



# Water Monitoring

## Monitoring Standard for Freshwater Blue-Green Algae

October 2008

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## 1. Purpose and Scope

Planktonic cyanobacteria, or blue-green algae, are a common, naturally occurring and integral component of many aquatic ecosystems. As such they often cause no obvious ecological problems. A small group of genera, however, produce toxins (cyanotoxins) that have caused sporadic cases of animal poisoning, have been implicated in human hepatoenteritis, and have led to human fatalities. Adverse health effects of exposure to cyanobacteria during recreational water-related activities have also been recently described (Pilotto et al 1997). Hence, in order to reduce the risks of adverse health impacts caused by cyanobacteria, it is essential to routinely monitor water supplies for their presence on a quantitative basis and where necessary, test for the presence and concentration of their toxins. This document outlines a standard procedure for the monitoring of cyanobacteria and their toxins in surface freshwaters. The information presented here is based largely on the Draft version 5.0 National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Waters (Jones et al 2002), Phytoplankton Methods Manual for Australian Freshwaters (Hötzel & Croome 1999), and Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management (Chorus & Bartram 1999).

## 2. Permits and Approvals

A general fisheries permit is required for all work that involves 'fish' as defined in s5 of the *Fisheries Act 1994*. Note that early life stages such as eggs, spat or spawn of fish are considered as fish under the Act. Water Accounting and Management (WAM) have obtained a general fisheries permit, which covers the majority of work conducted by officers of the Department of Natural Resources and Water (NRW), who act under the direction of the General Manager, Water Accounting and Management. This permit is valid from 21 May 2007 to 21 May 2012 and is suitable for all activities authorised by the permit. Before undertaking any work, ensure the department's General Fisheries Permit is current and covers the work outlined in this method. Refer to the General Fisheries Permit Work Practice (NRW staff), or contact the Department of Primary Industries and Fisheries (DPI&F) for more information.

Under the *Queensland Nature Conservation Act 1992* (NCA), approval from the Environmental Protection Agency (EPA) is required to conduct activities involving protected wildlife and locations. Failure to obtain approval prior to the commencement of activities is a breach under the NCA. Refer to the EPA permits work practice (NRW staff), or contact the EPA for more information.

Under the *Queensland Animal Care and Protection Act 2001* (the Act), approval from an Animal Ethics Committee (AEC) is required for the use of animals for scientific purposes. Failure to gain approval is a breach of the Act. It is a legal obligation to receive the approval from the AEC in writing, before any project can commence. For more information refer to the Animal Ethics Work Practice (NRW staff), or the DPI&F for more information.

To ensure staff have all the correct permits and approvals in place before using this method, refer to the Permits and approvals checklist (NRW staff), or contact the EPA and DPI&F for more information.

## 3. Workplace Health and Safety

Risk assessment is of vital importance in reducing risks from potential hazards in the workplace. In section 27A of the *Workplace Health and Safety Act 1995*, five basic steps are outlined that must be followed to manage exposure to risks. The Workplace Health and Safety Queensland Risk Management Code of Practice 2007 outlines the obligations of both the employer and employee when carrying out risk assessment in the workplace. More information can be found at:

1. [Workplace Health and Safety Queensland Risk Management Code of Practice 2007](#)
2. *Workplace Health and Safety Act 1995*

NRW recommends a minimum of two persons per sampling party for all fieldwork. Ensure that all risk assessment and trip approval paperwork is completed and that a ring-in schedule has been arranged. When working in areas potentially inhabited by estuarine crocodiles, additional protective measures must be taken. Refer to the appropriate guidelines.

**Before following the methods contained in this document, a detailed risk assessment should be undertaken. Table 1 outlines the areas to be completed.**

**Table 1: Workplace health & safety risk for monitoring standard for freshwater blue-green algae (Cyanobacteria) (See [Appendix A](#) for NRW Risk Analysis Matrix and NRW Risk Scores)**

CONTEXT Define the context of what risks are to be covered & identify the specific activity to be assessed	HAZARDS Make a record of each hazard (a hazard is something with the potential to cause harm)	RISK List the risks to health & safety posed by each hazard	RISK RATING Evaluate the risks to health & safety posed by each hazard			CONTROL MEASURES List suitable control members to eliminate, substitute, isolate, reduce or engineer out the risks	MONITORING What monitoring is proposed or already in place, to ensure that controls are effective in reducing/eliminating risks of hazards	REVIEW DATE <b>List suggested times for the review/update of monitoring procedures</b>
			CONSEQUENCE	LIKELIHOOD	RATING			
FIELD OPERATIONS undertaking sampling on-site	Unpredictable water depths & velocities	Drowning Personal Injury	Major	Unlikely	Medium (14)	Life-jackets to be worn at all times Ensure staff are capable swimmers First Aid Qualifications	Life-jackets to be tested Updating of First Aid qualifications Appropriate call-in procedure in place	Annually
	Sun & heat exposure	Sunburn & heat exhaustion	Minor	Unlikely	Low (5)	Appropriate PPE to be utilised	Monitor condition of PPE	Annually
	Navigation of unstable terrain	Personal Injury	Minor	Possible	Medium (8)	Appropriate PPE to be utilised First Aid Qualifications Two staff members to travel together at all times	Monitor condition of PPE Updating of First Aid qualifications	Annually
	Collision Car Rollover Breakdown	Personal Injury	Catastrophic	Rare	Medium (15)	Recovery equipment to be carried at all times Drive with due care 4WD training qualifications	Condition of car & recovery equipment to be inspected prior to leaving office Updating of driving licences	Annually

Note: Table 1 has been filled out as an example only.

## 4. Skills/Competency and Experience Required

Blue-green algae sampling knowledge and/or experience. Staff skills, training and experience records should be kept up to date.

