table.)~~
Surveillance (green mode) **Situation 1:** The cell concentration of total cyanobacteria is **< 500 cells/mL** or **Situation 2:** The biovolume equivalent for the combined total of all cyanobacteria **< 0.5 mm^3/L**[a]	• Undertake weekly or fortnightly visual inspections[b] and sampling of water bodies where cyanobacteria are known to proliferate between spring and autumn.
Alert (amber mode) **Situation 1:** The cell concentration of total cyanobacteria is **> 500 cells/mL and** the cell concentrations for toxin-producing cyanobacteria (observed in New Zealand) are; *Cuspidothrix issatschenkoi* **< 100,000 cells/mL** *Raphidiopsis raciborskii* **< 5,000 cells/mL** *Microcystis* spp. **< 30,000 cells/mL** *Nodularia spumigena* **< 10,000 cells/mL**, or **Situation 2: 0.5 to < 10 mm^3/L** total biovolume of all cyanobacteria[d]	• Increase sampling frequency to at least weekly.[e] • Notify the public health unit. • If possible, multiple sites should be inspected and sampled. • If potentially toxic taxa are present (see Table A4.1), then consider testing samples for cyanotoxins or toxin production genes.[f]
Action (red mode) **Situation 1:** Cell concentration thresholds for toxin-producing cyanobacteria (observed in New Zealand);[c] *Cuspidothrix issatschenkoi* **≥ 100,000 cells/mL** *Raphidiopsis raciborskii* **≥ 5,000 cells/mL** *Microcystis* spp. **≥ 30,000 cells/mL** *Nodularia spumigena* **≥ 10,000 cells/mL**, or **Situation 2: ≥ 10 mm^3/L** total biovolume of all cyanobacteria,[d] or **Situation 3:** Cyanobacterial scums consistently present,[g] or **Situation 4:** Cyanotoxin concentration thresholds;[f] Anatoxins **≥ 60 µg/L** Cylindrospermopsins **≥ 6 µg/L** Microcystins / Nodularins **≥ 24 µg/L** Saxitoxins **≥ 30 µg/L**	• Continue monitoring as for alert (amber mode).[e] • If potentially toxic taxa are present (see Table A4.1), then consider testing samples for cyanotoxins or toxin production genes.[f] • Notify the public of a potential risk to health.

a) Biovolumes are useful when high concentrations of picocyanobacteria are present in a waterbody (described in more detail in Section X) ~~and Situation 2 applies when 'non-toxigenic' cyanobacteria taxa are abundant in samples~~.

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

c) Cell concentration thresholds for planktonic toxin-producing cyanobacteria observed in New Zealand were developed using toxin quotas and the 2020 World Health Organisation guideline values for cyanotoxins in recreational waters (anatoxins, cylindrospermopsins and microcystins). When multiple toxin-producing cyanobacteria are present in a waterbody at the same time, the cumulative effects should be accounted for using the ratio of each cell concentration to the relevant 'Action' level thresholds and summing the ratios. Should this sum exceed 1, then the 'Action' level is triggered. Example calculations are provided in Section X.

d) Situation 2 applies where high cell concentrations of 'non-toxigenic' cyanobacteria taxa are present and the 10 mm^3/L threshold is to protects human health from the risks associated with irritants produced by cyanobacteria.

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

f) Cyanotoxin testing is useful to provide further confidence on potential health risks when a health alert is being considered and to show that residual cyanotoxins are not present when a toxic cyanobacteria bloom subsides. Toxin concentration thresholds are based on the 2020 World Health Organisation guideline values for cyanotoxins in recreational waters (anatoxins, cylindrospermopsins, microcystins and saxitoxins).

g) As scums will likely contain cyanobacterial biovolumes > 10 mm^3/L, Situation 3 allows for quick enactment of measures to protect human health (see Section X.X).

6. CONCLUDING REMARKS

Whilst the planktonic cyanobacteria ALF was a key revision identified during the 2018 review of the Recreational Cyanobacteria Guidelines (Wood et al. 2018a), other end-user recommendations from the 2018 review included developing additional and / or more comprehensive guidance on benthic cyanobacteria in lakes, education and communication strategies, picocyanobacteria identification and enumeration, cyanobacterial biovolume conversions, and the integration of emerging technologies into the risk management framework. End-user feedback from the 2018 review also included the desire for improved functionality of the guidelines and the ability to update the guidelines more regularly. Several of these recommendations were reiterated in the end-user feedback received for the revised planktonic cyanobacteria ALF; e.g., that emerging technologies need to be better integrated into the guidelines and that up-to-date knowledge on cyanobacteria management is greatly appreciated.

