National Environmental Monitoring Standards

Planktonic Cyanobacteria

Sampling and Processing of Planktonic Cyanobacteria from Lakes and Rivers

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The National Environmental Monitoring Standards

The current suite of National Environmental Monitoring Standards (NEMS) documents, Best Practice Guidelines, Glossary and Quality Code Schema can be found at http://www.nems.org.nz/

Implementation

When implementing the Standards, current legislation relating to health and safety in New Zealand (at the time of publication) and subsequent amendments shall be complied with. The NEMS Codes of Practice also provide guidance on Health and Safety issues and also structural design.

Limitations

It is assumed that, as a minimum, the reader of NEMS documents has undertaken industry-based training and has a basic understanding of environmental monitoring techniques. Instructions for manufacturer-specific instrumentation and methodologies are not included in this document.

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Development

The National Environmental Monitoring Standards (NEMS) Steering Group has prepared a series of environmental monitoring standards on authority from the regional chief executive officers (RCEOs) and the Ministry for the Environment (MfE).

The development of this Standard involved consultation with regional and unitary councils across New Zealand, industry representatives, and the National Institute for Water and Atmospheric Research Ltd (NIWA). These agencies are responsible for the majority of phytoplankton environmental-related measurements within New Zealand.

This standard has been prepared by a core working group comprising Karl Safi (lead technical writer – NIWA), Eloise Ryan (WRC), Andy Hicks (HBRC), Manas Chakraborty (NRC), Hugo

Borges (ORC). Rachel Herbert (ECan) provided oversight as the NEMS Steering Group representative.

The working group gratefully acknowledge:

- the laboratory taxonomic specialists who attended a workshop with a subset of the working group and generously contributed their time and expertise to add to and review draft material: Hugo Borges (ORC), Helen Brider (NIWA), Marina Fisher (Watercare), Catherine Moisan (Cawthron), James Dare (BOPRC), Katie Blakemore and Darin Sutherland (TRC).

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- Otago Regional Council Environment (ORC),
- Taranaki Regional Council (TRC)
- Horizons Regional Council (HRC)

Review

This document will be reviewed by the NEMS Steering Group within one year of its release and thereafter will be assessed for review approximately once every two years. Further details on the review process can be found at www.nems.org.nz. Users of this document are encouraged to provide feedback via the NEMS website.

Change Register

Version Number	Date		Section	Significant Change
0.0.1				Initial draft
0.0.2	April/May 2022			Updated draft
0.0.3	November 2022			Final Draft
1.0.0	July 2023			Publication of Version 1.0.0

FEEDBACK

If you wish to provide any feedback regarding this version of the document please provide it to:- https://www.nems.org.nz/feedback/

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Terms, Definitions and Symbols

Relevant definitions and descriptions of symbols used in this Standard are contained within the NEMS *Glossary* available at www.nems.org.nz.

Note: The definitions below will be removed from this document and transferred to the NEMS Glossary at the first revision of this document.

For the specific purposes of this document, the following definitions apply:

Phytoplankton	Microscopic free-floating aquatic plants (algae).
Cyanobacteria	Blue-green algae. These are potentially toxic. They can adjust their depth in the water column using small gas bladders (gas vacuoles), and some species can use (i.e. fix) atmospheric nitrogen for growth when nutrient nitrogen in the water column is depleted.
Biomass	The living mass of the phytoplankton or zooplankton populations.
Thermal stratification	Separation of a water column into two layers by temperature (warmer water on top).
Thermocline	The boundary zone or temperature gradient between the two layers in a thermally stratified water column.
Epilimnion	The upper water column in a thermally stratified water column.
Hypolimnion	The lower water column in a thermally stratified water column.
Metalimnion	The thermocline zone (of variable thickness).
Euphotic zone	The upper water column in which there is sufficient light for photosynthesis and hence phytoplankton growth.
Euphotic depth	Lower limit of phytoplankton growth where light levels are 1% of surface irradiance.
SoE	State of the Environment (SoE).
CTD	A profiling instrument that measures conductivity, temperature and depth.
TLI	Trophic Level Index.
FOV	Field of view.

Normative References

This Standard should be read in conjunction with the following references:

- NEMS *Glossary*
- NEMS *Quality Code Schema*
- NEMS *Safe Collection of Environmental Data*: *Guidelines for safe working when undertaking environmental monitoring,*
- NEMS Water Quality Part 2 of 4: *Sampling, Measuring, Processing and Archiving of Discrete River Water Quality Data*
- NEMS Water Quality Part 3 of 4: *Sampling, Measuring, Processing and Archiving of Discrete Lake Water Quality Data.*

About this Standard

Introduction

Monitoring of planktonic cyanobacterial communities is carried out in lakes and rivers across New Zealand for many purposes. Phytoplankton and algal monitoring is also often undertaken, as algae can be key indicators of ecosystem health. Planktonic cyanobacteria, however, are unique in that some can produce toxins that can affect human, animal and ecosystem health in freshwater systems. While, because of their ability to produce toxins, planktonic cyanobacteria are often assessed for focused compliance with drinking water standards or guidelines for recreational use, they also remain a fundamental measurement of aquatic ecosystem health. The monitoring of long-term changes in planktonic cyanobacteria for 'Human Contact' is also now mandatory under the National Policy Statement for Freshwater Management (NPS-FM). Cyanobacteria, like other algae, are also integral to aquatic food webs and are affected by physical, chemical and biological conditions, responding to changes in water quality, habitat and invasive species.

Planktonic cyanobacteria may also show the cumulative impacts of pollution and other stressors present within catchments and may reflect climate change, with many cyanobacterial species better suited to warming conditions. Assembling a valuable long-term freshwater planktonic cyanobacteria data record is underpinned by a consistent approach to sample collection, sample processing and data management. This is because information from planktonic cyanobacterial monitoring may be influenced by both sampling approach and the performance of laboratory staff who process, identify and count the taxa present.

This Standard has been prepared principally for field technicians, programme managers and environmental scientists who collect, quality control or report on freshwater planktonic cyanobacterial data. The primary focus is on data acquisition (sampling and processing), quality assurance (QA), and preparation of data associated with long-term State of the Environment (SoE) monitoring programmes. The principal purpose of SoE planktonic cyanobacterial monitoring in water bodies is to assess state (condition) and trends in ecosystem health through time. As mentioned above, planktonic cyanobacterial monitoring is carried out by many agencies for purposes other than long-term monitoring for SoE reporting, namely, to assess compliance with human drinking water standards[1] (following the associated guidelines[2)] and potential impacts on recreational use of waterbodies[2]. Wider monitoring may also occur for the characterisation of biodiversity. While sampling for these additional purposes is not addressed in detail in this document, some guidance is provided, and the underlying principles of sample collection and processing are relevant. This Standard, therefore, provides an up-to-date normative reference for most planktonic cyanobacterial monitoring carried out in lakes and rivers across New Zealand.

[1] The Water Services (Drinking Water Standards for New Zealand) Regulations 2022 came into force on 14 November 2022 and the Drinking-water Standards for New Zealand 2005 (revised 2018) was revoked.
[2] *New Zealand Guidelines for Cyanobacteria in Recreational Waters (Interim Guidelines)* (Ministry for the Environment and Manatu Hauorta - Ministry of Health, 2009). [3] dwg-ch9-cyanobacterial-compliance (available online).

Objective

The objective of this Standard is to ensure planktonic cyanobacterial sample collection, processing, numeric calculation of cell concentration and biovolume, and their associated quality assurance and quality control practices are consistent across New Zealand. This document comprises the Standard (presented first) followed by supporting information that describes the procedures practitioners are required to implement to achieve the Standard.

It is recommended that this Standard is adopted throughout New Zealand and all data collected be processed and quality coded appropriately to facilitate data sharing. The degree of rigour with which the Standard and associated best practice may be applied will depend on the quality of data sought.

Scope

The Standard covers planktonic cyanobacterial sampling in freshwater lakes and rivers (usually large, lake-fed rivers). The focus is primarily on obtaining representative samples of the planktonic cyanobacteria from the sampled water body (i.e. a lake or large river). Guidance is also provided on wadeable shoreline sampling where boat access may not be available. This Standard focuses on sampling to determine state and detect long-term changes in planktonic cyanobacterial populations in a water body as a whole (e.g. SoE monitoring). However, for completeness, some guidance is also provided on planktonic cyanobacterial monitoring conducted to assess compliance with drinking water standards and recreational use guidelines.

The Standard addresses:

- fieldwork preparation
- sampling site selection
- sampling equipment preparation and use
- field observations and associated measurements
- chain of custody (CoC)
- sample collection methods
- sample handling, preservation, storage and transport
- laboratory processing, taxonomic resolution and reporting
- calibration of equipment
- validation of calculations
- quality assurance (QA) and quality control procedures
- data quality coding
- data archiving.

If planktonic cyanobacterial samples are collected for a defined monitoring objective(s) this will influence the selection of sampling site(s), the timing and frequency of sampling, and the sample

collection and processing methods. It is beyond the scope of this document to address all monitoring objectives and sample design elements pertaining to a long-term monitoring programme, but a robust monitoring design is essential for establishing a valuable long-term planktonic cyanobacterial data record.

Identification, cell counts and biovolume of planktonic cyanobacteria are addressed in this Standard. Cell count and biovolume are the two measures typically calculated from routine (SoE) planktonic cyanobacterial samples.

Relationship with Existing Guideline Documents

Protocols for sampling planktonic phytoplankton and planktonic cyanobacteria in lakes and rivers have existed for decades (Graham et al., 2008; Burns et al., 1999, 2000; Hötzel and Croome, 1999). For example, the Trophic Level Index (TLI) lake sampling methods introduced in Burns et al. (1999, 2000) are an example of methods developed specifically for wider SoE monitoring programmes. More recently, specific methods have been developed to address 'recreational contact' with cyanobacteria and these are covered in the *New Zealand Guidelines for Cyanobacteria in Recreational Waters* (Ministry for the Environment and Ministry of Health, 2009) and their subsequent review (Wood et al., 2018). Ongoing developments have also occurred for cyanobacterial analysis in relation to drinking water standards (Ministry of Health, 2022. However, Taumata Arowai still refers specifically to 'Chapter 9: Cyanobacterial compliance' for drinking water guidelines.

Monitoring of cyanobacteria for recreational contact is focused on the water surface (top 50 cm), often around the wadeable margins of a water body, rather than obtaining a representative concentration of the bulk of the water body. Monitoring for drinking water standard compliance is focused on the point of abstraction. In addition, these types of monitoring focus on compliance with maximum values, rather than monitoring long-term changes in cyanobacteria. This Standard differs from other guidelines in that it focuses on monitoring long-term state and changes in cyanobacteria for a water body overall.

We recognise that resourcing may not allow for all recommended sampling protocols and therefore we also provide guidance on the grade (quality code) given to different data collection methods in terms of its suitability for long-term SoE monitoring; i.e. an integrated, middle water body sample will be assigned a higher quality code than an edge grab sample. This does not mean the data collected for purposes other than SoE monitoring are not good quality; it simply means that it may not be as representative of the larger water body.

Principles for NEMS

This Standard requires all collected data to be filed with its required metadata, including a quality code.

Data that are collected, processed, and archived in a verifiable and consistent manner according to this Standard can meet the highest quality code (QC 600). Data that do not meet QC 600 shall be coded appropriately following the Quality Matrices (see Table 3) and Quality Code Schema (see Figure 7).

Note: Enduring use – It is important to note that data that are coded lower than QC 600 may be restricted in their use for a wide range of (yet unknown) purposes in the future.

The Standard – Planktonic Cyanobacteria

As a means of achieving planktonic cyanobacterial data of the highest quality under this Standard (i.e. QC 600), the following requirements shall apply:

<table>
<tr><th colspan="3">Field measurements and sample collection</th></tr>
<tr><td>Training</td><td colspan="2">Planktonic cyanobacterial sampling, processing of samples in the field and collection of associated metadata shall be undertaken by trained staff following a procedure documented in an Office and Field Manual (or equivalent) that is consistent with the supporting information contained in this Standard.</td></tr>
<tr><td>Quality Assurance</td><td colspan="2">The planktonic cyanobacterial sample collection equipment (such as Van Dorn or tube samplers) and materials (such as bottles and preservative) shall be checked before sampling.

All sampling staff shall be initially trained by experienced personnel who are competent in these procedures, with training records to be maintained.</td></tr>
<tr><td rowspan="2">Sampling Equipment Specifications</td><td>Planktonic cyanobacterial sampling options</td><td>When selecting the type of sampler, consideration shall be given, where practical, to the type of lake system and whether it is mixed or stratified. Sampling equipment will be either a;

Van Dorn sampler: Van Dorn bottles (2–10 L capacity) are designed to capture water at selected depths below the surface. Samples may be composited or analysed per depth.

or a;

Tube sampler: a PVC tube (at least 25 mm diameter) of 2 m to 10 m length, with a weight and a rope attached to one end. This is used to provide an integrated surface water sample.</td></tr>
<tr><td></td><td>The sampling instrument shall be cleaned between sites and inspected for damage before each use.</td></tr>
<tr><td rowspan="2">Site Location and Changes</td><td colspan="2">The sampling site shall be chosen with characteristics that are representative of the broader lake or river environment. Consideration shall be given, where practical, of the type of lake system and whether it is mixed or stratified.</td></tr>
<tr><td colspan="2">The location for sample collection shall be clearly identifiable and retained through time as far as is possible.

Note: A permanent record shall be kept of any necessary changes to the site location (e.g. because of change in access or flood disturbance).</td></tr>
<tr><td>Site Metadata</td><td colspan="2">Site metadata should include, at a minimum, site name/unique identification ID (matching CoC), location (GPS location as latitude and longitude), elevation</td></tr>
</table>

	(NZVD 2016 or other known vertical datum) and the prescribed sampling method used (Tube, Van Dorn).	
Visit Metadata	Field Record Form	A Field Record Form shall be completed on each site visit that includes name of the water body, site name/unique identification ID (matching CoC), location (GPS location), date/time of sampling, the prescribed sampling method used (Tube, Van Dorn, name(s) of field personnel, and sample identification number(s). Associated data may also be recorded such as sample identification number(s); environmental conditions (e.g. weather), any unusual conditions at the site (e.g. atypical water clarity), and associated supporting measurements (e.g. water depth and velocity, water temperature, conductivity, pH).
Timing of Measurements	Records	The time of sample collection shall be recorded in New Zealand Standard Time (NZST) to the nearest 15 minutes. *Note: Do not use New Zealand Daylight Time (NZDT). If supporting discrete water quality measurements are made, record the sample collection time to align with the timing of these measurements.*
Planktonic cyanobacterial sampling	Sample collection method	Planktonic cyanobacteria samples shall be collected using either the Van Dorn or tube sampling method.
	Sample handling & preservation	Samples shall either be preserved at the time of sampling with Lugol's iodine. The use of ~5 mL of Lugol's solution per litre of sample is standard. However, it should be noted that for meso- and especially oligotrophic waters no more than 2 mL may be required. See Annex D for use and preparation of Lugol's iodine. or; If not preserved immediately (see section 1.6) unpreserved samples shall be transferred to the laboratory within 24 hours of collection, maintained in the dark, at less than 10°C, and once at the laboratory they should be preserved with Lugol's iodine (Annex D). If required, the sample may be split and a fresh sample can be kept for taxonomic reference only.
	Sample storage	Samples shall be preserved and stored in the dark until analysed.

	Sample traceability and integrity	Labelling	Planktonic cyanobacterial samples shall be clearly and permanently labelled on the side of the sample container with, as a minimum: • site name (and unique number where applicable) • sampling date and time • sample type (Cyanobacteria) It is also recommended to add: • sampler name or initials, • container number if applicable.
		Chain of Custody Form	Samples shall be accompanied by a completed Chain of Custody Form (CoC) that provides sample traceability from the field to the laboratory. CoC should at a minimum include analysis required (cyanobacteria), site name (and /or unique identification number that is consistent with Site ID), date and time of sampling, and identify the sampler. Settling time, date and date of analysis should also be recorded.
Laboratory measurements			
Certification	Planktonic cyanobacterial samples shall be processed by staff trained in taxonomic identification, enumeration (cell numbers) and biovolume assessment with evidence of the following: • adherence to formal standard protocols (accredited) or subsequently implemented protocols (i.e. this Standard), that include internal and external QA and quality control practices, and • regular (at least yearly) external quality control of planktonic cyanobacteria identification accuracy, enumeration accuracy and biovolume determination. Trained staff must follow a documented procedure in an Office Manual (or equivalent) that is consistent with the supporting information contained in this Standard.		
Sample arrival at the laboratory and pre-testing preparation	Documentation	Laboratory staff shall record the date and time of receipt of samples on the accompanying Chain of Custody (CoC) Form, together with: • sample temperature check • sample preservation check • any anomalies in sample condition with the potential to impact the sample (e.g. damaged sample container)	

	Temperature	Fresh samples shall be less than 10°C, unfrozen and free of ice crystals.	
	Processing and testing timeframes	Preservation of fresh samples shall occur within 24 hours of sample collection. {Preserved samples should be processed as soon as possible and ideally within seven days or a maximum of three months after sample collection (Annex D)}.	
	Pre-treatment	Appropriate pre-treatment for buoyancy and colony disaggregation shall be carried out as necessary as described in the supporting information to this Standard	
Test Method and Measurement Requirements	**Variable**	**Measurement unit**	**Test methods**
	Cell numbers	Cells per mL	Microscope analysis using Utermöhl chambers (10–100 mL subsamples) or Sedgwick-Rafter chamber (1 mL subsample)
	Biovolume	$mm^3\ L^{-1}$	Estimated from literature or direct microscope measurements based on geometric shapes (see 5.3.2, Annex J and Measurement Resolution Biovolume below).
	Taxa	Genera	Cyanobacteria shall be identified to genus level, and if possible, species level.
Measurement Resolution	Cell numbers	A minimum of fifty units of the dominant genera/species shall be counted, giving a 95% confidence limit of 28% (Edler, 1979). The precision is usually expressed as the 95% confidence limit as a proportion of the mean. Some species may not be sufficiently abundant, which will decrease the overall precision. To maintain an acceptable precision for the entire sample a total of at least 250 units should be counted and ideally at least 500 units (EPA 2010, Reavie 2013; Edler, 1979) A Sedgwick-Rafter settled sample of 1ml will give a detection limit of 1 cell per ml (sufficient for algal cell densities between 10,000 and 100,000 cells L^{-1}). For low concentration samples larger settling volumes should be	

		used increasing (10-100mls) the detection limit and number of cells available to count (ie. an Utermöhl chamber sample of 10ml will give detection limit of 0.1 cells per ml). The counting error (precision) can be estimated with a 95% confidence limit by: $=2/\sqrt{N} \times 100$ = counting error (± %) (see Annex F) For example, if 100 units are counted, the error is ± 20%; if 400 units are counted the error is reduced to ± 10%.
	Biovolume (see 5.3.2, Annex J).	The biovolume of cells shall be derived in μm^3 from measured dimensions of at least 20 cells (if ≥20 cells are present) using the appropriate geometric shape (see Annex J). In taxa that contain specialised cells, such as akinetes and heterocytes, volume measurements shall be of vegetative cells only. The total biovolume (BV) in mm^3/L of each species shall be calculated using the following formula: $BV = (n \times vol) / 1 \times 10^6$ where: n = number cells in a sample (cells/mL) vol = volume of a single cell (μm^3) 1×10^6 is a conversion from $\mu m^3/mL$ to mm^3/L.
	Taxa	It is recommended that species level taxonomy be reported where possible.
Data Records	The laboratory measurements shall be provided in a report that specifies: • the dates and times of sample collection, the date of receipt at the laboratory and the date of analysis at the laboratory • the measurement method including details of any modifications made to these, and • any anomalies in the condition of the sample upon receipt (e.g. temperature on arrival, sample outside recommended analysis timeframes) or the subsequent measurement value.	
Quality Coding	All data shall be quality coded as per the Quality Codes flowchart (Figure 7).	