## 5. Chemicals

Lugol's Iodine Solution - Made by mixing 20 g of potassium iodide (KI) with 200 mL distilled water, then dissolving 10 g of pure iodine in this solution. Glacial acetic acid (20 g) is added a few days before use. This stock solution must be stored in the chemical storage cupboard, in the dark, with the date of preparation, name of the analyst who prepared the solution, and a used by date clearly marked on the bottle's label. This solution shall be used for no longer than 12 months after the date of preparation.

Users should refer to the appropriate Material Safety Data Sheets prior to using any chemicals <http://www.msds.com/>.

## 6. Equipment

The following equipment is needed in order to take samples:

3. integrated hose-pipe sampler – 5 m length of 2.5 cm diameter plastic piping with a weighted collar at one end (Figure 1)
4. a cord attached to the hose and boat
5. a rubber cork to fit one end of the hose
6. a bucket
7. Lugol's iodine preservative solution
8. 200 mL amber Polyethylene terephthalate (PET) plastic bottle and lid

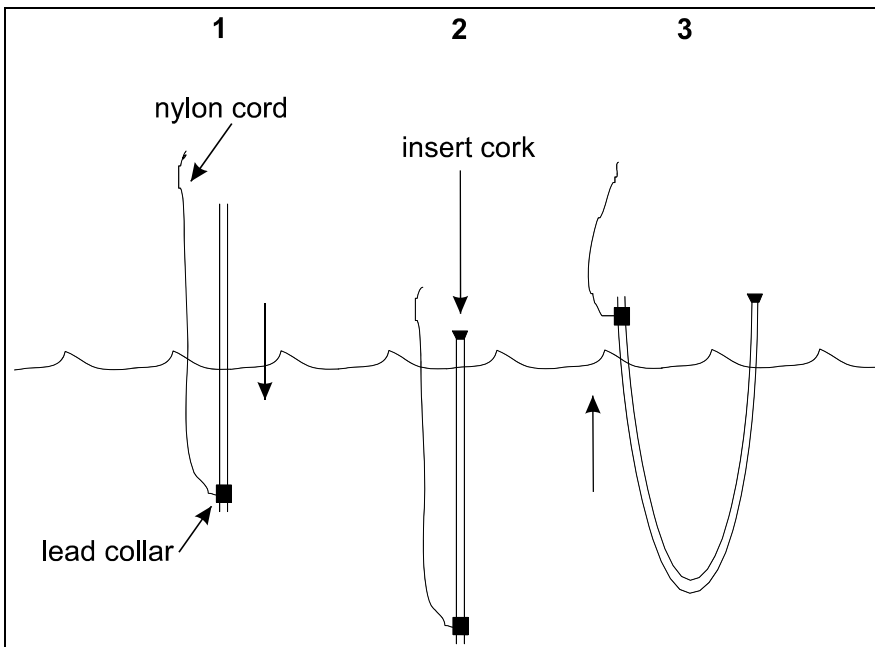


Figure 1: Procedure for use of the integrated hose-pipe sampler.

## 7. Method

### 7.1 Monitoring program design

Cyanobacteria pose a potential health risk through the consumption of water supplies contaminated by cyanotoxins, and through direct exposure to cyanotoxins during water-based recreational activities (i.e. skiing, bathing, wading, boating, etc.). Cyanotoxins have been isolated, identified, and characterised from a number of cyanobacterial taxa, and the ones reported from Australian freshwater to date are presented in Table 2.

**Table 2: Potentially toxic cyanobacteria reported from Australian freshwaters**

Toxic Species	Cyanotoxin	Target Organ
<i>Anabaena circinalis</i>	saxitoxins	nervous system
<i>Aphanizomenon ovalisporum</i>	cylindrospermopsin	liver
<i>Cylindrospermopsis raciborskii</i>	cylindrospermopsin	liver
<i>Microcystis aeruginosa</i>	microcystins	liver
<i>Nodularia spumigena</i>	nodularins	liver
<i>Nostoc cf. linkea</i>	unidentified	liver

The Australian Drinking Water Guidelines (NH&MRC/ARMCANZ 1996) currently provides guidance for one of the four major classes of cyanotoxins found in Australian freshwaters. The guidelines recommend that the concentration of total microcystins in drinking water not exceed 1.3 µg L<sup>-1</sup> (expressed as microcystin-LR toxicity equivalents). Due to the lack of adequate data, no guideline values have been set for the other three classes of cyanotoxins. Given the presence of cyanotoxins other than microcystins in Queensland freshwaters, NRW recommends a conservative approach be adopted wherein a value of 1.0 µg L<sup>-1</sup> be used for cylindrospermopsin and PSPs as an interim guidance level until sufficient data has been collected to allow for the development of individual guidelines for these two cyanotoxins.

The risk of adverse health effects through recreational contact in water containing cyanobacteria varies with the concentration of cyanobacteria and the nature of the activity. The risk is derived from contact with both the known cyanotoxins produced by the species listed in Table 3, as well as from a group of yet unidentified, non-specific compounds produced by cyanobacterial taxa which have been recorded to produce symptoms ranging from mild skin irritations to nausea, and pneumonia-like symptoms. The provisional guidelines for cyanobacteria in bathing water as adopted by NRW are presented in Table 4. These guidelines are based on the World Health Organization (WHO) guidelines for safe practice in managing bathing waters which may produce or contain cyanobacterial cells (Chorus & Bartram 1999). Rather than providing a single threshold value, these guidelines are framed as a series of three guidelines, which reflect incremental severity and probability of adverse effects. These guidelines can be assessed using cell concentrations or cell biovolume concentrations. The later measure is preferred, as it takes into account the huge size range of cyanobacterial cells that occur in natural populations and recognises that the contribution of cyanobacterial cells to the hazard is proportional to their cell volume.