Prior to releasing the revised cyanobacteria guidelines, testing laboratories providing cyanobacteria enumeration results should be engaged about the changes to the ALF for planktonic cyanobacteria. This would allow the testing providers to adapt their result reporting systems and to potentially develop user-friendly summary reports that would assist water managers to interpret data more easily (e.g., highlighting results for the confirmed toxin-producing cyanobacteria present in a sample and summing cell concentration results for *Microcystis* spp.). Other strategies to improve the usability of the revised ALF for planktonic cyanobacteria could include the development of a web-based calculator to evaluate the combined risk from multiple toxin-producing cyanobacteria and multiple cyanotoxins in a waterbody.

Because of the toxin quota data deficits described in Section 2.2, continuing to build the toxin quota dataset for New Zealand toxin-producing cyanobacteria would improve the science supporting the ALF for planktonic cyanobacteria in recreational freshwaters (as well as the ALF for planktonic cyanobacteria in drinking-water supplies). This would include isolating new cyanobacteria strains and evaluating their toxin production capacity (i.e., measuring toxin quotas) for integration in the framework. Several strains including *R. raciborskii*, *C. issatschenkoi* and *N. spumigena* have been identified as important in the New Zealand context.

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9. APPENDICES

Appendix 1. Methodology followed for determining cyanotoxin quotas in cyanobacteria cultures (Section 2.1).

Culture Sampling

Cultures in the growth phase were sampled. Duplicate subsamples were collected for cell enumeration (5 mL in a Falcon tube and preserved with Lugol's iodine) and toxin concentration (5 mL concentrated on a GF/C filter and frozen until extraction).

Cell Enumeration

Cell enumeration was undertaken on an Olympus CKX41 inverted microscope. An aliquot of each cell count sample (0.5-3 mL) was settled in 12-well plates with milli-Q water. The volume to be settled and the dilution factor were adjusted in order to have between 20 and 60 cells per field of view (FOV) for the *Microcystis* spp. and *Nodularia spumigena*, and between 5 and 20 filaments per FOV for the *Cuspidothrix issatschenkoi*. Samples of *Microcystis* spp. and *N. spumigena* were assessed at either 400× or 800× magnification where cell counts within a FOV were conducted on ten random FOVs. Samples of *C. issatschenkoi* were assessed at 400× magnification where the total filament length within a FOV were measured on ten random FOVs. The average total filament length was then converted into a cell concentration using the average cell length for *C. issatschenkoi* (determined by measuring the length of 50 *C. issatschenkoi* cells at 1,000× magnification under oil immersion).

Anatoxins Toxin Analysis

Samples of *C. issatschenkoi* were analysed for anatoxins. The toxin samples were extracted in 0.1% formic acid (v/v; 1 mL) using freeze-thaw cycles interspersed with sonication (30 min, 53 kHz, 100% power; in a bath sonicator; three freeze-thaw-sonicate cycles in total). Extracts were clarified by centrifugation (12,000 × g; 5 min, 18 °C) and the supernatant (0.8 mL) was transferred into a glass autosampler vial and stored at −20 °C until analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Sample components (5 µL) were separated on a Thermo Hypersil Gold-Aq column (1.9-µm; 50×2.1 mm) at 40 °C using a gradient of Milli-Q water to acetonitrile (both containing 0.1% formic acid; v/v) at a flow rate of 0.6 mL/min. Quantitation was performed in positive ion mode using multiple reaction monitoring channels for anatoxin-a (ATX), homoanatoxin-a (HTX), dihydroanatoxin-a (dhATX) and dihydrohomoanatoxin-a (dhHTX). A five-point mixed external calibration curve in 0.1% formic acid (v/v; 0.2–10 ng/mL) was prepared from a certified reference material for ATX (National Research Council, Canada) and an in-house standard for dhATX (calibrated using quantitative nuclear magnetic resonance spectroscopy). The ATX standard was used to quantify ATX and HTX, and the dhATX standard was used to quantify dhATX and dhHTX. A response factor of 1 was used for each set of compounds.

Microcystin and Nodularin Toxin Analysis

Samples of *Microcystis* spp. and *N. spumigena* were analysed for microcystins (MCs) and nodularins (NODs), respectively. The toxin samples were extracted in 80% methanol + 0.1% formic acid (v/v; 1 mL) using sonication (60 min, 53 kHz, 100% power; in a bath sonicator cooled with ice). Extracts were clarified by centrifugation (12,000 × g; 5 min, 18 °C) and the supernatant (0.8 mL) was transferred into a glass autosampler vial and stored at −20 °C until analysis by LC-MS/MS.

