National Environmental Monitoring Standards

Periphyton

Sampling and Measuring Periphyton in Wadeable Rivers and Streams

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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 nems.org.nz.

Implementation

When implementing the standards, current legislation relating to health and safety in New Zealand and subsequent amendments and the NEMS Code of Practice shall be complied with.

Limitations

It is assumed that, as a minimum, the reader of these 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.

The information contained in these NEMS documents relies upon material and data derived from a number of third-party sources.

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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 hydrological and continuous environmental-related measurements within New Zealand.

It is recommended that these Standards are 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 Standards and associated best practice may be applied will depend on the quality of data sought.

The development of this particular standard involved consultation with regional and unitary councils across New Zealand, the National Institute for Water and Atmospheric Research Ltd (NIWA), the Cawthron Institute and several analytical laboratory industry representatives. Collectively, regional and unitary councils are responsible for a significant amount of periphyton monitoring in wadeable rivers and streams across New Zealand. It is recommended that this Standard is adopted throughout New Zealand and all data collected are 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.

This document has been prepared by a working group comprising Phillip Downs (Steering Group representative), Shirley Hayward (lead technical writer – Environment Canterbury), Mark Heath (Greater Wellington Regional Council), Michael Patterson (Horizons Regional Council), and Roger Hodson (Environment Southland). Summer Greenfield (formerly of Greater Wellington Regional Council) was a member of the original working group and prepared an initial draft document. Cathy Kilroy (NIWA) and Juliet Milne (NIWA) contributed from early 2020 to review and finalise a draft for external release. The working group was selected from the regional sector's Surface Water Integrated Management (SWIM) Special Interest Group (SIG).

The extended working group would like to express their thanks to:

- laboratory staff who provided information on laboratory testing procedures: Jane Sherrard (Hill Laboratories), Catherine Moisan, Sally Robinson and Natsumi Nishikawa (Cawthron Institute)

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- the NEMS Steering Group for their oversight and review of this document.

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- National Institute of Water and Atmospheric Research Ltd (NIWA)
- Northland Regional Council
- Otago Regional Council
- Taranaki Regional Council
- Tasman District Council
- West Coast Regional Council
- Waikato Regional Council

Review

This document will be reviewed by the NEMS Steering Group within one year of its release and thereafter once every two years. Further details on the review process can be found at http://www.nems.org.nz. .

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Terms, Definitions and Symbols

Please note that this section will be removed out into the NEMS Glossary in the next version of this Standard.

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

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

- Aliquot: In the NEMS *Periphyton*, a portion of a subsample taken for filtering and subsequent analysis. A minimum of three aliquots are usually removed from each sample to make up a subsample for analysis.
- Ash-free Dry Mass (AFDM): In the NEMS *Periphyton*, a measure of total organic material (biomass) in a periphyton sample once the water content has been removed by drying the sample in an oven and organic material burnt off by ashing in a furnace at high temperature. Also sometimes called ash-free dry weight (AFDW) or loss-on-ignition.
- Biomass: The total quantity or weight of plants or animals in a given area or volume. For streambed periphyton, see "standing crop".
- Chlorophyll *a*: The primary photosynthetic pigment found in algae, cyanobacteria and plants. The pigment reflects green/yellow light and therefore gives algae and plants their green colour, when not masked by other pigments. In the NEMS *Periphyton*, chlorophyll *a* represents a measure of the total amount of live periphyton in a sample and is the most commonly used measure of periphyton biomass.
- Detritus: Dead particulate organic matter in a stream, including leaves and animal parts.
- Dry Mass: In the NEMS *Periphyton*, a measure of total organic and inorganic material in a periphyton sample (biomass) once the water content has been removed by drying the sample in an oven.
- Flushing flow: In the context of biological sampling in rivers and streams, a flow that is sufficient in magnitude to scour or otherwise remove periphyton and 'wash' invertebrates from the streambed.
- Hard-bottomed stream: A stream in which the bed substrate comprises more than 50% particles of gravel size or greater (i.e. dominated by gravel, cobble, boulder and bedrock substrates). Riffle and run habitats are common in these streams.
- Mesohabitat: Instream habitats (e.g. run, riffle, pool) when viewed at a middle range scale.
- Periphyton: A complex community of organisms, including algae, cyanobacteria, heterotrophic microbes (e.g. fungi) and detritus, that is attached to the bed or submerged surfaces in rivers, streams and other waterbodies.
- Pool: An area of deep, slow-flowing or standing water with a smooth water surface; usually found where the stream widens and/or deepens.
- Reach: A section of a stream or river selected for monitoring.

- Riffle: A typically short and shallow segment of a stream or river characterised by moderate to fast water velocity, with mixed currents, and water surface rippled and largely broken. The substrate is often larger than that found in a run.
- Run: A reach intermediate in character between a riffle and a pool, with low to moderate depth, slow to moderate water velocity, uniform to slightly variable current, and water surface smooth to rippled and unbroken.
- Soft-bottomed stream: A stream in which the bed substrate comprises more than 50% sand/silt/mud/clay. These streams are typically low-gradient and often dominated by macrophytes in unshaded reaches and woody debris in shady, forested reaches.
- Standing crop: In the NEMS *Periphyton,* the net amount of periphyton, measured as either percentage cover or biomass (as chlorophyll *a* or AFDM), on the streambed at any one time. Standing crop depends on the balance between growth processes (controlled by, for example, nutrients and light) and loss processes (such as invertebrate grazing or abrasion by fine sediment).
- Stream habitat: The place or environment where aquatic organisms, such as periphyton and invertebrates, live. Typically assessed by physical features such as substrate size and composition, and water depth and velocity.
- Visit metadata: Field observations and other information captured when visiting a sampling site (e.g. date, flow state, water colouration, weather conditions).
- Wadeable (stream reach): A river or stream reach in which someone can safely wade and reach down to retrieve a stone from the streambed at a sufficient number of suitable locations to collect a representative periphyton sample. In general, this means a water depth no greater than 0.6 m but other factors, such as water velocity, substrate type and sampler confidence, also need to be taken into account to assess safety.

Normative References

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

- NEMS *Glossary*
- NEMS *Quality Code Schema*
- NEMS *Code of Practice Safe Acquisition of Field Data In and Around Fresh Water,* and
- NEMS *Water Quality – Part 2 (Rivers).*

About this Standard

Introduction

Periphyton is the community of organisms, dominated by algae and cyanobacteria, that forms slimes, mats and filaments on rocks, wood and other substrates on river and stream (hereafter stream) beds. Periphyton is the primary food source for invertebrates, which in turn are food for fish and birds. However, too much periphyton can impair habitat quality for invertebrates and fish and have other negative impacts on cultural, recreational and amenity values. Periphyton is thus an important indicator of ecosystem health in streams dominated by hard substrate and has formed a core component of many regional State of the Environment (SOE) monitoring programmes across New Zealand. While the earliest monitoring focused largely on estimates of periphyton cover on the streambed, monitoring of standing crop biomass has become more common in recent years. In 2014, establishing objectives and water quality limits to manage periphyton biomass (measured as chlorophyll *a*) became mandatory under the National Policy Statement for Freshwater Management (NPS-FM).

This Standard has been prepared principally for field technicians, programme managers and environmental scientists who collect, quality check or report on stream periphyton cover and/or biomass. It is intended to be used primarily by field technicians and environmental scientists involved in SOE or NPS-FM-related monitoring but is also relevant to resource consent monitoring and aquatic ecological studies.

The Standard concerns periphyton standing crop (i.e. abundance of biomass at a particular time), which varies over time and space in response to stream flow and other environmental conditions. Periphyton standing crop can be quantified using a range of methods including visual assessments of periphyton cover on the streambed, laboratory analysis of biomass samples for chlorophyll *a* content or ash-free dry mass (AFDM), and sample processing using a microscope to obtain cell counts and biovolumes.

Assembling a valuable long-term freshwater periphyton data record is underpinned by a consistent approach to field observations, sample collection, sample processing and data management. This is because information yielded from periphyton measurements is influenced by sampling location, technique and effort, as well as the performance of laboratory staff processing the samples.

Periphyton standing crop estimates for use in the assessment of ecosystem health are undertaken by a wide range of agencies for several purposes, including assessing the effectiveness of regional plan policies, assessing the effects of consented activities and informing models of stream ecosystem processes. Depending on study objectives, periphyton sampling for these purposes may require different methods or other requirements from those documented in this Standard (e.g. habitat sampled, flow conditions prior to sampling, number of replicate samples collected). However, much of the guidance provided in this Standard will still be applicable. This Standard, therefore, provides an up-to-date normative reference for most periphyton cover and biomass monitoring carried out in streams across New Zealand.

Objective

The objective of this Standard is to ensure that periphyton cover estimates and periphyton sample collection, processing, and associated quality assurance practices are consistent and

comparable across New Zealand. This document comprises the Standard (presented first) followed by supporting information that describes the procedures practitioners are required to implement in order to achieve the Standard.

Scope

This Standard focuses on periphyton standing crop in the permanently and intermittently flowing freshwater reaches of streams. The scope is restricted to reaches with hard substrate (i.e. gravels, cobbles or boulders), where periphyton dominates primary production. Reaches comprising soft-sediment habitat are likely to be dominated by other primary producers, such as macrophytes and/or phytoplankton.

This Standard addresses both in-situ visual assessments of periphyton cover and laboratory analysis (for chlorophyll *a* content or AFDM) of periphyton biomass. Guidance on determining biomass through cell counts and biovolumes is provided in Biggs and Kilroy (2000).

This Standard targets long-term monitoring programmes (e.g. SOE monitoring, or monitoring of the NPS-FM periphyton attribute). However, the methods presented in the supporting text should also satisfy most Assessment of Environmental Effects (AEE) or resource consent compliance monitoring.

This Standard does not provide guidance on monitoring of cyanobacteria abundance to assess risk to human health. Guidance in this area can be found in *New Zealand Guidelines for Cyanobacteria in Recreational Fresh Waters – Interim Guidelines* (Ministry for the Environment and Ministry of Health, 2009) and subsequent amendments.

The Standard addresses:

- fieldwork preparation, including sampling equipment
- sampling site (reach) selection
- site and visit metadata
- collection of periphyton cover measurements and biomass samples
- periphyton sample handling, preservation, storage and transport
- laboratory analysis and reporting
- quality assurance (QA) and quality control procedures
- data quality coding, and
- data archiving.

Periphyton measurements are collected for a defined monitoring objective(s). The specific monitoring objective(s) will influence the selection of sampling site(s) and the timing and frequency of sampling. It is beyond the scope of this document to address 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 record of freshwater periphyton.

The following periphyton measures (variables) are addressed in this Standard. These variables are typically measured on a routine, ongoing basis as part of long-term stream monitoring programmes.

Metric/index	Recommended Abbreviation for Data Archiving and Reporting
Periphyton chlorophyll *a*	PERI_CHLA
Periphyton cover	PERI_COVER
Ash-free dry mass (also known as ash-free dry weight)	PERI_AFDM

Relationship with Existing Guideline Documents

This Standard has drawn heavily on the methods documented in the *Stream Periphyton Monitoring Manual* (Biggs and Kilroy, 2000), which have provided the foundation guidance for most periphyton monitoring in New Zealand. Where assessment of periphyton cover or measurement of chlorophyll *a* or AFDM are carried out for the purpose of long-term monitoring to assess ecosystem health this Standard updates and supersedes the methodology set out in Biggs and Kilroy (2000).

This Standard also updates and supersedes national SOE periphyton sampling recommendations provided in *Freshwater Monitoring Protocols and Quality Assurance* (Davies-Colley et al., 2012), prepared as part of the Ministry for the Environment's National Environmental Monitoring and Reporting (NEMaR) project.

Data Fit for Purpose

This Standard requires all collected data to be assigned 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.

Note: Enduring use – It is important to note that data that are coded QC 500 or QC 400 may be restricted in their use for a wide range of (yet unknown) purposes in the future.

The Standard – Periphyton

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

Field measurements and sample collection		
Certification	Periphyton cover measurements and/or periphyton biomass samples, and associated metadata, shall be collected 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.	
Quality Assurance	As a minimum, in-situ periphyton cover assessment and periphyton biomass sample collection procedures are checked in the field: • Internally, by experienced personnel within the monitoring agency holding a training day at least annually, and • Externally, every three years using suitably qualified and experienced personnel from an external agency.	
Equipment Specifications	Periphyton cover	Periphyton cover shall be assessed using an underwater viewer (bathyscope) with a clear bottom and a viewing diameter of at least 300 mm, clean and free of scratches.
Equipment Maintenance	Periphyton cover	Viewers shall be wiped clean with a soft cloth after each day of use and shall be stored appropriately to prevent damage to the viewing window.
Site Location and Stationarity	The site shall be a stream reach of length at least five times the stream width up to a maximum length of 50 m, located within a run that is representative of runs in the broader stream reach at that location. *Note: Runs will generally represent the dominant stream mesohabitat but it is acceptable to sample riffles where runs are lacking.*	
	The site location shall be clearly identifiable and retained through time as far as is possible.	
Site Metadata	Site metadata, including site name, location, altitude, photographs and access details, shall be recorded, with access details reviewed at least annually. *Note: A permanent record shall be kept of any necessary changes to the site location (e.g. because of flood disturbance or change in access).*	
Visit Metadata	Field Record Form	A Field Record Form shall be completed on each site visit to record the location, date and time of periphyton cover assessments and/or biomass sample collections; sample identification number(s); equipment used; width of stream assessed; environmental conditions, such as

		weather and shade; 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).
	Photographs	Photographs of the site in both upstream and downstream directions shall be taken at least annually.
Timing of Measurements	Antecedent flow conditions	Periphyton cover estimates and/or biomass sample collection shall be made irrespective of antecedent or current stream flow conditions, except where current flow conditions pose a health and safety risk.
	Records	The time of cover estimates and/or biomass sample collection shall be recorded in New Zealand Standard Time (NZST) to the nearest hour, *Note: Do not use New Zealand Daylight Time (NZDT). If supporting spot water quality measurements are made, record the sample collection time to align with the timing of these measurements.*
Streambed Periphyton Cover (PERI_COVER)	Assessment method	Periphyton cover estimates shall be made at defined points across at least two transects within the survey reach, starting at the downstream end of the site (reach). The streambed shall be viewed through the viewer window positioned horizontally under water to up to 20 cm depth. The percentage cover of surface periphyton in the viewed area shall be estimated and recorded to enable reporting, at a minimum, in terms of the following categories: • No algae (bare substrate) • Films • Mats, and • Filaments. The total number of point estimates shall be at least 20. Points shall be equally spaced along transects out to a maximum water depth of 0.6 m, or across the width of the stream where the depth is < 0.6 m.
	Unit	Percentage cover of the streambed.
	Resolution	Individual point cover estimates shall be made to the nearest 5% (or 1% for rare but notable periphyton forms).
Periphyton Biomass	Sample collection method	Samples shall be collected from fixed areas (e.g. defined by a circle 30–70 mm in diameter) on the upper surface of rocks collected at 10 or more points located at evenly spaced intervals across at least two transects to a maximum water

<table>
<tr><td rowspan="5">(PERI_CHLA and PERI_AFDM)</td><td></td><td colspan="2">depth of 0.6 m. Each subsample (from one rock) shall be collected by thoroughly scraping and collecting all periphyton material from within the fixed area into a primary container and rinsing this with stream water into a screw-cap, watertight sample container. The sample shall consist of pooled material from all 10 or more rocks.

Note: If small-sized substrate is dominant (e.g. large gravels rather than cobbles), then a smaller total area may be sampled comprising a minimum of 15 subsamples.</td></tr>
<tr><td>Sample handling</td><td colspan="2">Sample containers shall be promptly transferred to storage bins with ice to maintain a cool sample temperature and limit potential degradation of chlorophyll a by sunlight.</td></tr>
<tr><td>Sample storage</td><td colspan="2">Samples shall be transferred to the laboratory within 24 hours of collection, maintained at less than 10°C, OR

Samples shall be stored frozen at -20°C or lower for laboratory analysis as soon as possible and within three months for chlorophyll a or 12 months for AFDM.</td></tr>
<tr><td rowspan="2">Sample traceability and integrity</td><td>Labelling</td><td>Samples shall be clearly and permanently labelled on the side of the sample container with:
site name and number
sampling date and time
sampler initials, and
container number (for samples in multiple containers).
The sample identification number shall be included on both the Field Record Form and the Chain of Custody Form.</td></tr>
<tr><td>Chain of Custody Form</td><td>Periphyton samples shall be accompanied by a completed Chain of Custody Form that provides sample traceability from the field to the laboratory. This form shall include:
the date(s) of sample collection
whether samples were dispatched fresh or frozen, and
the total surface area (m^2) of substrate scraped for each sample.</td></tr>
<tr><td colspan="4">Laboratory measurements</td></tr>
<tr><td>Certification</td><td colspan="3">Periphyton samples shall be processed and analysed by trained staff with evidence of adherence to either formal standard protocols (i.e. Biggs and Kilroy, 2000) or subsequently implemented protocols (i.e.</td></tr>
</table>

	this Standard), including internal and external QA and quality control practices.			
Sample Arrival at the Laboratory	Documentation	Laboratory staff shall record the date and time of receipt of samples on the accompanying Chain of Custody Form, together with: • sample temperature on arrival (°C) • any anomalies in sample condition with the potential to impact laboratory testing (e.g. damaged sample container), and • where fresh samples are provided, whether these will be processed immediately or stored frozen.		
	Temperature	Fresh samples shall be less than 10°C, unfrozen and free of ice crystals. Frozen samples shall not show any signs of thawing.		
	Processing and testing timeframes	Testing of fresh samples shall commence within 24 hours of sample collection or the samples shall be placed in a freezer at -20°C or lower. All frozen samples shall be processed: • for chlorophyll *a* as soon as possible and within three months of sample collection, and • for AFDM within 12 months of sample collection.		
Test Method and Measurement Requirements	**Variable and nomenclature**	**Measurement unit**	**Test method**	**Method detection limit[1]**
	Chlorophyll *a* (PERI_CHLA)	mg/m^2	Extraction in boiling ethanol (78°C) for 10 mins followed by spectrophotometry in accordance with Biggs and Kilroy (2000) as modified in Annex G	0.1 mg/m^2
	AFDM (PERI_AFDM)	g/m^2	Dry 24 h at 105°C, ash at 400°C, with weighing of dry samples at each step, in accordance with Biggs and Kilroy (2000) as modified in Annex H	0.1 g/m^2

Measurement Resolution	PERI-CHLA	All measurements shall be reported as milligrams (mg) chlorophyll *a* per sample, to at least one decimal place, which shall then be converted to mg per unit area of substrate sampled (in m^2).
	PERI_AFDM	All measurements shall be reported as grams (g) of AFDM per sample, to at least one decimal place, which shall then be converted to g per unit area of substrate sampled (in m^2).
Data Records	The laboratory measurements shall be provided in a report that specifies: • the dates and times of both sample collection and receipt at the laboratory • whether samples were processed fresh (without any prior freezing) or thawed • the total surface area sampled (supplied by the sampling agency) • the measurement value in units of concentration per sample, and concentration per area • the measurement method and standard method detection limit, 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.	