Cyanotoxins have also been demonstrated to pose serious adverse effects on mammals, birds and fish and as such are being increasingly recognised as a potent stress and health hazard factor in aquatic ecosystems. Exposure of aquatic organisms may occur both orally by uptake of toxin-contaminating cells as food, or through the surface tissues of organisms submerged in water containing dissolved cyanotoxins. Despite a growing body of evidence on the ecological effects of these compounds, there are currently no accepted guidelines for cyanobacteria and their toxins relating to the protection of aquatic ecosystems.

#### 7.1.2 Storage classification

A national approach to cyanobacterial monitoring taking into account the varying needs and objectives of

government agencies, councils, community groups, and members of the public was developed with the creation of a Draft National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Waters (Jones et al 2002). It recognised a number of monitoring classes which decrease in effort and cost, and therefore precision and certainty of monitoring and analysis outcome, from that termed “class A1” monitoring, to that termed “class C” monitoring.

The scheme is three tiered, with each tier providing a different level of sampling and analytical precision, and overall certainty of monitoring outcome. Storage operators should determine the appropriate sampling regime based on this monitoring class classification system. A summary of the monitoring classes is given in Table 4.

**Table 3: WHO Guidelines for safe practice in managing bathing waters which may produce or contain cyanobacterial cells (after Chorus & Bartram 1999)**

Hazard Status	Guidance level or situation	Health risks	Recommended action
High	Cyanobacterial scum formation in contact recreation areas or > 100,000 cells total cyanobacteria mL <sup>-1</sup> or > 50 µg L <sup>-1</sup> chlorophyll-a with dominance of cyanobacteria or > 12.5 mm <sup>3</sup> L <sup>-1</sup> cyanobacterial biomass	Short term adverse health outcomes such as skin irritations or gastrointestinal illness following contact or accidental ingestion  Severe acute poisoning is possible in worst ingestion cases	Immediate action to prevent contact with scums  Signs to indicate HIGH alert level - warning of danger for swimming and other water contact activities
Moderate	20,000 - 100,000 cells total cyanobacteria mL <sup>-1</sup> or 10 - 50 µg L <sup>-1</sup> chlorophyll-a with dominance of cyanobacteria or 2.5 - 12.5 mm <sup>3</sup> L <sup>-1</sup> cyanobacterial biomass	Short term adverse health outcomes e.g. skin irritations, gastrointestinal illness, probably at low frequency	Signs to indicate MODERATE alert level - increased health risk for swimming and other water contact activities
Low	< 20,000 cells total cyanobacteria mL <sup>-1</sup> or < 10 µg L <sup>-1</sup> chlorophyll-a with dominance of cyanobacteria or < 2.5 mm <sup>3</sup> L <sup>-1</sup> cyanobacterial biomass	Short term adverse health outcomes unlikely	Cyanobacteria either absent or present at low levels - continue monitoring

**Table 4: Outline of monitoring classes for national cyanobacterial sampling protocol (after Jones et al 2002)**

Monitoring class	Recommended use category	General method description	Surety of Results
A1	Public health surveillance of drinking water supplies  Condition and trend monitoring – high priority water bodies	Open water sampling from boat  Detailed visual surveillance (for scums)  High counting precision (< 30% counting error)	High to Very High
A2	Public health surveillance of recreational water bodies  Condition and trend monitoring – moderate priority water bodies	Shoreline or bank sampling  Detailed visual surveillance (for scums)  High counting precision (< 30% counting error)	High
B1	Condition and trend monitoring – moderate priority water bodies	Open water sampling from boat  Low to moderate counting precision (> 30% counting error)	Moderate to High
B2	Condition and trend monitoring – low priority water bodies  Community group monitoring	Shoreline or bank sampling  Low to moderate counting precision (> 30% counting error)	Moderate
C	Public health surveillance for scum formation in bathing waters  Community group monitoring of cyanobacterial growth	Visual surveillance only	High  (for scum surveillance only)  Low

### 7.1.3 Monitoring sites

Buoyant cyanobacteria tend to accumulate near or at the shoreline at the down wind or down stream end of reservoirs or river reaches. Therefore for high priority public health surveillance monitoring, “depth integrated”, open water sampling is preferred. The selection of sampling sites will depend on a number of factors including prevailing winds, the position of stream inflows and the proximity to potential nutrient input sites. Open water sampling provides, in general, a better representation of the true or average cyanobacterial population of the water body. Open water or mid-stream sampling is normally carried out by operations staff working from a boat. For drinking water supplies, sampling the appropriate depth next to, or from the water off-take tower is desirable.

In general, one open water site in the vicinity of each recreational area, and one sampling site at the water supply off-take tower should give adequate coverage for both the water supply and recreational use health issues posed by cyanobacteria. It is desirable that both sites are marked with a buoy or similar device to ensure that sampling occurs in exactly the same area, no matter who samples

## 7.2 Sample collection

In order to obtain a representative sample for species identification and cell count over the surface depth range, each water sample should be collected at the clearly marked sampling point using the 5 m long, 2.5 cm diameter integrated hose-pipe sampler (Figure 1). Sampling should be carried out in the middle of the day, preferably about 1:00 pm.