The following summarises additional best practice measures. These measures are recommended but are not required to meet QC 600.

Field Documentation	Visit metadata and any associated measurements/observations are recorded electronically in the field.
Sampling locations	Include selected sampling sites based on hydrographic properties of the water body and depth resolved sampling based on conductively temperature depth profiles (CTD) and /or oxygen profiles.
Metadata / Supporting Variables	These may include profile measurements of fluorescence (Chlorophyll *a*, Phycocyanin or phycoerythrin), temperature and density. Sampling for nutrients and /or oxygen profiles.
Quality Control	Interagency comparisons are conducted on annual basis between laboratories.
Sample Storage	Storage temperature of < 10°C is used for all samples including Lugol's preserved. It is also preferred Lugol's iodine be added immediately at the time of collection.
Sample Retention	Preserved samples are kept for 3 months should reanalysis be required.
Laboratory Reporting	It is required that the laboratory reports cyanobacterial results in a fixed .pdf report format (as a permanent unalterable record) but it is also recommended that a .csv or equivalent format be provided to enable data to be directly added to a database with detection limits where practical.
Reporting and Data Checks	Data reported by the laboratory are checked by the collection agency against any other estimates of biomass made at the time of sample collection within three weeks of receipt to enable re-testing if necessary.
Data Archiving	File, archive indefinitely, and back up regularly in a time-series database: • site and field visit metadata • other biomass assessment methods, including the planktonic cyanobacteria categories recorded • point data for related variables, such as those associated with a CTD and/ or dissolved oxygen probes • biomass analysis method(s) • date and condition of sample receipt at the laboratory, and • any sample anomalies or analytical method modifications, including quality checks performed on these.
Data Auditing	Auditing requirements will be developed in the future.

1 Before You Leave the Office

In this section

This section outlines matters that need to be addressed prior to leaving the office, including health and safety considerations, sample record (field) forms, sampling equipment, sample bottle requirements and preservatives/pre-treatment, sample handling, and quality assurance.

1.1 Office and Field Manual

It is important that everyone involved in a planktonic cyanobacterial monitoring event/programme understands the objective(s) and has received sufficient training to carry out all assigned tasks and procedures correctly. The objectives and procedures shall be documented in an Office and Field Manual (or equivalent) that also addresses, in detail, the matters set out in the remainder of this section, and other in-house ancillary procedures.

1.2 Health and safety

Collection of planktonic cyanobacterial samples from lakes and rivers involves some elements of danger that shall be considered in a Health and Safety Plan, prepared in accordance with the monitoring agency's organisational processes. Safe access to monitoring sites is particularly important. Only trained personnel shall be involved in fieldwork and suitable lone worker procedures are required if lone work is unavoidable. Appropriate personal protection equipment, such as high-visibility clothing and flotation aids, should be worn to ensure safety. Field personnel should wear gloves when sampling in streams likely to present a significant health and safety risk (e.g. known to contain toxic cyanobacteria or sewage contamination).

Planktonic cyanobacterial sampling can be done using several methods that have different safety issues; these methods include:

- Boat sampling – Sampling from boats safely requires multiple trained personnel.
- Edge or wharf sampling – Sampling from a bridge or wharf may be conducted safely by a trained individual. Extra care should be taken when working from heights.
- Wading (which can be carried out in waters up to 0.6 m deep that can be waded safely). Wading can be hazardous depending on the water body and prevailing conditions and can require more than one individual to be present. Special care is needed to avoid slipping on substrate and when sampling during swift and/or turbid river flow.

For all types of sampling, the monitoring agency's procedures must be followed. Appropriate safety procedures and safety equipment must be provided.

For further guidance on safety precautions when collecting planktonic cyanobacterial samples refer to the NEMS *Safe Collection of Environmental Data: Guidelines for safe working when undertaking environmental monitoring*.

1.3 Quality assurance

Field sampling is a critical step in collection of reliable data on planktonic cyanobacterial communities. Any errors made during sample collection, transport or storage cannot be corrected later. Quality assurance (QA) procedures are therefore necessary to monitor the effectiveness of the field methodology and demonstrate that the various stages of fieldwork are adequately managed to minimise errors.

The QA procedure should comprise all the steps described in this Standard to ensure that valid data are produced. This includes documented evidence that sampling personnel are trained and competent, sampling equipment is well maintained, appropriate sample collection methods are used and the correct preservation and handling methods are employed. In particular, sampling personnel should be given training in different water body sampling strategies, sample collection techniques, in-field sample processing and correct completion of Field Record Forms (either with an experienced member of staff or through a suitable external agency). This is best done through a combination of annual internal training exercises and periodic interagency audits (outlined in subsection 1.3.1). QA (and quality control) programmes are mandatory components of laboratory practice to ensure measurements are accurate. Refer to subsection 4.3 for details on sample storage, handling and transport.

1.3.1 External 'field audit'

As a requirement of this Standard, staff training and performance shall be reviewed at least every three years through an interagency sampling exercise. This QA exercise shall:

- Collect and deliver to the laboratory samples that are representative of the cyanobacterial community at the site and are adequately stored or preserved, and

The QA field exercise should target specific practices relating to:

- selecting appropriate methods to sample the selected water body
- collecting and handling cyanobacterial samples, including adequate mixing and preservation of samples and labelling of sample containers, and
- completing the Field Record Form/ Chain of Custody Form, including site visit data.

The exercise should involve two or more staff simultaneously, but independently, completing the tasks above.

1.3.2 Other checks

Other checks can be made by the sampling agency to assess field practice and various aspects of the laboratory testing that could affect data accuracy. For sampling, this may be as simple as sending experienced observers to assess field performance and/or checking of routine labelling of containers and the details provided in Field Record and COC forms. To assess laboratory performance, the sampling agency could request that a planktonic cyanobacterial sample be split and a subsample sent to another laboratory for analysis. As samples can degrade with time, care would be needed to ensure that both laboratories proceed with analysis in the same timeframe to ensure a meaningful inter-laboratory comparison. In general, the sampling agency will need to rely on internal laboratory quality control or formal inter-laboratory testing (subsections 5.5 and 6.3.5).

1.4 Field Record Form

A standard Field Record Form (or/and CoC form) shall be used to record field visit data (both observations and any field measurements that may be collected). This form provides a vital record that verifies the location, timing, methods and conditions under which sampling was carried out, along with other factors that may influence the data being collected (for example, heavy rain, wind direction). This record is also essential for later reconciliation with cyanobacterial data received from the laboratory (section 6).

Electronic Field Record Forms are becoming increasingly common and have the advantage of offering more efficient data capture and the ability to build in automated calculations and checks to ensure records are correct at the time they are made.

If paper forms are used, waterproof paper and/or a suitable pen or pencil is recommended to protect the integrity of the record.

A CoC form may be a separate record but should have all primary information for tracking and analysis of the sample and must accompany the sample to the laboratory for analysis.

Useful metadata that shall be recorded when collecting cyanobacterial samples are outlined in subsection 3.2 and an example Field Record Form is provided in Annex B.

Field personnel shall be trained on how to correctly complete Field Record Forms.

Note: Use of customised Field Record Forms with pre-printed site names and site numbers, and standardised text options for sampling method, habitat types and weather conditions, will reduce time spent filling out the form on site and provide for consistent record keeping.

1.5 Field equipment

The core monitoring equipment that will generally be required includes:

- life jackets, waders, and other personal protection equipment (as required)
- gloves and personal protective equipment to avoid exposure to cyanotoxins
- field forms to record site and visit metadata and field measurements
- equipment for planktonic cyanobacterial sample collection –
    - Van Dorn sampler (see sections 1.5.1 and 4.2.2) or tube sampler (see sections 1.5.2 and 4.2.3)
    - rope or wire with depth increments
- waterproof marker pens and pencils
- cool, dark storage for samples (e.g. chilly bin with ice packs or ice cubes)
- disinfectant and associated gear for completing “Check, Clean, Dry” procedures (Ministry for Primary Industries, 2022)
- a camera, and
- a GPS receiver.

A calibrated multivariable hand-held field meter or CTD can be used if spot measurements or profiles of water quality variables, such as florescence, water temperature and conductivity, are required.

It is essential that all equipment and devices are properly maintained and cleaned to avoid contamination of phytoplankton cyanobacterial samples and to prevent transfer of unwanted freshwater organisms.

1.5.1 Van Dorn sampler

The Van Dorn sampler provides a means of obtaining water samples at selected depths below the surface. Van Dorn bottles were first developed by Scripps Institute of Oceanography and include vertical and horizontal bottles. These samplers are usually used to obtain composite samples from several depths or to pool samples from one depth and thus can be used for both horizontal and vertical sampling (Figure 1).

Vertical bottles are useful for obtaining an integrated sample over the bottle length, which is useful when targeting a feature that may be missed if the exact depth is not known.

Horizontal bottles are often used for sampling features such as the thermocline, at other stratification levels, or just above the bottom. Because they collect whole water samples, all size classes of phytoplankton/cyanobacteria are obtained.

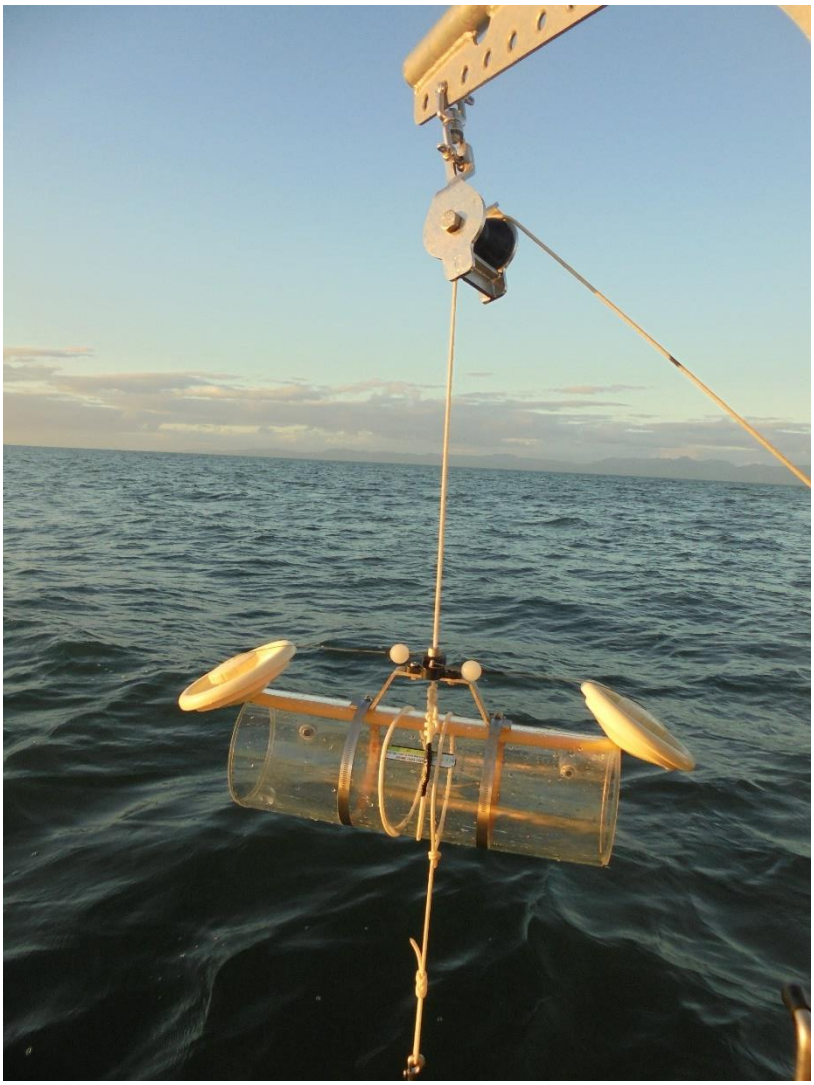

Figure 1. Set Van Dorn bottle ready to sample

Instruction for using a Van Dorn bottle are given in subsection 4.2, 'Sampling Procedures'.

A Van Dorn bottle may also provide a platform to which other instruments, such as thermometers, can be attached to record other water quality parameters during the deployment of the Van Dorn bottle. To collect samples from multiple depths, multiple Van Dorn bottles can be attached to the same line and lowered to different pre-selected depths before being triggered.

1.5.2 Tube sampler

The tube sampler provides a means of obtaining an integrated surface water sample. This is less time consuming than obtaining samples from multiple depths. It is best when used in well mixed or isothermal waters. Its capacity to integrate surface water, however, allows it to represent a larger depth range. The sampling tube's length should ideally cover the depth of the

photic zone to ensure active subsurface concentrations of cyanobacteria are incorporated within the sample.

1.5.3 Short tube/grab composite sampling

This type of sampling is designed to measure maximum cyanobacterial populations for recreational contact around lakes or reservoirs and it is the standard for contact recreation. It may be less representative of long-term water body concentrations for SoE monitoring especially if used at only selected shoreline sites where, for example, wind concentration has occurred. However, it may be fit for purpose if used at selected representative sites in well-mixed water bodies (see subsection 4.2.4). QC 600 cannot be assigned to data obtained from short tube / grab composite sampling, however, this does not exclude this data from a QC500 rating if taken from a well-mixed waterbody at a representative site .

The rationale for this sample type is that:

- the 50 cm integrated column or tube covers the surface zone (0.5 m) that recreational users are most likely to be exposed to
- the sampling of this surface zone represents the accumulation of buoyant cyanobacteria near the surface under calm conditions
- the recommendation for five pooled sub-samples accounts for spatial variability within a single site.

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

1.5.4 Sample containers and labels

Sample containers should be clean, non-toxic glass or PET plastics of 50–1000 mL volume, to avoid absorption of Lugol's iodine solution or deterioration of samples (Annex D, Figure 2).

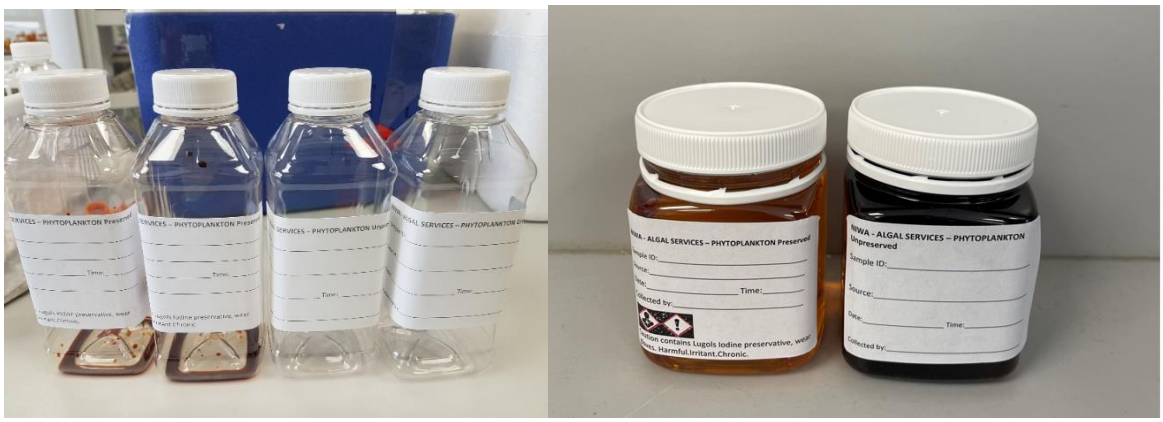

Figure 2. Examples of PET bottles used for sampling

Note: Use of dark or opaque sample bottles will reduce the potential for light to degrade samples.

All sample containers shall be uniquely labelled as outlined in subsection 3.2. Waterproof labels are recommended to avoid labels coming off the bottles if they get wet. On-site labelling is recommended to prevent accidental switching of pre-labelled bottles.

Note: Use of customised sample bottle labels with pre-printed site names and numbers can reduce labelling time in the field.

1.6 Sample storage and transport

Labelled planktonic cyanobacterial samples collected for laboratory analysis shall be preserved on site with Lugol's iodine (Annex D) or shall be firmly sealed and placed in a storage container (e.g. chilly bin), with ice or ice packs, promptly and not left exposed to daylight where they may be subject to warming and degradation.

Unpreserved samples should be transported to the laboratory as soon as possible after collection (subsection 4.3 and shall arrive at the laboratory within 24 hours of the time of collection to be preserved for analysis or assessed for species composition immediately (i.e. fresh).

Note 1: Fresh cyanobacterial samples carry the risk of transferring live cells of the unwanted organism didymo across regions. Once samples are preserved there is no risk of such transfer. To minimise risk, and to avoid having to take required biosecurity precautions, monitoring agencies and laboratories may prefer to routinely preserve samples, which is also required for analysis.

Note 2: Preserved cyanobacterial samples intended for analysis should only be stored for 3 months but may be kept longer if maintained (additional Lugol's iodine is added and the sample is kept in a cool, dark environment; see Annex D).

1.7 Chain of Custody

A Chain of Custody (CoC) form (Annex C and section 5) shall accompany cyanobacterial samples to provide an audit trail from sample dispatch to sample arrival at the laboratory, and a record of sample condition on arrival at the laboratory (subsection 5.2). The CoC information shall be used to provide a quality assurance check on sample integrity (subsection 6.5).

2 The Monitoring Site

In this section

This section outlines monitoring site considerations, with a specific focus on identifying lake and river sites for planktonic cyanobacterial sampling. Site metadata requirements are also addressed.

2.1 Site selection and number of sites

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

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

Due to these factors, monitoring programmes for planktonic cyanobacteria should be tailored to the characteristics of each water body (also see section 4). Collection of historical information on blooms and growth conditions, and the identification of patterns of cyanobacterial growth, can be used to help focus the monitoring programme on critical periods and locations in the water body of interest.

It is now possible to use satellite imagery and the Google Earth Engine to look at historical time series of predicted chlorophyll *a* in lakes to assist with future sampling plans (Allan et al., 2015) and site selection.

The aims of the sampling protocols include being able to assess long-term change in planktonic cyanobacteria in different waters bodies. Phytoplankton, and therefore cyanobacteria, should be collected from open water sites, so that tychoplankton or periphyton growing in the littoral region do not contaminate the samples and interfere with long-term data interpretation.

The number of cyanobacterial sampling sites required within a water body depends largely on the variability between sites and on how well the open water community is to be described. To obtain a precise estimate of cyanobacterial/phytoplankton abundance in the field it is recommended to (in the absence of specific information):

- sample no fewer than two widely separated sites but if sampling is to be limited to one site in a lake, then this shall be at an index site. Index sites are usually located at a mid-lake position, as far from nearest shoreline as practical
- if the surface area of a lake is between 2 and 4 km^2 then it is recommended that 3 sites are sampled, if between 4 and 10 km^2, 5 sites, and if > 10 km^2 then 10 sites should be sampled if possible.