Sample components (5 µL) were separated on a Waters Acquity BEH-C_{18} column (1.7-µm; 50×2.1 mm) at 40 °C using a gradient of 10% acetonitrile to 90% acetonitrile (v/v; both containing 100 mM formic acid and 4 mM ammonia) at a flow-rate of 0.4 mL/min. Quantitation was performed in positive ion mode using multiple-reaction monitoring channels for NOD-R, desmethyl-NOD-R (dmNOD-R), MC-RR, dm-MC-RR, didm-MC-RR, MC-YR, MC-LR, dm-MC-LR, didm-MC-LR, MC-AR, MC-FR, MC-WR, MC-RA, MC-RAba, MC-LA, MC-FA, MC-WA, MC-LAba, MC-FAba, MC-WAba, MC-LY, MC-LW and MC-LF. A five-point mixed external calibration curve in 80% methanol + 0.1% formic acid (v/v; 0.5–100 ng/mL) was prepared using standards for NOD-R, MC-RR, MC-YR and MC-LR (either certified reference materials from National Research Council or calibrated in-house by spectrophotometry). The NOD-R standard was used to quantify NOD-R and dmNOD-R; the MC-RR standard was used to quantify MC-RR, dmMC-RR and didm MC-RR; the MC-YR standard was used to quantify MC-YR; and the MC-LR standard was used to quantify the remaining microcystin congeners. A response factor of 1 was used for each set of compounds. When concentrations were outside of the calibration curve, samples were diluted in 80% methanol + 0.1% formic acid (v/v) and re analysed.

Determining Toxin Quotas

The average of the duplicate cell concentration and duplicate toxin concentration for each cyanobacterial strain was used to determine the toxin quota. Toxin quotas (in pg/cell) were calculated by dividing the cellular toxin concentration (in ng/mL) by the cell concentration (in cells/mL) and multiplying by 1,000 (to convert ng/cell into pg/cell).

Appendix 2. Anatoxin toxin quota values from *Cuspidothrix issatschenkoi* (previously called *Aphanizomenon issatschenkoi*).

Country	Source	Toxin Quota (pg/cell)	Sample Information	Reference
Germany	Culture	0.10	Strain SP33 (Lake Stolpsee)	Ballot et al. (2010)
New Zealand	Culture	0.40	CAWBG-002 (Lake Hakanoa)	Wood et al. (2007)
New Zealand	Culture	0.09	CAWBG-002 (Lake Hakanoa)	Selwood et al. (2006)
New Zealand	Culture	0.41	CAWBG-002 (Lake Hakanoa)	Wood et al. (2008)
New Zealand	Culture	0.03	CAWBG-002 (Lake Hakanoa)	This Report
New Zealand	Culture	0.06	CAWBG-031 (Lake Hakanoa)	This Report

Appendix 3. Microcystin toxin quota values from *Microcystis* spp.

Country	Source	Toxin Quota/s (pg/cell)	Sample Information	Reference
New Zealand	Culture	0.270	CAWBG-013 (Lake Horowhenua)	MfE and MoH (2009)
New Zealand	Culture	0.210	CAWBG-014 (Lake Horowhenua)	MfE and MoH (2009)
New Zealand	Culture	0.810	CAWBG-015 (Lake Horowhenua)	MfE and MoH (2009)
New Zealand	Culture	0.570	CAWBG-016 (Lake Horowhenua)	MfE and MoH (2009)
New Zealand	Culture	1.270	CAWBG-017 (Lake Horowhenua)	MfE and MoH (2009)
New Zealand	Culture	0.890	CAWBG-016 (Lake Horowhenua)	Wood et al. (2008)
New Zealand	Culture	5.949	CAWBG-011 (Lake Hakanoa)	Puddick et al. (2016)
New Zealand	Bloom	2.144, 1.634 1.144, 0.539 0.306, 0.279 0.266, 0.122 0.070, 0.066 0.065, 0.062 0.059, 0.058 0.058, 0.055 0.037, 0.034 0.032, 0.030 0.028, 0.024 0.024, 0.024 0.022, 0.020 0.014, 0.010 0.008, 0.007 0.006	Environmental samples containing *Microcystis* from long-term study at Lake Rotorua (Kaikōura)	Wood et al. (2017a)
New Zealand	Bloom	0.644	*Microcystis* dominated environmental sample from Lake Rotorua (Kaikōura)	Wood et al. (2011)
New Zealand	Bloom	1.379	*Microcystis* dominated environmental sample from Lake Rotorua (Kaikōura)	Wood et al. (2012)
New Zealand	Bloom	3.034, 4.223	*Microcystis* and *Dolichospermum* dominated environmental samples from Lake Waitawa	Steiner et al. (2017)
New Zealand	Bloom	2.825	*Microcystis* dominated environmental sample from Lake Rotorua (Kaikōura)	Wood et al. (2021)
New Zealand	Bloom	4.210	*Microcystis* dominated environmental sample from Lake Rotorua (Kaikōura)	Wood et al. (2021)