The procedure for collecting the sample is as follows:

1. Attach a cord to one end of the hose and the boat to prevent accidental loss of the hose
2. Holding the hose at the top end, rapidly drop the weighted end of the hose-pipe into the water to a depth of ca. 5 m
3. Return hose to the boat without inserting the rubber cork
4. Rinse the hose
5. Repeat the procedure, but this time insert the cork into top end of the hose (so that the end is held in the hand)
6. Pull the bottom end of the hose to surface using the cord, so that the tube is in a U-shape (see Figure 1)
7. Lower the weighted end of the hose into a bucket and remove the cork. Ensure that the entire contents of the hose are emptied into the bucket
8. Mix the contents of the bucket and then transfer part of the contents into a 200 mL amber PET plastic bottle, leaving a 25 mm gap at the top of the bottle. Discard the rest of the contents of the bucket

Algal scums should not be included in the water sample for routine identification and enumeration, however if they are present, a note should be taken indicating their nature and extent. This is particularly important in bathing or water recreation areas. Additional scum samples can be collected and submitted for qualitative analysis if extensive.

NOTE: Blue-green algae can cause skin irritation. If sampling from an area that has a high level of blue-green algae, minimise your contact with the water during sampling by wearing appropriate dress, in particular gloves. Normal hygiene precautions such as washing off any splashes and washing hands before eating or drinking should be observed at all times. When not in use, the hose-pipe sampler and bucket should be kept clean and stored in a dark shed or cupboard.

### **7.3 Sample storage and preservation**

To ensure the sample remains in a condition suitable for identification and enumeration, a sufficient volume of Lugol's iodine preservative solution should be added to the sample to render the sample a colour resembling weak tea (i.e. 1.0 mL Lugol's iodine solution to 200 mL sample). Once Lugol's is added to the sample, it requires no additional treatment prior to analysis (e.g. chilling etc). Lugol's iodine solution is made by mixing 20 g of KI with 200 mL distilled water, then dissolving 10 g of pure iodine in this solution. Glacial acetic acid (20 g) is added a few days before use. The solution must be stored in the dark in a glass bottle and remains effective for at least 12 months.

### **7.4 Sampling for cyanotoxin analysis**

Cyanotoxin analysis will generally be required in one of the following circumstances:

1. Action Level 1 status (i.e. > 2 000 cells mL<sup>-1</sup>) predominated by *Microcystis aeruginosa*, or when concentrations of other potentially toxic taxa (see Table 1) exceed 15 000 cells mL<sup>-1</sup>.
2. Action Level 2 status where numbers of a cyanobacterial taxa not previously recorded as toxic exceed 100 000 cells mL<sup>-1</sup> (recommended toxicity analysis by mouse bioassay or comparative method).

Samples for toxin analysis should be collected using the 5 m integrated hose-pipe sampler (as described in section 8.2) and a 2 L chilled sample sent to the laboratory for analysis (see [Appendix B](#) for laboratory contact details).

In Australia, and internationally, guidelines for cyanotoxins in drinking water supplies are being set based on the concentration of toxins in water ( $\mu\text{g toxin L}^{-1}$ ). Hence it is recommended that for those cyanobacterial species where the toxins are well known and characterised i.e., *Anabaena circinalis*, *Aphanizomenon ovalisporum*, *Cylindrospermopsis raciborskii*, or *Microcystis aeruginosa*, routine analysis of toxins should be carried out by High Performance Liquid Chromatography (HPLC). HPLC analysis enables the exact concentration of individual toxins in cyanobacterial samples to be quantified, with toxin concentration reported either in terms of the mass of toxin per unit mass of cyanobacteria, or mass of toxin per litre of water. Mouse bioassay should only be used if taxa other than the ones listed above are suspected of producing toxin.

## 7.5 Sampling frequency

Monitoring class A1 and A2 storages are recommended to be sampled on a fortnightly basis, until the total blue-green algae cell count exceed 2000 cells mL<sup>-1</sup>, after which they should be sampled weekly. Sampling can return to fortnightly after cell numbers fall below 2000 cell mL<sup>-1</sup>. Ideally sampling frequency should be determined on a storage by storage basis using historical records of blue-green algal dynamics.

## 7.6 Sample analysis and reporting

### 7.6.1 Analysis precision

A suitably qualified laboratory with appropriate quality systems in place should conduct the sample analysis. When requesting the analysis it is important to state the minimum precision required of the analysis both in respect to the identification (i.e. genus or species level) and enumeration (provides the level of confidence in the result). The precision associated with the analysis of samples is directly related to the amount of analytical effort with respect to the laboratory equipment, counting effort and therefore time, and staff expertise. Therefore there is likely to be a higher cost associated with higher levels of precision. For A and B monitoring classes, it is recommended that the minimum taxonomic precision be at the genus level with species identification essential for potentially toxic species (see Table 2).

The counting precision is an estimation of the error associated with the estimation of abundance. It is defined as the ratio of the standard error to the mean (expressed as a percentage) for replicated counts and assumes a Poisson distribution of counting units in the counting chamber (Laslett et al 1988). The precision (counting error) can be calculated from the total number of units (n) using the formula derived by Laslett et al (1998):

$$\text{Counting error } (\pm \%) = 100 \times \sqrt{\frac{2}{n}}$$

Therefore the more units counted, the lower the counting error. A summary of counting errors based on this formula is shown in Table 5. The minimum acceptable level of precision for public health monitoring is  $\pm 30\%$ . It is also recommended that a minimum precision of  $\pm 30\%$  be specified for potentially toxic species.

**Table 5: Minimum counting error after Laslett et al (1998)**

Total units counted	Counting error ( $\pm \%$ )
1	140
2	100
4	75
8	50
13	40
23	30
50	20
200	10

### 7.6.2 Sample analysis

Sample analysis is conducted by examining a sub-sample under a microscope and systematically identifying

and counting algal units. Specialised counting chambers of a known volume are used in conjunction with a grid system to enable examination of a known area of the chamber, and corresponding volume of sample. The two most commonly used chambers are the Sedgwick-Rafter chamber and the Lund Cell. Both are used with an upright microscope. Both are similar in that they contain a fixed volume of sample, however the Sedgwick-Rafter chamber is etched with a calibrated grid of 50 x 20 equal sized squares (1 mm<sup>2</sup>), whereas the Lund Cell is unmarked and is used in conjunction with a Whipple Grid, which is inserted in the microscope eyepiece.