The location of cyanobacterial sampling sites should include sites selected for water chemistry to ensure the maximum correlation of findings. Many papers and reports on phytoplankton

sampling design have been written (Graham et al., 2008; Hötzel and Croome, 1999; Burns, 1999, 2000; Pridmore, 1987; Irish and Clarke, 1984).

Fixed sites can be sampled within a broader monitoring programme to provide linear time series, but these can be supplemented by sampling where cyanobacterial scums are visible during sampling.

The selection of sampling sites is a key factor in collecting representative samples. The monitoring site shall therefore be easily identifiable, by GPS location or with a permanent location marker (buoy or physical location in space, i.e. a location point on a bridge). This is to allow for consistency in the location of field measurements and sample collection through time.

2.1.1 Wadeable sites

Safe access to larger rivers from bridges and boat access to lakes may not always be possible. However, it may be appropriate to carry out cyanobacterial monitoring in wadeable areas, or from wharfs, if these positions meet the general requirements for monitoring as outlined in subsection 2.1.

Note: The requirement for representativeness of the broader river or large water body (subsection 2.1) may be difficult or impossible to achieve. In these cases, agencies may choose to monitor planktonic cyanobacteria from a wadeable area or wharf location with the data recorded eligible for QC 400 or QC 500 depending on the degree of departure from non-representative conditions. Information should be provided in site and visit metadata to clarify the conditions at the time of such sampling.

2.2 Site changes

Site access may change over time, either by limitation of boat access (weather extremes) or limitation of location access (e.g. for rivers due to changes to access to private property or erosion that make tracks too dangerous to negotiate).

In these cases, a cyanobacterial monitoring site may need to be relocated to another site that remains representative of the water body. Ideally, the new monitoring site will also match the key features of the previous site and will be close to the original site.

Changes to features such as water depth (lakes and rivers), flow velocity (rivers) or wind mixing exposure (lakes) shall be clearly identified and new GPS co-ordinates recorded in the site metadata.

2.3 Site metadata

The following site metadata shall be recorded in the Office and Field Manual (or equivalent) at the time the monitoring is established:

- specific health and safety considerations
- lake or river name
- site name and number/unique code

- site location – e.g. GPS units or NZTM latitude/longitude coordinates, expressed to a minimum of six decimal places for the monitoring site.

Adequate mechanisms shall be put in place to store all relevant site-related metadata with the actual data records (see subsection 6.1.1).

Site metadata shall be reviewed, and records updated as necessary, annually. If a new monitoring site is required, the site metadata shall be managed accordingly.

3 Monitoring Principles and Procedures

In this section

This section covers general principles and procedures of planktonic cyanobacterial monitoring and those that apply to collection of samples for laboratory biomass analyses. These principles and procedures are divided into those related to monitoring programme design and those that apply once field personnel are on site to collect planktonic cyanobacterial samples.

3.1 Monitoring programme requirements

The timing and frequency of cyanobacterial monitoring depends on the purpose of the monitoring programme. This Standard primarily focuses on long-term SoE monitoring, which is often combined with monitoring related to the planktonic cyanobacterial attribute under the NPS-FM.

However, the methods used to assess samples for biomass (as biovolume) can also apply to monitoring carried out to assess compliance with drinking water standards and recreational use guidelines (Ministry of Health, 2020; Ministry for the Environment and Ministry of Health, 2009). These forms of monitoring are therefore briefly mentioned here.

This Standard concerns planktonic cyanobacterial standing crop (i.e. abundance of biomass at a particular time) and is limited to assessment of cyanobacterial standing crop by microscope identification of genera, cell counts and biovolume (biomass) estimates. Planktonic cyanobacterial standing crop can, however, be quantified using other methods, which are becoming more common but are not yet comparable to the Standard. These methods include:

- laboratory analysis of biomass samples for phycocyanin or phycoerythrin
- in situ analysis of phycocyanin or phycoerythrin
- 16S rRNA and other genetic profiling for cyanobacteria and their toxin-producing genes
- for small cyanobacteria (< 2 µm picophytoplankton), analysis by Flow Cytometry may be preferable.

These methods are discussed in Annex I.

3.1.1 Cyanobacterial identification and biomass

This Standard addresses identification of cyanobacterial genera and measurement of cell numbers (per mL) and biovolumes (mm^3 per L). This Standard follows a format using microscope identification, cell counts and biovolume estimates as these are the most commonly used measures of planktonic cyanobacterial biomass around the world at this time (Vasconcelos et al., 2011; Pobel et al., 2011; Graham et al., 2008; Hötzel and Croome, 1999).

3.1.2 SoE and NPS-FM monitoring

The National Policy Statement for Freshwater Management 2020 (NPS-FM) stipulates cyanobacterial biovolume will be used for the assessment of the cyanobacterial attribute bands (see Table 1 below). This also aligns with the principal purpose of SoE planktonic cyanobacterial monitoring: ability to assess changes in cyanobacterial populations through time. SoE assessments can then be used to measure the effect of changing cyanobacterial populations on human, animal and broader ecosystem health.

Table 1. Cyanobacterial attribute bands in the NPS-FM

Value	Human contact
Freshwater body type	Lakes and lake fed rivers
Attribute unit	Biovolume mm^3/L (cubic millimetres per litre)
Attribute band and description	**Numeric attribute state**
	80th percentile
A (Blue) Risk exposure from cyanobacteria is no different to that in natural conditions (from any contact with freshwater).	≤0.5 mm^3/L biovolume equivalent for the combined total of all cyanobacteria
B (Green) Low risk of health effects from exposure to cyanobacteria (from any contact with freshwater).	>0.5 and ≤1.0 mm^3/L biovolume equivalent for the combined total of all cyanobacteria
C (Yellow) Moderate risk of health effects from exposure to cyanobacteria (from any contact with freshwater).	>1.0 and ≤1.8 mm^3/L biovolume equivalent of potentially toxic cyanobacteria OR >1.0 and ≤10 mm^3/L total biovolume of all cyanobacteria
National bottom line	**1.8 mm^3/L biovolume equivalent of potentially toxic cyanobacteria OR 10 mm^3/L total biovolume of all cyanobacteria**
D (Orange/Red) High health risks (for example, respiratory, irritation and allergy symptoms) exist from exposure to cyanobacteria (from any contact with freshwater).	>1.8 mm^3/L biovolume equivalent of potentially toxic cyanobacteria OR >10 mm^3/L total biovolume of all cyanobacteria
The 80th percentile must be calculated using a minimum of 12 samples collected over 3 years. Thirty samples collected over 3 years is recommended.	

3.1.3 Managing changes in methods

Ideally, long-term planktonic cyanobacterial monitoring programmes should retain the same field and laboratory methods over time to avoid the potential for 'step' changes. Even when methods are prescribed (as in this Standard) there is scope for changes, such as through adopting a new type genera name due to changes in nomenclature, or biovolume due to recalculation. Changes in sampling and laboratory equipment, such as samplers, microscopes

and counting chambers, also need to be considered. This highlights the need for good documentation of field and laboratory methods.

Subsection 5.5.4 addresses changes in laboratory methods.

3.1.4 Other types of cyanobacterial monitoring

Other types of cyanobacterial monitoring beyond the Standard are described in brief in Annex I, including:

1) monitoring for assessing the planktonic cyanobacterial risk to public health associated with recreational use of a water body
2) monitoring for compliance with drinking water standards
3) monitoring biomass using phycocyanin or phycoerythrin (laboratory and in situ)
4) 16S rRNA and advances in genetic profiling of cyanobacteria
5) picophytoplankton analysis by 'Flow Cytometry'.

3.2 At the site

3.2.1 On-site risk assessment

Field personnel should carry out a rapid personal risk assessment upon arrival at the monitoring site. This assessment should be used to determine if it is safe to carry out sampling and/or field measurements in the usual manner.

If conditions are considered unsafe for conducting field measurements, sampling should not be attempted. Unsuitable conditions include flood flow and adverse weather, such as strong wind and heavy rain or other hazardous conditions.

Record any health and safety issues on the Field Record Form and update the Health and Safety Plan (subsection 1.2) as appropriate.

3.2.2 Site and visit identifiers

Each planktonic cyanobacterial sample or composite sample (derived from a range of depths or within a designated area, i.e. along-shore sampling) shall be referenced against existing unique identifiers for the monitoring site (e.g. WRC20202/01) and the monitoring occasion and recorded as visit metadata. This ensures that data collected at the assessment site and any laboratory sample analysis results can be related to each other as a single visit record.

3.2.3 Time records

The planktonic cyanobacterial sample collection times shall be recorded to within 15 minutes of the deployment of the sampler.

All times shall be recorded in New Zealand Standard Time (NZST). Accurate time recording can be assured by using a device that is regularly synchronised with reference time, for example, a smartphone or smartwatch.

Note: If water quality measurements or samples are collected alongside planktonic cyanobacterial samples, the planktonic cyanobacterial samples shall be given the same timestamp as the water quality measurements.

3.2.4 Visit metadata

A standard Field Record Form shall be used to record visit metadata (observations) on each visit to the site to carry out any planktonic cyanobacteria sampling. The visit metadata shall include:

- the name of the lake or river
- the name of the site
- any unique sample identification number(s) assigned
- the name(s) of field personnel
- date and time (in NZST) of sample collections
- protocols used, including any deviations from standard field measurement and/or sampling protocols, and
- GPS location (subsection 2.3).

Other data that are recommended to collect, but not required, include:

- weather conditions at the time of sampling and 24 hours prior to sampling (including wind direction and strength)
- water transparency (use a Secchi disc if available)
- any discolouration of the water or signs of blooms or mats
- water temperature
- dissolved oxygen
- phycocyanin or phycoerythrin and chlorophyll *a* sensor data
- photographs of the site
- directions for accessing the site
- flow state (for river sites; rated, gauged or estimated – at least as 'low', 'moderate', 'high' flow).

An example Field Record Form is provided in Annex B.

Note: If a sample collection cannot be made, it is important to record an uncollected sample (data gap) and the conditions at the time (e.g. rough water conditions for lakes, elevated flows following rainfall, turbid water as a result of upriver activity).

3.2.5 Supporting measurements

Water column dissolved oxygen (DO), CTD and fluorescence data may be correlated with cyanobacterial/phytoplankton biomass in some regions and could be useful to measure as a supporting variable at the time of planktonic cyanobacterial sample collection. Other water

quality variables of relevance to planktonic cyanobacteria biomass include soluble inorganic nutrients. Refer to:

- NEMS *Water Quality Part 3 of 4: Sampling, Measuring, Processing and Archiving of Discrete Lake Water Quality Data*
- *NEMS Water Quality Part 2 of 4: Sampling, Measuring, Processing and Archiving of Discrete River Water Quality Data.*

3.2.6 Decontamination

All measuring equipment and sampling devices should be properly cleaned to avoid transfer of biological pests (e.g. *Didymosphenia geminata*) and contaminants between monitoring sites. This is particularly important when multiple sites are visited on the same day.

Note the principles of 'Check, Clean Dry' for decontamination (Ministry for Primary Industries, n.d.): https://www.biosecurity.govt.nz/travel-and-recreation/outdoor-activities/check-clean-dry/

Note: Complete drying of equipment (following Ministry for Primary Industries guidelines) is the best form of decontamination.

4 Planktonic Cyanobacterial Sample Collection

In this section

This section focuses on the collection of planktonic cyanobacterial samples for subsequent laboratory analysis.

4.1 General approach

The aim of planktonic cyanobacterial sampling is to obtain a sample that is representative of the planktonic cyanobacteria community at a site, which itself is representative of the broader lake, river or other water body, (subsection 2.1).

Cyanobacterial samples shall be taken throughout the euphotic zone (i.e. using an integrated tube sampler) to minimise bias caused by unequal distribution with depth or using Van Dorn sampling following the protocols below. An integrated tube sampler is used because some groups of cyanobacteria have the ability to control their buoyancy and populations can concentrate at a depth that favours their growth. The depth of their buoyancy, however, may vary with conditions (such as light) and therefore integrated samples (or multiple Van Dorn sampling) will give a good representation through time.

Some councils currently use, or intend to implement, the sampling protocols used in the Trophic Level Index (TLI) lake sampling methods introduced by Burns et al. (1999, 2000). This type of sampling fits well with wider SoE monitoring programmes and cyanobacterial samples may also be collected using these protocols. Not all programmes will have the facilities or equipment to undertake this type of sampling; although recommended as part of a larger lakes monitoring programme, it is not required in this Standard. The TLI method from Burns et al. (1999, 2000) is described here in brief.

- TLI sampling sites:

  At least two sampling sites are usually chosen on each lake, one at the deepest point and the other at another deep part of the lake at some distance from the first site (see section 4.1 for additional guidance). On arrival at a sampling site, a Secchi depth measurement is taken and a temperature/dissolved oxygen profile is obtained to determine the depths of the bottom of the epilimnion and top of the hypolimnion for the sampling purposes.

- TLI sampling in mixed isothermal lakes

  If a lake is not stratified, samples should be collected using a Van Dorn bottle in the euphotic zone and also at three-quarters of the lake depth at the sampling site.

  (Note that use of an integrated tube sampler in surface water is also acceptable for cyanobacterial analysis).

  At the upper sampling point, multiple samples may be required to obtain enough volume for multiple analyses. If more than one Van Dorn bottle is required the contents

can be mixed, and then subsampled for algal (cyanobacteria) and other parameters required (nutrients and Chlorophyll *a* are usually taken as a minimum).

The deep sample is not required for cyanobacterial analysis. A single Van Dorn sample is usually collected at the lower sampling point for nutrient analysis. This lower sampling point is changed if the bottom waters are anoxic – the lower sample would then be taken from the mid-point of the anoxic layer.

- TLI sampling in stratified lakes

  Epilimnion sampling

  Because a relatively thin, turbid layer of detritus is often present near the thermocline, it is recommended to only sample the top 80% of the upper mixed layer depth. It is recommended (if using a Van Dorn bottle) that one sample is collected from just below the surface and one at 80% of epilimnion depth. If the lake is deep enough (see section 4), then two other samples should be collected, equally spaced between these depths. All samples can then pooled in a large container and subsequently subsampled for analysis. Again, a similar representative sample of the integrated upper water column can be achieved using a tube sampler.

  Hypolimnion sampling (not required for cyanobacteria)

  Samples are collected at one-third and two-thirds of the depth between the top of the hypolimnion and the lake bottom. These shall be pooled and a single subsample shall be taken for nutrient analysis

N*ote: Representativeness may be difficult to achieve in some situations. Refer to subsection 2.1.*

4.1.1 Number of samples

- At each site at least one integrated tube sample or multiple (Van Dorn) samples that represent the euphotic zone shall be taken (see sections 4.2 and 2.1)
- at a minimum take one 200 to 1000 m measure from each site sampled for cyanobacterial analysis.

4.1.2 Sampling points

Because planktonic cyanobacteria cover can be highly spatially variable, multiple sites may be required. These should be determined before arrival at the sampling location as identified in subsection 2.1.

4.2 Sampling procedure

Planktonic cyanobacterial samples shall be collected throughout the euphotic zone with either a PVC tube (25 mm diameter), which provides a depth-integrated sample, or a Van Dorn bottle, which allows samples to be collected from discrete depths.

4.2.1 Sampling depth (or depths)

The depth of the sampling tube will be determined by the depth of the euphotic zone. Usually, a tube of between 2 and 10 m will be required. If a Van Dorn bottle is used, the first sample should be taken 0.5 m below the lake surface; the remainder should be collected at intervals of 1.5 m or at five equal depth intervals, whichever provides the smallest number of samples. Those collected from different depths with a Van Dorn bottle should be combined (500–1000 mL from each) to provide a depth-integrated sample, similar to that obtained with a PVC tube. When taking samples at multiple depths it is good practice to start at the surface so as not to disturb any layering through the water column before sampling at those depths.

4.2.2 Van Dorn bottle sampling

The Van Dorn bottle consists of an open-ended plastic cylinder that can be attached to a rope line (which can be hand pulled or winched) or hydrographic wire (generally winched) and lowered to any desired depth. Each end of the cylinder is fitted with a rubber cover (Figure 3).

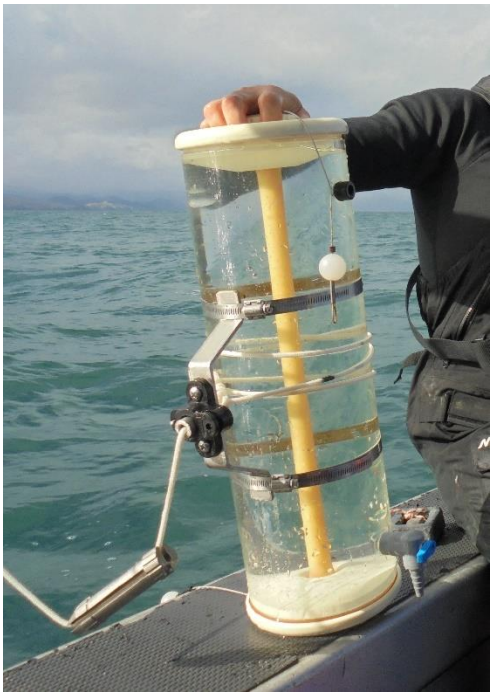

Figure 3. Van Dorn bottle after sampling

1. The Van Dorn bottle is attached to the line with the covers pulled out and twisted back and around to the side, and the covers are held in place on a tether or catch that may be remotely triggered (Figure 4).

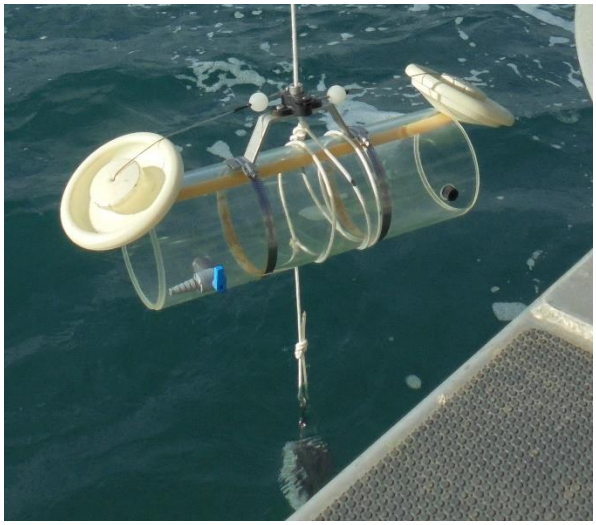

Figure 4. Set Van Dorn bottle being lowered into the water.

2. Once armed and attached to the line the bottle is lowered to a pre-selected depth. At this time the line should be allowed to straighten to ensure the correct depth is reached.
3. A metal weight called a 'messenger' is then attached above the upper bottle (Figure 5).
4. The water sample is taken by dropping the messenger down the wire.
5. When the messenger (weight) hits the catch on the Van Dorn bottle, the catch releases the rubber end covers.