[1] The actual detection limit depends on the size and concentration of sample. Practical limits are 0.1 mg/m^2 and 0.1 g/m^2 for chlorophyll a *and AFDM, respectively.*

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

Underwater Viewer	The viewing face is divided into quadrants (e.g. using thin black tape or paint) to improve accuracy of periphyton percentage cover estimates.
Field Documentation	Visit metadata (e.g. stream flow conditions) and periphyton visual assessment data are recorded electronically in the field.
Viewing/Sampling Points	Where both periphyton cover and biomass measurements are to be collected, temporary markers are placed on the streambed to indicate the locations of the periphyton observation and sample collection points.
Periphyton Cover	Additional definition of periphyton cover categories is made (e.g. short and long filaments, cyanobacteria mats).

Metadata / Supporting Variables	Photographs of the site, including the streambed, are taken on each sampling occasion using a camera with geo-referencing capability and, preferably, a polarising filter.
Quality Control	At least one sample in each batch of 10 samples processed together should be analysed in triplicate.
Sample Storage	If possible, for samples stored frozen, a sample storage temperature of lower than -20°C is used (e.g. -80°C).
Sample Retention	The laboratory freezes any surplus periphyton sample immediately following the subsampling process and retains the sample for at least one month.
Laboratory Reporting	The laboratory reports the raw measurements along with any censored measurements.
	Where replicate subsamples are analysed, the laboratory provides the result for each replicate as well as the mean result.
Reporting and Data Checks	Periphyton biomass data reported by the laboratory are checked by the collection agency against any visual cover estimate made at the time of sample collection within three weeks of receipt to enable re-testing if necessary. *Note: Periphyton chlorophyll a (but not AFDM) degrades over time when stored frozen.*
Data Archiving	File, archive indefinitely, and back up regularly in a time-series database: • site and field visit metadata • visual cover assessment method, including the periphyton categories recorded • point data for visual cover assessments • 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	*Quality assurance requirements are under development.*

1 Preparatory work in 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 periphyton monitoring programme understands the programme's 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.

1.2 Health and Safety

Periphyton surveys in streams (including periphyton cover assessments and periphyton biomass sampling) involve 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 in all weather conditions is particularly important. Periphyton monitoring necessarily involves wading and is generally not possible during high flows with swift water velocities and/or turbid conditions. Periphyton surveys can be carried out only when waters up to 0.6 m deep can be waded safely. 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 floatation aids, should be provided to ensure safety. Gloves should be worn when sampling in streams likely to present a significant health risk (e.g. due to known sewage contamination, toxic cyanobacteria).

For further guidance on safety precautions when collecting periphyton samples refer to the NEMS *Code of Practice Safe Acquisition of Field Data In and Around Fresh Water*.

1.3 Quality Assurance

Field sampling is the most critical step in collection of reliable data on periphyton cover and biomass. Any errors made while making cover estimates or during sample collection, transport or storage cannot be corrected. 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. QA procedures ensure that data obtained both directly (e.g. periphyton cover estimates) and indirectly (e.g. laboratory measurements on periphyton samples) from the field are robust.

The QA procedures should comprise all the steps described in this Standard to ensure that valid data are produced. This includes documented evidence that personnel carrying out the surveys are trained and competent, and appropriate sample collection and handling methods are employed. In particular, personnel carrying out the periphyton cover estimates should be given training in identification of periphyton cover types specified in this Standard (either with an experienced member of staff or through a suitable external agency). In addition, field inter-operator comparison exercises with experienced personnel within the monitoring agency should be carried out on a regular basis (at least annually) to ensure consistency in periphyton visual cover assessments and biomass sample collection and to check correct recording of cover data and completion of Field Record Forms. Field inter-operator comparison exercises should also be conducted with another experienced monitoring agency at least every three years, as outlined in subsection 1.3.1.

QA and quality control programmes are mandatory components of laboratory practice to ensure that measurements are accurate. Refer to subsection 6.3.5 for details.

1.3.1 External 'Field Audit'

As best practice, staff training and performance should be reviewed at least every three years through an interagency QA field exercise. This QA exercise shall seek to:

1. Obtain accurate field measurements (e.g. of periphyton cover), and
2. Deliver to the laboratory samples that are representative of the periphyton biomass at the site and are adequately preserved.

The QA field exercise should target specific practices around:

- selecting suitable monitoring reaches
- collecting and handling periphyton samples, including technique for obtaining random subsamples, rock scraping, bottle labelling, and sample handling after collection
- visually assessing periphyton cover, and
- completing the Field Record Form, including site and visit metadata.

The exercise should require two or more staff to simultaneously, but independently, complete 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 field practice, photographs of views of streambed periphyton cover could be taken for cover to be estimated back in the office; these estimates would then be compared with the viewer estimates recorded in the field.

To assess laboratory performance, the sampling agency could request that a composite periphyton biomass sample be split and a subsample sent to another laboratory for analysis. As chlorophyll *a* degrades with time, care would be needed to ensure that both laboratories proceeded 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 (see subsection 6.3.5).

1.4 Field Record Form

A standard Field Record Form shall be prepared to record visit metadata. This form provides a record that verifies the site location (as defined in site metadata), date and time of periphyton cover measurements and biomass sample collection, methods and conditions under which periphyton cover measurement and/or biomass sample collection were made, and details of other factors that may influence the data being collected (e.g. whether periphyton has been dislodged in the sample reach by, for example, a vehicle crossing the stream). The record is also needed for later assignment of a quality code to the periphyton data (subsection 7.2.1.2).

Electronic sample 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 of inputted data to ensure records are correct at the time they are made. If paper forms are used, waterproof paper is recommended to protect the integrity of the record.

Metadata that shall be recorded during site visits are outlined in subsection 3.2.4. 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, substrate 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:

- waders, life jackets and other personal protection equipment
- field forms to record site and visit metadata and field measurements
- underwater viewer (Figure 1) for assessing periphyton cover
- equipment for periphyton sample collection –
    - a circular ring(s) or equivalent (e.g. sample container lid) of known diameter(s) between 30 and 70 mm
    - a thin sheet of stiff plastic or a metal spatula large enough to completely cover the sampling ring when sampling gravelly and/or sandy substrate
    - blade(s) and scissors for scraping/cutting off thick algae
    - small scrubbing brushes for scrubbing thin, tightly attached algae (e.g. wire brush or firm toothbrushes)
    - two or three open containers (e.g. 2-litre square food containers, deep-sided laboratory trays)
    - squirt bottle containing stream water
    - small disposable pipettes, and

    - sample containers of 400 mL to 1,000 mL volume with watertight lids (subsection 1.5.2)
- 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, n.d.)
- a camera, and
- a GPS receiver.

A calibrated multivariable hand-held field meter may also be useful if spot measurements of water quality variables, such as water temperature and conductivity, are required.

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

1.5.1 Underwater Viewer

This Standard requires periphyton cover assessment to be carried out using an underwater viewer (bathyscope or aquascope) with a clear plastic or glass viewing window at least 300 mm in diameter (Figure 1). These viewers allow a clear view of the streambed with no interference from surface turbulence. They also enable definition of a more-or-less standard area of the streambed at each observation point (i.e. equivalent to a quadrat in terrestrial ecology). The exact area viewed will vary with water depth, but variation will be small over the range of water depths defined as wadeable (0.1 to 0.6 m). It is recommended as best practice that quadrant or grid lines are added to the base of the viewer using thin black tape or waterproof permanent marker to aid estimation of periphyton cover.

Note: This Standard does not recommend the use of black disk viewers to estimate periphyton cover owing to their small field of view. Periphyton cover estimates made using such a viewer will qualify for a maximum quality rating of QC 400.

It is important that the viewing window is kept clean and free of scratches. A purpose-made storage bag or box is recommended for both transporting and storing the viewer.

Figure 1 – Example of a suitable underwater viewer

Source: www.envco.co.nz

1.5.2 Sample Containers

Periphyton samples for chlorophyll *a* or AFDM analysis shall be stored in polyethylene containers or jars with a wide opening and a watertight screw-cap. Container size will depend on the size and nature of the sample collected. Containers generally need to hold at least 100 mL of sample. Containers of 400 mL to 1 L in volume should be appropriate for samples collected using the method described in subsection 5.2. For large samples (e.g. from sites with very high biomass and/or many replicates) multiple sample containers can be used to hold portions of the same sample, provided that each container is clearly labelled (e.g. container 1 of 2).

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

1.5.2.1 Labels

All sample containers shall be uniquely labelled as outlined in subsection 5.2.1. Waterproof labels are recommended to avoid labels coming off the bottles if they get wet. A second label, written in pencil on waterproof paper and placed inside the container, is also recommended. 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 periphyton samples collected for laboratory analysis 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 photochemical degradation.

Samples should be transported to the laboratory as soon as possible after collection (subsection 5.3) and 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 frozen at -20°C or lower and dispatched to the laboratory for analysis within three months of collection.

Note 1: Fresh periphyton samples carry the risk of transferring live cells of the unwanted organism didymo across regions. Once samples are frozen there is no risk of such transfer. To avoid any risk, and to avoid having to take required biosecurity precautions, monitoring agencies and laboratories may prefer to routinely freeze samples prior to analysis.

Note 2: Periphyton samples intended for AFDM analysis only can be stored frozen for 12 months.

1.7 Chain of Custody

A Chain of Custody (CoC) form (Annex C and subsection 5.3.1) shall accompany periphyton 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 6.2). The CoC information shall be used to provide a quality assurance check on sample integrity (subsection 7.2.1.2).

2 The Monitoring Site

In this section

This section outlines monitoring site considerations, with a specific focus on identifying stream reaches within which the periphyton cover estimates and biomass sample collection will occur. Site metadata requirements are also addressed.

2.1 Site (Reach) Location

This Standard applies to hard-bottomed reaches, which are characterised by a bed substrate comprising more than 50% particles of gravel size or greater (i.e. dominated by gravel, cobble, boulder and bedrock substrates). Around 60–80% of New Zealand's streams are hard-bottomed, and riffle and run habitats are common in these streams.

Note: Stream reaches characterised by soft substrate (> 50% cover by sand or silt) or by significant cover by macrophytes are not appropriate for application of this Standard as the only measure of aquatic plant growth. Measures of macrophyte abundance may also be needed to adequately assess aquatic plant growth in such reaches.

To account for heterogeneity in periphyton abundance in stream environments, periphyton cover estimates and periphyton biomass sample collection shall be carried out over a reach about 50 m long or at least five times the stream width (whichever is shorter).

The monitoring reach shall be representative of the broader stream reach, including consideration of water depth and velocity, substrate type and size, stream slope, degree of braiding, and shading.

To standardise periphyton monitoring to areas with similar habitat and flow characteristics, this Standard requires periphyton cover estimates and biomass sample collections to be carried out in run habitat where possible (Figure 2). This is because:

- runs are the most common mesohabitat in most streams, and
- periphyton standing crop in runs tends to be more variable and more responsive to the effects of both high flows and nutrient supply than that in riffles. Stable substrata in riffles can lead to persistent high standing crop over a range of conditions (Biggs, 2000).

Note: It is acceptable to sample riffle mesohabitat where runs are lacking or short in length.

All periphyton cover estimates and biomass sample collections shall be made in wadeable water depths, generally between 0.1 and 0.6 m deep. Initial site selection should ideally be carried out under baseflow conditions and should include consideration of the extent of the site that will be wadeable under a range flow conditions. Other factors that affect wadeability include water velocity and substrate type.

The monitoring reach should be well away from the influence of bridges and river crossings (either vehicle or stock). Upstream confluences with point source discharges, tributary streams or drains should be far enough away that their waters will be completely mixed in the monitoring reach.

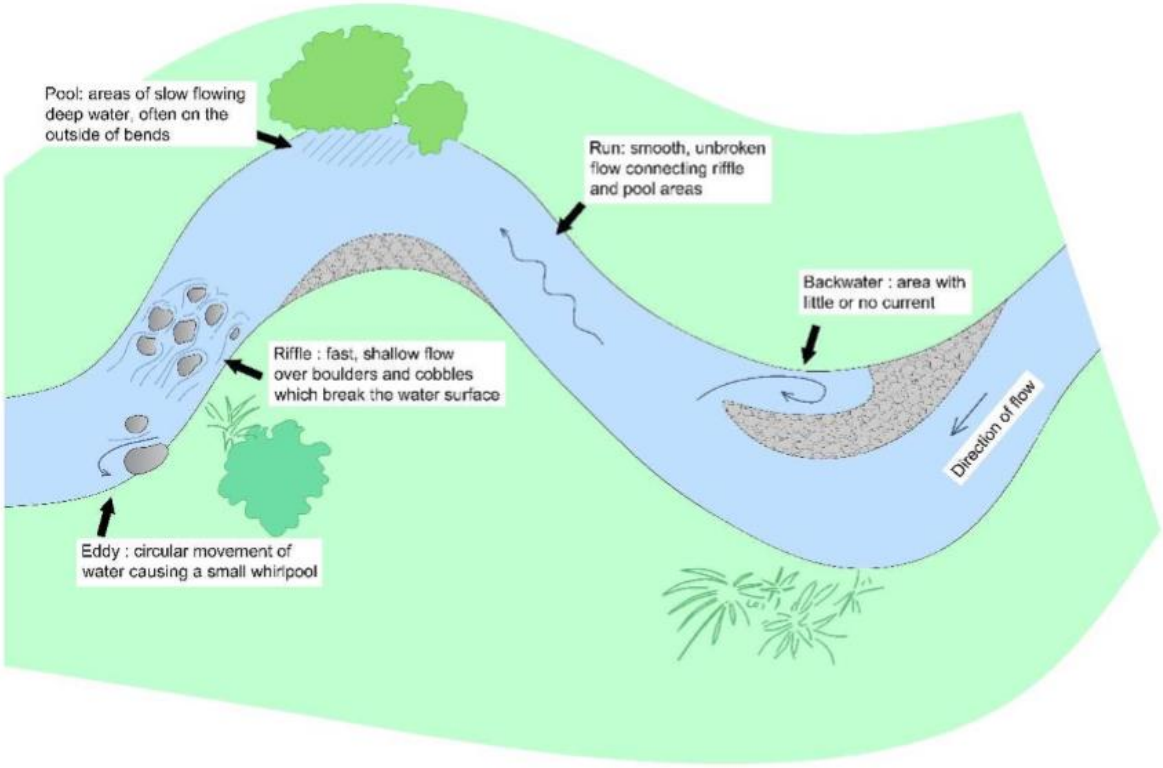

Figure 2 – Schematic of a hard-bottomed stream showing run, riffle, pool and other hydrology-related mesohabitat features

Figure: Quentin Gilkinson, after Biggs et al. (1999).

The monitoring reach shall be easily identifiable, preferably with a permanent location marker (e.g. waratah) at the downstream end of the site to ensure consistency in the location of field measurements and sample collection through time.

Note: Markers such as waratahs should be installed in locations that will remain unaffected by high flows and that do not cause any obstruction or hazard to the public (if applicable).

2.1.1 Partially Wadeable Reaches

Safe access across the entire width may not be possible in larger streams (rivers) at baseflows or in streams at higher flows. However, it may be appropriate to carry out periphyton monitoring in the wadeable portions of large rivers if this portion meets the general requirements for a monitoring reach as outlined in subsection 2.2. Ideally, the wadeable reach should extend to a width of at least 10 m in the flowing channel and comprise a reasonable proportion (e.g. > 40%) of the total river width at baseflow.

2.2 Site Stationarity

Stream channel morphology can change, for example, because of streambank erosion over time or after large floods, and a reach selected for periphyton surveys may become unsuitable. Other disturbances, such as gravel extraction or changes in public access, may also affect suitability for

periphyton monitoring. Site access may also change over time (e.g. due to changes to access to private property or erosion that make tracks too dangerous to negotiate).

In all these cases, a periphyton monitoring reach will need to be relocated to a reach that remains representative of those in the stream in that part of the catchment. Ideally, the new monitoring reach will also match the key mesohabitat and flow characteristics of the previous site and will be close to the original site, such as within the same NZREACH.

Features such as water depth and velocity, substrate type and size, stream slope, degree of braiding, and shading are important characteristics that determine comparability of monitoring reaches. Any changes in monitoring reach shall be clearly identified 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 reach is established:

- Stream or river name
- Site name and number
- Site location – e.g. GPS units or NZTM latitude/longitude coordinates, expressed to a minimum of 6 decimal places, for the downstream boundary of the monitoring site
- Related sites and records, including the nearest climate station, stream flow/level recorder and water quality monitoring site(s)
- Landowner contact details and, where relevant, directions for accessing the site
- Specific health and safety considerations
- Relevant environmental characteristics and features applicable to the whole site, including:
    - degree of shade and whether shade varies over seasons (e.g. shading by deciduous versus evergreen trees)
    - water depth and velocity at defined flow conditions (e.g. median flow) (optional)
    - substrate composition (noting that substrate composition may also be recorded as part of visit metadata)
    - habitat description (e.g. run)
- Photographs of the site taken looking both upstream and downstream, and
- Start date of monitoring.

Note: A photo of the streambed substrate may also be useful. A camera with a polarising filter will reduce reflection and improve capture of instream substrate and periphyton cover.

A Field Record Form for visit metadata (subsection 3.2.4) shall be used to capture any changes in the site characteristics during each visit.

Adequate mechanisms shall be put in place to store all relevant site-related metadata with the actual data records (see subsection 7.1.3).

Site metadata shall be reviewed, and records updated as necessary, annually. If a new monitoring reach 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 periphyton monitoring that apply to both visual assessments of periphyton cover and collection of samples for laboratory biomass analyses. These principles and procedures are divided here into those related to monitoring programme design and those that apply once field personnel are on site to collect periphyton data.

3.1 Monitoring Programme Requirements

The timing and frequency of periphyton 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 periphyton attribute under the NPS-FM. However, the methods used to assess periphyton cover and collect samples for biomass analyses can also apply to monitoring carried out to assess recreational suitability and the effects of proposed and consented activities (e.g. wastewater discharges). These forms of monitoring are therefore briefly addressed here, along with SOE and NPS-FM monitoring.

The Standard concerns periphyton standing crop (i.e. abundance of biomass at a particular time). Periphyton standing crop can be quantified using a range of methods, including visual assessments of periphyton cover on the streambed, laboratory analysis of biomass samples for chlorophyll *a* content or ash-free dry mass (AFDM), and sample processing using a microscope to obtain cell counts and biovolumes. Because periphyton standing crop varies over time in response to river flows and other environmental conditions, regular monitoring is required under a range of conditions to enable calculation of appropriate metrics for assessing stream condition. Whichever measure is used, the critical step is collection of data or samples in the field. Periphyton cover can be highly spatially variable. Ideally, periphyton cover estimates and samples will provide a realistic representation of cover or biomass on the streambed within the reach of interest.