There is no Australian Standard for the analysis of planktonic microalgae; subsequently laboratories will vary slightly with respect to their preferred method. An Australian “benchmark” or recommended approach for the enumeration of cyanobacteria is given in Jones et al (2002), based on the “Phytoplankton Methods Manual for Australian Freshwaters” (Hötzel & Croome 1999). A generic method for the identification and enumeration of planktonic microalgae using a Sedgewick-Rafter chamber based on the benchmark approach is given in [Appendix C](#) as an example.

### 7.6.3 Reporting

Phytoplankton density is a concentration measure and should be reported as cells per millilitre. Algal biomass can also be reported as cell biovolume which takes into account the contribution of species based on their relative size. Cell biovolume is measured by calculating an average volume for each species using formulae for geometrical shapes closest to the cell’s shape. The average volume for each species is then multiplied by the cell count for the species and all the products summed to gain a biovolume per sample in mm<sup>3</sup> per litre. Advice on developing a cell biovolume method can be found in APHA (1992) and Hillebrand et al (1999). Cell biovolume measurements are recommended for the calculation of recreation hazard risk after the WHO guidelines in Table 3.

## 7.7 Contingency plan framework

Contingency Plans provide an action framework that specifies appropriate management actions in response to blue-green algal cell level thresholds. Whilst the Queensland Water Quality Task force created a generic action framework in 1993, agencies operating storages are encouraged to develop site specific contingency plans to address their particular situation.

An Action Level Framework is a monitoring and management action sequence that water treatment operators and storage managers can use to provide a graduated response to the onset and progress of a cyanobacterial bloom. The managerial response model presented as a “decision tree” in Figure 2 provides for the assessment of a potentially toxic cyanobacterial bloom, with appropriate actions and responses, through three “threshold” stages. The action framework is based on the Australian National Alert Level scheme, and reflects recent developments in the risk assessment and monitoring of cyanobacteria released by the World Health Organisation (Chorus & Bartram 1999).

**Table 6: Generic contingency plan framework**

<b>Vigilance Level</b>
Threshold Definition: cyanobacterial cell numbers 500 – 2 000 cells mL-1
The vigilance level encompasses the early stages of bloom development, when cyanobacterial cells are first detected in un-concentrated lake or raw water samples.
9. When vigilance level is exceeded, it is recommended to increase the sampling frequency to at least once a week, so that potentially rapid changes in cyanobacterial biomass can be monitored
10. Visual inspection for algal scums or accumulations of all water intakes and water recreation areas should be conducted on at least a weekly basis
<b>Action Level 1</b>
Threshold Definition: cyanobacterial cell numbers > 2 000 cells mL-1. Persistently high cyanobacterial cell numbers throughout the storage increasing or remaining high as per threshold definition
Action Level 1 threshold (cyanobacterial cells > 2 000 cells mL-1) is derived from the WHO guideline

for microcystin-LR and the highest recorded microcystin content for cyanobacterial cells. The threshold level assumes that the species present is a microcystin producer, where raw water microcystin concentration could exceed the WHO guideline value of 1 µg L<sup>-1</sup>.

11. If *Microcystis aeruginosa* is present at concentrations > 2 000 cells mL<sup>-1</sup> or other known toxin producing taxa (see Table 2) at > 15 000 cells mL<sup>-1</sup>, the conditions require a quantitative analysis of the concentration of cyanotoxin in the raw water supply, and an assessment of whether the water treatment processes available are effective in reducing toxin concentrations to acceptable levels. Ongoing analysis of algal toxins in the raw water is necessary if values exceed 1 µg L<sup>-1</sup>
12. Continue routine weekly monitoring of raw and treated water to ensure adequate removal of algal cells and toxins
13. Implement use of alternative water supplies and consult health authorities if toxin concentrations in treated water exceed 1 µg L<sup>-1</sup>
14. Visual inspection of all recreation areas should be conducted prior to entering the water – bathers should avoid contact with cyanobacterial scums. See Table 3 for appropriate water recreational hazard status based on latest analysis results