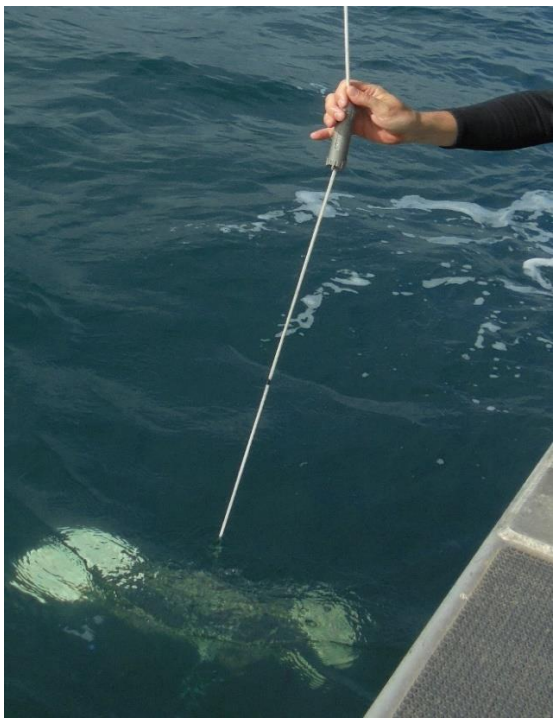

Figure 5. Messenger attached to line ready to send to trigger bottle to close.

6. The two ends snap around and seal off the ends, capturing the water sampled at the required depth.
7. The sampler is then retrieved, the sample bottle/receptacle rinsed with a small amount of sample and then the contents transferred to sample bottles/receptacle via the spigot or by opening one end of the Van Dorn bottle's caps. All sample bottles should be rinsed with the sample at least once.
8. The sample may be collected as a discrete sample or added to a larger composite sample (derived from samples collected from multiple depths).
9. The filled sample bottle is then placed in the cooler with ice for transport to the laboratory for analysis.

To sample multiple depths, multiple Van Dorn bottles can be attached to the same line and lowered to different pre-selected depths; otherwise sampling can be repeated.

If other water quality readings are required, the contents can be transferred into a sampling receptacle/bucket, but as with any sampling this should first be rinsed with the sample and then the rest of the contents of the Van Dorn transferred. Subsamples for phytoplankton or water chemistry should then be taken before testing with any instruments to reduce any risk of the instrument affecting the sample quality.

4.2.3 Tube sampler sampling procedure

Use a tube sampler made from a clear PVC pipe (25 mm diameter) of 2 m to 10 m length, depending on the depth of the river or lake, with a weight and a rope attached to one end.

1. The weighted end of the pipe is lowered into the water until the whole pipe is in the water. Make sure the boat does not move, so that the pipe enters the water vertically. In a lake it is best to deploy the sampling tube on the windward side of the boat so as not to drift over the sampling tube. Similarly, in a large river you would deploy on the upstream side of the boat.

2. Close the top end with a rubber stopper and pull up the bottom end until a U-shape is formed. Place the weighted end in a container and remove the stopper from the other end. Allow sample to empty into a bucket large enough to contain entire sample, i.e. do not fill from tube directly into bottle as the sample needs to be mixed/integrated in the sample bucket.

3. Mix and then take required subsamples from the bucket.

The tube should be rinsed with clean water, or with the water to be sampled, when arriving at the next site.

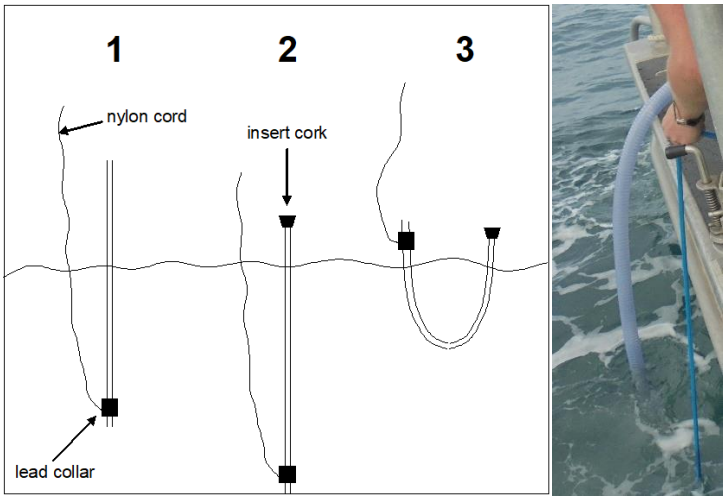

Figure 6: Diagram of tube sampler with an image of a tube sampler in use

4.2.4 Short tube/grab composite sampling

Short tube/grab composite sampling is generally used for assessing public health risk of cyanobacteria to recreational water users but could also be used for SoE monitoring in a well-mixed system however, a QC 600 cannot be achieved with this method. These samples can be collected using a rigid or flexible plastic tube with an inner diameter of at least 25 mm; a rigid PVC or acrylic plastic pipe is more practical than a flexible pipe for depths of around 1 m or less.

A sample representing an area of shoreline will be a composite of five 50 cm depth-integrated column subsamples collected evenly along a 20–30 m transect parallel to the lake shore, and mixed into a single container (e.g. a bucket). A composite sample is taken from the bucket for the cell counts and biomass estimation.

4.2.5 Sample labelling

All sample containers shall be uniquely labelled with, at minimum, the site name or number, sample number as appropriate, sample date, and sampler's name. Waterproof labels are recommended to avoid labels coming off the bottles if they get wet. On-site labelling is recommended to prevent accidental switching of pre-labelled bottles.

Labelling should be on the side of the container and not on the lid (unless labelling on the lid is additional to that on the side).

4.3 Sample preservation, storage, handling and transport

To maintain planktonic cyanobacterial sample integrity for subsequent laboratory testing:

- samples should be preserved with Lugol's iodine at the time of collection
- samples shall be placed in the dark in a chilly bin with crushed ice, or ice packs, to reduce the temperature to less than 10°C (4°C is optimum)

- if required, two planktonic cyanobacteria subsample bottles may be collected at the site – one to be preserved with Lugol's iodine as soon as possible, while the other will be kept fresh to assist with identification in the laboratory.

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As noted in subsection 1.6, unpreserved samples shall arrive at the laboratory within 24 hours of the time of collection if they are to be analysed immediately (i.e. fresh). Alternatively, samples shall be preserved with Lugol's iodine at the time of collection and submitted to the laboratory for analysis within three months of collection.

When packing samples (fresh or preserved) into chilly bins, use crushed ice or ice packs to keep bins cool. Pack any spaces with crumpled newspaper or equivalent so that samples do not move about while in transit. Ensure that there is no risk of damage to sample labels from direct contact with ice or water (e.g. samples can be placed in a plastic bag before placing on ice).

Record the courier ticket number(s) on the field sheet to assist with prompt tracing of any chilly bins lost or delayed in transit to the laboratory.

Note: Preserved samples left exposed to daylight may be subject to warming and photochemical degradation; this will impact subsequent measurements.

4.3.1 Chain of Custody

A Chain of Custody (CoC) form (Annex C) shall be completed and inserted (within a sealed waterproof bag) inside the chilly bin. As a minimum, the CoC form shall include:

• analysis required (cyanobacteria)

• site name (and unique number where applicable)

• sampling date and time.

It is also recommended to add:

• settling date and time (to be completed by the analysing laboratory)

• date of analysis (to be completed by the analysing laboratory)

• sampler name or initials (to allow any questions that may arise about the sample/sampling to be followed up), and;

Ideally, a CoC form will track the sample from collection through to reporting; this is why it is recommended that the CoC form includes both sampling information and analysis tracking. If the CoC form is also used as the Field Record it should **also,** at a minimum, contain location (GPS coordinates) and sampling method.

If more than one chilly bin is dispatched, either place a copy of the CoC form into each bin or include a waterproof note confirming the number of bins and which contains the CoC form (e.g. "Bin 2 of 2 – see CoC in Bin 1").

5 Laboratory Processing and Reporting

In this section

This section focuses on activities undertaken by the processing laboratory. It addresses laboratory credentials, receipt of planktonic cyanobacterial samples at the laboratory, sample preparation (preservation and settling), sample processing (taxonomic identification and enumeration), biomass assessment by biovolume, planktonic cyanobacterial reporting (encompassing the aforementioned variables), and managing changes in taxonomic guides and laboratory providers, quality control, and quality control audits. These procedures include sample receipt, sample preparation, sample analysis, reporting and all aspects of quality assurance.

5.1 Laboratory credentials

There is currently a laboratory accreditation process in New Zealand through IANZ that applies to planktonic cyanobacterial sample processing. Accredited laboratories will already be following the protocols for laboratory analysis outlined in this Standard. We, however, recognise that not all laboratories will seek to gain accreditation and therefore provide the following guidance.

Under this Standard, planktonic cyanobacterial samples shall be processed at a laboratory that can provide evidence of the following:

- adherence to either formal standard protocols recognised under IANZ accreditation or subsequently implemented protocols (i.e. this NEMS) for the processing and reporting of planktonic cyanobacteria. This may include, but is not necessarily limited to, documenting all stages of sample processing, including internal and external quality assurance and quality control (QA/QC) practices, and
- that staff are suitably qualified/trained in carrying out planktonic cyanobacterial analyses and reporting.

Note: It would be desirable to establish some form of New Zealand interlaboratory comparison in the future to provide additional confidence in data quality.

5.2 Sample receipt

Planktonic cyanobacterial samples shall be registered on arrival at the laboratory. This shall include completion of the Chain of Custody (CoC) form that accompanied the samples.

The laboratory may promptly (same day, if possible) return a copy of this form to the monitoring agency if requested, with a laboratory reference or job number, or include all relevant information (including CoC and processing times) in the final report.

5.2.1 Date of arrival

Laboratory staff shall record the date of arrival of samples at the laboratory on the CoC form and record the time the sample(s) were settled for analysis.

5.2.2 Sample condition and temperature on arrival

Once in the laboratory, samples should be unpacked and the sample condition checked:

- any visible anomalies in sample bottles with the potential to impact processing (e.g. split or leaking containers) shall be recorded on the CoC form and in the laboratory's sample notes for inclusion in reporting of planktonic cyanobacterial data (see subsection 5.4)
- laboratory staff shall measure the temperature of samples on arrival at the laboratory (e.g. through the use of a calibrated probe thermometer or infrared thermometer inside each chilly bin) and note any significant variance on the CoC form. If the sample is unpreserved, it must also be <10 ^{0}C.
- fresh samples shall be checked to ensure they have been received within 24 hours of collection
- adequate sample preservation (by Lugol's iodine) should be assessed by checking the sample colour (should be at least the colour of weak tea), as outlined in subsection 4.3
- for any evidence the sample has been frozen (if there is then sample analysis should not proceed)

Following this assessment, a decision must be made whether to proceed with analysis or if the sample should be rejected. If the sample is analysed, its condition shall be noted in the report.

5.3 Methods overview

The Standard requires that laboratory analysis of planktonic cyanobacterial samples for biomass as biovolume is carried out using the methods set out below and in Annexes F, G and J. To be eligible for the highest quality rating of QC 600:

- the analyses performed on preserved samples shall commence within 3 months of collection, and the analyses involve the following general steps:
    1. Sample preparation and pre-treatment (subsection 5.3.1)
        a. Pre-treatment <u>if required:</u>

            - Removal of cell buoyancy

            - Disaggregation treatment of colonial samples

        b. Chamber selection and filling, and
        c. Settling protocols.
    2. Microscope analysis (subsection 5.3.2)
        a. Introduction and handling
        b. Visual density and distribution check
        c. Algae counting protocols – whole-settling chamber or field-of-view count
        d. Selecting an appropriate magnification for counts

e. Cell counts for colonies and filaments:
    - Total cell numbers to be counted and measurement uncertainty
    - Recording the number of algal cells
    - Validation of electronic calculations.

f. Taxonomic identification and resolution:
    - Taxonomic accuracy
    - Naming conventions
    - Taxonomic resolution
    - Nomenclature for identified taxa
    - Grouping taxonomic ranks.

g. Biovolume calculation.

3. Laboratory reporting (subsection 5.4)
    a. Sending client reports
    b. Reporting formats (.pdf, .csv).
4. Quality control (subsection 5.5)
    a. Internal quality control
    b. External quality control
    c. Sample re-analysis
    d. Managing changes in laboratory methods

The following subsections focus on critical parts of the four procedures above.

Detailed procedures and further reference material are provided in Annex D to H and J.

5.3.1 Supporting calibrations required

Supporting calibrations are required for equipment used in the preparation of samples. These calibrations are beyond the scope of this document but are required for proficient laboratory practice. These include:

- settling chamber calibration
- pipette calibration
- microscope eyepiece graticule calibration.

5.3.2 Sample preparation and pre-treatment

5.3.2.1 Sample pre-treatment - removal of cell buoyancy

Species containing gas vesicles will settle more consistently if their gas vesicles are collapsed prior to settling. While Lugol's iodine should disrupt these vesicles (given adequate concentration and time), a further precautionary measure to ensure full collapse may also be taken.

This procedure should be followed when:

- species containing gas vesicles are known to be present in a sample (e.g. a sample has been analysed from the same site recently)
- settled samples show evidence of unsettled buoyant algae after visual inspection of the aliquoted sample surface prior to adding the cover glass, or
- species containing gas vesicles are observed in a settled sample above background levels. In this instance the sample should be treated and resettled before repeat analysis; however, some untreated sample may also be kept for algal identification purposes.

One method that can be used to collapse the gas vesicles involves applying pressure to the sample as follows (other referenced methods may be used):

1. Draw up ~15 mL of mixed sample into a clean 20 mL plastic syringe.
2. Seal the outlet of the syringe and bang the syringe plunger with force several times.

After treatment the sample is settled (sedimented) as usual.

> Note: The distinction between certain cyanobacterial taxa is compromised after gas vesicles have been collapsed. Therefore, the untreated sample is used to perform identification and estimate the proportions of the different cyanobacteria present, so that cell counts can be apportioned accordingly. These methods are based on Hötzel and Croome (1999).

5.3.2.2 Sample pre-treatment – disaggregation treatment on colonial samples

The reason for disaggregation of cyanobacterial colonies is that their formation can make reliable estimates of algae abundance by microscopy difficult. For example, species of *Microcystis* commonly form large colonial aggregates constrained by an amorphous mucilage or sheath (Holt et al., 1994). The number of cells per colony can reach tens of thousands in a three-dimensional matrix (Bernard et al., 2004).

Studies have reviewed various techniques to disrupt cyanobacterial colonies to enable more acuate counting of cells (Bernard et al., 2004, 2007; Joung et al., 2006). Disruption for counting is best achieved through the grinding technique, which effectively and rapidly (1–2 min treatment required) disassociates all types of colonies of *Microcystis*, fresh or fixed, without loss of sample material that heating techniques can cause (Bernard et al., 2007).

Below is one disaggregation treatment method that can be used. Other referenced methods may also be used.

For disaggregation by grinding treatment:

- samples in capped glass tubes are gently mixed by inversion 20 times
- 2–10 mL subsamples are placed in the grinding chamber of a tissue grinder with ~100 µm of clearance between chamber walls and tissue grinder surface.
- each aliquot is then homogenised in a manual and constant grinding motion with a Teflon® pestle for 1–2 min
- after treatment the sample is settled (sedimented) as usual in preparation for counting.

5.3.2.3 Chamber selection

There are two main types of settling chamber used for planktonic analysis in New Zealand – Utermöhl chambers (1–100 mL samples, see Annex G) or Sedgwick-Rafter chamber (1 mL, see Annex H).

5.3.2.4 Settling protocols

The sample is mixed by gently inverting the sample bottle for at least 30 seconds or a minimum of 20 times before sub-sampling and aliquoting into the preferred settling chamber (Annex G and H). An archive sample should also be taken at this time.

An archive sample should be taken at the time of settling following the same procedures as filling a settling chamber. Archive samples are to be stored in the dark. Archive samples can be discarded after 3 months.

Sample settling times

Settling time is dependent on the height of the settling chamber and the preservative (e.g. Hasle, 1978; Rott, 1981) but a rule of thumb is 30 minutes of settling time per ml of water column depth (APHA, 2017). The times given in Annex G are the minimum. If vibration (movement disturbing the sample) is a problem, the minimum time given in Annex G should be significantly exceeded otherwise it is suggested that counting be performed within four days. Sedimented samples not counted within a week should be discarded.

Cleaning settling chambers

Settling chambers should be cleaned and dried between uses. For best results, this will include a detergent rinse and drying with a clean tissue, followed by a distilled water rinse and drying with a tissue. Where samples have been concentrated or the chamber has dried out an additional rinse with isopropanol, followed by a rinse with distilled (or similar, i.e. reverse osmoses) water may also be required.

5.3.3 Microscope analysis

5.3.3.1 Introduction and handling

Once a known volume of sample has been settled for the required time (30 minutes settling time per millimetre of column depth), analysis of the sample may begin.

It is important that the settling chamber be moved gently to the microscope as so not to disturb the settled particles. When a full settling chamber with a glass cover is used, mixing is minimised (settled algae in an open or partially filled sedimentation chamber are more easily disturbed).

5.3.3.2 Visual density and distribution check

The quality of the analysis depends on the density and distribution of the subsample over the base of the settling chamber. A visual inspection must be performed to ensure the number of cells is not excessive and they are evenly distributed (this check is typically carried out at low magnification). If algal density is too high to allow adjacent algae colonies or filaments to be distinguished, then a smaller volume will also need to be settled. If the volume settled was less than approximately 10 mL and, after settling, the density of plankton is too low to achieve a statistically significant count (Lund et al., 1958), then a larger volume of sample should be resettled. High concentrations of sediment can also necessitate resettling of smaller volumes.

If algae are not evenly distributed across the base of the chamber, there are two options:

- resettle a new subsample for the count, or
- count the whole chamber.

5.3.3.3 Algae counting protocols

Whole-settling chamber or field-of-view count

When cyanobacteria numbers are low (< 100 units/cells observed in 20 fields of view), the entire chamber is scanned. This is done by traversing transects back and forwards across the settling chamber from top to bottom/side to side (or vice versa).

Whole-settling chamber counts are neither necessary nor feasible when cyanobacteria numbers are high. If you encounter cyanobacteria in at least four out of five fields of view (FOV) then scanning the entire chamber is not necessary. Instead, FOV counts will provide an adequate number of cells for a statistically significant count (see Annex G). Randomly selected FOV can be used if algae have settled evenly across the base of the chamber (checked by scanning the whole settling chamber at low magnification). If algae are not settled evenly, the chamber should be resettled. FOV are selected along these transects at approximately equal spacing. The number of FOV required will vary with algae density (see Annex G).

Random fields of view are taken across the chamber. If bias is visually detected in the initial scan, the chamber should be resettled.

Cells must appear to be viable (dead cells are often clear or collapsed). Cell fragments are not counted. Cells that are partially included in a counting field on the right half of the FOV are counted, but those on the left are omitted.

Selecting an appropriate magnification for counts

The magnification used for counting algae varies according to the density and size of the algae. Cells or colonies > 200 µm are identified and counted using the 10x objectives. For dense samples and/or smaller cells, a higher magnification may be appropriate.

Cell counts for colonies and filaments

Colonies are a cluster of cells (often spherical), whereas filaments are strands of cells connected end-to-end. When large numbers of colonies or filaments are present, counting every cell

observed is not practical. For colonies, a portion of the colony is counted, and the total number of cells is estimated by scaling the number of cells counted by the proportion of the colony counted. When the filament concentration is high, filaments may be counted and converted to cell numbers using the following protocol:

- Measure the average length of at least 20 but ideally 30–50 filaments and calculate the number of cells per filament length. Using these figures, convert number of filaments to number of cells and use these for subsequent calculations (see subsection 5.3.2.4).