3.1.1 Periphyton Variables

This Standard addresses measurements of periphyton cover (PERI_COVER), chlorophyll *a* (PERI_CHLA) and ash-free dry mass (PERI_AFDM).

Visual assessments of periphyton cover on a streambed provide useful indicators of both stream ecosystem health and aesthetic/recreational values. Visual assessments can often be carried out rapidly, incur no laboratory costs, and often correlate with direct measurements of biomass. Chlorophyll *a* is the most commonly used measure of periphyton biomass because all types of algae (including cyanobacteria) contain this photosynthetic pigment (albeit in different quantities). Chlorophyll *a* represents the total amount of live periphyton in a sample. AFDM (also known as ash-free dry weight (AFDW) or loss-on-ignition) is a measure of total organic material (biomass) in a periphyton sample. AFDM is usually correlated with chlorophyll *a* and it is generally unnecessary to carry out both measures. However, AFDM can provide useful additional information about a periphyton community in specific situations. For example, AFDM can be combined with chlorophyll *a* data from the same periphyton sample to calculate the

autotrophic index (AI = AFDM/CHLA, with both biomass measures in mg/m^2). The AI can indicate differences in stream health between sites due to organic enrichment and other stressors (e.g. Delgado et al., 2017). The AFDM method also returns dry mass (DM), which can be a useful additional measure in ecological studies.

Note: The NPS-FM specifies periphyton biomass as chlorophyll a.

3.1.2 SOE and NPS-FM Monitoring

The NPS-FM stipulates monthly monitoring of periphyton biomass (as chlorophyll *a*) for a minimum of three years with the monitoring schedule being pre-planned so that it is random in relation to river flows. When high flows or extremely turbid conditions mean a site cannot be safely waded on a scheduled sampling date, this will result in a missing data point for that month. In the event of a high flow, sampling should be carried out on the next scheduled monthly sampling date rather than returning to the site as soon as flows have subsided. The missing data point should be recorded on the Field Record Form so that it can be recorded in the time series.

Note: Failure to account for a missing data point can lead to bias in a time series because, at least in the case of high flows, the missed data point would generally have been low biomass. A missing data point shall be coded QC 100 in accordance with the NEMS Quality Code Schema *(subsection 7.2.1) or a surrogate value determined (subsection 3.1.2.2).*

3.1.2.1 Monitoring During High Stream Flows

When carrying out periphyton cover estimates or biomass sample collections at times of elevated flows, exclude any wadeable areas of streambed that are outside the area of the streambed that is normally wetted (under lower flows).

3.1.2.2 Chlorophyll a Estimates and Surrogates

It may be possible to identify the flow at which periphyton is effectively removed from the streambed at the monitoring site and use this information to infer a periphyton cover or chlorophyll *a* value for occasions when high flow prevents sampling. Alternatively, chlorophyll *a* concentrations of less than 5 mg/m^2 could be assigned to the monitoring event.

Estimating chlorophyll *a* concentrations may be more difficult when sampling was missed due to turbid conditions during a period of stable flows (e.g. as a result of upstream river works). It may be possible to derive an estimate by applying estimated accrual rates to periphyton results from the preceding or following months (if no significant freshes have occurred in the intervening period). Accrual rates could be estimated using historical periphyton records for the same time of year.

Monitoring guidance for the NPS-FM permits substitution of "quicker and less costly visual inspection methodologies" for collection of samples for chlorophyll *a* analysis (Ministry for the Environment, 2017). The approach assumes that where periphyton abundance is low it may be possible to estimate periphyton chlorophyll *a* from visual assessments of periphyton cover. For example, if cover of chlorophyll *a*-rich classes (e.g. periphyton filaments or mats) is less than 5%, conversion factors may be used to estimate periphyton chlorophyll *a* concentration from periphyton cover data (see Annex D for more information). When periphyton abundance is low, visual assessment of periphyton cover is generally quicker than collecting a periphyton sample for chlorophyll *a* analysis. Use of estimates derived from periphyton cover also mean that laboratory analysis costs can be avoided.

Note: It is recommended that periphyton cover–chlorophyll a relationships are established and validated for local conditions before applying conversion factors.

Surrogate results represent indirect measurements of periphyton chlorophyll *a* biomass and, in accordance with the NEMS *Quality Code Schema*, shall be assigned QC 300 (synthetic data).

3.1.3 Monitoring of Recreational Suitability

Monitoring of periphyton cover or biomass at stream sites used for recreation is likely to be seasonal and more frequent (e.g. weekly) than SOE monitoring if carried out at the same time as monitoring of microbiological water quality. Assessing nuisance periphyton cover (i.e. lengths of filaments and thickness of mats) in accordance with the Biggs (2000) periphyton guidelines can be accommodated by using the thick mats and long filaments subcategories in Table 1 (see subsection 4.1.4). The reader is referred to Ministry for the Environment and Ministry of Health (2009) for monitoring of benthic cyanobacteria in recreational waters.

Note: As indicated in subsection 4.1.4, it is possible to collect data to satisfy periphyton cover classifications for recreational waters alongside SOE classifications. However, the various guidelines for monitoring recreational waters are in need of review, with increasing emphasis now being placed on techniques that will improve the spatial extent of monitoring (e.g. through the use of unmanned aerial vehicles) and forecasting of cyanobacteria blooms (e.g. Milne et al., 2017).

3.1.4 Assessment of Environmental Effects and Resource Consent Monitoring

Unless a regional plan or resource consent requires otherwise, monitoring of periphyton cover or biomass to assess the impacts of a proposed or consent activity, such as a discharge or water take, should target stable flow periods (generally summer or autumn) that are expected to lead to maximum biomass at the site. This is particularly important when comparing periphyton biomass above and below the point of discharge or water abstraction. In general, periphyton accrual is expected to occur during long periods of declining or stable flow from a starting point of median flow or lower. The time required by periphyton to attain maximum biomass varies widely (e.g. 2 to 12 weeks or more; Biggs, 2000) and is likely to be site- and season-specific. As a practical guide, at least three weeks should have elapsed since the most recent flushing flow (e.g. > 3 x median flow, or a flow magnitude appropriate for the site, Hoyle et al., 2017) before carrying out monitoring.

3.1.5 Managing Changes in Methods

Long-term periphyton monitoring programmes should ideally retain the same field and laboratory methods 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 of brush for sample collection or a laboratory upgrading its spectrophotometer. This highlights the need for good documentation of field and laboratory methods.

Subsection 4.1.6 and Annex E address reconciliation of periphyton cover measurements when the cover categories have changed through time. Subsection 6.5 addresses changes in laboratory methods.

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 or sampling they should not be attempted. Such conditions include streams in flood flow and adverse weather, such as heavy rain or snow that make ground conditions hazardous.

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 set of periphyton cover estimates and/or periphyton sample shall be referenced against existing unique identifiers for the monitoring site (e.g. TDC_20202/01) and the monitoring occasion and recorded as visit metadata. This ensures that the visual assessment data and the laboratory sample analysis results can be related to each other as a single visit record.

3.2.3 Time Records

The periphyton cover estimates and sample collections shall be recorded as a single site visit time to the nearest hour at the start of recording the site visit data.

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 periphyton samples, the periphyton samples shall be given the same time-stamp 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 periphyton cover estimates (Section 4) and/or collect periphyton samples for biomass analyses (Section 5). This form shall include:

- the name of the stream
- the name of the site
- any unique sample identification number(s) assigned
- the name(s) of field personnel
- date and time (in NZST) of periphyton cover estimates and/or sample collections
- the weather conditions at the time
- flow state (rated, gauged or estimated – at least as 'low', 'moderate', 'high')
- Protocols used, including any deviations from standard field measurement and/or sampling protocols (e.g. samples collected from a depth > 0.6 m or from multiple habitat types)

- length of river reach assessed/sampled
- the diameters of the areas scraped for collection of each periphyton subsample so the total area sampled can be calculated later
- the number of transects over which the assessment and/or sample collection took place
- in partially wadeable reaches, an estimate of the proportion of total stream width (to nearest 10%) in the wetted stream channel assessed/sampled for each transect
- notes of any factors that may influence the data (e.g. recent vehicle or stock crossing, gravel extraction works)
- notes of significant changes in reach characteristics since the previous site visit (e.g. loss of run habitat, channel deepening, increased fine substrate), and
- GPS location of reach if the site needs to be relocated (see subsection 2.2).

An example Field Record Form is provided in Annex B. Photographs of the site looking both upstream and downstream can provide useful additional metadata.

Note: If an observation and/or sample collection cannot be made, it is important to record the conditions at the time (e.g. elevated flows following rainfall, turbid water as a result of upstream activity) to enable determination of an estimate or a surrogate data point later (see subsection 3.1.2.2).

3.2.5 Supporting Measurements

Water column conductivity (in µS/cm at 25°C) may be correlated with periphyton biomass in some regions (Biggs, 1990; Kilroy et al., 2018) and could be useful to measure as a supporting variable at the time of periphyton cover estimation and/or sample collection. Other water quality variables of relevance to periphyton biomass include water temperature and soluble inorganic nutrients. Refer to the NEMS *Water Quality – Part 2 (Rivers)* for measurement method details.

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 where multiple streams 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 Periphyton Cover Measurements

In this section

This section sets out how to visually estimate periphyton cover within a stream reach. It provides an overview of the monitoring approach, including selection and layout of viewing points and the different periphyton cover categories to be recorded, followed by a detailed procedure for carrying out point estimates of cover.

4.1 General Approach

As outlined in subsection 1.5.1, this Standard requires the use of an underwater viewer for visual assessments of streambed periphyton cover. The aim is to obtain data on periphyton cover that is representative of the wadeable portion of run habitat in a stream reach, which itself shall be representative of the broader stream environment (subsection 2.1).

Because periphyton cover can be highly spatially variable, multiple observations (viewing points) are essential. A minimum of 20 views can achieve reasonable representation (i.e. within 10% of the true mean cover as determined from 120 views in the same reach) unless cover is highly heterogeneous (i.e. where periphyton cover is unevenly and patchily distributed) (Kilroy et al., 2013). A minimum of 20 views is required under this Standard as a compromise between adequate representation and practicality (i.e. time spent in the field). Increasing the number of views to 25 or more in heterogenous reaches will increase confidence that the mean of views will be closer to the true mean.

4.1.1 Viewing Points

Assessments shall be made in at least 20 defined areas (hereafter 'points') within the stream reach. The points should be pre-selected at equal intervals on transects that span the stream width or part-width out to a maximum depth of 0.6 m (Figure 3). Pre-selection of viewing points is equivalent to random selection of points across a variable streambed. Spacing viewing points on transects ensures that all parts of the wadeable channel are represented (i.e. edge habitat is included as well as the central channel). The transects should typically be 8–15 m apart within the recommended reach length of ~50 m (see subsection 2.1). Under this Standard, viewing points should span two transects as a minimum.

When identifying transects, take care to avoid areas of the streambed where periphyton has been dislodged by people or stock, or where vehicles have been driven on the streambed. Details of any sampling that occurs in a non-standard way shall be recorded on the Field Record Form (subsection 3.2.4).

Note: Temporary markers can also be useful for locating points for periphyton sample collection (see subsection 5.1.1) and ensuring that samples are collected from areas of undisturbed streambed.

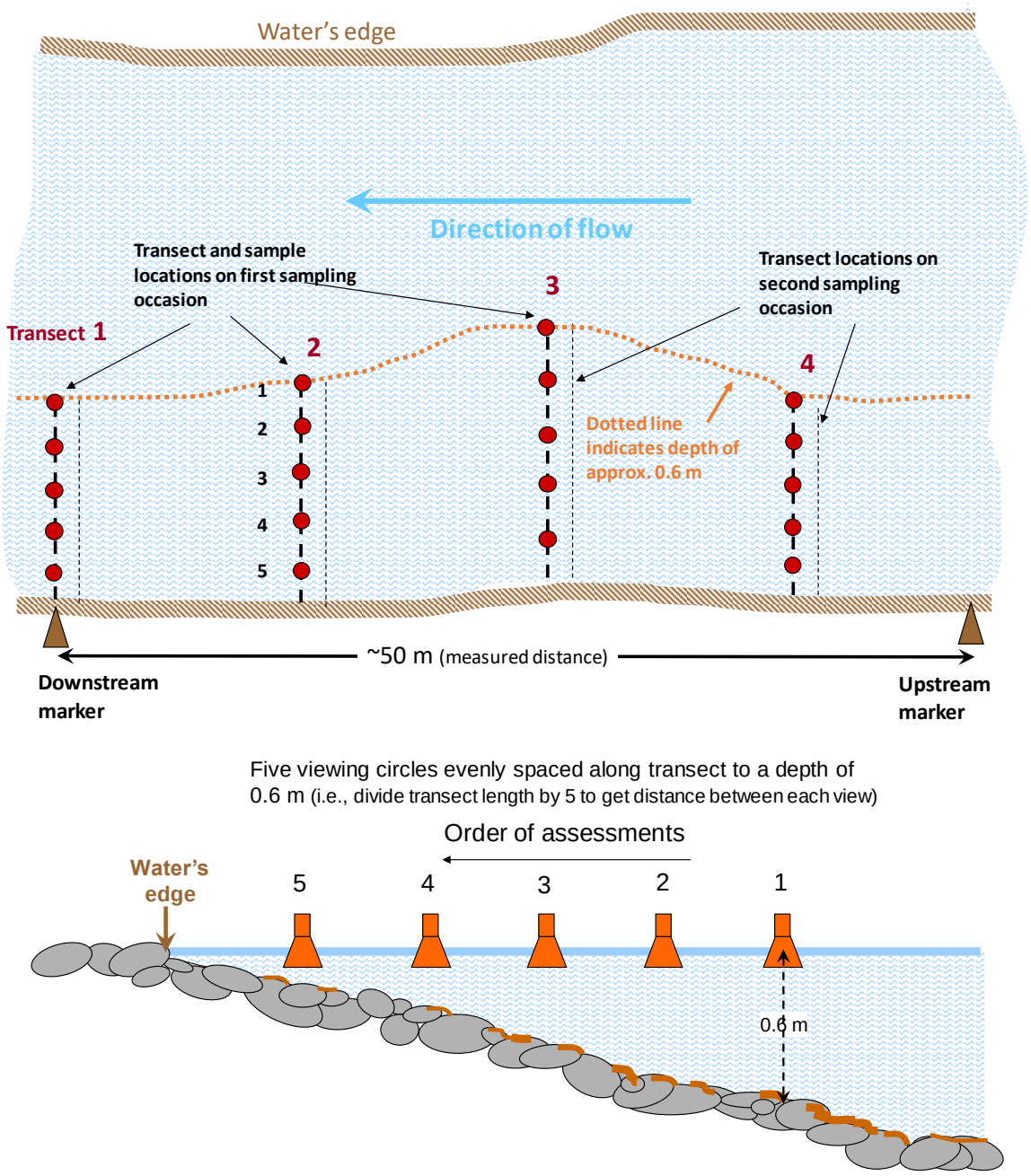

Figure 3 - Location of viewing points across a partially wadeable reach for visual assessment of streambed periphyton cover

Top: Plan view; Bottom: Cross-section (From C. Kilroy in Ministry for the Environment and Ministry of Health, 2009)

4.1.2 Viewing Point Markers

Viewing points may be marked with temporary or semi-permanent markers on the streambed (e.g. Figure 4) to help field personnel avoid trampling in areas that may be viewed later. Temporary markers can be, for example, painted rocks or coloured tags (e.g. forestry tape) tied to fishing sinkers, which are placed on the streambed at the time of monitoring (subsection 4.2).

Note: Temporary markers can also be useful for locating points for periphyton sample collection (see subsection 5.1.1) and ensuring that samples are collected from areas of undisturbed streambed.

Figure 4 - Example of markers (painted rocks) on the bed of a stream to indicate periphyton cover observation (and/or sampling) points

Photo: Anika Kuczynski

4.1.3 Variations to Viewing Point Layout

The example in Figure 3 is of five viewing points on each of four transects. The layout of viewing points on transects may need to be varied depending on stream width and reach length. For example, in many streams a reach of 50 m long is unrealistic. In that case, where the stream is wide and < 0.6 m deep, a substantial area of streambed can be covered by using two transects each with 10 viewing points, rather than four transects each with five viewing points. The transects could be up to 20 m apart. One way to think about using two transects only is that they are equivalent to four part-transects, but with two on each side of the stream.

In narrow streams, only two or three viewing points per transect may be possible (as the viewing area is 0.3 m in diameter). Therefore, views could be spaced along a reach length of at least five times the stream width using alternating three and two views per transect with transects more closely spaced (e.g. Figure 5).

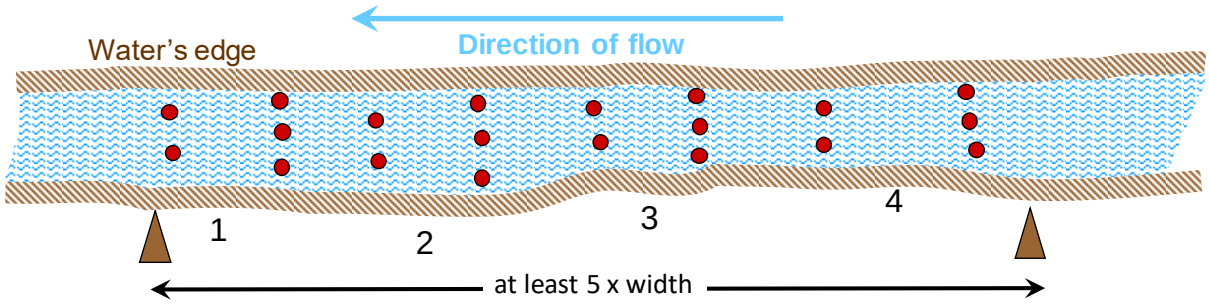

Figure 5 - Possible layout of viewing points in a very narrow stream. The numbers 1 to 4 indicate groups of five points equivalent to transects on the Field Record Form

Figure: Cathy Kilroy

4.2 Periphyton Cover Categories

This Standard requires percentage cover estimates of streambed periphyton to be made that enable reporting, at a minimum, against each of the four categories listed in Table 1. Further definition of cover types, in the form of subcategories, may be desirable to satisfy different SOE monitoring and information needs. Example pictures of periphyton from each category, with subcategory noted, are shown in Figure 6.

Table 1 - Periphyton categories that shall be used for documenting streambed periphyton cover under this Standard (modified from Kilroy et al., 2013)

Note: The four categories represent the minimum requirement for obtaining a quality rating of QC 600 under this Standard. Further discrimination in the form of subcategories may be made as needed.