Country	Source	Toxin Quota/s (pg/cell)	Sample Information	Reference
New Zealand	Culture	0.776	CAWBG-617 (Lake Rotorua, Kaikoura)	This Report
New Zealand	Culture	2.903	CAWBG-624 (Lake Rotorua, Kaikoura)	This Report
New Zealand	Culture	0.660	CAWBG-011 (Lake Hakanoa)	This Report
New Zealand	Culture	0.444	CAWBG-563 (Lake Pauri)	This Report
New Zealand	Culture	0.208	CAWBG-570 (Lake Pauri)	This Report
New Zealand	Culture	0.129	CAWBG-706 (Lake Waitawa)	This Report

Appendix 4. Nodularin toxin quota values from *Nodularia spumigena*.

Country	Source	Toxin Quota (pg/cell)	Sample Information	Reference
New Zealand	Culture	0.35	CAWBG-020 (Wairewa / Lake Forsyth)	Wood et al. (2008)
New Zealand	Culture	0.26	CAWBG-021 (Wairewa / Lake Forsyth)	This Report
New Zealand	Culture	0.50	CAWBG-703 (Whakakī Lake)	This Report
New Zealand	Culture	1.86	CAWBG-704 (Whakakī Lake)	This Report
New Zealand	Culture	3.96	CAWBG-709 (Whakakī Lake)	This Report
New Zealand	Culture	2.43	CAWBG-710 (Whakakī Lake)	This Report
New Zealand	Culture	1.50	CAWBG-711 (Whakakī Lake)	This Report
New Zealand	Culture	2.80	CAWBG-712 (Wairewa / Lake Forsyth)	This Report
New Zealand	Culture	2.79	CAWBG-713 (Wairewa / Lake Forsyth)	This Report
New Zealand	Culture	0.85	CAWBG-714 (Wairewa / Lake Forsyth)	This Report
New Zealand	Culture	1.50	CAWBG-715 (Wairewa / Lake Forsyth)	This Report

Appendix 5. Cylindrospermopsin toxin quota values from *Raphidiopsis raciborskii* (previously called *Cylindrospermopsis raciborskii*).

Country	Source	Toxin Quota/s (pg/cell)	Sample Information	Reference
Australia	Culture	0.026	Strain AWT205	Hawkins et al. (1997)
Australia	Bloom	0.009, 0.009, 0.021, 0.015, 0.019, 0.023, 0.026, 0.033, 0.017, 0.056, 0.036, 0.031, 0.024, 0.015	*Cylindrospermopsis raciborskii* dominated environmental samples (with some *Aphanizomenon ovalisporum*)	Orr et al. (2010)
Australia	Bloom	0.004	*Cylindrospermopsis raciborskii* dominated environmental sample	Saker et al. (1999)
Australia	Culture	0.004	No strain identifier	Saker et al. (1999)
Australia	Culture	0.014	Strain CS506; Low CO_2	Pierangelini et al. (2015)
Australia	Culture	0.019	Strain CS506; High CO_2	Pierangelini et al. (2015)
Australia	Culture	0.006	Strain CYP 030A	Carneiro et al. (2013)
Australia	Culture	0.025	Strain CYP 011K	Carneiro et al. (2013)
Australia	Culture	0.210	Strain CQU FR001	White et al. (2006)
Australia	Culture	0.013	Strain NPD	Willis et al. (2017)
Australia	Culture	0.031	Strain AWT205	Willis et al. (2017)
Saudi Arabia	Culture	2.010	Coiled Morphotype	Mohamed & Al-Shehri (2013)
Saudi Arabia	Culture	3.270	Straight Morphotype	Mohamed & Al-Shehri (2013)
Saudi Arabia	Bloom	14.600, 11.900, 2.060, 1.440, 0.650, 0.600, 0.620	*Cylindrospermopsis raciborskii* dominated environmental samples	Mohamed & Al-Shehri (2013)

Appendix 6. Hypothetical calculation examples for assessment of multiple cyanotoxins in waterbodies for inclusion in the cyanobacteria guidelines.

Below is text included in section 3.3.3 of the recreational cyanobacteria guidelines.

In Situations 1 and 3, when there are multiple toxin-producing cyanobacteria taxa present (Situation 1) or different types of cyanotoxins present (Situation 3) the combined risk needs to be evaluated when determining if the action level (red mode) is triggered. The cumulative effects from multiple cyanotoxins should be accounted for using the ratio of the cell concentration for each toxin-producing taxa or each toxin concentration to the relevant threshold and summing the ratios. If the ratio exceeds 1, then the action level is triggered. Hypothetical example calculations using cell concentration thresholds (Situation 1) and cyanotoxin concentrations (Situation 3) are provided below:

Situation 1 example, if 7,500 cells/mL for *Microcystis* spp. and 6,000 cells/mL for *Nodularia spumigena* were detected in a lake, the ratio for these toxin-producing cyanobacteria would be:

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

As this value is < 1, the action level (red mode) threshold is not breached, and this lake would remain in alert level (amber mode).