Total cell numbers to be counted and measurement uncertainty

- A whole-settling chamber count is used when cell counts are relatively low. At least 50 units cells of the dominant cyanobacteria shall be counted if present in the sample. Accuracy of the result will not be as great when the number of cells counted is low, but a higher margin of error is tolerable for low cell counts as the maximum value (total + error margin) falls short of levels that cause concern for drinking water.
- For a FOV count, a minimum of 250 units/unicells should be enumerated (EPA, 2010) to ensure that the count is representative of the sample (see Annex F).
- If a count is near or around either drinking water standards or recreational use guidelines, then consider either increasing the volume settled or increasing the number of cells counted to increase the accuracy to the ± 20% confidence level (see Annex F).
- For other phytoplankton (non-cyanobacteria), such as species with individual cells or small colonies, it is recommended to set the level of precision to ± 28% error for the most abundant taxa following Lund et al. (1958) and as described in Hötzel and Croome (1999). This level of precision is reached by counting a minimum of 50 algal units of the taxon in question per sample. The counting precision achieved for less common taxa will always be lower than that for common taxa since the required minimum of 50 algal units will not be found in the sample.

  The counting error (precision) can be estimated with a 95% confidence limit by:

  Counting error (± %) = $2/\sqrt{N} \times 100$

  Where N is the number of units counted, if the algal units follow a Poisson distribution for both subsampling steps.

  For example, if 100 units are counted, the error is ± 20%; if 400 units are counted the error is reduced to ± 10%. Thus, a four-fold increase in counted units is required to halve the error. Generally, 100 to 150 units of the common taxa are counted, giving an approximate error of ± 20%.

Recording the number of algal cells

After scanning at low magnification to check cell distribution and density, select the appropriate cell counting methodology and magnification following sections 5.3.1 and 5.3.2.

Calculation fields on the Electronic Calculation Sheet to be completed include:

- the quantity of the subsample analysed (volume)
- the microscope used
- the lens selected for cell counts (e.g.~ x20, x40, x60)
- the chamber diameter (usually 24–26 mm) or units counted in Sedgewick-Rafter
- the number of FOV or grids units counted (Sedgewick-Rafter method)

5.3.3.4 Calculations

The transformation of the microscopic counts to the concentration or density of phytoplankton of a desired water volume (usually litre or millilitre) can be achieved using Equations 1 & 2:

Equation 1.

$$Cells\ L^{-1} = N * \left(\frac{At}{Ac} \right) * \frac{1000}{V}$$

Equation 2

$$Cells\ L^{-1} = N * \left(\frac{At}{Ac} \right) * \frac{1}{V}$$

V: volume of counting chamber (mL)

At : total area of the counting chamber (mm^2)

Ac : counted area of the counting chamber (mm^2)

N: number of units (cells) of specific species counted

C: concentration (density) of the specific species.

Validation of electronic calculations

These calculations may be set up electronically, for example, in a spreadsheet. These calculations should be protected within the electronic sheet and validated externally (i.e. using a calculator).

5.3.3.5 Taxonomic identification and resolution

Planktonic cyanobacterial taxonomic resolution shall be at least to genus level and consistent with that outlined below and with examples provided in Annex E.

- Where practical, cyanobacteria are identified to genus (and/or species) level.
- Identification of common species and genera is conducted using reference to keys, guides and floras relating to the taxa reported; records should be kept of this reference material.

- The Taxon list in Annex E depicts many common taxa identified and can be used as a general guide to common species in New Zealand.

NOTE: Validated historical data from the same site and a scan of the unpreserved sample (if available) can aid identification.

Naming conventions

Due to the diversity of algae and cyanobacteria typically encountered during a sample analysis, it is unlikely that all individuals will be identifiable to the desired taxonomic level.

The naming convention that follows uses the rules of the International Code of Nomenclature (Turland et al., 2018; McNeill et al., 2012) and sets out the rules and terms to be used in the naming and reporting of algal taxa.

Nomenclature for identified taxa

The base taxonomic unit required for the standard is genus. Where possible, species can be given. Species name is a combination of the superior generic name and the species epithet. For example, "*Chroococcus turgidus*" is the taxon of the category "species"; "*Chroococcus*" is taxon of the category genus. In laboratory reports, both species- and genus-level taxa names should be indicated with italics with the first letter of the genus name as a capital and the species epithet in lower case. An example of common taxon reporting units across the nomenclatural hierarchy is given in Table 2.

Table 2. Nomenclatural hierarchy for identified taxa

Taxonomic rank	Example
Species	*Chroococcus turgidus*
Genus (a single species reported at the genus level)	***Chroococcus* sp.**
Family	Chroococcaceae
Order	Chroococcales
Phylum	Cyanobacteria

Nomenclature for unidentified or partially identified taxa

Examples of this type of identification are "unidentified Chroococcales" or "unidentified cyanobacterial filament". We recognise that these terms may differ between organisations. These types of description are generally only used for rare cyanobacteria where genus identification is not possible due to missing features. These may not need to be included in database records or may be included under a broad categorisation ("unidentified cyanobacteria").

Note: Standardisation of terms to be used in a national database will require general agreement among analysts.

Grouping taxonomic ranks

It is common for analyses to be performed at designated taxonomic ranks, i.e. species level, genus level, etc. Under these circumstances, analysts may group a number of taxa under a higher taxonomic rank and report them as such. An example of how to indicate a genus-level taxonomic grouping (i.e. a number of species of the same genus are present in the sample but are grouped for reporting purposes) is:

Chroococcus spp. or *Chroococcus*

where the genus name (indicated in italics) is followed by either 'spp.' (indicating multiple species of that genus), or by no species-level epithet.

Managing changes in taxonomy and laboratory

Because the objective of most long-term planktonic cyanobacterial monitoring programmes is detection of changes through time, long-term monitoring programmes would ideally retain the same taxonomy and processing laboratory to avoid the potential for inconsistencies that can arise when a change is made to either of these. However, this may be unrealistic because both taxonomy and laboratory contracts change through time. The effects of these changes can be minimised by ensuring that:

1. only laboratories that meet this Standard are utilised, and
2. good documentation of sample processing details is maintained, including consistent use of taxonomic names in accordance with Annex E and the International Code of Nomenclature (Turland et al., 2018; McNeill et al., 2012).

5.3.3.6 Biovolume calculations

Measurement of biovolume as an estimate of biomass of individual taxa in a sample provides valuable additional information to the usual cell counts.

The biovolume of a species can be regarded as more representative of the relative contribution to the total phytoplankton population. Biovolume can also be used as a measure or 'surrogate' for the potential toxicity or toxin hazard for species and taxa in a sample, where no toxin determinations have been made.

The method for determining cyanobacteria cell biovolume is:

1. Determine the appropriate geometric shape and measure the dimensions of cyanobacterial cells using 600x, or preferably 1000x, magnification with an oil immersion objective.

2. Measurements for the closest geometric shape can generally only be made to approximately 0.5 µm. The majority of cyanobacterial cells have a shape that approximates to either a sphere or a cylinder (cells within a filament).

3. Determine the mean cell dimension for the taxa based upon a minimum of 30 cells per sample and up to 100 cells, depending on size variability.

4. Calculate the mean cell volume using the mean cell dimension and the appropriate calculation for that shape.

5. The average cell volume is then multiplied by the total cell number to obtain biovolume in cubic microns per millilitre ($\mu m^3 mL^{-1}$), which should then be converted to cubic millimetres per litre ($mm^3 L^{-1}$) by dividing by 10^6

This process should be repeated individually for each cyanobacterial species.

A couple of caveats need to be considered when using biovolumes:

1. When considering biovolume calculations for cyanobacteria taxa that contain specialised cells, such as akinetes and heterocytes, volume measurements shall be only of vegetative cells.

2. Consideration should be given to the preservation of samples with Lugol's iodine as it can cause shrinkage rates of up to 40 per cent of the live biovolume (Hawkins et al., 2005). Using a lower concentration (~0.3-0.5% final concentration) of Lugol's iodine and analysing samples within 24–48 hours of collection will minimise shrinkage.

More specific references and details for calculations are provided in Annex J.

5.4 Laboratory reporting

The laboratory shall, as a minimum, report for each planktonic cyanobacterial sample analysed:

- the name of the sample
- the date and time it was collected
- the date of receipt of the sample at the laboratory
- the variables measured, measurement value and unit of measurement
- the measurement method, including details of any modifications made to these
- comments on any anomalies in the condition of the sample upon receipt and supporting commentary from the analyst in the advent of difficulties with testing the sample or an unusual result.

All laboratory analytical reports shall be checked by a second laboratory analyst prior to release to the monitoring agency. Reports shall be supplied to the monitoring agency digitally, locked to prevent inadvertent editing, usually as a .pdf. Unofficial reports may also be supplied in the form of spreadsheet (.csv) and/or database (.xml) files for convenience. Results contained in these unofficial files shall be checked by the monitoring agency against the official report.

5.5 Quality control

5.5.1 Internal quality control

To ensure high quality results, all steps of the method must be validated.

Ideally this is performed on natural samples, but in some instances, it may be helpful to spike the sample with a cultured cyanobacteria. Steps in the use of settling chamber methods to validate are:

• homogenisation of sample

• sedimentation/sinking

• distribution on chamber bottom

• repeatability and reproducibility.

Ultimately, the quality of the result from microscope counts is dependent on the skill of the analyst.

The variation of parallel samples counted by the same analyst and the variation in parallel samples counted by different analysts are two of the most important considerations in quality assurance. When possible, laboratories should take part in interlaboratory comparisons.

As a minimum, the following practices are expected:

- intralaboratory testing (6 monthly) – to test the accuracy and consistency of cell count, taxonomic identification and biovolume analysis for cyanobacteria between different analysts using standardised methods
- check the calibration and volume setting on the pipette used for subsampling regularly (3 monthly) by weighing subsamples of distilled water on a precision balance
- calibrate balances (3 monthly); calibrate balances and thermometers externally at least annually
- have a second laboratory analyst check and sign off all results, calculations, and transposition of data. All errors should be logged in a laboratory registry of errors; this helps to isolate problem areas in the analytical process and ensure the quality of the analysis, and
- review, at least yearly, the sample processing performance of individual laboratory staff.

Note: The monitoring agency may wish to request evidence documenting the internal quality control carried out on its samples and or external quality control the laboratory is involved in (for example, IANZ accreditation).

5.5.2 External quality control

It is required that the laboratory undertake:

- Interlaboratory testing – to test the accuracy and consistency of cell count, taxonomic identification and biovolume analysis for cyanobacteria between the laboratory and other laboratories using standardised methods.
- External or fully documented internal calibration of equipment, including balances and thermometers, at least annually.
- Servicing of microscopes at least annually.

As noted in Subsection 1.3, internal quality control is the primary measure of laboratory performance.

5.5.3 Sample re-analysis

Although the laboratory performs internal checks, it does not hold the long-term history of a particular monitoring site or any metadata that was not included on the CoC form. It is recommended that the monitoring agency, which holds all metadata, systematically checks laboratory results as soon as possible (e.g. against previous results or corresponding planktonic cyanobacteria data). If this is completed within a reasonable timeframe (preferably two weeks up to a maximum interval coinciding with the laboratory's maximum sample holding time of three months) it is possible for the monitoring agency to query unusual measurements, which may trigger re-analysis. Re-analysis should be done within three months.

5.5.4 Managing changes in laboratory methods

Changes within laboratories are expected to be limited to substitution of updated or replacement instruments (microscopes or chambers, for example). The laboratory is expected to ensure that the setup and calibration of such new instruments produces results that are consistent with the original instruments.

6 Data Processing and Quality Assurance

In this section

This section contains information on the processing, storage and quality assurance of cyanobacterial data and associated metadata. It includes a flow chart outlining the quality coding process and an associated matrix to assist with assigning quality codes.

6.1 Data types

Data from planktonic cyanobacterial monitoring programmes can be split into several categories:

- site metadata (subsection 2.3), which are specific to the site location and may change with time but generally not on each site visit
- visit metadata (subsection 3.2.4), which are specific observations recorded on a Field Record Form about where, when and how the planktonic cyanobacterial sample was collected, and
- planktonic cyanobacterial data, which comprise sample condition on arrival, planktonic cyanobacterial laboratory identification, enumeration, and calculation of biovolume.

All three data types shall be stored in a database.

6.1.1 Site metadata storage

Adequate mechanisms shall be put in place to store, indefinitely, all relevant site-related metadata listed in subsection 2.3.

6.1.2 Visit metadata storage

Adequate mechanisms shall be put in place to store, indefinitely, all relevant visit-related metadata listed in subsection 3.2.4, together with the planktonic cyanobacterial laboratory measurements (subsection 6.1.3).

6.1.3 Planktonic cyanobacterial data and associated metadata

Each planktonic cyanobacterial measurement shall be stored with:

- the measurement date and time
- analytical laboratory name and location
- analytical method (including modifications, if applicable)
- all relevant visit-related metadata (subsection 3.2.4), including the name(s) of personnel who conducted the sampling and collected any associated field measurements
- any relevant comments from the sampling or laboratory report (subsection 5.4), and
- an associated quality code (assigned in accordance with subsection 6.2.1).

6.2 Data processing

Processing of planktonic cyanobacterial data involves several components: sample condition assessment, taxonomic identification, cell number calculation and biovolume calculation. It also involves assigning quality codes through consideration of field practices, sample handling and storage, documentation, and laboratory practices. Any adjustments to data should be documented.

6.2.1 Quality coding

6.2.1.1 Performance

All individual planktonic cyanobacterial measurements shall be quality coded in accordance with the NEMS *Quality Code Schema* (Figure 7) and matrices (Table 3) that form part of this Standard. The schema comprises seven 'parent' codes and permits valid comparisons within and across multiple data series.

In most cases, planktonic cyanobacterial data collected for long-term (e.g. SOE) monitoring will fall under one of QC 400, QC 500 or QC 600. Original data is classed as QC 0. The matrices in Table 3 provide a framework for assigning one of these quality codes to individual planktonic cyanobacterial variable measurements. The matrices are:

Matrix A: General procedures (sampling agency)

Matrix B: Sampling and sample condition (sampling agency and/or laboratory)

Matrix C: Taxonomic identification, cell counts and biovolume (laboratory)

To assign a quality code follow Matrices A, B and C then sum to determine the total number of 'demerit' points.

Total 'Demerit' Points
(All matrices)

QC 600	< 3 points
QC 500	3–11 points
QC 400	12+ points

Note 1: If 3 or more criteria are in the '3 points' column of the matrices in Table 3 the data are deemed to be QC 400.

Note 2: Child codes (e.g. QC 510 or QC 550) may be used to provide for a more detailed breakdown of data quality. Refer to the NEMS Quality Code Schema*.*

QC 0, QC 100, QC 200 and QC 300 should also be used as appropriate. These four quality codes should be applied as follows:

- QC 0 should be utilised for original data

- QC 100 should be utilised for data that are missing for whatever reason (Note: this is unlikely to be utilised for discrete sampling such as that associated with cyanobacteria)
- QC 200 should be utilised for data that cannot be resolved, or have a high level of unquantifiable uncertainty associated with it, or is not rectifiable for whatever reason
- QC 300 should be utilised for synthetic/correlated data (Note: this is unlikely to be utilised for discrete sampling such as that associated with cyanobacteria).

Figure 7 – NEMS *Planktonic Cyanobacteria* Quality Coding Schema. Use the flowchart as a framework to assign quality codes to individual planktonic cyanobacteria variable measurements using the associated matrix (Table 3).

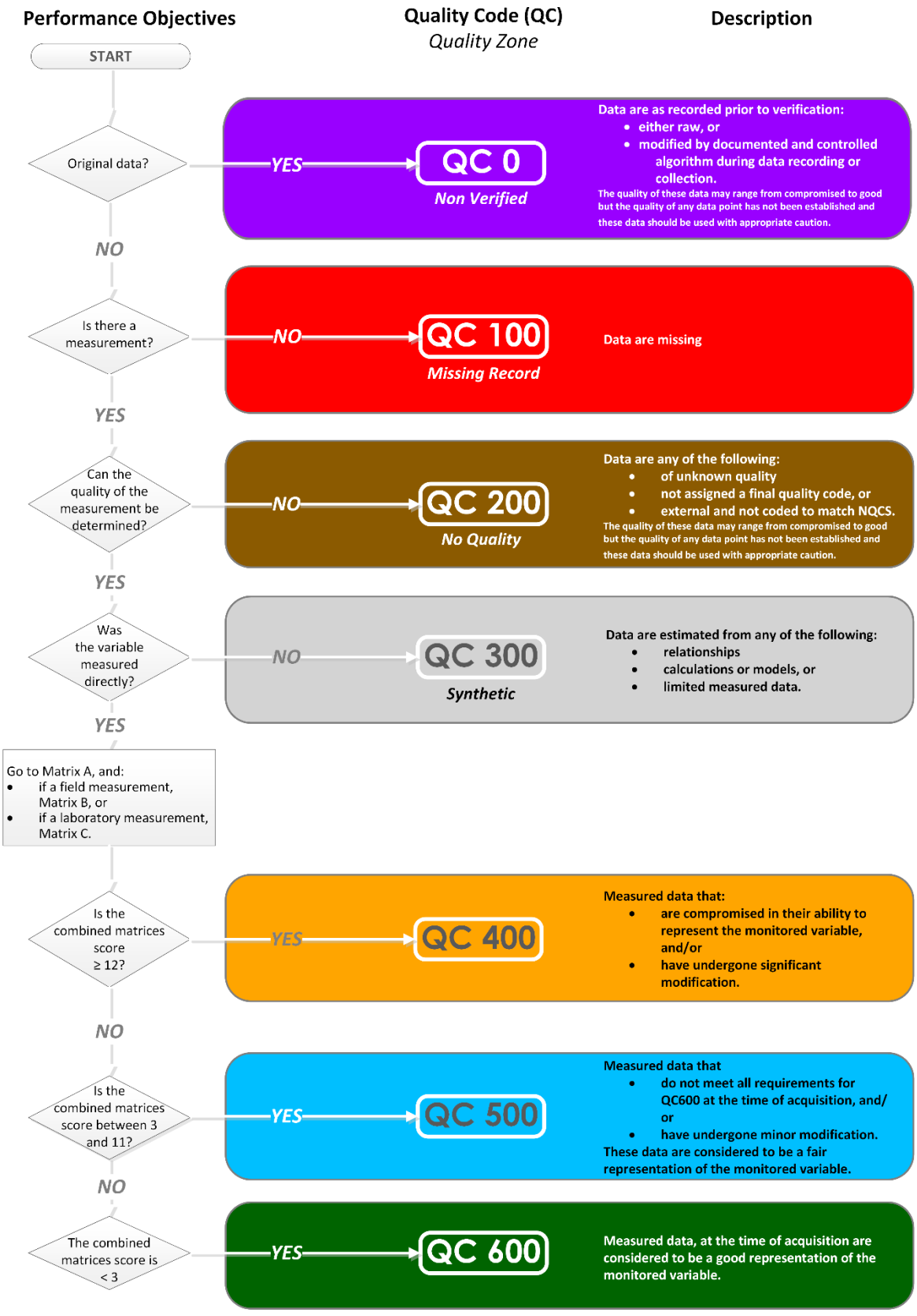

Table 3: Criteria for assigning quality codes to planktonic cyanobacterial variable measurements under this Standard

Matrix A: General procedures

Quality coding of all planktonic cyanobacteria data collected as part of long-term monitoring programmes shall firstly address the following criteria.