Category	Subcategories (examples)	Description / identification	Comments (including notes on taxa present)
No algae	No algae	Stone surface not slippery or slimy; bare substrate with no algae visible	May be calculated by difference (i.e. 100% - total % of other categories)
Films	Green film Brown film Black film	Thin, slightly to very slimy coating, various colours; rock generally visible through film	May include cyanobacteria, diatoms and green algae, but also bacteria and organic matter
Mats	Sludge	Loose, unconsolidated algae, easily dislodged; often in slow-flowing areas between large substrate and along stream margins	Examples are mucilage-rich accumulations of diatoms such as *Cymbella,* or loose remnants of detached algae
	Thick mats	Any type of mat that is > 0.3 cm thick (when out of the water)	Many taxa. Category used in monitoring to maintain recreation / aesthetics values
	Cyanobacteria – *Microcoleus*	Distinctive black, brown or whitish flecked with black/brown, smooth surface	May be included in observations because of health risk (potentially toxic)
	Other distinctive cyanobacteria	For example, *Nostoc*: firm, jelly-like nodules, from pale brown to dark green-brown.	*Nostoc* can be toxic and can be abundant at times.
	Mixed algal mats (greenish to light brown to bark brown, or a mixture)	Consolidated layer with defined edges or covering whole stones. The thinnest mats form a visible blob when scraped for 5 cm along stone surface with fingernail. May resemble very short filaments (< 1 cm) when viewed under water	Often dominated by diatoms (many different taxa) but can be a mixture of diatoms, cyanobacteria, Rhodophyta, Chlorophyta

	Red algae mats	Slimy nodules up to 5 mm across, or coalescing. Reddish to pale yellow-green colour	Includes the filamentous alga *Audouinella,* which forms mat-like growths
	Didymo mats	Distinctive nodules/mats, brown surface and white/pale brown inside; tough, woolly texture; firmly attached. Can be > 3 cm thick	The bloom-forming diatom *Didymosphenia geminata;* South Island only (as of 2020)
Filaments	Long filaments	Any type of filament that is > 2 cm long	Many taxa. Category used in monitoring to maintain recreation / aesthetics values
	Green slimy filaments	Filamentous algae, usually forming long filaments. Range of shades from pale to yellowish to bright green	Includes *Oedogonium*, *Spirogyra*, *Ulothrix*, *Microspora* etc. (Chlorophyta) and Xanthophyceae (Ochrophyta) such as *Tribonema*
	Short green filaments	Tufts of deep green or paler green filaments, typically < 2 cm long	*Stigeoclonium* (deep green, often in enriched waters); *Cladophora* sp. (paler, often in unenriched waters)
	Brown slimy filaments	Golden brown to dull brown filamentous masses in slow-flowing water	Usually filamentous diatoms such as *Diatoma* (golden brown) or *Melosira* (dull brown)
	Coarse filaments	Thick filaments ranging from pale rope-like strands to thick reddish brown filaments with irregular thickness	Includes Rhodophyta (e.g., *Compsopogon*), and Chlorophyta (usually *Cladophora*) covered with epiphytic diatoms

4.2.1.1 Periphyton Cover Subcategories for Other Monitoring Purposes

The four categories listed in Table 1 are the minimum classification required to qualify for a quality rating of QC 600 under this Standard. However, further subdivision of categories may be necessary to satisfy some other monitoring purposes. For example:

- some regional plans and resource consent conditions specify outcomes or limits for periphyton cover in terms of defined lengths of filaments or thickness of mats in accordance with specifications in Ministry for the Environment (2000) to maintain recreation/aesthetics values, and
- when data are required for describing ecological conditions in more detail or for the purpose of developing models, agencies may subdivide the four categories into additional subcategories that are appropriate for their region and monitoring purpose.

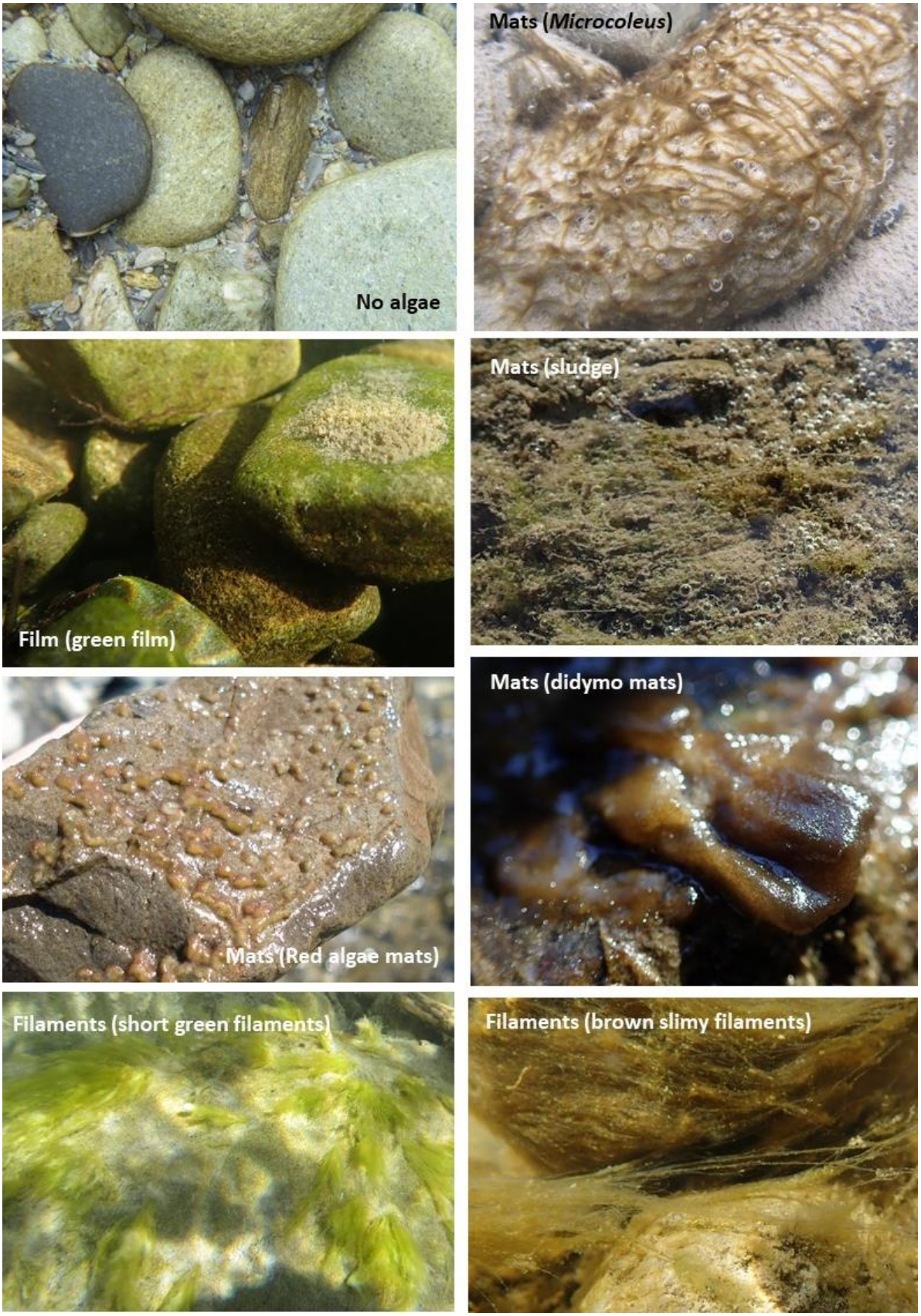

Figure 6 - Examples of the periphyton categories (with subcategory descriptions in brackets) listed in Table 1 that shall be used to document streambed periphyton cover under this Standard

Photos: Anika Kuczynski and Cathy Kilroy

4.2.2 Measurement Units and Resolution

Under this Standard, streambed periphyton cover shall be recorded as a percentage to the nearest 5%, or to 1% for unusual but notable types of periphyton such as low, but highly visible, cover by short green filaments. When cover of < 5% is recorded, percentage cover of another category with higher cover must be adjusted so that the total cover adds to 100%.

4.2.3 Stationarity of Record

Because visual cover estimates may have in the past been carried out using a range of criteria to separate periphyton categories, some agencies may wish to maintain continuity of records by linking the categories or the subcategories listed in this Standard (Table 1) to existing categories they use. Suggestions on how to align different periphyton cover categories are provided in Annex E.

Note: There is no requirement to use the subcategories listed in Table 1 but some organisations may wish to align their records with these.

4.3 Monitoring Protocol

The following protocol assumes that four transects are to be used in the monitoring reach. Different numbers of transects may be required, depending on the wadeable stream width. Irrespective of the number of transects, always start the monitoring at the most downstream transect and work upstream. This ensures that viewed areas are not disturbed by material dislodged from upstream.

1. Mark, at the stream edge, the locations of four transects up to 15 m apart. Use temporary markers such as piles of rocks. Transects may also be optionally marked at viewing points on the streambed (e.g. using painted rocks as shown in Figure 4).
2. With the underwater viewer (and attached datasheets if working alone), wade into the stream at 90° to the water's edge on the most downstream transect and go to the first observation point. This point will be at the opposite edge of the stream if the transect is fully wadeable or at the deepest point that can be safely waded if the transect is only partially wadeable.

   Note: If working alone, you will need a means of attaching a clipboard or data collection device to your person, so that you always have one hand free to hold the viewer upright. In addition, the viewer could be tethered to your waist or arm.
3. Roughly estimate the distance required for five assessment points to be equally spaced along the transect or part-transect. An easy way to measure the distance is to count strides as you wade into the stream.
4. Where possible, place temporary markers (see subsection 4.1.2) on the streambed to indicate the locations of the observation (viewing) points and ensure that the next point to be viewed is not one you have just walked over.

   Note: The use of temporary markers is not a requirement to be eligible for QC 600, but it is recommended best practice, particularly if periphyton samples are also being collected on the same site visit.

5. Turn to face upstream and hold the underwater viewer so that the viewing window is up to 20 cm below the water's surface on the transect line. Holding the viewer steady and as vertical as possible (Figure 7), estimate the percentage cover by periphyton in the categories in Table 1 to the nearest 5% (or 1% if cover is 1 to 3%). Typical examples of each periphyton category are shown in Figure 8.

Figure 7 – Demonstrating the correct technique for use of an underwater viewer to estimate streambed cover (left) and the streambed as seen from looking down the viewer

Photos: left, Anika Kuczynski; right, Charlotte Woods

6. Record the estimated percentage cover of each periphyton category in the appropriate boxes for transect 1 on the Field Record Form. The total percentage of all categories combined should add up to 100.

   Note: If periphyton cover categories overlap (e.g. filaments overlaying mats), record the total percentage cover of the overlying category (i.e. filaments). If overlapping algal types are of interest they may be recorded, and the total cover may be > 100%. The observer should indicate as metadata on the Field Record Form which categories are overlapping.

7. When you have finished the assessment at the first point, take the appropriate number of strides towards the water's edge or go to the next marker (see step 3 above). Lower the viewer into the water and repeat the assessment.

8. Repeat for the remaining three points on transect 1. The fifth point (near the water's edge) should ideally be at a depth of 0.1–0.15 m, although this depth will vary according to the type of stream. For example, if the streambank is incised (channelised) the closest survey point may be deeper.

   Note: When carrying out periphyton cover estimates or biomass sample collections at times of elevated flows, exclude any recently wetted wadeable areas of streambed that are outside the area of the streambed that is normally wetted (under lower flows).

9. Move upstream to transects 2, 3 and 4, and repeat steps 2 to 8 above.

10. Upon completion of the assessment, wipe clean the viewer with a soft cloth and store appropriately to prevent damage to the viewing window. In addition, ensure that the viewer is disinfected before use in a separate stream (see subsection 3.2.6).

    Note: A purpose-made storage bag or box is recommended for transporting and storing the viewer.

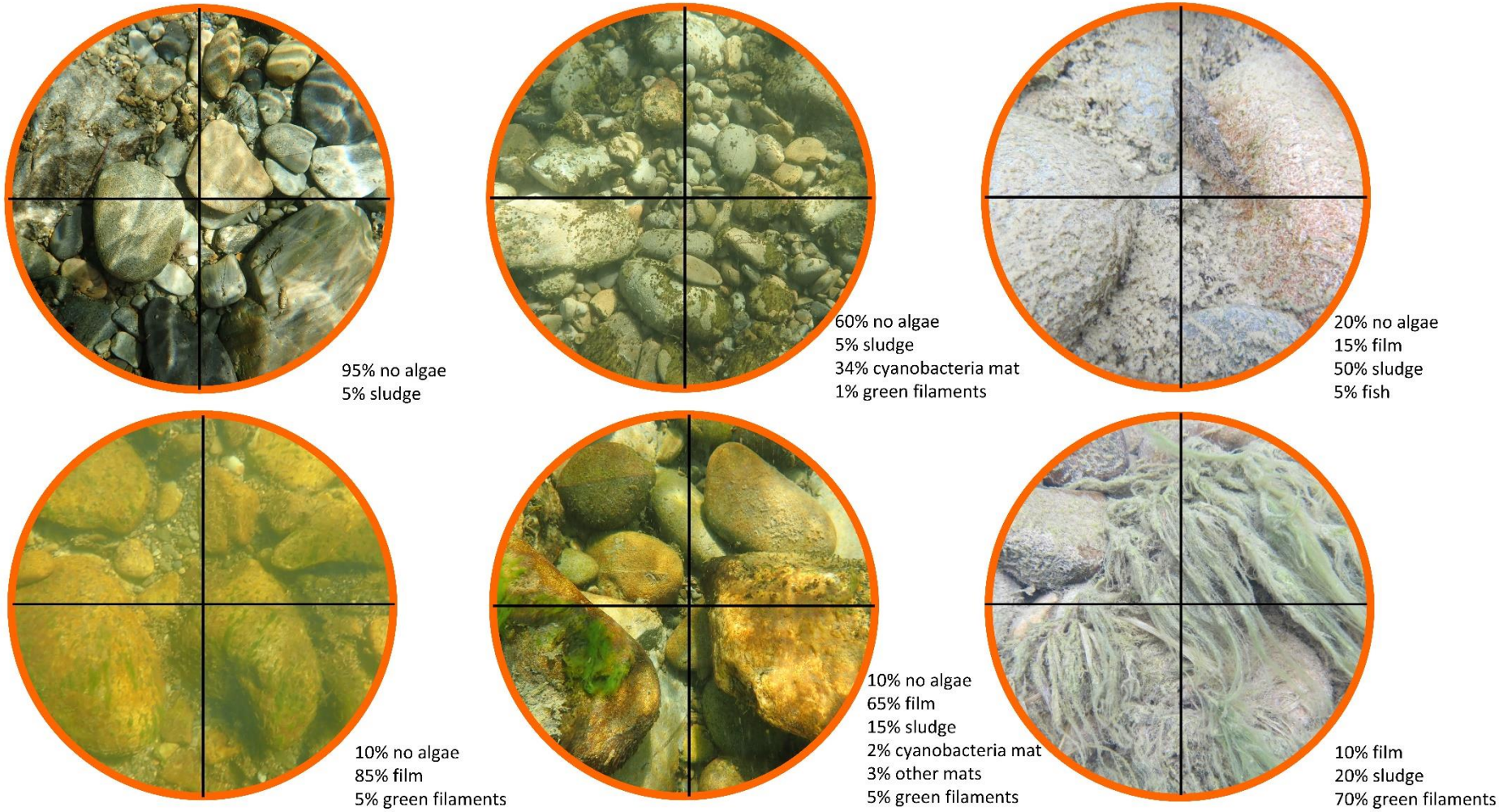

Figure 8 – Examples of different amounts of streambed periphyton cover

Photos: Cathy Kilroy

5 Periphyton Sample Collection

In this section

This section focuses on the collection of periphyton samples for subsequent laboratory analysis for periphyton biomass as either chlorophyll *a* or ash free dry mass (AFDM). It covers the equipment needed and sets out the required protocol for sample collection.

5.1 General Approach

The aim of periphyton sampling is to obtain a sample that is representative of the periphyton in the wadeable portion of a river reach, which itself is representative of the broader river environment (subsection 2.1). Because periphyton cover can be highly spatially variable, multiple subsamples are essential. A minimum of 10 subsamples can achieve reasonable representation of chlorophyll *a* concentration within a stream reach, but up to 40 subsamples are required for the best representation when periphyton cover is patchy (Kilroy et al., 2013). However, collection of more than 10 subsamples can be unacceptably time-consuming. This Standard therefore requires at least 10 subsamples to be collected as a compromise between adequate representation and practicality. These subsamples are then pooled to produce a single composite sample per site.

5.1.1 Sampling Points

Ten or more replicate subsamples shall be collected at random points within the monitoring reach. At each sampling point, a rock or stone is retrieved from the streambed. Periphyton is collected from the surface (top and sides) of the rock exposed to the overlying water column.

A random distribution of replicate subsamples within the monitoring reach can be achieved by pre-selecting points on transects that span the stream width or part-width out to a depth of 0.6 m (see Figure 3).

Note: Temporary markers (refer to subsection 4.1.2 and Figure 4) can be useful for identifying points for sample collection and ensuring that samples are collected from areas of undisturbed streambed.

5.1.2 Sampling Area

Periphyton samples shall be collected from a known area to enable standardisation of the results to the area sampled (i.e. as mg chlorophyll *a* per m^2). There are two general methods (Biggs and Kilroy, 2000) for defining the surface area:

1. Collection a subsample from a known area of the rock, usually defined by a circle, or
2. Collection of each periphyton subsample from an entire rock, and estimation of each rock's area from its dimensions.

This Standard requires the use of method 1 on the primary basis that it introduces the least amount of error:

- For fixed-area sampling under (1), the main source of error is selection of a representative area for sampling on the selected rock. This error is controllable by the observer, who can make a judgement about the area of the rock that best represents periphyton cover on the entire exposed surface (although a disadvantage is that subjectivity is involved).
- In contrast, in (2) the formula for calculating rock surface area can introduce large errors, depending on rock shape and the proportion of the rock exposed to the overlying water. Once the data are collected the errors cannot be checked. There are methods for determining the surface area of whole rocks, but all are laborious and time-consuming, taking at least as long as the initial sample collection.

Note: A review of methods by Bergey and Getty (2006) concluded that method 1 is appropriate for field use and requires no additional laboratory processing to determine rock size.

Under this Standard, the fixed area of each rock or stone for periphyton removal shall be defined by a ring (typically, a container lid) of known diameter (30–70 mm). The diameter of the sampling ring shall be recorded as visit metadata on the Field Record Form.

Note: Ideally, have lids in a range of diameters to suit the stone size. If the ring diameter is required to be at or less than the minimum specified (30 mm) (e.g. where substrate is consistently large gravel rather than cobble) a minimum of 15 subsamples shall be collected to increase the total area sampled.