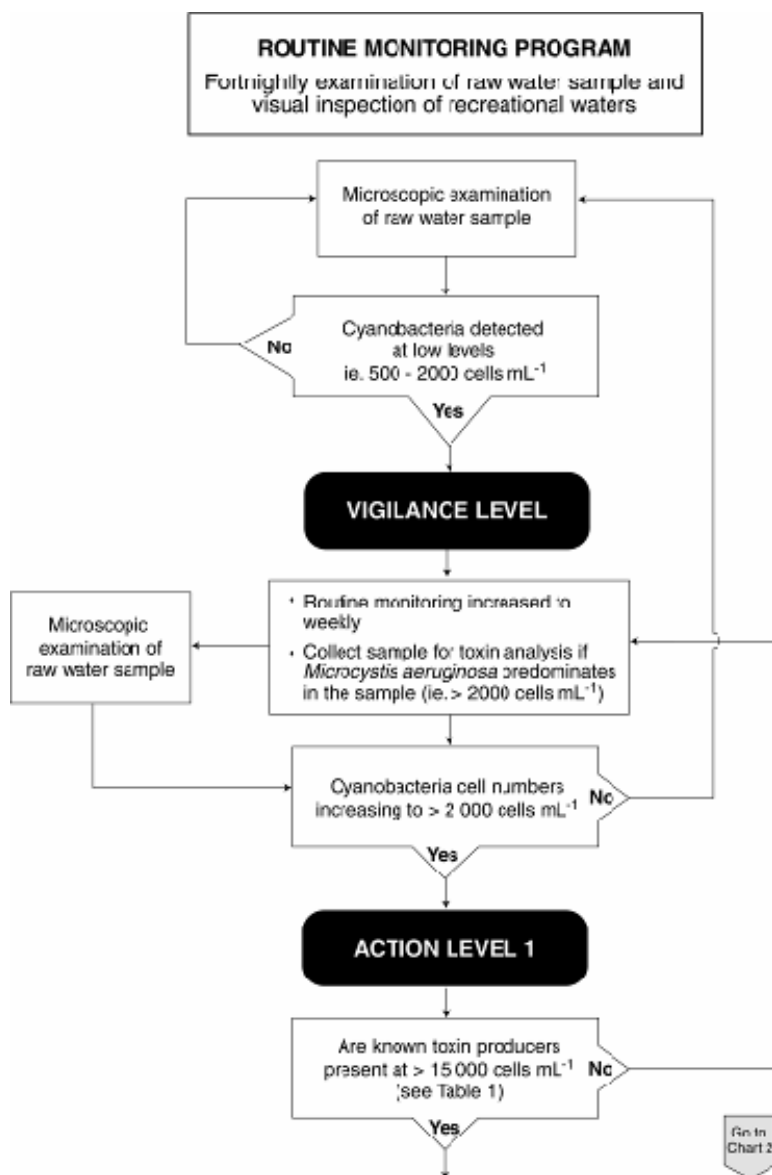
### Action Level 2

Threshold definition: cyanobacterial cell numbers > 100 000 cells mL<sup>-1</sup>. Persistently high cyanobacterial cell numbers throughout the storage increasing or remaining high as per threshold definition

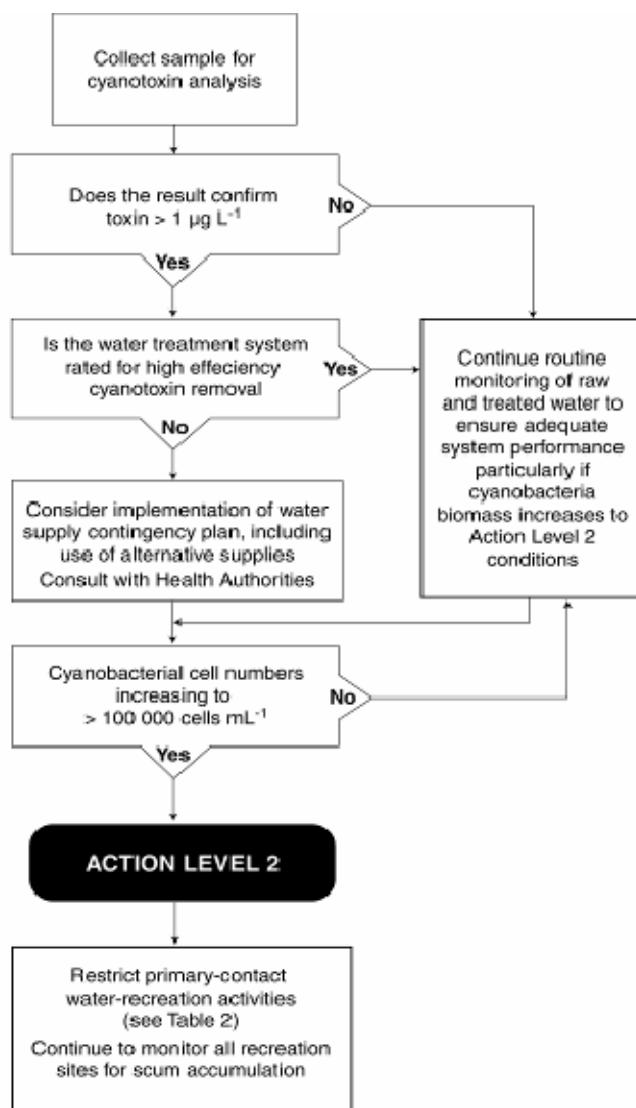
The threshold for Action Level 2 (cyanobacterial cells > 100 000 cells mL<sup>-1</sup>) describes an established bloom with high biomass with the possibility of localised scums. Conditions in Action Level 2 are indicative of a significant increase in the risk of adverse health effects from the supply of water that is untreated or treated by an ineffective system or through primary contact water recreation or bathing activities.

15. Maintain weekly or bi-weekly sampling (depending on the dominant cyanobacterial taxa present) including all sites and visual inspection of all water recreation areas for scum formation. Ensure warning signs indicate current recreation hazard status (see Table 3) or direct access to storage is restricted
16. Implement use of alternative water supplies and consult health authorities if toxin concentrations in treated water exceed 1 µg L<sup>-1</sup>

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**Figure 2: Decision tree incorporating the model Action Levels framework for monitoring and management of cyanobacteria in drinking and recreational waters.**

### 7.8 Variation to method

There are no variations identified.

### 7.9 Limitations of Use

This method is applicable to monitoring blue-green algae.

## 8. Quality Assurance

Complete and accurate completion of field sheets.

## 9. References

APHA & AWWA 1992, Standard methods for the examination of water and wastewater, 18th edn, prepared and published jointly by American Public Health Association, American Water Works Association, Water

Environment Federation, Washington.