Situation 3 example, if 15 µg/L of saxitoxins and 45 µg/L of anatoxins were detected in a lake, the ratio for these cyanotoxins would be:

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

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

Appendix 7. Updated toxicity calculations to derive the Action / Red mode thresholds for planktonic cyanobacteria for inclusion in the cyanobacteria guidelines.

Below is an updated version of Appendix 2 in the 2009 interim recreational cyanobacteria guidelines (now Appendix 5 in the revised guidelines). Appendices and sections mentioned below are from the cyanobacteria guidelines rather than this report. The use of [REF] below indicates where a reference citation would be included in the cyanobacteria guidelines. To avoid confusion with the references included in this report, these have not been included here.

Appendix 5: Derivation of guideline values

Planktonic cyanobacteria

The action level (red mode) – Situation 3 cyanotoxin concentration thresholds are based on the 2020 WHO recreational guideline values for anatoxins (60 µg/L) [REF], cylindrospermopsins (6 µg/L) [REF], microcystins (24 µg/L) [REF] and saxitoxins (30 µg/L) [REF]. These WHO guideline documents provide a review of toxicological information on each class of cyanotoxin and the calculations used to derive each guideline value. These calculations are also provided in Boxes A5.1-A5.4 below. Thresholds for nodularins (described in these guidelines for NZ) are based on the guideline value for microcystins (due to the similar structure, toxicity and mode of action shared by the two toxin classes).

Box A5.1: Calculation of guideline value for anatoxin-a in recreational waters.

This calculation is for the 2020 WHO provisional recreational water health-based reference value for anatoxin-a (Section 8.1 of the WHO background document for anatoxin-a and analogues; pg 15 [REF]).

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

Where:

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

The calculation is based on toxicology data for anatoxin-a, which is very limited. Dihydroanatoxin-a has been demonstrated to have higher oral toxicity than anatoxin-a [REF], therefore, it was recommended during a review of New Zealand's maximum acceptable values for cyanotoxins in drinking-water that a toxicity equivalence factor of 3 is used for dihydroanatoxin-a [REF]. Because no robust toxicology data was available for homoanatoxin-a and dihydrohomoanatoxin-a, a toxicity equivalence factor of 1 was suggested for these anatoxin congeners [REF]. Testing providers may be able to provide more up-to-date information on toxicity equivalence factors for anatoxins.

Box A5.2: Calculation of guideline value for cylindrospermopsin in recreational waters.

This calculation is for the 2020 WHO provisional recreational water guideline value for cylindrospermopsin (Section 8.1 of the WHO background document for cylindrospermopsins; pg 21-22 [REF]).

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

Where:

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

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

Box A5.3: Calculation of guideline value for microcystin-LR in recreational waters.

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

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

Where:

GV	=	guideline value for recreational waters
NOAEL	=	no-observed-adverse-effect level (40 µg/kg bw/day; based on liver toxicity in the study of Fawell et al, 1999 [REF])
bw	=	body weight (15 kg for a child)
UF	=	uncertainty factor (100 = 10 for interspecies variation × 10 for intraspecies variation)
C	=	daily incidental water consumption (0.25 L for a child)

The calculation is based on toxicology data for microcystin-LR. In the absence of oral toxicity data for other microcystin congeners, the WHO recommends that total microcystins are assessed as gravimetric or molar equivalents (pg 40 of the WHO microcystins guideline document [REF]). Although not explicitly stated in the WHO guidance, nodularins should also be assessed in the same manner. A TEF of 1 should be used for all microcystin and nodularin congeners unless new oral toxicity information becomes available. Testing providers may be able to provide more up-to-date information on toxicity equivalence factors for microcystins.

Box A5.4: Calculation of guideline value for saxitoxins in recreational waters.

This calculation is for the 2020 WHO recreational water guideline value for saxitoxins (Section 8.1 of the WHO background document for saxitoxins; pg 18 [REF]).

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

Where:

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

The calculation is based on human poisoning data for saxitoxins reported as STX-equivalents. Saxitoxin measurements in recreational freshwaters should also be assessed as STX-equivalents. Toxicity equivalence factors for saxitoxin congeners assessed in New Zealand's regulatory monitoring for saxitoxins in bivalve molluscan shellfish can be found in Cawthron Report 3219 [REF]. This includes updates recommended in the 2016 FAO/WHO technical paper [REF], as well as TEFs adopted for other saxitoxin congeners (not included in the 2016 FAO/WHO technical paper) in New Zealand's regulatory monitoring for saxitoxins in bivalve molluscan shellfish. In the absence of a saxitoxin TEF and with no oral toxicity data to base it on, a TEF of 1 should be used. This aligns with the advice provided by the WHO; to either evaluate total saxitoxins as gravimetric or molar equivalents, or as toxicity equivalents relative to saxitoxin (pg 18-19 of the WHO saxitoxins guideline document [REF]). Testing providers may be able to provide more up-to-date information on toxicity equivalence factors for saxitoxins.