5.2 Sampling Procedure

1. Select locations for at least two transects. Roughly estimate the distance required for 10 sampling points to be equally spaced across the transects. An easy way to measure distance is to count strides as you wade into the stream.
2. The number of sampling points on each transect will be five points on two transects or fewer points on more than two transects (e.g. alternating between two and three points on four transects, as in Figure 9).

   Note: transects may be varied further in very small streams. See Section 4.1.3.
3. If sample collection is being carried out in the same reach and during the same site visit as measurements of periphyton cover, then sample collection points may coincide with cover viewing points, if the latter are marked on the streambed (see subsection 4.1.2).

   Note: Sample collection at pre-marked points is best practice because placing markers allows field personnel to avoid trampling in areas from which samples may be collected.
4. Move to the first sampling point by wading into the stream at 90° to the water's edge, about 1 m downstream of the most downstream transect. If the stream is not wadeable for the full width, wade out to the point of maximum wadeable depth (0.6 m), and this will be the first sample point.

5. Bend down and lightly touch the streambed sediments without looking. The substrate touched identifies the first subsample. If the substrate (rock) is too big or heavy to pick up, find the closest adjacent stone that can be picked up.

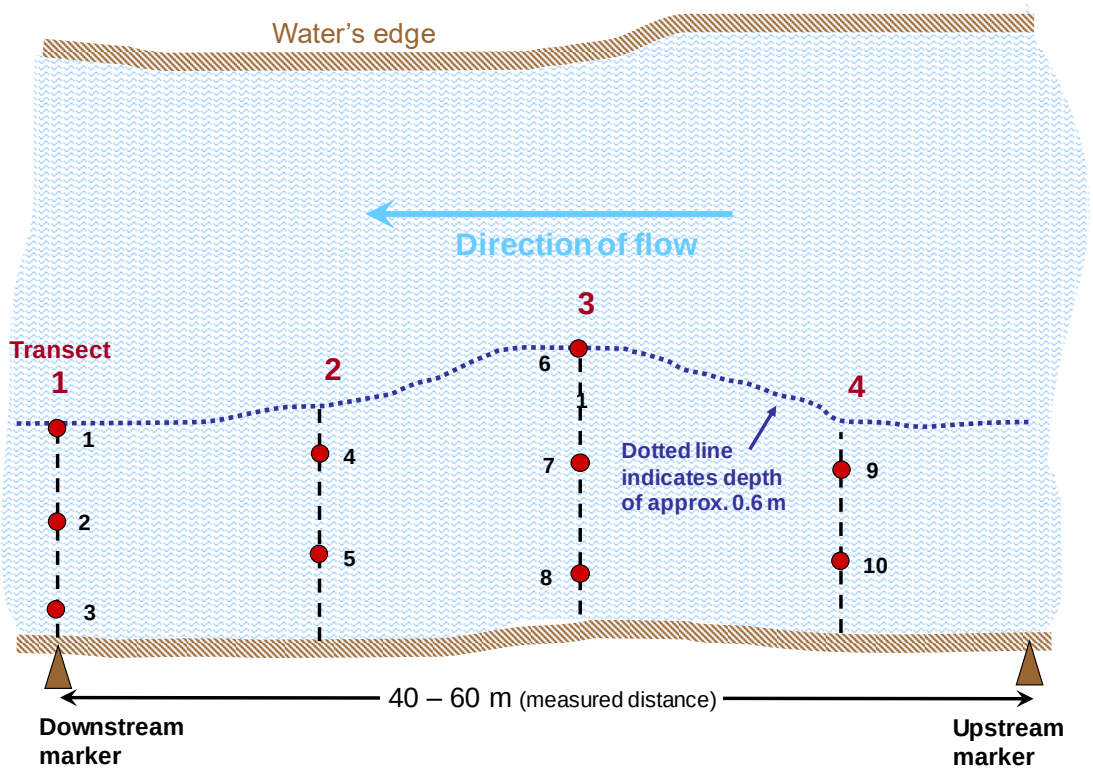

Figure 9 - Location of 10 sampling points across four part-transects (in plan view)

Figure: Cathy Kilroy

6. If all substrate is too large to handle, or is bedrock, look for the nearest retrievable rock that has similar periphyton cover. The periphyton sample can be collected from this adjacent rock.

   Note: A double syringe underwater sample for sampling periphyton from underwater surfaces is described in section 6.5.5 of Biggs and Kilroy (2000). Biggs and Kilroy (2000) cited earlier studies showing that periphyton samples collected using underwater samplers underestimate biomass in streams. It was recommended that these samplers should be used only for entire studies rather than one-off samples. Therefore, underwater samplers are not recommended for use under this Standard.

7. If the substrate is too small to sample directly (i.e. smaller than your smallest sampling circle), or the area touched is a patch of sand or silt, the following method can be used:

   - Place the lid directly on the substrate and push in until level with the substrate surface. Slide a stiff plastic sheet or metal spatula under the lid full of sediment and lift the lid plus sediment out of the water column. Smooth off the sample surface so that it just fills the lid. This is the substrate to be sampled for periphyton.

8. Without disturbing its periphyton cover, place the rock in a tray and either return to the bank or move to the next sampling point on the transect.

9. Repeat the above process until all retrievable rocks and/or substrate from that transect's sampling points are collected. Return to the bank.

10. Ensure that collected rocks/substrate are kept cool and moist during collection of the remaining subsamples. Gently drizzle stream water over the periphyton surface and shade the substrates from direct light.

11. Collect the periphyton subsamples from each rock. Place the sampling lid over an area of representative periphyton cover on the upper surface of the rock (i.e. the surface that was exposed to the water column in the stream). Holding the lid firmly, use a knife or brush to scrape/brush away all the algae on the rest of the rock (Figure 10). Rinse away the surplus using a squirt bottle filled with stream water, then remove the lid to expose the circular subsample. Rinse brushes or knife thoroughly with stream water or a squirt bottle.

    Note: If periphyton cover consists of long streaming filaments, use scissors to cut around the lid to obtain a subsample. Do not include filaments streaming outside the lid in the subsample.

Figure 10 - Periphyton subsamples still attached to rock, ready to scrape into the primary container

Photos: Cathy Kilroy

12. Scrape/brush the sample into the primary sample container (laboratory tray). Rinse off remaining periphyton into the tray using the squirt bottle. Use a transfer pipette to suck up any sample in crevices or depressions in the rock. Minimise water use to ensure that the sample size is no more than the watertight sample container capacity.

13. Rinse residual periphyton from the knife and/or brush using a small amount of stream water and transfer this residual material to the primary container.

14. For subsamples from fine substrate (step 7 above), collect the periphyton using the following method:

    - Transfer the subsample to a tray (separate from the primary container for subsamples from larger substrate) with a small amount of stream water and mix together thoroughly. Individually scrub any larger particles using a brush before rinsing into the tray and discarding.

- Drain the supernatant (which may be a brown or greenish colour) into the sample container (if larger substrate is also sampled, subsamples from both will go into the same container).
- Add a little more stream water to the subsample in the tray, mix again and transfer the supernatant to the sample container. Repeat one more time. Discard the small substrate once it has been sufficiently rinsed.

15. Repeat the stone/sample collection and sampling procedure for all remaining points on the transect(s), ensuring that substrate is not collected from any areas of the streambed that have been walked on.
16. Rinse the entire contents of the primary collecting tray into the sample container. No periphyton should remain stuck to the sides or bottom of the primary tray. Sufficient air space should be left in the sample container to allow for expansion if the sample is to be frozen. Ensure that the container lid is screwed on tightly.
17. Transfer the sample to a chilly bin as soon as it is collected. It is important to keep the samples in a dark, cool environment until they are ready for processing in the laboratory.

    Note: Recent investigation into alternative sampling methods has seen the development of a vacuum pump system. This system appears to cleanly remove all periphyton from a defined area (Figure 11).

Figure 1 - A vacuum pump system (left) and rocks from which periphyton has been removed

Photos: Taranaki Regional Council

5.2.1 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). It is good practice to also place a waterproof paper label, with sample details, inside the sample container at the time of sample collection as a back-up in case the outer labelling is damaged or lost while samples are in transit.

5.3 Sample Storage, Handling and Transport

To maintain sample integrity for subsequent laboratory testing, all periphyton 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).

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

As noted in subsection 1.6, 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 frozen at -20°C or lower and dispatched to the laboratory for analysis within three months of collection.

When packing samples (fresh or frozen) 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).

Note: When sending frozen samples to the laboratory, to avoid thawing in transit add ice packs and ensure that the samples are not in transit for more than 18 hours (Biggs and Kilroy, 2000).

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.

5.3.1 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:

- the date and time of sample collection and dispatch
- whether the samples were dispatched fresh or frozen
- analysis required
- the total surface area (m^2) of substrate scraped for each sample, and
- anything unusual about the samples.

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”).

6 Laboratory Processing and Reporting

In this section

This section describes key steps in procedures for the laboratory analysis of periphyton samples for chlorophyll *a* and ash-free dry mass (AFDM). These include sample receipt, sample preparation, sample analysis, reporting and quality assurance.

6.1 Laboratory Credentials

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

- adherence to either formal standard protocols (i.e. Biggs and Kilroy, 2000) or subsequently implemented protocols (i.e. this NEMS) for the processing of periphyton samples for chlorophyll *a* and/or AFDM. 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
- staff are suitably qualified/trained in carrying out chlorophyll *a* and/or AFDM analyses.

Note: It would be desirable to establish some form of laboratory processor accreditation in the future to provide a level of confidence in data quality.

6.2 Sample Receipt

Periphyton samples shall be registered on arrival at the laboratory. This shall include completion of the Chain of Custody (CoC) Form that accompanied the samples and prompt (same day) return of this to the monitoring agency with a laboratory reference or job number.

6.2.1 Date of Arrival

Laboratory staff shall record the date and time (in NZST) of arrival of samples at the laboratory and record this on the CoC Form.

6.2.2 Sample Temperature and Condition on Arrival

Laboratory staff shall measure the temperature of any samples received in a fresh (unfrozen) state on arrival at the laboratory (e.g. through the use of a calibrated dual-beam infrared thermometer, or 'laser gun', inside each chilly bin) and record this on the CoC Form. Laboratory staff shall also inspect the condition of samples and record any anomalies on the CoC Form and in their sample notes for inclusion in reporting of sample test results (see subsection 6.4). For example, anomalies noted shall include:

- damaged or leaking sample containers, and
- whether or not samples were fully frozen (if dispatched frozen).

Fresh samples shall be checked to ensure they have been received within 24 hours of collection.

6.3 Methods Overview

The Standard requires that laboratory analysis of periphyton samples for biomass as chlorophyll *a* and AFDM is carried out using the methods set out in Biggs and Kilroy (2000), as modified in Annexes F and G. To be eligible for the highest quality rating of QC 600:

- analyses performed on fresh samples shall commence within 24 hours of collection (or the samples shall be frozen at -20°C or lower), and
- analyses performed on frozen samples shall commence within three and 12 months of collection for chlorophyll *a* and AFDM, respectively.

The analyses involve the following general steps:

1. Homogenisation of the raw composite periphyton sample provided by the monitoring agency, to enable transfer of representative subsamples with minimal water to non-combustible glass-fibre filters.
2. For chlorophyll *a*:
    a. extraction of chlorophyll *a* from the subsamples on filters into a solvent
    b. reading the absorbance of the colour in the solvent at a wavelength appropriate for chlorophyll *a*, using a spectrophotometer
    c. conversion of absorbance readings to concentration of chlorophyll *a* in the sample using published conversion factors and allowing for the volumes of solvent, periphyton subsamples and the original sample, and
    d. calculating the amount of chlorophyll *a* in the original sample.
3. For AFDM:
    a. drying the pre-weighed filter plus subsample to drive off all the water in the periphyton, then weighing
    b. ashing the filter plus subsample to burn off all organic material in the periphyton, then re-weighing, and
    c. calculation of AFDM in the sample as the difference between the dried weight and ashed weight, multiplied up using the volumes of the periphyton subsamples and the original sample.

The following subsections focus on critical parts of the three procedures above. Detailed procedures (modified from Biggs and Kilroy, 2000) are provided for reference in Annex F (chlorophyll *a*) and Annex G (AFDM). Refer to Biggs and Kilroy (2000) for additional background to the recommended methodology.

6.3.1 Sample Preparation

Obtaining a representative subsample is critical for accurate measurement of periphyton biomass. Most periphyton samples will contain clumps of filamentous algae or diatoms, creating difficulties in subsampling representative portions for analysis. Homogenising the sample with a simple, hand-held laboratory or kitchen blender can significantly reduce variability across

subsamples (Biggs, 1987). Sharp scissors can be useful for breaking up tough filaments that may become wrapped around the blender shaft.

Once blended, and before removing subsamples, the homogenised sample either shall be made up to a known volume using distilled water or the volume of sample recorded with no dilution. This volume is the volume of the original sample, which is used in later calculations and shall be recorded on the laboratory worksheet.

6.3.2 Chlorophyll *a* Analysis

Chlorophyll *a* is analysed as follows:

1. Concentrate subsamples of homogenised periphyton onto glass-fibre filters with a pore size of 1.2 µm (e.g. Whatman GF/C 47 mm filters). At least two, and preferably at least three, aliquots should be taken and filtered from each sample (i.e. pooled onto the same filter). The aliquots together make up the subsample. Aliquots are typically 5 mL. Record the total volume filtered on the laboratory worksheet.

Note 1: If the original sample is too dense to allow filtration of multiple aliquots, all or part of the sample should be diluted to the point where multiple aliquots are possible. Record the dilution (and the revised original sample volume) on the laboratory worksheet. Alternatively, at least duplicate (and preferably triplicate) single 5 mL subsamples should be filtered onto separate filters so that a mean value can be calculated.

Note 2: The filtering (subsampling) step is critical for accurate results. It is recommended as best practice under this Standard to always remove aliquots (using a calibrated pipette) from the homogenised composite sample when it is in motion (but not a swirling motion) and to take at least three aliquots to help to average out variability among aliquots.

Note 3: To minimise degradation of chlorophyll a*, work in muted light conditions and keep the samples as cool as possible during filtering (e.g. hold them in a chilly bin with ice).*

2. Extract the chlorophyll *a* in a solution of 90% boiling ethanol (i.e. 78°C). Boiling increases extraction efficiency and helps to "fix" the chlorophyll by destroying enzymes thus making the chlorophyll relatively stable when in storage.

Note: This Standard specifies 90% boiling ethanol as the extractant because (a) ethanol has been shown to be generally more efficient at extracting chlorophyll a *than other extractants, including acetone, and (b) ethanol presents a lower health and safety risk than other extractants (Biggs and Kilroy, 2000; Ritchie, 2008). Extraction efficiency may vary between ethanol and acetone depending on the taxonomic composition of the algal sample (Wasmund et al., 2006). An inter-laboratory comparison of chlorophyll* a *analysis of periphyton samples (covering steps 2 to 7 in this section) has been carried out across six New Zealand laboratories (Kilroy and Daly in prep.). Chlorophyll* a *concentrations returned from four laboratories using the method specified in this NEMS showed minor differences (on average ~±5% difference between laboratories). Differences between results from these four laboratories and two laboratories using acetone as an extractant were larger (on average ~±14%). Furthermore, the two laboratories using acetone returned results on average 23% different from each other. It was concluded that the ethanol method provided generally consistent results across laboratories. The results from the two laboratories using acetone may require further investigation. It was noted that results from all the laboratories were strongly correlated to the overall mean value (calculated across all laboratories) enabling results to be converted to an estimated mean value using a laboratory-specific equation. To*

promote national consistency, ethanol shall be used as the extractant for chlorophyll a measurements to be eligible for a QC 600 quality rating.

3. Leave to complete extraction in a refrigerator for at least three hours.

Note: Overnight extraction in a fridge is often convenient. Laboratory trials indicate that extraction for three hours is sufficient.

4. Centrifuge to remove particulates from the solution.

Note: The extracted chlorophyll a should be uniformly dispersed in the solution after the centrifugation step (Figure 12).

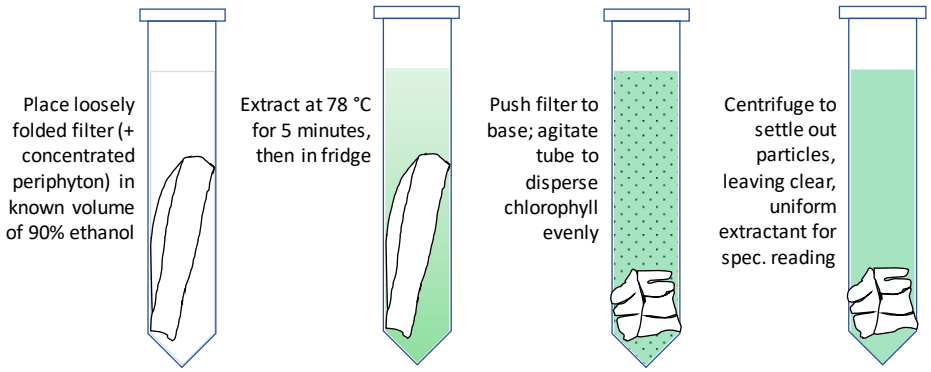

Figure 12 – Steps in preparing periphyton on glass-fibre filters for reading of the concentration of chlorophyll a (extracted into 90% ethanol) on a spectrophotometer

Figure: Cathy Kilroy

5. Read background turbidity and pigment concentration on a spectrophotometer.

Note 1: The absorbance peak for chlorophyll a is narrow and varies slightly among spectrophotometers, so it is important that you locate the wavelength of the peak exactly by scanning absorbance of a standard solution. Refer to Annex F for details.

Note 2: Ethanol solutions that appear extremely deep green may need to be diluted to ensure that readings are not taken at concentrations outside the range of the linear relationship between absorbance and chlorophyll a concentration. Refer to Annex F for details.

6. Acidify the ethanol solution to convert the chlorophyll *a* to phaeopigments and re-read absorbances.

Note: The acidification step is required to enable correction for the presence of phaeophytins in degraded periphyton in the sample. See Annex F for details.

7. Calculate chlorophyll *a* concentration by multiplying by an absorbance coefficient, including a correction for turbidity and the presence of phaeopigments (refer to Annex F for details). The concentration is then converted to the amount of chlorophyll *a* (in mg) in the original sample supplied by the monitoring agency, from the original sample volume (see subsection 6.2) and the total volume of aliquots pipetted onto the filter (Step 1 above).

6.3.2.1 Quality Checks

Sources of error and quality checks during analysis of samples for chlorophyll *a* are described in detail in Biggs and Kilroy (2000), and a modified version is included in Annex F. Key steps for minimising error are:

- Ensure that subsamples concentrated onto filters are composed of at least two 5 mL aliquots
- Use a calibrated pipette with the correct techniques for aliquot withdrawal and release
- Establish a systematic routine for the filtering process to avoid mixing up subsample numbers
- Keep all subsamples as cool as possible and in the dark during filtering
- Prevent evaporation of ethanol during the hot extraction phase, and
- Locate the correct peak in absorbance for chlorophyll *a* on the spectrophotometer.