Criteria	12 points	3 points	1 point	0 points
Office and Field Manual *(subsection 1.1)*	Manual lacks quality assurance procedures for sampling and data management	Manual lacks quality assurance procedures for some aspects of sampling or data management that could partially affect sample quality (e.g. missing requirements for essential training or recording of information that may affect sampling)	Manual lacks quality assurance procedures for an aspect of sampling or data management that is unlikely to affect sample quality (e.g. training records out of date)	Manual maintained in accordance with the Standard, including quality assurance procedures for all aspects of sampling and data management
Field Record and Chain of Custody *(subsection 1.4)*	No Field Record Forms and/or Chain of Custody supplied	Field Record Forms and/or Chain of Custody are supplied but missing essential information to ensure sample integrity (e.g. sampling time)	Field Record Forms and/or Chain of Custody are missing information, but this will not compromise the sample (e.g. date analysed)	Field Record Forms and Chain of Custody are in accordance with the Standard
Subtotal Score				

Matrix B: Sampling and sample condition

Quality coding of all planktonic cyanobacterial data collected as part of long-term monitoring programmes shall then address the following criteria.

Note: The first two criteria represent system 'sampling and sample condition' checks may be reported by those collecting the samples or by the analysing laboratory.

Criteria	12 points	3 points	1 point	0 points*
Sample collection *(subsection 4.2)*	Sampling equipment leaked (e.g. Van Dorn bottle or tube) or was incorrectly used, or damaged, compromising the sample	Sampling equipment or sample containers were not clean or rinsed and low-level contamination was possible	Depth of samplings was not measured but was representative (e.g. Van Dorn depths were not recorded)	Tube or Van Dorn sample collection followed documented procedures in accordance with those in this Standard
Site representativeness *(subsection 2.1)*	Site was not accessible, and monitoring was conducted at a site that was not representative of the broader lake or river (e.g. high concentration of wind-blown scum)	Site was not accessible, but monitoring was conducted at a site that was partially representative of the broader lake or river (e.g. lake front integrated edge sampling)	Sampling site was not fully representative of the broader lake or river (e.g. samples were only taken from 1 site on large lake > 5 km^2)	The site sampled met all requirements of this Standard, including characteristics representative of the broader lake or river
Sample container and containment *(subsection 1.5.4 & 5.2)*	The container used was not of the correct type and is likely to affect sample quality (e.g. chemical residue in container) and/or Evidence that the sample container has compromised the sample (e.g. leakage)	The container used was not of the correct type and could have partially affected the sample quality (e.g. not clean and/or not rinsed prior to sampling)	The container used was not of the correct type but is unlikely to affect the sample quality (e.g. clean but wrong plastic, should be replaced in the laboratory)	Container type, material and integrity (will not leak etc) are in accordance with the Standard
Visit metadata *(subsection 3.2.4)*	No metadata recorded other than the site name and date of sample collection	Visit metadata generally recorded in accordance with the Standard but missing information that could affect the sample integrity (e.g. specific equipment used etc)	Visit metadata generally recorded in accordance with the Standard but missing some non-essential information that will	Visit metadata captured on a Field Record Form in accordance with the Standard.

			not affect the sample (e.g. name of sampler, bloom information)	
Preservation (in field and checked in laboratory) *(subsections 1.6 & 4.3)*	Sample is/was not preserved or was compromised before preservation (e.g. high or low temperature)	Sample was not fully preserved (low coloration by Lugol's)	The sample was not preserved in the field but was kept cool and in the dark and was received within 12 hrs of sampling and preserved	Preservation was checked, correct and documented in accordance with the Standard
Temperature *(subsection 2.2)*	Fresh sample temperature was more than 10°C OR Sample was frozen and/or had ice crystals			Sample temperature was checked and was not greater than 10°C for an unpreserved sample in accordance with the Standard
Subtotal Score				

*IANZ accredited results will be assumed to have meet the Standard unless a variance is indicated by the laboratory or sampler prior to sample receipt.

Matrix C: Taxonomic identification and cell counts of planktonic cyanobacteria

In addition to the criteria in Matrix A and B, quality coding of planktonic cyanobacterial measurements shall address the following criteria. The total score that determines the final Quality Code for each measurement is the sum of the total scores from (A+B) added to C.

Note: Matrix C criteria represent system 'Analysis' checks.

Criteria	12 points	3 points	1 point	0 points *
Documentation of laboratory procedures *(section 5)*	No documentation of laboratory procedures	Records of sample analysis are incomplete (e.g. missing some sample information, receipt date or time settled)	Documented laboratory procedures were not followed but the changes will not affect the sample analysis (e.g. date analysed was not reported but can be deduced and does not compromise the sample)	All documented laboratory procedures were followed.
Equipment specification/calibration *(subsections 5.3 & 5.4)*	Equipment was out of specification at the time of analysis and data cannot be verified (e.g. chamber leaked, sample bottle leaked)	Equipment was out of specification at the time of analysis, but either calculations have been revised or the equipment used is unlikely to have affected the sample. There is still some uncertainty (e.g. microscope or pipette was shown to be out of calibration, and/or incorrect bottle material used).	Equipment was out of specification at the time of analysis, but calculations have been revised (e.g. a new calibration applied).	All equipment was within specification at the time of analysis (correct equipment was used as specified).
Taxonomic level assessed *(subsection 5.3.2)*	Dominant cyanobacteria have not been identified to the genus level	There is some uncertainty about the taxonomy and data cannot be verified (e.g. some colonies/ filaments analysed were in poor condition and/or lacked diagnostic features so could not be identified to genus level).	There was some uncertainty about the taxonomy, but the data have now been verified (e.g. a synonym/ historical name was used instead of the most recent)	The taxonomic level assessed is at or above the standard (to genus or higher to species level)

Cell counts *(subsection 5.3.2)*	There is uncertainty about validity of calculations for cell counts and data cannot be verified .	There is uncertainty about validity of calculations for cell counts and recalculated data still have some uncertainty (e.g. magnification was not recorded but is likely to have been X)	There was uncertainty about validity of calculations for cell counts but these have been recalculated.	There is clear tracking of calculations that means they can be checked and follow the requirements of the Standard.
Measurement units and resolution *(subsection 5.3.2)*	Less than 50 units of the dominant cyanobacteria were counted or less than 250 units for the entire sample and/or Volume analysed was too low to detect cyanobacteria at ≥1 cell per ml.	There are no records available to track the number of cells counted or the detection limits, but they are likely to be near or above the standard.	There was uncertainty about the number of cells counted or the detection limits, but through tracking they can be shown to be above the standard.	The number of cells counted was ≥ 50 units of the dominant cyanobacteria were counted or less than 250 units for the entire sample and/or Volume analysed was above the detection limit of 1 cell per ml.
Biovolume calculations *(subsection 5.3.2)*	No biovolume was given.	The biovolume was not calculated and when derived from the literature there was a large range of uncertainly (e.g. species was not identified, and different species vary largely in size)	The biovolume was not calculated but could be derived from the literature with some uncertainty (e.g. species identified, and cell size variation is limited)	The biovolume calculated was derived from the measurement of at least 20 cells using shapes for biovolume that meet the Standard
Total Score **=(A +B + C)**				

*IANZ accredited results for cyanobacteria counts will be assumed to have met the Standard unless a variance is indicated by the accredited laboratory.

6.2.1.2 Considerations

The following records shall be utilised in the quality coding process:

- Office and Field Manual (subsection 1.1)
- Field Record Form (subsections 1.4 and 3.2.4 and Annex B)
- Chain of Custody Form (subsections 4.3.1 and 5.2, and Annex C), and
- Laboratory report (subsection 5.4).

Site metadata (subsection 2.3) will also need to be consulted from time to time.

Field measurements:
The initial quality code for field measurement data should be assigned by the field personnel who made the measurements.

Laboratory measurements:
Quality coding of laboratory measurement data is a two-step process. The first step involves the laboratory conducting checks identified in subsection 5.2 (time, temperature, age and condition of samples on arrival at the laboratory) and subsection 5.5 (quality checks), and providing comments on these checks to the monitoring agency.

The second step is best carried out by a data analyst within the monitoring agency who collates all information about each sample, including that provided from the laboratory, to assign the final quality code to the measurement value. Measurements should ideally be checked within two to three weeks of receipt from the laboratory to enable sample re-testing if necessary (see subsection 5.5.3).

6.2.1.3 Batch coding

Quality coding in batches is recommended. The original data archive should be copied into a processing/batch file. Any edits to the data and changes to codes should only occur in this file. When applying a quality code, a comment should sit alongside that code to enable other staff to determine why that code has been assigned. Original data sitting in the processing/batch file will have QC of 0 until an alternate quality code has been assigned.

Once a batch file is completed, a different staff member should QA the batch file before it is committed to the final archive.

6.2.1.4 Data that do not meet the Standard

Measurement data that do not meet this Standard shall not be assigned QC 600 but assigned a quality value from QC 100 to QC 500.

Note: A quality value of QC 600 shall only be assigned where the requirements of this Standard are met.

6.3 Data preservation and storage

The planktonic cyanobacterial data and visit metadata shall be stored together (ideally using time-series management software), linked with a single sampling date and time. A link to the following records should also be considered:

- site metadata
- quality assurance, and
- any legal requirements, confidentiality agreements and/or restrictions related to data access.

All original records shall be retained indefinitely by the monitoring agency.

6.3.1 Database comments

Comments, including visit metadata, are useful to explain unusual features that are not easily quality coded, of which data users should be aware.

Note: Current software packages provide several ways to build a database of comments. Comments can be entered into an unstructured file of text. If the comments are entered into an ODBC (Open Database Connectivity) database, they can be accessed by any ODBC-compliant software. Therefore, the latter method is recommended.

6.3.2 Data files

Any data that have been subsequently edited or adjusted shall be retained separate from the original data. This ensures that data can be traced back to the original values. Examples of editing or adjustment include:

- correction of sample date or time
- correction of the planktonic cyanobacteria taxa, cell count of biovolume measurement due to errors associated with its calculation
- correction of a laboratory planktonic cyanobacteria value following re-analysis of planktonic cyanobacterial sample, and
- averaging replicate measurement values to report a single final value.

6.4 Data archiving

The archiving procedures, policies and systems shall consider:

- future data format changes
- off-site duplication of records, and
- disaster recovery.

6.5 Quality assurance

All agencies should implement a standard methodology for data audit and review.

Note: This is to ensure standardisation of data sets that enable meaningful analyses and comparison of data within regions, between regions and nationally.

6.5.1 Audit cycle

Quality assurance processes shall include an audit of the data:

- at a frequency appropriate to the needs of the monitoring agency and end users, or
- as defined by the monitoring agency's quality management system or documented procedures.

This work shall be carried out by a suitably qualified and experienced practitioner.

Unaudited data that are released for use shall be identified as "unaudited".

Note: Datasets other than those under review may be included in the audit to help with comparisons. Where available, reliable data records held by other agencies may be used.

6.5.2 Minimum audit report requirements

As a minimum, analyses and information required for an audit report shall cover:

- site and deployment metadata details, including site details
- comments and quality coding attached to the records
- data tabulations, and
- data plots.

6.5.3 Site and analysis details

The following shall be included in the audit report:

- a site details summary, and
- a location map, with monitoring sites identified.

The site details summary shall:

- identify the lake, river, or reservoir
- identify other planktonic cyanobacterial data utilised in the audit report for comparison purposes or for generating synthetic values to fill a missing record (where relevant), and
- for each individual planktonic cyanobacteria variable record (measurement), identify:

  – the date and time of record collection

  – the site name and number

  – GPS reference (lat, long.)

  – sample collection method details

  – laboratory analytical method details

–the laboratory report, and

–comments of observation noted in the laboratory report or associated correspondence.

6.5.4 Comments and quality coding

The following shall be included in the audit report:

- for each planktonic cyanobacterial record being reviewed, a copy of the filed comments for the record, and
- a copy of the quality codes of all the data being audited.

6.5.5 Other requirements

6.5.5.1 Outputs

Recommended QA audit report outputs include:

- a hard copy report
- an electronic report, or
- at a minimum, an electronic document that identifies which records have passed the audit.

6.5.5.2 Audit Certification

The completed audit shall contain the name and signature of the auditor and the date that the audit was completed.

Annex A – List of Referenced Documents

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Annex B – Example Field Record Form

Note: The following form is intended to serve as a guide only.

Example field sampling sheet for planktonic cyanobacteria

Contact Information:

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

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

Sample Information:

Date:..... /..... /.....Time: ..... Sample location.....

GPS. Latitude …….. Longitude……,..

Sampling method (no. of samples, 5m Tube sample, Van Dorn sampling):
.....

Depth of sample/s (m):.....

Distance from shore (m):.....

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

Potable (drinking) Stock drinking Irrigation Oxidation pond

Recreation (please state activities; eg, swimming/sailing/fishing): .....

Other (please state):.....

Weather Conditions:

Weather at time of collection (circle): Clear, Drizzling, Light rain, Moderate rain, Heavy rain

Cloud cover: 1–8 (1 = clear):.....Wind strength: 1–8 (1 = calm): ……. Wind direction:.....

Weather conditions for 24 hours prior to sampling: .....

Bloom Information:

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

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

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

Limnological Data:

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

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

Associated samples taken (CTD profile, DO profile, Nutrients)

……

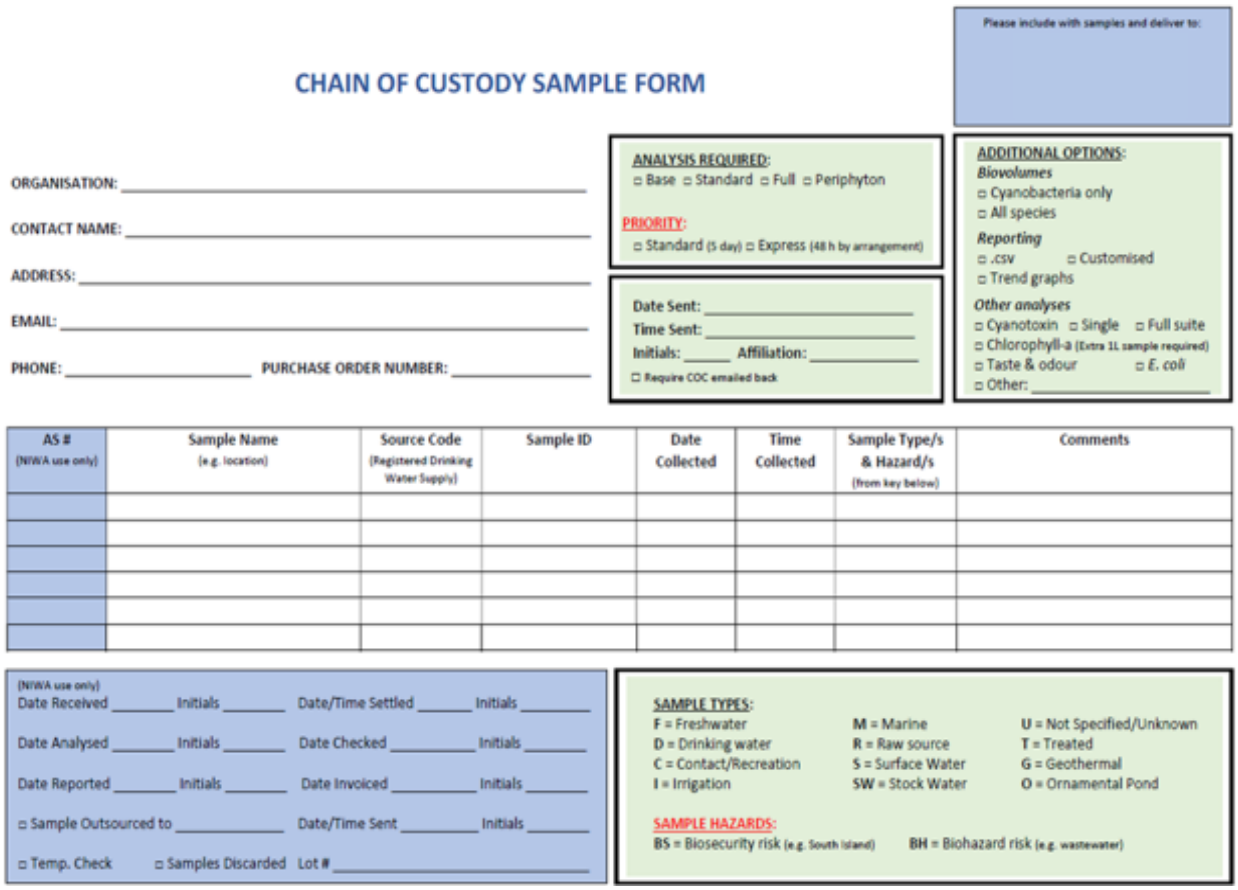
Predominant catchment cover (eg, farmland):... .....

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

Additional Comments:

.....

Annex C – Example Chain of Custody Form



Please include with samples and deliver to:

CHAIN OF CUSTODY SAMPLE FORM

ORGANISATION: _____

CONTACT NAME: _____

ADDRESS: _____

EMAIL: _____

PHONE: _____ PURCHASE ORDER NUMBER: _____

ANALYSIS REQUIRED:
□ Base □ Standard □ Full □ Periphyton

PRIORITY:
□ Standard (5 day) □ Express (48 h by arrangement)

Date Sent: _____
Time Sent: _____
Initials: _____ Affiliation: _____
□ Require COC emailed back

ADDITIONAL OPTIONS:
Biovolumes
□ Cyanobacteria only
□ All species
Reporting
□ .csv □ Customised
□ Trend graphs
Other analyses
□ Cyanotoxin □ Single □ Full suite
□ Chlorophyll-a (Extra 1L sample required)
□ Taste & odour □ *E. coli*
□ Other: _____

AS # (NIWA use only)	Sample Name (e.g. location)	Source Code (Registered Drinking Water Supply)	Sample ID	Date Collected	Time Collected	Sample Type/s & Hazard/s (from key below)	Comments

(NIWA use only)
Date Received _____ Initials _____ Date/Time Settled _____ Initials _____
Date Analysed _____ Initials _____ Date Checked _____ Initials _____
Date Reported _____ Initials _____ Date Invoiced _____ Initials _____
□ Sample Outsourced to _____ Date/Time Sent _____ Initials _____
□ Temp. Check □ Samples Discarded Lot # _____

SAMPLE TYPES:
F = Freshwater
D = Drinking water
C = Contact/Recreation
I = Irrigation
M = Marine
R = Raw source
S = Surface Water
SW = Stock Water
U = Not Specified/Unknown
T = Treated
G = Geothermal
O = Ornamental Pond

SAMPLE HAZARDS:
BS = Biosecurity risk (e.g. South Island)
BH = Biohazard risk (e.g. wastewater)

Annex D – Lugol's iodine

Samples for later counting can be preserved immediately at the sampling site or upon arrival at the laboratory. Preservation is achieved by adding Lugol's iodine solution at a ratio of 1:100 (Vollenweider, 1969). This gives the sample a weak tea colour; drops should be added until this is achieved.