For each of the cyanotoxins produced by planktonic cyanobacteria in New Zealand (anatoxin-producing *Cuspidothrix issatschenkoi*, cylindrospermopsin-producing *Raphidiopsis raciborskii*, microcystin-producing *Microcystis* spp. and nodularin-producing *Nodularia spumigena*), cell concentration thresholds were developed using toxin quota data. Depending on the data available, the toxin quota datasets were either based entirely on New Zealand data (microcystins and nodularins), based entirely on international data (cylindrospermopsins) or based on a mixture of New Zealand and international data (anatoxins). The summary statistics for this toxin quota data can be found in Box A5.5 and more information can be found in Cawthron Report 3726 [REF].

The action level (red mode) – Situation 1 cell concentration thresholds for cyanotoxin-producing planktonic cyanobacteria observed in New Zealand were then derived using the 2020 WHO cyanotoxin guideline values and the toxin quota data. For cylindrospermopsins, microcystins and nodularins the mean toxin quota was used and for anatoxins the maximum toxin quota was used. The threshold derivation can be found in Box A5.6 and more information can be found in Cawthron Report 3726 [REF]. The surveillance level (green mode) – Situation 1 cell concentration threshold (which triggers alert level - amber mode) was retained at 500 cells/mL to maintain continuity with the previous version of cyanobacteria guidelines. Calculations indicated that this still provided safety as cell concentrations for each cyanotoxin class were still > 500 cells/mL at 10% of the recreational guideline values (Box A5.6).

Note that action level (red mode) – Situation 1 cell concentration thresholds were not developed for saxitoxin-producing cyanobacteria because no planktonic saxitoxin-producing cyanobacteria have been recorded in New Zealand to-date. The saxitoxin action level (red mode) – Situation 4 cyanotoxin concentration threshold has been retained for the eventuality that saxitoxin-producing planktonic cyanobacteria are observed in New Zealand in the future and for situations where saxitoxin-producing benthic cyanobacteria need to be managed (see Appendix 4).

Box A5.5: Summary of toxin quota data from a literature review of studies on New Zealand and international planktonic cyanobacteria.

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

[a] A mixture of New Zealand and international data was used. [b] Because no New Zealand data was available, international data was used. [c] Because sufficient data was available, only New Zealand studies was used. [d] Because no international data was available, only New Zealand data was used. [e] Unable to calculate a 95th percentile value due to insufficient data. * These toxin quota values were used for formulating cell concentration thresholds in the alert-level framework.

Box A5.6: Calculation of cyanobacteria cell concentration thresholds for each toxin type using the mean or maximum toxin quota values.

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

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

Appendix 8. End-user feedback on the revised alert-levels framework for planktonic cyanobacteria in recreational freshwaters.

Permission to include feedback in this report was received from all survey participants by email. Where applicable, identifying information was removed from responses.

Online Survey Information

An information packet and online survey was prepared and distributed to regional councils and public health officers. The information packet included links to the Interim Cyanobacteria Guidelines and the report on the 2018 guidelines review, and a revised version of the Planktonic Cyanobacteria section of the guidelines (Section 3 - Part A of the Cyanobacteria Guidelines) including the revised ALF.

Questions Surveying General Thoughts and Feelings on the Revised ALF

The first four questions gauged general feelings on the revisions made to the ALF for planktonic cyanobacteria and the accompanying guidance, and allowed end-users to supply comments. Responses were numerically coded (see below) to determine the median and average response.

Number key for coding responses:

Strongly Disagree	=	0
Disagree	=	1
Neutral	=	2
Agree	=	3
Strongly Agree	=	4
No Comment	=	Not included in the analysis

Q1 - The revised alert-level framework for planktonic cyanobacteria is an improvement from that currently included in the Interim Guidelines.

Response	Code	Comment
Neutral	2	I like that you've redressed problems your seeing in the existing framework. But it does not address the main problem with the existing guidelines - delayed and reactionary monitoring and warning system, and limited systems for dealing with spatio-temporal variability.
Agree	3	The way different species are being dealt with is much clearer and some of the common sense approaches we have taken in the past around when to go up and down and action levels appear to have been incorporated.
Agree	3	
Agree	3	
Agree	3	I think is a good direction to revise all the different cyanobacteria results/response we are having across the different regions to understand if more adjustments needs to be done.
Agree	3	
Agree	3	

Q2 - The revised alert-level framework for planktonic cyanobacteria will likely lead to less unnecessary alert level escalations in my region.