6.3.3 AFDM Analysis

Analysis of AFDM (and dry mass, or DM) in a sample involves the following steps:

1. Concentrate subsamples of homogenised periphyton onto pre-weighed glass-fibre filters with pore size of 1.2 µm (e.g. Whatman GF/C 47 mm filters). At least two (and preferably at least three) aliquots should be withdrawn and filtered from each subsample (i.e. pooled onto the same filter). Aliquots are typically 5 mL. Record the total sample filtered on the laboratory worksheet.

   Note: It may be necessary to pre-ash the glass-fibre filter to ensure that no organic material in the filter adds to the initial dry weight of periphyton. Pre-weighing the filter is essential if also measuring DM.

   Refer to notes under Step 1 in subsection 6.3.2.

2. Dry the subsamples at 105°C for 24 hours to drive off all water, and re-weigh.

   Note: It is critical to hold samples in a desiccator while cooling to room temperature and during weighing, after drying and ashing. Absorption of water from the atmosphere can significantly increase the weight of a sample.

3. Ash the subsamples in a muffle furnace at 400°C to burn off organic material, and re-weigh.

   Note: Use of a higher temperature (e.g. 500°C) leads to chemical changes that reduce the ashed weight and therefore increase AFDM estimates. Rehydration can remedy this. Refer to Annex G for further explanation. Laboratory trials on periphyton samples with AFDM concentration of 7–72 g/m² showed that ashing at 500°C with no rehydration resulted in AFDM on average 12% (± 6%) greater than ashing at 400°C (NIWA, unpublished data). An ashing temperature of 400°C shall be used for AFDM measurements to be eligible for a QC 600 quality rating.

4. The difference between the dry weight and the ashed weight gives the ash-free dry mass (AFDM) value.

6.3.3.1 Quality Checks

Sources of error and quality checks during analysis of samples for AFDM are described in detail in Biggs and Kilroy (2000), and a modified version is included in Annex G. Key areas for minimising error are:

- Ensure that subsamples concentrated onto filters are composed of at least two 5 mL aliquots

- Use a calibrated pipette with the correct techniques for aliquot withdrawal and release
- Establish a systematic routine for the filtering process to avoid mixing up subsample numbers
- Ensure that subsamples are weighed at room temperature, and
- Hold subsamples in a desiccator before and during the weighing process. Make sure that the silica gel in the desiccator is fully dry.

6.3.4 Detection Limits and Measurement Resolution

The detection limit for chlorophyll *a* is determined by the spectrophotometer. A typical value of 0.08 mg/L has been cited (Hambrook Berkman and Canova, 2007). This detection limit by volume applies to the original, homogenised sample (see subsection 6.3.1). As an example, assuming analysis of a 15 mL subsample from an original sample volume of 100 mL, with material collected from 0.03 m^2 of streambed (i.e. subsamples scraped from 10 circles of 62 mm diameter, a typical sampling area), the concentration of 0.08 mg/L equates to 0.4 mg chlorophyll *a* per m^2. The precision of the measurement in samples with very little periphyton can be increased by increasing the volume of the subsample at the filtration stage (subsection 6.3.2, step 1). The "detection limit" therefore depends on the amount of periphyton collected in a sample. In practice, chlorophyll *a* concentrations of < 0.1 mg/m^2 represent extremely low periphyton on the streambed; therefore, a measurement resolution of 0.1 mg/m^2 is required under this Standard.

The detection limit for AFDM is determined by the precision of the balance used to weigh samples. The precision of weights for samples with low AFDM can be increased by concentrating a larger volume onto the filter at the filtration stage (see subsection 6.3.3, step 1). AFDM of < 0.1 g/m^2 represents very low periphyton on the streambed; therefore, a measurement resolution of 0.1 g/m^2 is required under this Standard.

Note: Laboratories shall also supply the 'uncensored' data (i.e. raw, unrounded data).

6.3.5 Quality Control

6.3.5.1 Internal Quality Control

As a minimum, the following practices are expected:

- For at least 10% of samples, take duplicate (preferably triplicate) subsamples (i.e. two or three sets of at least three 5 mL aliquots, each on a separate filter) at Step 1 in subsections 6.3.2 and 6.3.3. This provides an additional check on the accuracy of laboratory subsampling for both chlorophyll *a* and AFDM;
- Check the calibration and volume setting on the pipette used for subsampling regularly by weighing subsamples of distilled water on a precision balance;
- Calibrate balances, ovens and muffle furnaces (the latter two for temperature) 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 ensure the quality of the analysis and isolate problem areas in the analytical process; and

- Review, at least two-yearly, the sample processing performance of individual laboratory staff.

Note: The monitoring agency may wish to request a report documenting the internal quality control carried out on its samples. The internal quality control report would document the individual measurement values for each replicate subsample analysed and comment on any unusual variation across replicates.

6.3.5.2 External Quality Control

As noted in subsection 1.3, internal quality control is the primary measure of laboratory performance given that chlorophyll *a* in periphyton samples degrades over time and with each repeated thawing and freezing. However, if desired, the monitoring agency could request that a composite periphyton biomass sample be split and a subsample sent to another laboratory for analysis, assuming both laboratories could analyse subsamples under the same conditions and same timeframes to ensure a meaningful inter-laboratory comparison.

6.4 Laboratory Reporting

The laboratory shall, as a minimum, report for each periphyton measurement:

- The name of the sample and the date and time it was collected
- The date and time of receipt of the sample at the laboratory
- The variable measured, measurement value and unit of measurement
- The measurement method and standard limit of detection, including details of any modifications made to these
- Comments on any anomalies in the condition of the sample upon receipt (see subsection 6.2.2) and supporting commentary from the analyst in the advent of difficulties with testing the sample (e.g. very low or very high chlorophyll *a* relative to the appearance of the sample) or an unusual result, and
- The maximum time the laboratory will hold the sample to provide for re-testing, if requested (if longer than the minimum requirement of one month).

All laboratory analytical reports shall be checked prior to release to the monitoring agency. Reports shall be supplied to the monitoring agency digitally, locked to prevent inadvertent editing. 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.

As noted in subsection 6.3.4, laboratories shall also supply the 'uncensored' data (i.e. raw, unrounded data).

6.4.1 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, who holds all metadata, systematically checks laboratory results as soon as possible (e.g. against previous results or corresponding periphyton cover data). If this is completed within a reasonable timeframe (preferably two weeks but coinciding with the laboratory's maximum sample holding time of one month, or longer), it is possible for the monitoring agency to query unusual measurements which may trigger re-analysis.

Note: The one-month timeframe applies to samples analysed for chlorophyll a *and some sample degradation will occur and impact subsequent measurements. Samples for AFDM analysis can be re-analysed at any time up to 12 months following collection and will not be affected.*

6.5 Managing Changes in Laboratory Methods

Changes within laboratories are expected to be limited to substitution of updated or replacement instruments (e.g. spectrophotometer, balances, ovens). The laboratory is expected to ensure that the setup and calibration of such new instruments produces results that are consistent with the original instruments. In the case of a change in spectrophotometer, the laboratory should employ a period of duplicate measurement of subsamples using both the old and new instruments. Monthly parallel measurements for a period of time should provide sufficient data to enable a conversion factor to be derived to 'align' the old and the new instrument measurements.

7 Data Processing and Quality Assurance

In this section

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

7.1 Data Types

Data from periphyton monitoring programmes can be split into several types of data:

- 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 and when periphyton streambed cover was estimated and/or periphyton samples (and any associated field measurements, e.g. conductivity) were collected, and
- periphyton data, which comprise periphyton cover measurements and/or laboratory measurements of chlorophyll *a* and/or AFDM.

All three data types shall be stored in a database.

7.1.1 Site Metadata

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

7.1.2 Visit Metadata

Adequate mechanisms shall be put in place to store all relevant visit-related metadata listed in subsection 3.2.4, together with the periphyton (and associated) measurements (subsection 7.1.3).

7.1.3 Periphyton Data and Associated Metadata

Each periphyton measurement shall be stored with:

- the measurement date and time
- analytical laboratory name and location
- analytical method and any modifications to this (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 laboratory report (subsection 6.4), and
- an associated quality code (assigned in accordance with subsection 7.2.1).

7.2 Data Processing

Processing of periphyton data primarily involves assigning quality codes through consideration of field practices, sample handling and storage, documentation and laboratory practices. Any adjustments to data should be documented.

7.2.1 Quality Coding

7.2.1.1 Performance

All individual periphyton variable measurements shall be quality coded in accordance with the NEMS *Quality Code Schema* (Figure 13) and matrices (Table 2) that form part of this Standard. The schema comprises six 'parent' codes and permits valid comparisons within and across multiple data series.

In most cases, periphyton data collected from long-term (e.g. SOE) monitoring will fall under one of QC 400, QC 500 or QC 600. The matrices in Table 2 provide a framework for assigning one of these quality codes to individual periphyton variable measurements. The matrices are:

Matrix A: General procedures and site visit
Matrix B: Periphyton cover measurements, and
Matrix C: Chlorophyll *a* and AFDM measurements.

To assign a quality code to a periphyton cover measurement, follow Matrices A and B and determine the total number of 'demerit' points. To assign a quality code to a laboratory measurement of chlorophyll *a* or AFDM, follow Matrices A and C and 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 2 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.

Some periphyton data may be coded QC 300 or QC 100. QC 300 shall apply where a variable has not been measured directly (e.g. an estimate of chlorophyll *a* derived from periphyton cover using an existing established relationship between these two variables). A code of QC 100 may apply on occasions when stream conditions prevent safe access for sampling (e.g. turbid water or elevated stream flows) and a periphyton measurement cannot be reliably estimated given the flow conditions.

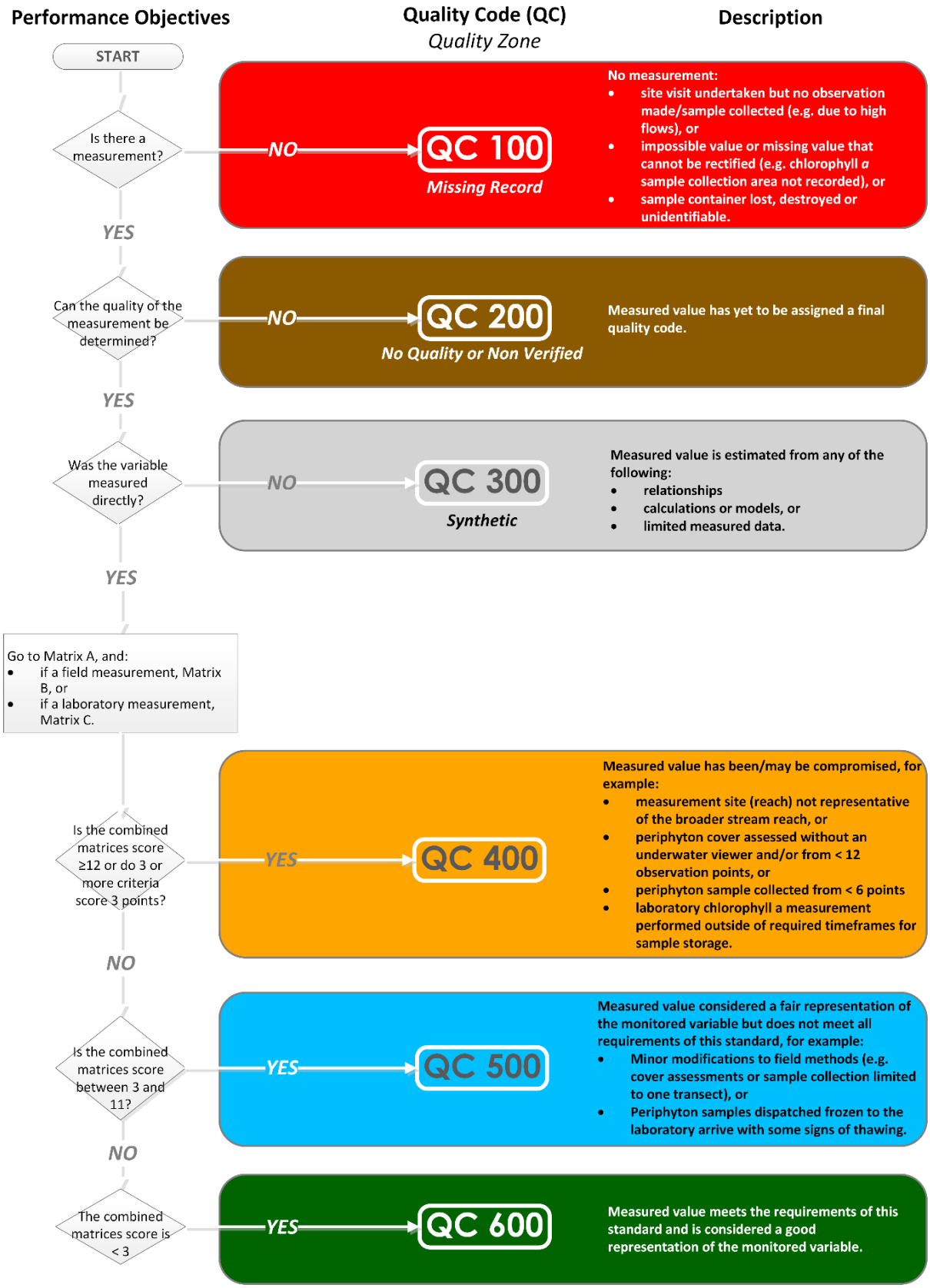

Figure 13 – NEMS Periphyton Quality Coding Schema

Use the flowchart as a framework to assign quality codes to individual periphyton variable measurements using the associated matrix (Table 2).

Table 2 - Criteria for assigning quality codes to periphyton variable measurements under this Standard

Matrix A: General Procedures and Site Visit

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

Note: The first two criteria represent system 'completeness and currency' checks.

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		Manual maintained in accordance with the Standard, including quality assurance procedures for all aspects of sampling and data management
Site Metadata *(subsection 2.3)*		Site metadata partially or not documented	Site metadata documented in accordance with the Standard but in need of updating	Site metadata documented in accordance with the Standard and checked/updated less than 12 months ago
Site Stationarity *(subsection 2.2)*		Different site location that is not truly representative of the broader stream reach		Site location consistency maintained or, if relocation is unavoidable (e.g. because of a flood or change in access), the new site location shall be close to the original site and remain representative of the broader stream reach.
Site (reach) Habitat and Representativeness *(subsection 2.1)*	Run habitat was available but monitoring reach was located in > 30% riffle or pool habitat, or soft sediment with macrophyte growth and/or was not representative of the broader stream reach	As for 0 points but part (< 30%) of the reach assessed/sampled comprised riffle habitat and/or soft sediment with macrophyte growth when sufficient run habitat was available for sampling	As for 0 points but some aspects of the habitat, such as substrate size or streambed shade, were not fully representative of	The site (reach) assessed/sampled met all requirements of this Standard, including comprising run habitat (where dominant) with substrate and shade characteristics representative of the broader stream reach at that location

			the broader stream reach	
Antecedent Flow Conditions *(subsection 3.1.2)*	Site visit for data collection restricted to at least two weeks without a bed-moving flood event or was otherwise non-random with respect to river flow.		Site visit date altered for Health and Safety reasons	Site visit for data collection made following a regular schedule irrespective of antecedent or current stream flow conditions
Visit Metadata *(subsection 3.2.4)*	Little metadata recorded other than the site and date of sample collection and, for periphyton biomass samples, the sample area was not recorded for all points of collection so a best guess needed to determine the total fixed area sampled	Little metadata recorded other than the site and date of sample collection, but diameter(s) of sampling area recorded for all samples collected	Visit metadata generally recorded in accordance with the Standard but the percentage of wadeable stream channel assessed/sampled for each transect is not recorded	Visit metadata captured on a Field Record Form in accordance with the Standard, including: • the location, date and time of periphyton cover assessment and/or sample collection • the number of transects and observation or sampling points, including the % of stream channel assessed/sampled for each transect and if any were located outside of run habitat • where a periphyton biomass sample was collected, the total area of streambed sampled, and • the environmental conditions at the time
Total Score				

Matrix B: Periphyton Cover Measurements

In addition to the criteria in Matrix A, quality coding of streambed periphyton cover measurements shall address the following criteria. The total score that determines the final Quality Code for each measurement (as in Figure 13) is the sum of the total scores from Matrix A and Matrix B.

Criteria	12 points	3 points	1 point	0 points
Equipment Specifications *(subsection 1.5.1)*	No underwater viewer was used to estimate periphyton cover.	The underwater viewer was of a diameter < 300 mm (e.g., a black disk viewer) or was dirty or scratched on the day of use, and the window was not divided into quadrants	The underwater viewer window was not divided into quadrants	An underwater viewer with a clean and clear bottom viewing diameter of at least 300 mm and divided into quadrants was used to estimate periphyton cover
Periphyton Cover Assessment Method *(subsections 4.1.1 and 4.2)*	Cover estimates were made at fewer than 12 points	Cover estimates were made at a minimum of 13 points but at fewer than 20 points OR Estimates (at least 20) were limited to a single transect within the monitoring site	Cover estimates were made at a minimum of 20 points but there was some bias in the location of these points	Cover estimates were made at a minimum of 20 points spaced at equal distances across at least two transects within the monitoring site to a maximum water depth of 0.6 m
Periphyton Cover Categories *(subsection 4.1.4)*	Periphyton cover was recorded in a manner that prevents aggregation of data for reporting according to the following categories: • No algae (bare rock) • Film • Mats, and			Periphyton cover was recorded in a manner that enables, at a minimum, reporting according to the following categories: • No algae (bare rock) • Film • Mats, and • Filaments

	• Filaments			
Measurement Units and Resolution *(subsection 4.1.5)*	The resolution of individual point cover estimates was > 10%	Individual point cover estimates were made to the nearest 10%	Individual point cover estimates were made to the nearest 5% for all forms of periphyton	Individual point cover estimates were made to the nearest 5% (and 1% for rare but notable periphyton forms)
Total Score				

Matrix C: Chlorophyll *a* and AFDM measurements

In addition to the criteria in Matrix A, quality coding of streambed periphyton samples collected for chlorophyll *a* or AFDM analysis shall address the following criteria. The total score that determines the final Quality Code for each measurement (as in Figure 13) is the sum of the total scores from Matrix A and Matrix C.