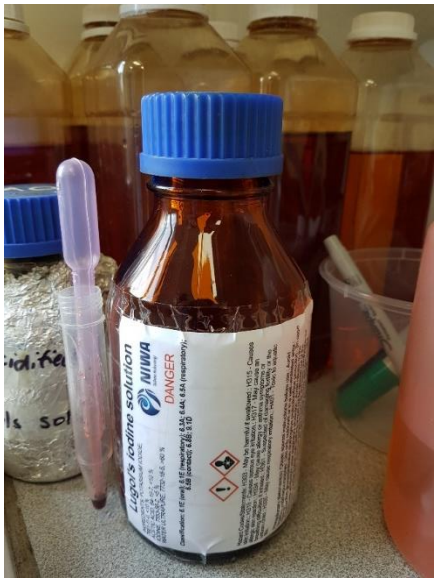
The use of around 5 mL of Lugol's solution per litre of sample is recommended, especially for samples that may be kept for longer periods. However, it should be noted that for meso- and especially oligotrophic waters more than 2 mL might cause over saturation rendering the algae difficult to identify.

If a higher ratio is needed to preserve the sample, this should be noted on the field data sheet and taken into account during enumeration when calculating cell concentrations.

Samples so preserved will keep for months if stored correctly and topped up with the preservative.

Lugol's solution

Add 20 mL of glacial acetic acid to approx. 100 mL of distilled water in a 200 mL volumetric flask. Wash 20 g potassium iodide and 10 g iodine crystals into the flask. Dissolve and make up to the mark. The stock solution must be stored in a dark and well-ventilated space (Vollenweider, 1969) in a glass bottle and remains effective for at least a year.



Annex E – Cyanobacterial taxa identified in New Zealand

Taken from Drinking Water Guidelines Table 9.2; dwg-ch9-cyanobacterial-compliance May 2020

Summary of known toxin-producing cyanobacteria identified internationally by isolation of cultured strains. Species in **bold type** are known to produce the associated toxin (also in bold type) in New Zealand.

Cyanobacteria Genus/Species	Cyanotoxin/s
Anabaena sp.	Microcystins
Anabaenopsis millenii	Microcystins
Annamia toxica	Microcystins
Aphanizomenon flos-aquae	Anatoxin-a, Cylindrospermopsins, Saxitoxins
Aphanizomenon gracile	Anatoxin-a, Cylindrospermopsins, Saxitoxins
Chrysosporum ovalisporum	Cylindrospermopsins
Aphanizomenon sp.	Anatoxin-a, Cylindrospermopsins, Saxitoxins
Aphanocapsa cumulus	Microcystins
Arthrospira fusiformis	Anatoxin-a,
Chrysosporum ovalisporum	Cylindrospermopsins
Coelosphaerium kuetzingianum	Microcystins
Cuspidothrix issatchenkoi	**Anatoxin-a**
Cylindrospermum stagnale	Saxitoxins
Cylindrospermum sp.	Anatoxin-a, Saxitoxins
Dolichospermum circinale	Anatoxin-a
Dolichospermum flos-aquae	Anatoxin-a, Anatoxin-a(S), Microcystins
Dolichospermum lapponica	Cylindrospermopsins
Dolichospermum lemmermannii	**Anatoxin-a**, Anatoxin-a(S), Microcystins
Dolichospermum sp.	Anatoxin-a, Anatoxin-a(S), Cylindrospermopsins, Microcystins
Dolichospermum spiroides	Anatoxin-a(S)
Dolichospermum subcylindrica	Microcystins
Dolichospermum ucrainica	Microcystins
Dolichospermum variabilis	Microcystins
Fischerella sp.	Microcystins
Geitlerinema amphibium	Saxitoxins

Cyanobacteria Genus/Species	Cyanotoxin/s
Geitlerinema carotinum	Anatoxin-a, Microcystins
Geitlerinema lemmermannii	Saxitoxins
Geitlerinema splendidum	Anatoxin-a, Microcystins
Gloeotrichia natans	Microcystins
Hapalosiphon hibernicus	Microcystins
Heteroleiblenia kuetzingii	Microcystins
Iningainema pulvinus	Nodularin
Leptolyngbya sp.	Microcystins
Limnothrix mirabilis	Microcystins
Microcoleus autumnalis	**Anatoxins**
***Microcoleus* sp.**	**Anatoxins**, Microcystins,
Microcystis aeruginosa	**Microcystins**, Saxitoxins
Microcystis botrys	Microcystins
Microcystis flos-aquae	Microcystins
Microcystis ichthyoblabe	Microcystins
Microcystis novacekii	Microcystins
Microcystis panniformis	Microcystins
***Microcystis* sp.**	Anatoxin-a, **Microcystins**, Saxitoxins
Microcystis viridis	Microcystins
Microcystis wesenbergii	Microcystins
Microseria wollei	Cylindrospermopsins, Saxitoxins
Nodularia spumigena	**Nodularin**
Nodularia sphaerocarpa	Nodularin
Nostoc commune	Microcystins
Nostoc linckia	Microcystins
Nostoc muscurum	Microcystins
***Nostoc* sp.**	**Microcystins**, Nodularin
Nostoc spongiaeforme	Microcystins
Oscillatoria agardhii	Microcystins
Oscillatoria limnetica	Anatoxin-a
Oscillatoria limosa	Microcystins
Oscillatoria margaritifera	Microcystins
***Oscillatoria* sp.**	**Anatoxin-a**, **Microcystins**

Cyanobacteria Genus/Species	Cyanotoxin/s
Phormidium ambiguum	Cylindrospermopsins
Phormidium corium	Microcystins
Phormidium favosum	Anatoxin-a
Phormidium sp.	Anatoxin-a, Microcystins
Phormidium splendidum (Syn. *Geitlerinema splendidum*)	Microcystins
Phormidium uncinatum	Anatoxin-a, Microcystins, Saxitoxins
Planktothrix agardhii	Microcystins
Planktothrix formosa	Anatoxins
Planktothrix rubescens	Microcystins
***Planktothrix* sp.**	Anatoxins, **Microcystins**, Saxitoxins
Plectonema boryanum	Microcystins
Pseudoanabaena frigida	Microcystins
Pseudocapsa dubia	Microcystins
Radiocystis fernandoi	Microcystins
Raphidiopsis brookii	Saxitoxins
Raphidiopsis curvata	Cylindrospermopsins
Raphidiopsis mediterranea	Anatoxins, Cylindrospermopsins
Raphidiopsis raciborskii	**Cylindrospermopsins**, Saxitoxins, Microcystins
Rivularia biasolettiana	Microcystins
Rivularia haematites	Microcystins
Schizothrix rivularianum	Microcystins
Scytonema* cf. *crispum	**Saxitoxins**
Scytonema drilosiphon	Microcystins
Snowella sp.	Microcystins
Synechococcus lividus	Microcystins
Synechocystis sp.	Microcystins
Tolypothrix distorta	Microcystins
Tychonema bourrellyi	Anatoxins
Umezakia natans	Cylindrospermopsins
Woronichinia sp.	Microcystins
Notes: This is a compilation of worldwide information. New toxic species continue to be identified, and all cyanobacteria should be regarded as potentially toxic until proven otherwise.	

Annex F – Cyanobacterial counting measurement uncertainty

A whole-Settling Chamber count is used when cell counts are relatively low. Accuracy of the result will not be as high when the number of cells counted is low, but a higher margin of error is tolerable for low cell-counts as the maximum value (total ± error margin) falls short of levels that cause concern (for example, sensitive standards, such as those required for drinking water, would be higher than those for SoE reporting).

For cyanobacteria, with individual cells or small colonies, it is recommended to set the level of precision to ± 20% error for the most abundant taxa, following Lund et al. (1958) and as described in Hötzel and Croome (1999) (Table 1). This level of precision is reached by counting a minimum of 100 algal units of the taxon in question per sample. The counting precision for less common taxa will always be lower than that for common taxa since the required minimum of 100 algal units will not be found in the sample.

The counting error (precision) can be estimated with a 95% confidence limit by:

$=2/\sqrt{N} \quad x\ 100$ = counting error (± %)

For example, if 100 units are counted, the error is ± 20%; if 400 units are counted the error is reduced to ± 10%.

Table 1: Cell Count Accuracy (Lund et al., 1958).

Cell no. counted	**Accuracy expressed as % of count (95 confidence limits)**	
4	±100	
16	± 50	
100	±20	= 100 units/unicells
400	±10	
1600	±5	

Annex G – The Utermöhl method

The methodology described here is simplified; for further details see:

Edler L, Elbrächter M. 2010. The Utermöhl method for quantitative phytoplankton analysis. Chapter 2 in: Karlson B, Cusack C, Bresnan, E. (Eds.) *Microscopic and molecular methods for quantitative phytoplankton analysis*. IOC Manuals and Guides no. 55, UNESCO, Paris.

Introduction

The Utermöhl method (Utermöhl, 1958) is used to both identify and enumerate algal cells, including cyanobacteria. Using this method, it is also possible to determine individual cell size, form, and biovolume. The method is based on sedimentation of a known volume of water sample in a chamber. Gravity causes the phytoplankton cells to settle on the bottom of the chamber. The settled cells can then be identified and enumerated using an inverted microscope; however, to quantify the result as cells per mL a conversion factor must also be determined (Edler and Elbrächter, 2010).

Materials

Equipment:

Sample bottles

It is preferable that PET (polyethylene terephthalate) plastic or glass bottles be used; however, if samples are analysed immediately or within a few days a variety of plastic vials/bottle may be used. Note that the Lugol's iodine may be absorbed by the plastic if it is not PET (polyethylene terephthalate). For long-term storage, PET or glass sample bottles should be used to minimise any chemical reaction with the preservative.

These samples must be stored in the dark to prevent the degradation of Lugol's iodine in light. It is important the bottle cap is securely tightened to avoid spillage and/or evaporation of the preservative. Utermöhl (1958) recommends that the bottle is filled to 75–80% of its volume as when mixed this facilitates the homogenisation of the sample before dispensing into the sedimentation chamber.

The most commonly used preservative is potassium iodine used in the form of "Lugol's iodine solution" – usually in its acidic form. If samples are stored for long periods, other preservatives can be used, such as neutral formaldehyde or glutaraldehyde. These, however, have more hazardous properties and are not suitable for all algal types (Edler and Elbrächter, 2010).

Sedimentation chambers

A range of Utermöhl sedimentation chambers are available, ranging in volume from 2 to 100 mL. The thickness of the coverslip should not exceed 0.2 mm, as this will affect the resolution achievable by the microscope. Alternatively, a range of plastic and glass "well plates" made for microscopy may also be suitable.

Counting chambers should be calibrated. This is achieved by first weighing the chamber while empty and then when filled with water, to confirm the volume.

Examples of settling chambers (Figure 1) that consist of four parts:

1. A plexiglass tube (internal 24, 25 or 26 mm diameter). This part of the chamber holds a given volume of sample (e.g. 10 mL for a 10 mL settling chamber).

2. A glass cover slip, which is placed over the bottom of the tube (with vacuum grease on its lip).

3. Metal rim (into which the tube and glass cover slip is screwed).

4. A clear, ground glass lid that allows light to pass through.

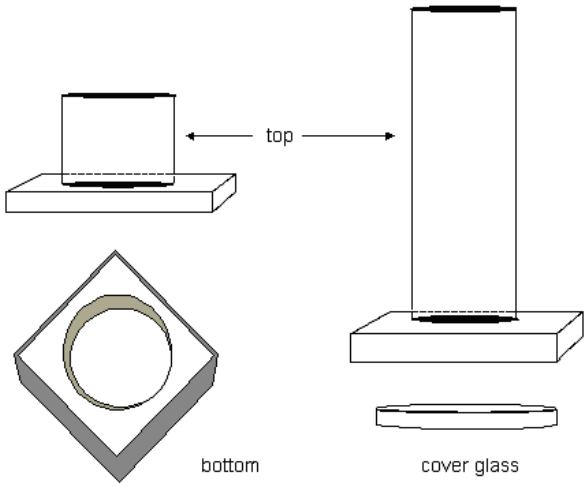


Figure 1: Settling chambers.

The inverted microscope

For quantitative analysis using sedimentation chambers, an inverted microscope is required (Figure 2). The optical quality of the microscope is crucial for facilitating phytoplankton identification. Phase- and/or differential interference-contrast is helpful for the identification of most phytoplankton.

- One eyepiece should be equipped with a calibrated ocular micrometer. The other eyepiece can be equipped with a grid, square or two parallel threads forming a transect.
- The eyepiece micrometer and counting graticule must be calibrated for each magnification using a stage micrometer.
- The microscope should have objectives of 5x, 10x, 20x and 40–60x. For detailed examination a 100x oil immersion objective may also be used.

In order to survey the entire bottom plate, the microscope must be equipped with a movable mechanical or electronic stage.

Figure 2: An inverted microscope with counting chamber.

Cell counters

A cell counter is recommended. It is also now common to have a computerised counting programme beside the microscope, so that the observed species are registered directly into a database.

Laboratory facilities

Laboratory facilities necessary for the quantitative analysis of phytoplankton require amenities for storing, handling (mixing and pouring samples) and washing of sedimentation chambers.

Preserved samples should be stored in cool and dark conditions.

Level settling

During sedimentation the chambers should be placed on a level, horizontal and solid surface. This will prevent any non-random distribution of settled phytoplankton cells.

Methods

Preparation of sample and preservation

Once the sample has been collected from the field and poured into the sample bottle it should be immediately preserved using 5 mL of Lugol's solution per litre of sample (standard). However, it should be noted that for meso- and especially oligotrophic waters no more than 2 mL of Lugol's solution may be required (see Annex D).

The advantage of Lugol's iodine solution is that it has an instant effect and increases the weight of the organisms, reducing sedimentation time.

Lugol's iodine solution will cause discolouration of some phytoplankton making identification difficult. To allow for this effect, a live sample may also be kept and examined. Alternatively, to reduce discolouration by Lugol's solution, the sample can be bleached with sodium thiosulfate prior to analysis (Edler and Elbrächter, 2010).

Storage of samples

Preserved phytoplankton samples shall be stored in cool and dark conditions.

When using Lugol's iodine solution, the colour of the sample should be checked regularly and if necessary, more preservative added. Preserved samples should be analysed without delay. Samples must be analysed within 3 months as if stored for more than a year they are considered of little use (Olenina et al., 2006).

Temperature adaptation

The first step in the analysis procedure is to adapt the phytoplankton sample and the sedimentation chamber to room temperature. This prevents convection currents and air bubbles forming in the sedimentation chamber. If this is not carried out, non-random settling of the phytoplankton cells may occur.

Chamber preparation

Sediment chambers must be clean and dust free to avoid contamination from previous samples. Many laboratories use a new base plate after every sample. Sometimes it is necessary to grease the chimney bottom with a small amount of Vaseline/vacuum grease to ensure the chamber parts are tightly sealed (Andersen and Throndsen, 2004). In studies where a succession of phytoplankton samples are examined over a period of time, it is important to use the same chamber volume for the analysis (Hasle, 1978a,b).

At times, the "standard" chamber size may be either too small (extreme winter situations) or too large (phytoplankton blooms) and another chamber size must be used.

Sample homogenisation

Before the sample is poured into the sedimentation chamber, the bottle should be rotated firmly, but gently, to homogenise the contents. The sample is mixed by gently inverting the sample bottle for at least 30 seconds or a minimum of 20 times before sub-sampling and aliquoting into the determined settling chamber. Violent shaking will produce bubbles, which can be difficult to eliminate. It is recommended to check that homogenous sample distribution is being achieved by counting 3 subsamples from the same stock sample several times per year.

Concentration/dilution of samples

Although it is possible to concentrate or dilute samples that are either too sparse or too dense, these practices are not recommended because additional handling steps may interfere with the sample contents. Instead, it is recommended that a sediment chamber of an appropriate size be used to allow accurate identification and enumeration of cells.

Filling the sedimentation chamber

After homogenisation, the sedimentation chamber is placed on a horizontal surface and gently filled from the sample bottle. The chamber is then sealed with a cover glass. It is important that no air bubbles are left in the chamber. It may be necessary to grease the cover glass with a little Vaseline to maintain a tight seal.

Sedimentation

The sedimentation should take place at room temperature and out of direct sunlight. To minimise evaporation, the sedimentation chamber may be covered with a plastic box, and a Petri dish containing water should be placed beside the chamber.

Settling time is dependent on the height of the chamber and the preservative used (Lund et al., 1958; Nauwerck, 1963). Recommended settling times for Lugol's preserved samples are shown in Table 1.

Table 1. Settling time is dependent on the height of the Settling Chamber and the preservative The times given are the minimum. If vibration is a problem, the minimum time should not be significantly exceeded otherwise it is suggested that counting be performed within four days. Sedimented samples not counted within a week should be discarded. Time are equivalent to 0.5h settling time per millimetre of column depth.

Settling Chamber volume (mL)	Height of chamber (cm)	Settling time required in Lugol's solution (Hours)
~ 2	1	3 h
~ 5	1	3 h
~ 10	2	8 h
~ 50	10	25 h
~ 100	20	50 h

Care should be taken not to introduce air bubbles when settling. The transfer of the chamber or bottom plate to the microscope will not affect the distribution of the settled phytoplankton cells if there are no air bubbles present. Larger settling chambers will require the top of the chamber to be removed – this slides aside, leaving a smaller undisturbed volume. The chamber or bottom plate is placed on the inverted microscope and the phytoplankton cells are identified and counted.

Counting procedure

- The quantitative analysis should start with a scan of the entire chamber bottom at a low magnification. This will help to give an overview of the density and distribution of phytoplankton.
- If the distribution is considered uneven, the sample must be discarded.
- During this scan it is also convenient to make a preliminary species list, which may help to select the counting strategy.
- Organisms should be identified to the lowest taxonomic level that time and skill permits.
- Ultimately, the objective of the study will determine the level of identification accuracy required.
- Counting begins at the lowest magnification, followed by analysis at successively higher magnification.

- For adequate comparison between samples, regions and seasons, it is important to always count the specific species at the same magnification.
- In some situations (such as bloom conditions), this may not be possible. Large species which are easy to identify (e.g. C*eratium* spp.), and which are usually relatively sparse, can be counted at the lowest magnification over the entire chamber bottom.
- Smaller species are counted at higher magnifications, and if needed, only on a part of the chamber bottom.
- Counting the whole chamber bottom is done by traversing back and forth across the chamber bottom. The eyepiece or parallel eyepiece threads/ grid or square delimit the transect where the phytoplankton are counted.

Counting part of the chamber bottom can be done in different ways. If half the chamber bottom is to be analysed, every second transect of the whole chamber is counted. If a smaller part is to be analysed, one, two, three or more diameter transects are counted.

When counting cells that fall only partly within a FOV (field of view) or transect line only half should be only counted. For example you would count only half the bordering area counting cells crossing two halves of a square or half a round FOV but omitting counting the other half.

To obtain a statistically robust result from the quantitative analysis, it is necessary to count a minimum number of counting units (cells, colonies or filaments) (See Table 1, Annex F).