Response	Code	Comment
Agree	3	You've given careful consideration to what the data has shown and worked to revise the recommendations accordingly.
Neutral	2	It might do in some circumstances but hard to be sure. Several of our lakes tend to be clearly one way or the other.
Strongly Agree	4	
Neutral	2	Haven't had the time to do in-depth analysis, but we predominantly get Microcystis and not the other species. Haven't had the chance to look at if the threshold for this will change our alert level, but I suspect we fall into situation 2 whereby the total biovolume of all cyanobacteria exceeds 0.5 mm^3/L, therefore wouldn't have much of an impact on the alert level reached.
Neutral	2	The common cyanobacteria species that are predominant in the lakes I visit are composed by the genera Microcystis sp., Aphanizomenon sp., Aphanocapsa sp., Pseudanabaena sp., Woronichinia sp. and Dolichospermum sp., which would not have a big change. I think in going forward it is necessary to identifying the main common species per region to understand the biota distribution and eventually standardize to a more integrated alert-level framework.
Agree	3	
Neutral	2	

Q3 - The additional guidance on using the revised alert-level framework for planktonic cyanobacteria was informative.

Response	Code	Comment
Agree	3	
Strongly Agree	4	More up to date science is always helpful.
Agree	3	
Agree	3	
Agree	3	I have been working in this for a year and I found all the information important. I would like to received more updates related to cyanobacteria on a daily basis or create a way to know/communicate what is the status on other regions.
No Comment	-	I am unsure what 'additional guidance' is referred to here, but if it is the text around the tables, then yes, it is informative and helpful. I did not have access to Appendix 1-5, which would have been helpful.
Agree	3	

Q4 - The ‘actions’ in the revised alert-level framework for planktonic cyanobacteria are reasonable and useful.

Response	Code	Comment
Disagree	1	The algae analysis based framework, which we have used for some decades now, I have come to realise is no longer fit for purpose. Even rapid lab analysis takes too long, and we can do better to empower people in their decision on if/where to go swimming.
Neutral	2	For us it doesn't change much at the moment.
Neutral	2	For Alert level I don't necessarily think that multiple sites should be inspected and sampled. Some lakes are quite small, or areas where people recreate are quite localised. I think it should read that multiple sites may need to be inspected or sampled if required but there should be some discretion whether additional sites need to be added to the survey.
Agree	3	We find it challenging to sample multiple sites if in the amber alert, so remain sampling at the primary access point during this alert level, but do increase to weekly sampling.
Strongly Agree	4	Yes I think is a very easy way to take decisions and with all the feedback it will improve over time.
Agree	3	
Agree	3	

Summary statistics for Questions 1-4 gauging general feelings on the revisions made to the ALF for planktonic cyanobacteria and the accompanying guidance (Min = minimum, Med = median, Max = maximum).

Question	Min	Med	Mean	Max
Q1 - The revised alert levels framework for planktonic cyanobacteria is an improvement from that currently included in the Interim Guidelines.	2	3	2.9	3
Q2 - The revised alert levels framework for planktonic cyanobacteria will likely lead to less unnecessary alert level escalations in my region.	2	2	2.6	4
Q3 - The additional guidance on using the revised alert levels framework for planktonic cyanobacteria was informative.	3	3	3.2	4
Q4 - The ‘actions’ in the revised alert levels framework for planktonic cyanobacteria are reasonable and useful.	1	3	2.6	4

Questions on Specific Aspects of the Revised ALF

Two questions (Questions 5-6) gathered feedback on specific queries relating to the guidelines (ease of use and naming of the thresholds).

Q5 - Taking into account the alert-level framework (Decision Chart 1) and the additional guidance provided around the framework (the associated text), do you feel that you could navigate more complex situations that might arise in lakes in your region (e.g., multiple toxin-producing cyanobacteria present, the presence of potential toxin-producing cyanobacteria)?