Criteria	12 points	3 points	1 point	0 points
Sample Collection Method *(subsections 5.1.1 and 5.2)*	Periphyton was collected from fewer than six points or periphyton was collected from whole rocks (rather than from a defined area as required under this Standard)	As for 0 points but there was some bias in the location of the sampling points or size of substrate sampled OR Periphyton was collected from at least 10 points but collection was limited to a single transect within the survey reach OR Periphyton was collected from between 7 and 9 points		A composite sample was collected by scraping periphyton from a fixed area comprising at least 10 points at equal intervals across at least two transects within the survey reach to a maximum water depth of 0.6 m
Sample Traceability *(subsections 5.3.1 and 6.2)*	Poor, damaged or missing labelling so that sample identity could not be verified with 100% certainty	Sample was identifiable but poorly labelled and/or received without a completed Chain of Custody Form	Sample was identifiable and accompanied by a Chain of Custody Form but some details specified in this Standard were missing	Sample was clearly identifiable and accompanied by a completed Chain of Custody Form in accordance with the specifications of this Standard
Sample Condition (Chlorophyll *a* only) *(subsection 6.2.2)*	Sample dispatched frozen arrived at laboratory	Sample dispatched frozen arrived frozen with some signs of thawing OR		Sample dispatched fresh and arrived at a temperature < 10°C

	completely thawed and with a temperature > 10°C	Sample dispatched fresh arrived at a temperature > 10°C		but unfrozen and free of ice crystals OR Sample dispatched frozen and arrived frozen with no signs of any thawing
Sample Processing Timeframe **(Chlorophyll *a* only)** *(subsection 6.3)*	Frozen sample processed more than 4 months after collection	Frozen sample processed between 3 and 4 months after collection	Frozen sample processed within 3 months of collection	Sample processed fresh within 24 hours of sample collection, OR Sample frozen in a freezer at -20°C, or lower and processed within 3 months
Sample Processing Timeframe **(AFDM only)** *(subsection 6.3)*	Frozen sample processed more than 2 years after collection	Frozen sample processed between 12 months and 2 years after collection	Frozen sample processed within 12 months of collection	Sample processed fresh within 24 hours of sample collection, OR Sample frozen in a freezer at -20°C, or lower and processed within 12 months
Analytical Method *(subsections 6.3.2 and 6.3.3)*	The laboratory report states that the method used is different or modified from that specified in this Standard but the modification is not documented on the laboratory report	The method is modified from that specified in this Standard and this modification is documented on the laboratory report. For example: • the subsample was based on only a single aliquot at the filtration stage, OR	Sample was analysed in accordance with the method specified in this Standard but the detection limit was not met	Sample was analysed in accordance with the method and detection limit specified in this Standard for the periphyton variable in question

		• for chlorophyll *a*, the sample was analysed using acetone as the extractant, OR • for AFDM, the subsample was analysed using a temperature(s) different from those specified under this Standard		
Measurement Units and Resolution *(subsection 6.3)*			Sample analysed in accordance with the method specified in this Standard but results were not reported at the resolution required	The measurement was reported as milligrams (mg) chlorophyll *a* or grams (g) AFDM per sample, to at least one decimal place, and was converted to mg or g per unit area of substrate sampled (in m^2) for chlorophyll *a* and AFDM, respectively.
Total Score				

7.2.1.2 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 5.3.1 and 6.2, and Annex C), and
- laboratory report (subsection 6.4).

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

Field measurements

The initial quality code for streambed periphyton cover data and any other supporting field measurements (e.g. conductivity) 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 6.2 (time, temperature, age and condition of samples on arrival at the laboratory) and subsection 6.3.5 (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 by the laboratory, to assign the final quality code to the measurement value. Measurements should ideally be checked within two to four weeks of receipt from the laboratory to enable sample re-testing if necessary.

Note: As noted in subsection 6.4.1, some sample degradation will occur and impact subsequent chlorophyll a *measurements.*

7.2.1.3 Batch Coding

Quality coding in batches is recommended. The raw 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.

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

7.2.1.4 Data That Do Not Meet The Standard

Any measurement data that do not meet this Standard shall be 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 achieved.

7.4 Data Preservation and Storage

The periphyton data and visit metadata shall be stored together using time-series management software, linked with a single 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.

7.4.1 Database Comments

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

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, this method is recommended.

7.4.2 Data Files

Any data that has been edited or adjusted shall be retained separate from the raw 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 streambed periphyton cover measurement value due to errors associated with its calculation from point observation data
- correction of a laboratory AFDM measurement value following re-analysis of the periphyton sample, and
- Averaging replicate measurement values to report a single final value.

7.5 Data Archiving

The archiving procedures, policies and systems shall consider:

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

7.7 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.

7.7.1 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 being 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.

7.7.2 Minimum Audit Report Requirements

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

- site and deployment metadata details, including catchment (if applicable) and site details
- comments and quality coding attached to the records
- data tabulations, and
- data plots.

7.7.3 Catchment and Site 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 stream and catchment
- identify other periphyton 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 periphyton variable record (measurement), identify:

  – the date and time of record collection
  – the site name and number
  – map reference
  – altitude

– sample collection method details, and
– laboratory analytical method details.

7.7.4 Comments and Quality Coding

The following shall be included in the audit report:

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

7.7.5 Other Requirements

7.7.5.1 Outputs

Recommended 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.

7.7.5.2 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.

Periphyton Cover and Sample Collection Field Record Form

Site Name: _____ Site ID: _____ Downstream GPS: E _____ N _____

Date: _____ Time (NZST): _____ Reach Length and Shade: _____ m ○ Full ○ Partial ○ None

Field Personnel: _____ Stream Flow Conditions: ○ Low ○ Moderate ○ High

Weather Conditions: ○ Fine ○ Overcast ○ Drizzle ○ Rain Digital Photos ○ Y ○ N Bank Entry: ○ TLB ○ TRB

PART A: PERIPHYTON COVER

	Transect 1					Transect 2					Transect 3					Transect 4				
View	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Start at most downstream transect; enter visual assessment of periphyton cover (to nearest 1-5%) on exposed surfaces of the streambed within the view.																				
No algae																				
Film																				
Sludge																				
Cyanobacteria mats																				
Other mats																				
Didymo mats																				
Green filaments																				
Other filaments																				
Other observations																				
PART B: SAMPLE COLLECTION *Tick at sampling points*																				

Substrate composition information

Substrate type	Transect 1	Transect 2	Transect 3	Transect 4
Bedrock				
Boulder				
Large cobble				
Small cobble				
Gravel				
Sand				
Silt				

PART C: WATER QUALITY MEASUREMENTS *(if collected)*

Conductivity: _____ µS/cm at 25°C **Meter:** _____

Other water quality measurements: _____

_____

Comments: *(e.g. scums, new hazards, changes to riparian vegetation, etc.)* _____

_____

_____

_____

Annex C – Example Chain of Custody Form

Chain of Custody Form for Periphyton Samples

Client Name _____ **Client Contact** _____

Client Reference _____ **Email*** _____

Quote/Order No. _____ ** For return of CoC*

Client to complete

Sample Dispatch Date _____ Time (NZST) _____

Name _____ Signature _____

Additional Notes _____

_____

_____

Laboratory to complete

Sample Arrival Date _____ Time (NZST) _____

Name _____ Signature _____

Sample Condition ○ Fresh ○ Frozen* ○ Damaged/leaking*

Temperature (if supplied fresh) _____ °C (measured inside chillibin)

*Comments *(e.g. sample shows some signs of thawing, label damaged/illegible)* _____

_____

_____

Job. No. _____

Sample Details *(Client to complete)*

Dispatched ○ Fresh ○ Frozen _____

Samples dispatched _____

Sample No./ID	Sample Site Name & Code	Collection Date	Sample Collector	No. of Containers	Total sample area (m^2)	Lab no.
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Annex D – Conversion of Periphyton Cover to Chlorophyll *a*

The following is a suggested procedure for substituting chlorophyll *a* derived from periphyton cover estimates for laboratory-measured chlorophyll *a* from samples. The procedure has been developed and validated using field data from the Wellington region (Greenfield, 2016).

The method relies on field observations that have shown that when the total cover on the streambed of chlorophyll *a*-rich periphyton cover categories (i.e. all categories except 'no algae' and 'films') is < 5%, there is a high probability that estimates of chlorophyll *a* from periphyton cover (see below) will be within 10 mg/m^2 of chlorophyll *a* determined from samples.

The method requires the following steps in the field:

1. Carry out rapid periphyton cover estimates, using the periphyton viewer, to identify whether chlorophyll *a*-rich cover categories make up > 5%, on average, of cover on the streambed (or, conversely, 'no algae' and 'film' categories make up ≥ 95%). This rapid assessment can be based on an initial instream cover assessment at five points across a single transect.

   Note: An average of 5% cover on the streambed over all 20 views is equivalent to 100% cover in one of the 20 views. Therefore, over five views, 25% cover in a single view is equivalent to 5% on average and more than 25% cover in one view indicates that that threshold is exceeded..

2. If the combined average cover of 'no algae' and 'film' categories is ≥ 95% in the first five views, then continue to carry out a full instream cover assessment (e.g. 20 views across four transects).

3. If the combined average cover of 'no algae' and 'film' categories in the first five views is < 95% (i.e. the summed percentage cover by chlorophyll *a*-rich cover categories exceeds 25%), then the visual assessment can cease and a periphyton sample should be collected for chlorophyll *a* measurement.

   Note: At step 2, if subsequent views after the quick assessment indicate an average of > 5% cover on the streambed by chlorophyll a-rich cover categories, then the visual assessment can be stopped at any time and replaced by sample collection.

4. Visual assessment results in the case where the 20 views return ≥ 95% combined cover of 'no algae' and 'film' categories are then used to derive chlorophyll *a* using conversion factors, as described below.

Conversion factors are chlorophyll *a* equivalents for 100% cover of the streambed by a single cover category. Conversion factors have been derived empirically for different regions in New Zealand and vary between regions. Two examples from Canterbury are shown in Table D1.

The second set of conversion factors in Table D1 had acceptable performance when applied to data from rivers in the Wellington region (Greenfield, 2016). However, other regions may require development of different conversion factors.

Derived chlorophyll *a* (mg/m^2) is calculated as:

((%cover category 1)/100 * conversion factor for category 1) +
((%cover category 2)/100 * conversion factor for category 2) +
((%cover category 3)/100 * conversion factor for category 3) +
(etc. for all categories observed, up to a total of 100%)

Table D1 – Examples of conversion factors for deriving chlorophyll *a* from periphyton cover estimates

Data used to derive factors	**Three Canterbury rivers**	**24 Canterbury rivers**
	Kilroy et al. (2013)	Kilroy et al. (2017)
No algae	1	0.3
Film	9	6.8
Sludge	72	27
Cyanobacterial mats	599	335
Other mats	118	147
Didymo	NA*	100
Green filaments	404	340
Other filaments	517	305

* No conversion factor is provided for didymo because none was encountered in the surveys

Annex E – Alignment of Periphyton Categories

Different systems have been used in the past for subdividing periphyton cover categories when visually assessing periphyton cover on the streambed. The simplest system was RAM-1 in Biggs and Kilroy (2000), which records only cover by green/brown filaments > 3 mm long to assess compliance with the guideline for recreational values in Biggs (2000). Other types of periphyton were not included. More comprehensive cover estimates were developed recognising the value of obtaining an overall picture of periphyton cover. However, these have evolved over time in light of experience in using the methods and the appearance of nuisance algae (e.g. didymo, *Microcoleus*) that were not a problem in New Zealand in 2000. In addition, different regions can have different algal assemblages, which may need to be recorded using region-specific descriptions.

Below, categories of two of the earlier methods are aligned with the categories used in this Standard. Others may be added.

Method reference	Category	Description		Equivalent in this NEMS	Comments
Biggs and Kilroy (2000) RAM-2	Thin mat/film	< 0.5 mm	Green	Green film	NB. "No algae" not recorded
			light brown	Brown film	
			black/dark brown	Black film	
	Medium mat	0.5 - 3 mm	Green	Mixed algal mats	
	Thick mat	> 3 mm	light brown	Sludge *or* Mixed algal mats	May not be possible to separate
			black/dark brown	Cyanobacteria	May also include "other mats"
			green/light brown	Sludge *or* Thick mats	May not be possible to separate
	Filaments, short	< 2 cm	Green	Green filaments	
			brown/reddish	Brown slimy filaments	
	Filaments, long	> 2 cm	Green	Long filaments	
			brown/reddish	Brown slimy filaments *or* Coarse filaments	Could also include long filaments

Note: The Biggs and Kilroy (2000) RAM-2 method was first used in the Stream Health Monitoring and Assessment Kit method (SHMAK; Biggs et al.*, 1998). Neither cyanobacteria nor didymo were recognised problems at that time. Note that no category for No algae or No cover was included in the RAM-2 method.*

Kilroy *et al.* (2008)	No cover	(clean stones)		No algae	
	Thin film	(green or brown colour, slimy texture)		Thin film	
	Loose "sludge"	(usually brown)		Sludge	First use of the "sludge" category

Cohesive mats	(usually brown/black, don't fall apart when handled)	Mixed algal mats	
Slimy, fragile filaments	(usually bright green but can be brown or dark coloured)	Green slimy filaments	May also include "brown slimy filaments"
Tough, coarse filaments	(usually green or brown)	Coarse filaments	

Note: This set of categories was developed specifically for use in the Manawatu-Whanganui region, in consultation with Horizons Regional Council. Cyanobacteria mats were not recognised as a problem at that time.

Annex F – Chlorophyll *a* Analytical Method

Overview

The method involves the following steps:

1. Concentrate a subsample of homogenised periphyton on a glass-fibre filter.
2. Extract the chlorophyll *a* in a solution of 90% boiling ethanol (i.e. 78°C. Boiling increases extraction efficiency and helps to "fix" the chlorophyll by destroying enzymes, thus making the chlorophyll relatively stable when in storage).
3. Leave to complete extraction in a refrigerator overnight.
4. Centrifuge to remove particulates from the solution.
5. Read background turbidity and pigment concentration on a spectrophotometer.
6. Acidify sample to convert the chlorophyll *a* to phaeopigments and re-read absorbances.
7. Correct for the presence of turbidity and phaeopigments.
8. Calculate chlorophyll *a* concentration by multiplying by an absorbance coefficient.

Note:

The absorbance peak for chlorophyll a is very narrow and varies slightly among spectrophotometers, so it is important that you locate the wavelength of the peak exactly by scanning absorbance of a standard solution. This is done as follows:

- *Make up a dilute standard solution of chlorophyll a in 90% ethanol (concentrated chlorophyll is available from some laboratory supply companies) and bring to the boil at 78°C for 5 minutes, and*
- *Cool the standard to room temperature and then measure the absorbance of the standard at wavelengths ranging from 660 to 668 nm. This will enable you to determine where the peak in absorbance is for your spectrophotometer. This will normally lie between 663 and 665 nm. Use this waveband for all future measurements on your spectrophotometer.*

Equipment

- Laboratory worksheets
- Ethanol (e.g. absolute, 95% or 96% ex-stock) diluted to 90% with distilled water
- Glass fibre filters, 47 mm Whatman GF/C
- Polythene centrifuge tubes, stoppered, numbered and stored in order in racks
- Forceps/tweezers
- Vacuum filtration apparatus
- Pipettes set to 5 mL and 0.1 mL (the 5 mL pipette needs to have a wide aperture ~ 2 mm diameter)
- Squirt bottle with distilled water

- 0.30 molar HCl
- Water bath, and
- Spectrophotometer.

Subsample filtering

- Prepare centrifuge tubes (e.g. 10 or 15 mL) by numbering each in order in racks.
- Place a second empty rack on ice in a chilly bin or fridge, or otherwise in cool conditions out of the light. Tubes with filtered subsamples will be transferred to this bin during the filtering process.
- Set up filtering apparatus.
- Place fresh filters in each filtering chamber.
- Apply suction pressure (be careful not to have a high pressure as this will rupture cells releasing the chloroplasts; < 10 mm Hg vacuum is recommended).
- The filtered subsample comprises at least two (preferably at least three) smaller aliquots of the blended sample, which are pooled. To take these aliquots, shake the bottle of blended sample and withdraw 5 mL with an automatic pipette from half-way down the solution while the liquid is still agitated. Release this solution into the filtering chamber. Repeat this twice more to give the full subsample (i.e. the total subsample volume = 15 mL) (see QA and Quality Control recommendations at the end of this Annex).
- If there is not an obvious colouring from periphyton on the filter, then you should filter more aliquots.
- Check for any fragments of leaves, mosses, invertebrates etc. on the filter paper and remove these with forceps.
- Record the volume of subsample (i.e. number of 5 mL aliquots used).
- Rinse the pipette by sucking up and discarding some clean water.
- Remove the filter from the filtering apparatus, fold in half, loosely roll up and place in its numbered tube and place in a rack in the chilly bin or other cool location.
- After each batch of samples (e.g. 10 samples) add 5 mL or 10 mL of chilled 90% ethanol (depending on sample size) to the tubes in the chilly bin. Make sure the filters are completely covered with the ethanol and the tube is firmly sealed.

Notes:

- *Always check that the intake to the pipette has not been blocked, for example, by clumps of algae or a leaf fragment.*
- *If the aliquots are taking a very long time to filter for each subsample, you probably need to dilute your sample or take a smaller volume aliquot (e.g. 2 mL). Ensure that you record the degree of dilution and aliquot volume so that this can be used in the calculations of chlorophyll* a *concentration.*

- *Remember to have sufficient sample solution for multiple analyses as required (particulate N and P content, species analysis, repeat analyses, etc.).*
- *Throughout the filtering process, periodically check that the centrifuge tube numbers you use correspond to the numbers and the sample identifiers marked on the data sheets.*

Extraction and Analysis

- Pre-heat the water bath to 78°C (boiling point of ethanol). Immerse the racks of tubes in the bath for exactly five minutes. (Make sure the tubes are held firmly in the racks.) Loosen the tops to prevent them popping off as the ethanol boils. But leave the tops on, otherwise you will lose ethanol through evaporation.
- Place the racks in the refrigerator overnight.

Notes:

- *At this stage, if any of the chlorophyll seems to be really concentrated (i.e. a very deep green), then dilute the extractant with 90% ethanol. The aim is to keep the absorbance readings to less than 1.000. Readings higher than this indicate concentrations that may no longer be linearly proportional to absorbance.*
- *To dilute, first add an extra 5 mL of 90% ethanol to the tube. Push down the stopper firmly and shake well. Note on the data sheet that the volume of extractant is 10 mL (instead of the usual 5 mL). If the solution still looks very concentrated, centrifuge the solution (as described above), then withdraw 5 mL from the tube, add another 5 mL of 90% ethanol and shake well. Note on the data sheet that the (effective) volume of extractant is now 20 mL.*

Absorbance Readings

Equipment

- Spectrophotometer, including cuvettes and printout paper
- 90% ethanol for washing out cuvettes and for blank solutions
- Pipettes set to 4 mL and 0.1 mL
- Tissues, and
- 0.30 molar hydrochloric acid (6.25 mL concentrated HCl made up to 200 mL with distilled water.

Procedure

The following steps are just one way to approach this analysis. Laboratories may follow modifications to this procedure provided that the critical steps are included (i.e. centrifugation to clear the samples, the first spectrophotometer reading is made on an unacidified sample, acidification to achieve a HCl concentration in the samples of about 0.008 M (Parker et al., 2016), and the second spectrophotometer reading is made on the acidified sample).