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## 10. Appendices

## Appendix A

Table 7: NRW Risk Analysis Matrix

LIKELIHOOD	CONSEQUENCES				
	Insignificant No measurable physical effects on staff/public, no medical treatment, incident of low level short term inconvenience.	Minor Minor effects on staff/public, minimal medical treatment, minor threat to safety systems, low level incident.	Moderate Serious harm to staff/public (<30%), medical treatment required, safety system breach, significant incident.	Major Single fatality or major physical harm to staff/public (30%), serious medical treatment/hospitalisation, safety systems major failure.	Catastrophic Multiple fatalities or significant irreversible damage to public/staff (>50%), large-scale medical attention/hospitalisation, complete breakdown of safety system.
Almost certain is expected to occur in most circumstances >90%	Medium (11)	Medium (16)	High (20)	Extreme (23)	Extreme (25)
Likely Will probably occur in many circumstances 70%	Low (7)	Medium (12)	High (17)	High (21)	Extreme (24)
Possible Will probably occur at some time 30-70%	Low (2)	Medium (8)	Medium (13)	High (18)	High (22)
Unlikely Could occur at some time but it is improbable 10-30%	Low (2)	Low (5)	Medium (9)	Medium (14)	High (19)
Rare May occur only in exceptional circumstances <10%	Low (1)	Low (3)	Low (6)	Medium (10)	Medium (15)

**Table 8: NRW Risk Scores**

Risk Level	Risk Score	Definition/Action Required
Extreme	23-25	Grave risk, risk not acceptable, immediate urgent action required. Stop activity immediately.
High	17-22	Risk not acceptable. Prompt management action required.
Medium	8-16	Prompt action is highly desirable. Scheduled management action required.
Low	1-7	Scheduled corrective actions as part of normal operations.

## Appendix B

Contacts for blue-green algae identification, enumeration and toxin analysis:

Queensland Government Laboratories available for blue-green algae identification and enumeration:

Organisation	Contact Name	Phone Number
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## Appendix C

### Method for the identification and enumeration of planktonic microalgae

#### METHOD SUMMARY

Microscopic examination of a 1 mL portion of Lugol's Iodine fixed sample (or part thereof) in a Sedgwick-Rafter counting cell. Concentration of the sample, if required, by Lugol's sedimentation. This methodology has been formulated in accordance with Jones et al (2002), Hötzel & Croome (1997, 1999).

#### 1.0 REAGENTS

Lugol's Iodine Solution is made by mixing 20 g of potassium iodide (KI) with 200 mL distilled water, then dissolving 10 g of pure iodine in this solution. Glacial acetic acid (20 g) is added a few days before use. This stock solution must be stored in a chemical storage cupboard, in the dark, with the date of preparation, name of the analyst who prepared the solution, and a used by date clearly marked on the bottle's label. This solution shall be used for no longer than 12 months after the date of preparation.

#### 2.0 EQUIPMENT

1. 250 mL Glass Measuring Cylinder (calibrated no less frequently than 12 monthly)
2. Plastic, disposable 1 mL pipettes
3. Glass Sedgwick-Rafter counting chamber (calibrated no less frequently than 12 monthly)
4. Disposable glass cover slips 24 × 60 mm
5. Glass microscope slides
6. Parafilm or alfoil
7. Compound Microscopes fitted with phase contrast and × 10, × 20, and × 40 (long working distance) objective lens, (photomicrography system optional)

#### 3.0 SAMPLE RECEIPT, PRESERVATION AND STORAGE

3.1 Samples should be assessed for condition prior to analysis. Samples which have not been preserved with Lugol's iodine at the time of collection should remain in the dark and chilled (< 10°C) prior to analysis. Unpreserved samples should be kept for not longer than 24 hours prior to fixation. Samples that have been frozen are not suitable for analysis.

3.2 Samples received by the laboratory in an unpreserved state require the immediate addition of Lugol's solution at a ratio of 1:100 (i.e. 1 mL Lugol's solution to 100 mL of sample) prior to examination. This gives the sample a weak tea colour.

3.3 Preserved samples should be stored in the dark, unpreserved samples retained to assist in taxonomic analysis should be refrigerated in the dark until required.

#### 4.0 SAMPLE CONCENTRATION

4.1 If the original sample cell density is low (i.e. < 100 units in 5 horizontal Sedgwick-Rafter counting chamber traverses), the sample needs to be concentrated prior to counting. This can be established by an initial assessment prior to the counting and enumeration procedure.

4.2 The Lugol's sedimentation method is the national benchmark method for sample concentration. After mixing the preserved sample by gently inverting 10 – 20 times, transfer 100 mL of sample to a calibrated 100 mL measuring cylinder and cover with parafilm or alfoil to prevent evaporation. A minimum settling period of two hours per cm of sample liquid height in the settling column is required. After allowing sufficient time for sedimentation, the top 90 mL of sample is carefully siphoned off without disturbing the sedimented algae. The remaining sample is shaken gently and transferred to a labelled 50 mL sample bottle.

## 5.0 SUB-SAMPLING TO THE SR-COUNTING CHAMBER

5.1 A wide bore disposable plastic pipette is used to sub-sample 1 mL of either the original sample or the concentrated sub-sample. Prior to sub-sampling mix the preserved sample by gently inverting 10 – 20 times, in order to avoid sub-sampling errors. This method minimises the break-up of filaments and colonies often associated with shaking the sample. Immediately pipette 1mL from the centre of the bottle with a large bore disposable plastic pipette and place into a calibrated glass Sedgwick-Rafter counting chamber (see Sedgwick–Rafter Chamber Calibration).

5.2 Before filling the cell with sample, place the disposable cover glass diagonally across the cell and transfer the sample from both sides as the cover glass rotates and the cell fills. Care should be taken to avoid over filling of the cell. No excess sample should remain outside the cell after the cover glass has been rotated into position. Before counting let the Sedgwick-Rafter cell stand for at least 20 minutes to ensure the sample has adequately settled.