Response	Comment
Yes	
Yes	
Yes	
Yes	Yes I think the guidance around thresholds for certain toxin producing species is a good addition. Although, in my opinion, the reporting of results from laboratories could be made clearer into the splitting of toxin-producing cyanobacteria etc. I have been guided that our results (NIWA) report that all cyanobacteria is potentially toxin producing, so for comparable results to be reported nationally it would be good to have the guidance feeding into how laboratories are reporting them as well. This would enable more consistency across councils (LAWA is not reporting on cyanobacteria grading as per NPS-FM), so comparable data is required for this process. LAWA has data collected for all cyanobacteria and toxic producing cyanobacteria biovolumes.
Yes	Although it would not make a big difference as only one of the four species mentioned are generally seen in the lakes we monitor.
Yes	I found the testing for the presence of toxin producing genes very helpful for managing blooms of species that may be toxin producers, but there is no reference in the text.
Yes	

Q6 - In the NZ recreational cyanobacteria guidelines, the alert levels (for both planktonic cyanobacteria and benthic cyanobacteria) are currently named according to a traffic light system (Green Mode, Amber Mode, Red Mode; Surveillance Level, Alert Level, Action Level). In the WHO recreational cyanobacteria guidelines and the NZ drinking-water guidelines for cyanobacteria, the alert levels are named numerically (Vigilance Level, Alert Level 1, Alert Level 2). Do you support renaming the alert levels in the NZ recreational cyanobacteria guidelines to align with the WHO guidelines (i.e., Vigilance Level, Alert Level 1, Alert Level 2)?

Response
No
No
No
Yes
No
No
Yes

Open Feedback on the Revised ALF

The final question inquired about any additional suggestions to improve the ALF for planktonic cyanobacteria.

Q7 - Do you have any additional suggestions for improving the alert-level framework for planktonic cyanobacteria in the NZ recreational cyanobacteria guidelines? Please specify:

Comment
We are developing a revised framework with local DHB's and TA's and would be keen to explore ways to incorporate some of those learnings into the national guideline. The changes we are developing would be complementary to the changes you are making based on a better understanding of toxin production in algae. We are focused more on how risk is communicated and steps required to do so.
We think that Green amber red or similar is more user friendly for a public conversation. We have spent a long time teaching the public about the traffic light system. The alert level system comes with new learning, bedding in time and confusion with the complexity of its use for other purposes such as Covid19.
"however, only three planktonic taxa are regularly observed; Microcystis sp.,Nodularia spumigena and Cuspidothrix issatschenkoi." My region most regularly observes Dolichospermum, and in particular D. circinale. I note that ID work undertaking by Cawthron which identified D. circinale in our samples listed it as potentially toxic (Contract 13650) but its not mentioned in Table 2. My generally concern is that with the higher biovolume of 10 and New Zealands incomplete knowledge of planktonic cyanobacteria and their toxin producing capabilities that situations might arise where water is not suitable for recreation but does not meet the Action level where we might close a lake to users.
I would be interested in knowing how this would correspond to NPS-FM (2020) grading since the previous guidelines tables are in Table 10 of Appendix 2A, and there has been some interpretation around the different languages used in grades A & B (biovolume equivalent for combined total of all cyanobacteria) and C & D (biovolume equivalent of potentially toxic cyanobacteria OR total biovolume of all cyanobacteria).
I think is a great idea for this revision and I would like to have more updates in this matter.
3.2 Alert-level framework: planktonic cyanobacteria: Dolichospermum lemmermannii is frequently observed in my region. It is unclear from the wording whether a) this species is not often observed in NZ or b) is has not been observed to produce toxins in NZ. Please clarify. I had assumed it has a high potential to produce toxins and we have been treating it as very likely toxin producer, so it would be good to know. The loss of 1.8 mm^3/L threshold for know toxin producers in NZ (but other than the named 4 species) such as Dolichospermum lemmermannii means that a bloom of Dolichospermum lemmermannii would trigger a warning now at only at $10mm^3/L$, which is a big difference to 1.8 mm^3/L. The same applies to other less common known toxin producers in NZ. Are these not a concern a biovolumes below $10mm^3/L$ or is this an oversight? How to we cover these less common species without extensive (and expensive) toxin testing? Scums have triggered action mode in the past, does this still apply? I cannot find a reference to this in the text. Would be great to see the information in Appendix 1-5 for a full review.

Separate Feedback Received via Email from a Public Health Officer

Toxicity - The guidance based around whether to test for toxins seems a little loose, but I understand that if you already have the species and the biovolume/cell density over time you probably wouldn't repeat the toxicity tests unless something changed – mass cell die off, new species etc. Still, it should include a minimum in my opinion (example - if exceeds Surveillance Amber then an initial test should be undertaken unless recent other tests show known species and previously measured toxicity risk) – so…. maybe an area with one predominant species and an annual event, then may not need to test, anything new with mod – high rec use they should test for toxicity.

3.3.1 Surveillance Green Mode – Sampling and Cell Counts undertaken weekly to fortnightly from spring to autumn. I agree with the frequency. Fortnightly appropriate for areas with lower usage and non-toxigenic species present (yes).

3.3.2 Alert Amber Mode - Requires notification and consultation with PHU (similar to my comments above for Surveillance Green mode – toxin analysis information would be very useful, less necessary for an annual event with known measured results on toxicity and species/biovolumes).