- Switch on the spectrophotometer; wait for initialization and for the machine to completely warm up (warm-up time will vary among brands).

- Set the wavelengths at the wavelength peak for chlorophyll *a* for your spectrophotometer and 750 nm.
- Ensure that the outside surfaces of your cuvette are clean and free of drips and dirt. Take blank readings using 90% ethanol to check this.
- Using a glass rod, or metal forceps, push the filter papers as far as possible to the bottom of the centrifuge tubes, and re-close firmly.
- Centrifuge at a speed of 6000 rpm for 10 minutes.
- Take the first centrifuged sample and pipette out 4 mL into the cuvette.
- Read absorbances at 665 and 750 nm.
- Remove the cuvette, inject 0.1 mL of 0.3 M HCl in the cuvette, place a cap on the cuvette and invert to mix. Wipe the side of the cuvette again with a clean tissue.
- Place the cuvette back into the spectrophotometer and re-read absorbances at 665 and 750 nm, leaving a 30-second delay.
- Remove the cuvette, discard the acidified sample and rinse the cuvette thoroughly with 90% ethanol. Invert and tap dry on a clean tissue.

Note:

- *Always make sure that the spectrophotometer is adequately warmed up and has stabilised before analyses. This may take up to an hour for some machines.*
- *Always use the same cuvette for each sample batch (or the same batch of disposable cuvettes), and always use it facing the same way (e.g. always place it in the compartment with the "L" facing to the left.) Make sure that the outside of the cuvette is clean and dry before each reading.*
- *Always hold the cuvette by the frosted side panels.*
- *Acidification occurs within a few seconds but ensure that there has been a delay of at least 30 seconds before re-reading to ensure that acidification is complete before taking the next reading.*
- *Make sure you clean the cuvette thoroughly after each sample has been acidified to avoid contaminating the next sample with an acidic solution. Use of disposable cuvettes avoids this issue.*
- *Be careful not to over-acidify the extract because this may cause the development of products that interfere with the phaeopigment absorbance peak.*
- *Always close the sliding door over the cuvette compartment before taking a reading.*
- *The optimal band pass width for the spectrophotometer is 1–2 nm, with a slit width of ~ 0.2 nm.*

Calculations

Chlorophyll *a* (mg per sample) =

[(absorbance 665 before – absorbance 665 after) x 28.66 x sample vol. x extractant vol.] / (filtered subsample volume)

where:

- absorbance 665 before and absorbance 665 after are the absorbance readings at a wavelength of 665 nm before and after acidification, respectively (having already subtracted the respective turbidity blanks read at 750 nm)
- 28.66 is the absorption coefficient for chlorophyll *a* as defined by Sartory and Grobbelaar (1984) (based on the specific absorption coefficient for chlorophyll in ethanol of 83.4 g/L/cm and an acid ratio for chlorophyll *a* in ethanol of 1.72; Sartory, 1982), and
- all volumes are in litres.

Phaeopigment concentrations may be calculated as follows:

Phaeopigments (mg per sample) =

[(1.72 x absorb. 665 after) – absorb. 665 before] x 28.66 x sample vol. x extractant vol.] / (filtered subsample volume)

where all volumes are in litres.

The chlorophyll or phaeopigment concentration needs to be normalised for the area from which the sample was collected. For example:

CHLA (mg/m^2) = Chlorophyll *a* (mg/sample)/area of sample (m^2).

Ensure that you have converted your sampling area from cm^2 to m^2 for this final calculation.

QA and quality control

The following notes and suggestions are modified from Biggs and Kilroy (2000) and apply to periphyton samples using the method specified in this Standard.

Sources of error

Errors in chlorophyll *a* analysis can occur during both subsampling and spectrophotometric analysis.

Variation associated with subsampling is generally < 10% (as % coefficient of variation) but may be as high as 25% for samples of communities that are difficult to break up during blending (Biggs, 1987). Higher levels of subsampling precision are expected with diatom-dominated communities than with filamentous communities (particularly mat-forming cyanophytes). Error can be minimised by paying attention to the following:

- Ensure that subsamples concentrated onto filters are composed of at least two (preferably at least three) 5 mL aliquots.
- Shake the blended sample thoroughly to ensure that a homogeneous mixture can be subsampled from approximately the middle of the container.
- Do not swirl the container as this distributes the heavier cells/filaments to the perimeter of the container.
- Ensure that 5 mL aliquots are accurate by:
    - Calibrating the pipette prior to starting each batch of samples, and

- Using the correct pipetting technique for withdrawing and releasing samples (check against pipette manufacturers' instructions).

During the filtering process:

- Ensure that numbers of aliquots are recorded accurately.
- Establish a systematic routine for the filtering process to avoid getting sample numbers mixed up. Do not attempt to carry out the subsampling/filtering procedure too fast.
- Ensure that the sample container labels are clearly readable on the container side and lid (but never on the lid only).
- Arrange bottles in order on the lab bench, and in the same order as they are listed on the laboratory bench sheet.
- Keep all samples as cool as possible and in the dark during filtering, including holding filters with subsamples in a chilly bin on ice, or in a fridge, as the filtration process proceeds.

Error associated with spectrophotometric analysis should, in most cases, be small relative to variability associated with the patchy distribution of mats on the streambed (i.e. sampling error). Error can be minimised by:

- Using a calibrated dispenser to ensure consistent and accurate aliquots of 90% ethanol.
- Allowing chlorophyll *a* to extract in boiling 90% ethanol for exactly 5 minutes and taking steps to prevent evaporation from the tubes (e.g. holding the loosened lids in place by placing a cover over all of the tubes in the rack).
- Allowing the spectrophotometer to warm up properly (as recommended/required by the manufacturer).
- Locating the correct the peak in absorbance for chlorophyll *a* on your spectrophotometer. Not doing this can introduce very large errors because the peak is very narrow.
- Allowing sufficient time for acidification.
- Double checking all calculations for dilutions.

For overall errors associated with subsampling and analyses, Biggs (1987) reported mean co-efficients of variation of 10.6% for chlorophyll *a* based on 258 sets of analyses of three subsamples. The samples covered a wide range of biomass and community types including diatoms, filamentous green algae and cyanobacteria. Ethanol dissolves ink from marker pens, so be sure that any drips created by pouring the chlorophyll extract from the centrifuge tube to the cuvette don't wash the identifying mark off the outside of the tube. If this does happen, then ensure that the drip with the associated ink doesn't land in the cuvette. Wipe the outside of the tube immediately and re-apply the identifier.

Formal quality control procedures

The following steps should be included in a formal QA and quality control system for chlorophyll *a* analysis:

- Check the location of the chlorophyll absorbance peak on the spectrophotometer using a pure chlorophyll *a* in 90% ethanol solution at least twice per year.
- Check the calibration, and volume setting, on the automatic pipette used for subsampling before each batch of analyses by weighing subsamples of distilled water on a precision balance.
- All field sample labels should be checked and signed off as being clearly readable.
- All samples should be registered in a properly prepared and maintained electronic or hard copy register as they arrive in the laboratory. Information should include unique sample identifiers (this would normally be a site + replicate sample number), date of collection, date of receipt in laboratory, method of preservation, analysis required, person who collected sample, person responsible for the job, and job number.
- Check the transcribing of sample numbers/labels onto the laboratory bench sheet and ensure that the samples on the bench are in the same order as on the laboratory bench sheet. Prepare data sheets before you begin the analyses. Enter each site/sample number in a logical order (e.g. sites in upstream to downstream sequence).
- Prepare racks of numbered centrifuge tubes for extraction of chlorophyll *a* and enter these numbers onto the data sheets. Start numbering from 1 for each sampling run and ensure that the tubes are arranged in their racks in the correct order.
- Results, calculations, and transposition of data should all be checked and signed off by someone other than the analyst. All errors should be logged in a laboratory registry of errors. Apart from helping ensure the quality of the analysis, this registry also helps isolate problem areas in the analytical process.
- For analyses where the results are likely to be used for a resource consent hearing or the Environment Court, then three subsamples should be analysed from each sample and the results averaged. If the value for any one subsample is greater than 2 x the mean of the two nearest subsamples, then the outlier is considered to be a subsampling or analytical error and the result of the outlier should be discarded.
- The scientist responsible for analysing the data and reporting must check the results and sign them off as being acceptable. The balance of all samples should be retained (frozen) until the data has been approved in case re-analysis is required.

Note:

- *It is difficult to develop Quality Control charts to allow "benchmarking" of batch analyses for chlorophyll* a *as has been suggested for AFDM (see Annex H). This is because we are, as yet, uncertain of the stability of frozen chlorophyll samples or ethanol extracts if the subsamples are kept for more than a few months. Preserving samples for later analysis and benchmarking is an essential component of a Quality Control chart.*
- *A good quality assurance system in a laboratory is dependent on feedback. Feedback between:*
    - *laboratory staff and laboratory manager, regarding problems with samples, techniques etc., and*
    - *laboratory staff and scientists regarding expected capabilities, variability, and expected levels for the results, and required levels of precision, etc.*

- *Feedback helps to ensure:*
    - *the objectives of the project are being met*
    - *variability of results is reduced and thus confidence in the results is maintained, and*
    - *all participants in the analyses know what is expected of them in the QA system.*

Annex G – AFDM Analytical Method

Overview

The method involves drying a subsample to drive off all water, determining the dry weight, ashing the sample and then re-weighing. The difference between these two weights gives the ash-free dry mass (AFDM) value (sometimes called loss-on-ignition or ash-free dry weight).

Equipment and materials

- Data sheets
- Glass-fibre filters, 47 mm Whatman GF/C
- Crucibles or aluminium foil weighing dishes, with number/ID mark, set out on metal trays
- Forceps/tweezers
- Vacuum filtration apparatus
- Glass beakers, stoppered bottles (marked at 50 mL intervals)
- Blender
- Automatic pipettes set to 5 mL (with a wide aperture ~ 2 mm diameter)
- Squirt bottle with distilled water
- Desiccator
- Muffle furnace, drying oven, and
- Balance capable of measuring to 0.1 mg.

Procedure

Pre-ashing of glass-fibre filters

Note: It may be necessary to pre-ash the glass-fibre filter to ensure that no organic material in the filter adds to the initial dry weight of periphyton.

- Place one filter in each crucible/dish. Do not fold or tear.
- Pre-ash in the muffle furnace at 400°C for two hours.
- Transfer the crucibles/dishes and filters to a desiccator to cool. Allow expanded hot air to escape for a few seconds through the desiccator lid valve, then close the valve to create a vacuum seal. About 30 minutes is usually enough for cooling.
- Record the weight of each crucible + filter paper, foil dish + filter paper, or filter paper alone (if preferred) after they have cooled. Record the weight in milligrams (mg).
- Note that the weight of foil dishes does not change after heating to 400°C. Trials have shown that the change is typically ±0.1%, which is within the error of the balance readings (NIWA, unpublished data).

Note: Always handle the crucibles, foil dishes and filters with forceps/tweezers to avoid contamination with grease etc. from your hands.

- Replace the lid on the desiccator between each weighing. Once out in the open, the filter papers start to absorb moisture, which increases their "dry" weight.
- If using crucibles, allow them to cool fully (e.g. 1 hour) and weigh the crucibles at room temperature. If they are warm or hot, you get spuriously low values. Foil dishes cool down rapidly (e.g. 15 mins).

Subsample filtering

- Set up the filtering apparatus.
- The filtered subsample comprises at least two (and preferably at least three) smaller aliquots of the blended sample, which are pooled. To take these aliquots, shake the bottle of blended sample and withdraw 5 mL with an automatic pipette from half-way down the solution while the liquid is still moving. Release this solution into the filtering chamber. Repeat this twice more to give the full subsample (i.e. the total subsample volume = 15 mL).
- If there isn't a significant accumulation of periphyton on the filter then you should filter more aliquots.
- Check for any fragments of leaves, mosses, invertebrates etc. on the filter and remove these with forceps.
- Record the volume of subsample (i.e. number of 5 mL aliquots used).
- Apply suction pressure to the filtering apparatus.
- Rinse the pipette by sucking up and discarding some distilled water.
- If you are subsampling for both AFDM and chlorophyll *a*, get into a routine of always doing them in the same order.
- Replace the filter in its crucible or foil dish when filtering is complete.

Note:

- *If the aliquots are taking a very long time to filter for each subsample, you will probably need to dilute your sample or take a smaller volume aliquot (e.g. 2 ml). Ensure that you record the degree of dilution and/or smaller aliquot volume so that this can be used in the calculations of AFDM concentration.*
- *Pipette tips can be widened slightly to overcome persistent blockage.*
- *Remember to have sufficient sample solution for multiple analyses as required (N and P content, species analysis, repeat analyses, etc.).*
- *Throughout the filtering process, periodically check that the crucible numbers you use correspond to the numbers and the sample identifiers marked on the data sheets.*

Ashing of samples

- Dry the subsamples (crucibles or foil dishes + filters with filtered material) for 24 hours at 105 ± 2°C.

- Weigh each subsample (crucibles or foil dishes + filters with filtered material) in mg after cooling in a desiccator, as described above.
- Ash for 4 hours at 400°C, cool in the desiccator, and weigh (in mg) for the final time.

  Note: Subsamples will dry in less than 24 hours but must be dried for a minimum of 2 hours. Drying for between 2 and 20 hours is acceptable but drying for less than 20 hours will require more steps as checks are needed to ensure that a constant weight is maintained between two successive series of heating and cooling to room temperature.

Calculations

AFDM is typically reported as grams per square metre of streambed (g/m^2).

Ash-free dry mass (g per sample) =

[{(weight of crucible + filter + sample after drying) – (weight of crucible + filter + sample after ashing)} x sample volume] / (1000 *[volume of filtered subsample])

Dry mass (g per sample) =

[{(weight of crucible + filter + sample after drying) – (weight of crucible + filter after drying)} x sample volume] / (1000 * [volume of filtered subsample])

All weights are in mg and all volumes are in litres. Dividing by 1000 converts mg to grams. AFDM is typically reported as grams per square metre of streambed (g/m^2).

Finally, calculate the AFDM or DM in g/m^2 as:

AFDM or DM (g/sample) = AFDM or DM (g)/area of sample (m^2)

Additional variables may be calculated as follows:

% organic matter = (AFDM x 100) / (DM)

Autotrophic index (AI) = AFDM (in mg/m^2) / chlorophyll *a* (mg/m^2)

QA and Quality Control

The following notes and suggestions are modified from Biggs and Kilroy (2000) and apply to periphyton samples using the method specified in this Standard.

Sources of error

Errors in AFDM analysis can occur during both subsampling and the drying and ashing process.

Variation associated with subsampling is generally < 10% (as % coefficient of variation) but may be as high as 25% for samples of communities that are difficult to break up during blending (Biggs, 1987). Higher levels of subsampling precision are expected with diatom-dominated communities than with filamentous communities (particularly mat-forming cyanophytes). Error can be minimised by:

- Ensuring that subsamples concentrated onto filters are composed of at least two (and preferably at least three) 5 mL aliquots.
- Including two blank filters in every batch of samples.

- Shaking the blended sample thoroughly to ensure that a homogeneous mixture can be subsampled from approximately the middle of the container.
- Not swirling the container as this distributes the heavier cells/filaments to the perimeter of the container.
- Ensuring that 5 mL aliquots are accurate by:
    - Calibrating the pipette at regular intervals (e.g. weekly, where analyses are routinely undertaken)
    - Using the correct pipetting technique for withdrawing and releasing samples (check against pipette manufacturers' instructions).

During the filtering process:

- Ensure that numbers of aliquots are recorded accurately.
- Establish a systematic routine for the filtering process to avoid getting sample numbers mixed up. Do not attempt to carry out the subsampling/filtering procedure too fast.
- Ensure that the sample container labels are clearly readable on the container side and lid (but never on the lid only).
- Arrange bottles in order on the lab bench, and in the same order as they are listed on the laboratory bench sheet.

During drying and ashing:

- Account for dehydration of the inorganic fraction, where appropriate. The ashing temperature in this method has been set at a relatively low level of 400°C to help reduce the effect of dehydration of the clay fraction in the sample. However, if clays are a large fraction of the sample and precise results are required, then it is recommended that the mineral fraction is re-hydrated after ashing (but before final weighing). This is done by cooling the sample after ashing, adding a few millilitres of water to the sample, allowing it to stand overnight, drying again at 105°C for 4 hours, cooling in a desiccator and then taking the final weight.
- Re-hydration of the organic component can occur after the initial drying step if the sample is not cooled in a desiccator. This can result in positive biases to the results. Ensure that the silica gel in the desiccator is fully dry.
- Weighing errors can occur. This is a particular problem when biomass is very low. Indeed, AFDM is not nearly as accurate as chlorophyll *a* as a measure of biomass at low levels. The magnitude of error introduced may be illustrated by taking the ratio of AFDM:chlorophyll *a* (both in mg/m^2, which gives the Autotrophic Index [AI] – see above) and making the assumption that the chlorophyll measurement is a more sensitive and accurate analysis. Healthy communities in unpolluted streams normally have an AI of 100–200. However, for healthy low-biomass communities dominated by, say, diatoms, the error associated with weighing will usually result in spurious AI values of 0.1–2,000. Occasionally there appear to be net gains in biomass during ashing. Therefore, if you are attempting to discriminate differences among sites or treatments in an experiment where biomass levels are all low, then use chlorophyll *a* as the measure of biomass. Autotrophic indices should not be calculated for samples where AFDM < 2 g/m^2.

- For overall errors associated with subsampling and analyses, Biggs (1987) reported mean coefficients of variation of 8.9% for AFDM based on 258 sets of analyses of three subsamples for each variable. The samples covered a wide range of biomass and community types including diatoms, filamentous green algae and cyanobacteria.

Formal quality control procedures

The following steps should be included in formal QA and Quality Control procedures for AFDM:

- Calibrate balances, ovens and muffle furnaces (the latter two for temperature) at least annually.
- The calibration and volume settings on the automatic pipette used for subsampling should be checked before each batch of analyses by weighing subsamples of distilled water on a precision balance.
- All sample labels should be checked and signed off as being clearly readable.
- All samples should be registered in a properly prepared and maintained electronic or hard copy register as they arrive in the laboratory. Information should include unique sample identifiers (this would normally be a site + replicate sample number), date of collection, date of receipt in laboratory, method of preservation, analysis required, person who collected sample, person responsible for the job, and job number.
- Check the transcribing of sample numbers/labels onto the laboratory bench sheet and ensure that the samples on the bench are in the same order as on the laboratory bench sheet. This checking should be done by another analyst and signed off.
- Results, calculations, and transposition of data should all be checked and signed off by someone other than the analyst. All errors should be logged in a laboratory registry of errors. Apart from helping ensure the quality of the analysis, this registry also helps isolate problem areas in the analytical process.