## 6.0 SAMPLE IDENTIFICATION AND ENUMERATION

6.1 Using the compound microscopes, on x 200 magnification approximately one square of the Sedgwick-Rafter chamber fills the field of view. As randomly as possible, select a starting column and move to the top of that column. Moving down the column, each square is examined and cells identified are counted and recorded for each unit (i.e. colony, filament or trichome). When all units in the square have been counted and identified, proceed to the next grid square and repeat the procedure. Identification should be conducted from the appropriate texts using keys, illustrations, and species descriptions relevant to the organism. For units that cross more than one square, count only those colonies, filaments or trichomes that cross the top and right boundaries of the square.

Continue this procedure until:

1. 50 units of the most common taxa are counted (yielding a counting error of  $\pm 20\%$ ), and
2. A minimum of 23 units are counted for all potentially toxic cyanobacteria species detected (i.e. *Anabaena circinalis*, *Aphanizomenon ovalisporum*, *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*).

*For un-concentrated samples:* a minimum of 1 horizontal column traverse (20 squares) should be examined, and counting stopped after examining 5 horizontal column traverses (100 cells) regardless of the number of algal units counted.

*For concentrated samples:* a minimum of 3 squares should be examined, and counting stopped after examining 5 horizontal column traverses (100 cells) regardless of the number of algal units counted.

6.2 Live (unpreserved) samples may be useful in the identification process but should not be used for enumeration.

## 7.0 RESULTS

7.1 Results should be recorded on a standard count sheet containing the relevant sample information. At the end of the count, all cell, colony, filament and trichome counts for individual species or genera are tallied, along with the total cell count. The raw count data is converted to cell concentrations in the original sample taking into account the number of squares counted and the concentration factor for the sample. Final results are to be expressed as cells mL<sup>-1</sup> of the original sample. The result can be calculated from:

$$\text{cells per mL} = \frac{C \times V_2 \times F_1}{V_1 \times F_2}$$

where:

C = number of organisms, colonies, or trichomes counted

V1 = initial volume of sub-sample concentrated or diluted

V2 = final volume of sub-sample concentrate or dilute

F1 = total number of fields of the SR-chamber (1000)

F2 = number of squares that were examined

## 8.0 LEVEL OF TAXONOMIC PRECISION

8.1 Cyanobacterial species that have previously been recorded as producing toxins in Australia (i.e. *Anabaena circinalis*, *Aphanizomenon ovalisporum*, *Cylindrospermopsis raciborskii*, *Nodularia spumigena*, *Microcystis aeruginosa*) should be identified to species level. It is generally acceptable to identify other microalgae and cyanobacteria to genus level, however identification to species level is encouraged wherever technically feasible.

8.2 Taxa that cannot be identified by the analyst using existing reference material or through consultation with other laboratory analysts should be photographed where possible, sketches made, and measurements taken of key morphological features (i.e. vegetative cells, end cells, akinetes, heterocytes, etc.). Observation of problematic taxa under high power magnification (i.e.  $\times 1000$ ) using a flat slide may reveal additional information that will aid in identification. Ultimately advice should be sought from the relevant algal specialist or local algal workers on the identity of unresolved taxa. In the interim, each unknown organism is to be given a unique identification code that is recorded on the photograph/sketch of the specimen, along with other relevant morphological details.

8.3 Unidentified or partially identified taxa should be noted on results sheets as follows:

1. Identification to genus only: e.g. *Anabaena* sp.
2. Identification to order only: e.g. Nostocales
3. Identification to class/group only: eg. Unidentified filamentous cyanobacteria (additional morphological descriptions should accompany and results such as these)

8.4 Taxa that are similar in most respects to a known species but may deviate sufficiently to place their designation in some doubt should be indicated as cf. or aff. eg. *Anabaena* cf. *circinalis*. This indicates to the recipient of the results that we consider the species closely aligned to *Anabaena circinalis*, however some doubt remains over its exact identity.

8.5 In some cases, the individual cells in each algal unit may not be visible under  $\times 400$  magnification (maximum magnification using the Sedgwick–Rafter Chamber). Under these circumstances, the algal unit is counted as one cell, and a cell per unit conversion is applied to the final results. The cell per unit conversion is derived by examination of a sub-sample under high power magnification (i.e.  $\times 1000$ ) using a flat slide. The cells per unit are counted for a minimum of 30 units, and the median value of these measurements used as the conversion to apply to the original sample. When individual cells cannot be resolved under high power magnification, it is acceptable to use average cell measurements from the published literature and apply this to the sample.

## 9.0 QUALITY ASSURANCE

9.1 Sedgwick-Rafter Counting Chambers and measuring cylinders used in the sedimentation and concentration of samples should be calibrated no less frequently than every twelve months (see section 10).

9.2 Counting and taxonomic precision assurance is to be assessed by participation in the national NATA Algal Proficiency Testing Program, and regular participation in an inter laboratory proficiency program.

## 10.0 SEDGEWICK–RAFTER CHAMBER MAINTENANCE AND CALIBRATION

10.1 Sedgwick–Rafter Chambers should be thoroughly cleaned after the completion of each sample. Abrasive cleaning brushes should not be used on the chambers as they may scratch the glass surface. Using your finger, gently rub the surface of the chamber with the detergent solution and rinse with a firm spray of clean water. Chambers should be dried using a low-linting absorbent tissue such as KimWipes®. After cleaning the chambers should be stored face side down to prevent accumulation of dust particles on the chamber surface.

10.2 All Sedgwick–Rafter Chambers are to be calibrated once every 12 months. Each chamber is individually numbered and a record of calibration maintained. Procedure for this calibration is as follows:

1. For calibration the chamber is filled in the usual manner using distilled water rather than a preserved sample. The SR-cell volume is then determined by weighing on a 4 place balance.
2. This step is repeated five times, determining the weight of the filled cell each time onto a SR-Cell Calibration Worksheet.
3. If the filled chamber weight differs by 5% or more, a factor is calculated to correct. This factor should be marked permanently on the chamber and used when calculating final counting results.