Recommended magnification for counting of different size classes of phytoplankton (Edler, 1979; Andersen and Throndsen, 2004):

- Size class
    - 0.2–2.0 µm (picoplankton)*: 1000 x
    - 2.0–20.0 µm (nanoplankton): 100–400 x
    - > 20.0 µm (microplankton): 100 x
      (* picoplankton are normally not analysed using the Utermöhl method)

Cleaning of sedimentation chambers

The cleaning of sedimentation chambers is a critical part of the Utermöhl method. The chambers should be cleaned immediately after analysis. A tissue and general-purpose detergent should be used. To clean the chamber margin properly a toothpick can be used. Usually it is sufficient to clean the chamber bottom coverslip without dissembling the bottom glass.

Sometimes, however, it is necessary to separate the bottom coverslip from the chamber, either to clean it or to replace it. This is easily done by disassembling the chamber. Care should be taken as the bottom glasses are very delicate. Counting chambers should be checked regularly to ensure that no organisms stick to the bottom glass. This can be achieved by filling the chambers with distilled water and viewing the chamber at suitable magnification.

Quality assurance

To ensure high quality results are routinely achieved, all steps of the method must be validated. Ideally this is performed on natural samples, but in some instances it may be helpful to spike the sample with cultured algae. Steps in the Utermöhl method that must be validated are:

• homogenisation of sample

• sedimentation/sinking

• distribution on chamber bottom

• repeatability and reproducibility.

Ultimately, the quality of the result from this method is dependent on the skill of the analyst. The variation of parallel samples counted by the same analyst and the variation in parallel samples counted by different analysts are two of the most important considerations in quality assurance. When possible, laboratories should take part in interlaboratory comparisons.

The precision is usually expressed as the 95% confidence limit as a proportion of the mean.

See Table 1, Annex F for the relationship between number of units counted and the uncertainty.

In many studies it has been decided that counting of 50 units of the dominant species, giving a 95% confidence limit of 28%, is sufficient. Increasing the precision to, for example, 20% or 10% would need a dramatic increase in counted units (100 and 400, respectively) (Edler, 1979).

It will often not be possible to count 50 units of all species present in a sample. Some species may not be sufficiently abundant, which will decrease the overall precision. To maintain an acceptable precision for the entire sample a total of at least 500 units should be counted (Edler, 1979).

The counting unit of most phytoplankton species is the cell. In some cases, this is not practical. For filamentous cyanobacteria, for instance, the practical counting unit is a certain length of the filament, usually 100 µm (Olenina et al., 2006).

In some colony-forming species and in colonies, it may be difficult to count the individual cells. In such cases the colony should be the counting unit. If desired, the calculation of cells per colony/coenobium can be approximated by a thorough counting and calculation of the mean value derived from analysis of several colonies. Colonial disaggregation may also be used (see section 5.3.1).

The transformation of the microscopic counts to the concentration or density of phytoplankton of a desired water volume (usually litre or millilitre) can be achieved using these equations:

Equation 1.

$$Cells\ L^{-1} = N * \left(\frac{At}{Ac} \right) * \frac{1000}{V}$$

Equation 2

$$Cells\ L^{-1} = N * \left(\frac{At}{Ac} \right) * \frac{1}{V}$$

V: volume of counting chamber (mL)

At: total area of the counting chamber (mm^2)

Ac: counted area of the counting chamber (mm^2)

N: number of units (cells) of specific species counted

C: concentration (density) of the specific species.

Annex H – Sedgewick-Rafter counting slide method

The methodology described here is simplified; for further details see:

LeGresley M, McDermott G. 2010. Counting chamber methods for quantitative phytoplankton analysis-haemocytometer, Palmer-Maloney cell and Sedgewick-Rafter cell. Chapter 4 in: Karlson B, Cusack C, Bresnan E. (Eds.) *Microscopic and molecular methods for quantitative phytoplankton analysis*. IOC Manuals and Guides no. 55, UNESCO, Paris.

Introduction

The Sedgewick-Rafter counting slide is a traditional counting method using a compound microscope (Figure 1). It follows many of the principles of the Utermöhl method but is a rapid method for quantifying samples with high cell numbers. The slide is comprised of a transparent base, which has a centrally mounted chamber (50 mm x 20 mm x 1 mm deep) and can hold 1 mL of sample.

The base of this chamber has a ruled 1 mm grid, so that the 1 mL sample is subdivided into single microlitres. This chamber is covered by a cover glass, which protects the sample from drying out and disturbances by air currents.

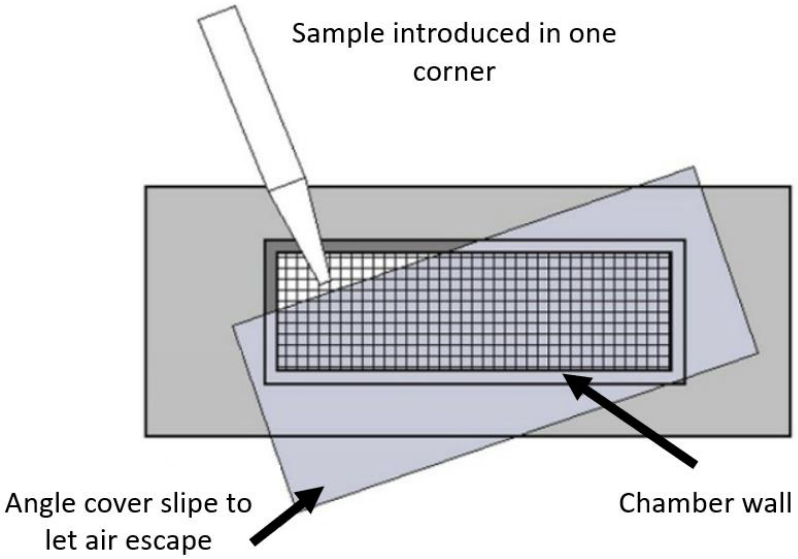


Figure 1: Sedgewick-Rafter counting slide and filling method.

The sample is then counted using a compound microscope.

Materials and equipment

- A standard, compound microscope with 10x and 20x objectives and brightfield and phase contrast. A 40x objective may not be able to be used for this analysis; this depends on the working distance of the objective lenses.

• A Sedgewick-Rafter slide: This can be made from plastic or glass.

• A tally counter is useful when counting high cell numbers.

• A pipette is needed to dispense the sample into the cell.

• Sample bottles for storing the samples.

• Solution for preservation - Lugol's iodine

Methods

1. Prior to analysis the sample should be homogenised.

   This is achieved using a combination of horizontally rolling and vertically turning the sample bottle as gently as possible to prevent the breakup of colonies and the accumulation of bubbles.

2. From a well-mixed sample, 1 mL is removed using a pipette. The pipette should have a wide opening that does not restrict the movement of larger phytoplankton species.

   This is especially important when using a 1 mL pipette with removable tips. The end of the tip should be cut off to widen the opening but care is needed not to alter the accuracy/repeatability of volume dispensed.

3. The cover glass should be placed carefully onto the counting slide, perpendicular to the long axis of the slide, so one corner is left open for filling and another for the escape of air.

4. The sample aliquot is then dispensed into the counting cell (Figure 1).

5. Slowly swing the cover glass so that it completely covers the sample.

   Careful alignment of the cover glass will prevent air bubbles from being introduced into the sample and will ensure that the sample holds its complete volume. If a bubble develops, refill the counting cell.

6. Preserved samples should be left to settle for 15 minutes before enumeration.

7. The sample is then examined using a compound microscope.

   The slide should first be scanned under low magnification to estimate the concentration of cells. Using this information, a counting strategy is decided upon as to whether the whole slide or a noted fraction is to be counted.

8. If the concentration of phytoplankton in the Sedgewick-Rafter slide is too dense and the cells are overlapping thus hindering identification, a dilution step should be performed using RO or DI water.

9. After analysis is completed the slide is washed and cleaned between samples to prevent cross-contamination. A detergent like a liquid soap is recommended.

Quality checks:

- General internal and external quality control measures that apply to planktonic cyanobacterial sample processing are outlined in subsection 5.5.

Preservation and storage (see section 5.3)

Formulas for calculating results:

The Sedgewick-Rafter slide has a volume of 1 mL with the base of the cell being divided into 1,000 squares (50 column by 20 rows), each representing 1/1,000 of the volume of the slide.

- To obtain a final result expressed as cells L^{-1}, the following equation is used to calculate the multiplication factor (F).

Equation 1.

$$F = \frac{(1000)}{Number\ of\ squares\ counted} * 1000$$

- *F* is dependent on the number of squares of the base of the cell counted during the analysis.

- For example, *F* for the Sedgewick-Rafter slide: 4 rows (200 squares) are counted:

$$F = \frac{1000}{200} * 1000 = 5 * 1000 = 5000$$

- For example, *F* for the Sedgewick-Rafter slide: 50 rows (1000 squares) (the entire slide) is counted:

$$F = \frac{1000}{1000} * 1000 = 1 * 1000 = 1000$$

Comments

The Sedgewick-Rafter slide is best used when analysing high-biomass blooms. As this method does not require an overnight settling period, it is rapid and can provide a quick assessment of a water sample. It has been proven to provide accurate results in samples with algal cell densities between 10,000 (ICES, 2006) and 100,000 cells L^{-1} (McAlice, 1971). The set-up cost is low due to the use of a compound microscope. The Sedgewick-Rafter slide tends to perform better with samples containing larger phytoplankton cells.

Another counting method may have to be used for samples with low cell densities. It may be possible to pre-concentrate cells using a filtering/settling step when the target organism is present in low concentrations.

Plastic Sedgewick-Rafter slides tend to scratch easily, and care must be taken when cleaning the cell. Scratches may hinder accurate identification of cells.

Due to the design of the Sedgewick-Rafter slide it may be difficult to use the 40x magnification.

This could prove a problem in the identification of smaller (10–15 μm) phytoplankton cells. In addition, extreme care must be taken to load the Sedgewick-Rafter slide correctly and avoid the introduction of air bubbles to ensure the even distribution of phytoplankton in the slide.

Annex I – Planktonic cyanobacterial monitoring for non-SoE purposes and future monitoring options

A) Monitoring for health risk to recreational use

The interim *New Zealand Guidelines for Cyanobacteria in Recreational Waters* (Ministry for the Environment and Ministry of Health, 2009) are available online:

Cyanobacteria Interim Guidelines (environment.govt.nz)

This sampling approach is designed to measure the maximum exposure risk for human health when in recreational waters and trigger testing of cyanobacterial toxins. A more recent review indicates the way this type of monitoring is changing (Wood et al., 2018):

Review of the 'New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters' - 2018 (environment.govt.nz)

B) Monitoring risk to drinking water

The Water Services (Drinking Water Standards for New Zealand) Regulations 2022 contain maximum acceptable values of toxins known to be produced by planktonic cyanobacteria. Monitoring for compliance with these regulations is focused on toxin levels and looks at cyanobacterial counts and biomass as precursors to toxin testing. Specific guidance can be found in guidelines:

Drinking-Water Guidelines - Chapter 9: Cyanobacterial compliance (health.govt.nz)

C) Monitoring biomass using phycocyanin or phycoerythrin (laboratory and in-situ)

Phycocyanin (phycoerythrin) fluorescence sensors and in-situ instruments are being developed as tools for providing fast and cost-effective measurements of cyanobacterial biomass. However, poor precision and low sensitivity in many of the probe sensors assessed to date have restricted their potential for cyanobacterial monitoring programmes. This, however, is a fast-developing area of research with new and better probes becoming available. More recent studies also suggest that the more sensitive laboratory systems that are becoming available may overcome some of the limitation of in-situ systems and maybe a useful monitoring tool. Some references are given below.

Almuhtaram H, Kibuye FA, Ajjampur S, Glover CM, Hofmann R, Gaget V, Owen C, Wert EC, Zamyadi A. 2021. State of knowledge on early warning tools for cyanobacteria detection. *Ecological Indicators 133*: 108442.

Thomson-Laing G, Puddick J, Wood SA. 2020. Predicting cyanobacterial biovolumes from phycocyanin fluorescence using a handheld fluorometer in the field. *Harmful Algae 97*: 101869.

Cotterill V, Hamilton DP, Puddick J, Suren A, Wood SA. 2019. Phycocyanin sensors as an early warning system for cyanobacteria blooms concentrations: a case study in the Rotorua lakes. *New Zealand Journal of Marine and Freshwater Research 53*(4): 555–570.

D) 16S rRNA and advances in genetic profiling of cyanobacteria

This is new area where groups are developing specific Gene Oligonucleotide Probes for genetic profiling of cyanobacterial abundance and diversity. Probes were designed for detecting specific cyanobacterial groups and potentially to detect specific species. In addition, there is ongoing work to look at the genes responsible for toxin production. Some references are listed below.

Castiglioni B, Rizzi E, Frosini A, Sivonen K, Rajaniemi P, Rantala A, Mugnai MA, Ventura S, Wilmotte A, Boutte C, Grubisic S. 2004. Development of a universal microarray based on the ligation detection reaction and 16S rRNA gene polymorphism to target diversity of cyanobacteria. *Applied and Environmental Microbiology 70*(12): 7161–7172.

Rudi K, Skulberg OM, Skulberg R, Jakobsen KS. 2000. Application of sequence-specific labeled 16S rRNA gene oligonucleotide probes for genetic profiling of cyanobacterial abundance and diversity by array hybridization. *Applied and Environmental Microbiology 66*(9): 4004–4011.

Kelly LT, Bouma-Gregson K, Puddick J, Fadness R, Ryan KG, Davis TW, Wood SA. 2019. Multiple cyanotoxin congeners produced by sub-dominant cyanobacterial taxa in riverine cyanobacterial and algal mats. *PloS one 14*(12): .e0220422.

Zhang W, Lou I, Ung WK, Kong Y, Mok KM. 2014. Application of PCR and real-time PCR for monitoring cyanobacteria, Microcystis spp. and *Cylindrospermopsis raciborskii* in Macau freshwater reservoir. *Frontiers of Earth Science 8*(2): 291–301.

Puddick J, Thomson-Laing G, Wood SA. 2019. Microcystins in New Zealand: a review of occurrence, congener diversity and cell quotas. *New Zealand Journal of Botany 57*(2): 93–111.

E) Picophytoplankton (Picoprokaryotes or picocyanobacteria) analysis by Flow Cytometry

Picophytoplankton (phytoplankton < 2 µm in size) can be very important, especially in oligotrophic waters. Due to their small size they are often overlooked in fresh waters when they are not colonial. In addition, it is not unusual for picophytoplankton biomass to peak at depth, often around a thermocline or pycnocline. Within the picophytoplankton, there are two recognised groups: the picoeukaryotes and picoprokaryotes, the latter of which is a class of cyanobacteria. Because of their size these cannot be counted or distinguished easily using light microscopy; however, this can be relatively easy using flow cytometry. Using this method, the two groups can also be successfully separated by their fluorescence. Below are some references that include examples of this methodology. The first of these references also looks at the comparative effort and the results using microscopy.

Salmi P, Mäki A, Mikkonen A, Pupponen VM, Vuorio K, Tiirola M. 2021: Comparison of epifluorescence microscopy and flow cytometry in counting freshwater picophytoplankton. *Boreal Environment Research 26*: 17–27.

Callieri C. 2008. Picophytoplankton in freshwater ecosystems: the importance of small-sized phototrophs. *Freshwater reviews 1*(1): 1–28.

Crosbie ND, Teubner K, Weisse T. 2003. Flow-cytometric mapping provides novel insights into the seasonal and vertical distributions of freshwater autotrophic picoplankton. *Aquatic Microbial Ecology 33*(1): 53–66.

Hall JA, Safi K. 2001. The impact of in situ Fe fertilisation on the microbial food web in the Southern Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography 48*(11-12): 2591–2613.

Annex J – Biovolume calculation

Biovolume calculation requires different cell types to be assessed using different geometric calculations of volume. Providing details of this methodology for individual species is beyond the scope of this Standard. However, we recommend referring to:

- Olenina I, Hajdu S, Edler L, Andersson A, Wasmund N, Busch S, Göbel J, Gromisz S, Huseby S, Huttunen M, Jaanus A, Kokkonen P, Ledaine I, Niemkiewicz E 2006. Biovolumes and size-classes of phytoplankton in the Baltic Sea. *HELCOM Baltic Sea Environment Proceedings 106.* 144p.

For specific New Zealand cyanobacterial examples see:

- Ministry for the Environment and Ministry of Health, 2009. *New Zealand Guidelines for Cyanobacteria in Recreational Waters – Interim Guidelines*. Prepared for the Ministry for the Environment and the Ministry of Health by S.A. Wood, D.P. Hamilton, W.J. Paul, K.A. Safi and W.M. Williamson. Wellington: Ministry for the Environment.

  (In particular, see Appendix 4, Tables A4.1 and A4.2)

- Wood SA, Paul WJ, Hamilton DP. 2008. *Cyanobacteria biovolumes for the Rotorua lakes*. Prepared for Environment Bay of Plenty. Cawthron Report No. 1505. 19p.

The method for determining cyanobacterial cell biovolume is:

- Determine the appropriate geometric shape and measure the dimensions of cyanobacterial cells using 600x or (preferably) 1000x magnification.

- Measurements for the closest geometric shape can generally only be made to approximately 0.5 µm. The majority of cyanobacterial cells have a shape that approximates to either a sphere or a cylinder (cells within a filament).

- Determine the mean cell dimension for the taxa based upon a minimum of ~30 cells per sample and up to 100 cells, depending on size variability.

- Calculate the mean cell volume using the mean cell dimension and the appropriate calculation for that shape.

The average cell volume is then multiplied by the total cell number to obtain biovolume in cubic microns per millilitre ($\mu m^3 mL^{-1}$), which should then be converted to cubic millimetres per litre ($mm^3 L^{-1}$) by dividing by 10^6.

The example below, based on *New Zealand Guidelines for Cyanobacteria in Recreational Waters – Interim Guidelines,* shows the working of biovolume calculations for two species.

For example, based on their shape:

Species	Cell shape	Formula
Microcystis wesenbergeii	Ovoid (round)	$V=((4/3)*\pi*(diam/2)^3)$
Dolichospermum planctonicum	Ovoid	$V=(4/3)*\pi*(width/2)^2*(length/2)$

Using a diameter of 6.9 µm for *Microcystis wesenbergeii* we get a biovolume of 182 μm^3

Using a length of 6.8 µm and width of 10.5 µm for *Dolichospermum planctonicum* we get a biovolume of 399 μm^3

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

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

Where:

n = number cells in a sample (cells/mL)
vol = volume of each cell (μm^3)
1×10^6 is a unit's conversion from μm^3/mL to mm^3/L.

The total biovolume (TBV) of each sample is calculated by combining the individual totals for each species.

For this example, the total biovolume in a sample containing 10,200 cells/mL of *Dolichospermum planctonicum* and 5600 cells/mL of *Microcystis wesenbergii* is calculated as follows:

BV (*D. planctonicum*) $= (10{,}200 \times 399^{\#}) / 1 \times 10^6 = 4.07\ mm^3/L$

BV (*M. wesenbergii*) $= (5600 \times 182^{\#}) / 1 \times 10^6 = 1.02\ mm^3/L$

Total BV $= 4.07 + 1.02 = 5.09\ mm^3/L$

Where # = the calculated biovolume in μm^3