National Environmental Monitoring Standards

Macroinvertebrates

Collection and Processing of Macroinvertebrate Samples from 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 http://www.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.

This standard has been prepared by a core working group comprising Juliet Milne (lead technical writer – NIWA), Alastair Suren (Bay of Plenty Regional Council), Alicia Williams (Waikato Regional Council), Brian Smith (NIWA), Carol Nicholson (formerly Northland Regional Council), Duncan Gray (Environment Canterbury) and Evan Harrison (Greater Wellington Regional Council). Glenn Ellery (Bay of Plenty Regional Council) provided oversight as the NEMS Steering Group representative.

The working group gratefully acknowledge:

- members of the 'macroinvertebrate subgroup' of the regional sector's Surface Water Integrated Management (SWIM) Special Interest Group (SIG) who prepared a paper for the NEMS Steering Group in early 2019 that set the scene for the development of this Standard: Michael Pingram (Waikato Regional Council), Alton Perrie (Greater Wellington Regional Council), Duncan Gray, Darin Sutherland (Taranaki Regional Council), Dean Olsen (formerly Otago Regional Council), Roger Hodson (Environment Southland) and Alastair Suren
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- Northland Regional Council
- Otago Regional Council
- Ryder Environmental Ltd
- Stark Environmental Ltd
- Taranaki Regional Council
- Tasman District Council
- West Coast Regional Council
- Waikato Regional Council

Review

This document will be assessed for revision by the NEMS Steering Group within one year of its release and thereafter approximately once every two years. Further details on the review process can be found at 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:

- Detritus: Dead particulate organic matter in a river or stream, including leaves and dead organisms.
- %EPT$_{\text{-HA}}$ abundance: A biotic index of stream condition based on the percentage of individual macroinvertebrates present in a sample that belong to the orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies). These orders are known to be sensitive to pollution and/or habitat disturbance.

  Note: In the NEMS Macroinvertebrates*, the abundance of both hydroptilid caddisflies Oxyethira and Paroxyethira are excluded from calculations of %EPT abundance (as denoted by $_{\text{-HA}}$) because some common species within these genera are considered tolerant of pollution/habitat disturbance.*
- EPT$_{\text{-HA}}$ taxa richness: A biotic index of stream condition based on the total number of distinct Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) taxa present in a sample, often expressed as the percentage of EPT macroinvertebrate taxa present in a sample (%EPT taxa richness). These orders are known to be sensitive to pollution and/or habitat disturbance. Taxa are identified to the level listed in Annex H.

  Note: In the NEMS Macroinvertebrates*, both hydroptilid caddisflies Oxyethira and Paroxyethira are excluded from calculations of %EPT taxa richness because some common species within these genera are considered tolerant of pollution/habitat disturbance.*
- Elutriation: In the NEMS *Macroinvertebrates*, the process of separating macroinvertebrates from gravels and fine inorganic material collected in a macroinvertebrate sample. Usually performed in the field prior to sample preservation using a large bucket of water to suspend macroinvertebrates which are then tipped into a sieve, leaving the heavier inorganic material in the bucket. This process is repeated a few times until most of the fine smaller organic material has been collected, leaving coarser material and gravel behind. This remaining inorganic material is quickly scanned for any larger or heavy macroinvertebrates (e.g. snails).
- Fixed count: A method for processing macroinvertebrate samples. In the NEMS *Macroinvertebrates*, this involves removing a fixed-fraction subsample from the collected sample (a sufficient amount of subsample to identify at least 200 individuals). This is then followed by a scan of the remaining sample residue for any additional 'missed' taxa not present in the subsample (traditionally termed 'rare taxa' but better described as 'missed taxa').
- Full count: A method for processing macroinvertebrate samples where all individual macroinvertebrates present in a sample are identified and counted. Variations to the method include an option to perform a full count on a representative subsample.

- Genera: Plural for genus, which is a taxonomic category used in biological classification that ranks above species and below family.
- Hard-bottomed stream: A stream or river in which the bed substrate comprises at least 50% particles of 2 mm or greater in size (i.e. dominated by gravel, cobble, boulder and bedrock substrates). Riffle and run mesohabitats are common in these streams.
- Hess sampler: A device with a circular-shaped mesh net that is generally used for quantitative sampling of aquatic macroinvertebrates in wadeable (and typically hard-bottomed) streams and rivers. The NEMS *Macroinvertebrates* specifies a mesh size of 0.5 mm.
- Intermittent stream: A stream or river that ceases to flow for some period of time during the year, typically in very dry periods.
- Kick-net: A triangular or D-framed mesh hand net with a pole handle that is used to collect aquatic macroinvertebrates in a river or stream. The NEMS *Macroinvertebrates* specifies a mesh size of 0.5 mm.
- Macrophyte: A vascular aquatic plant growing in or near water. Typically classified in a stream as emergent (i.e. with upright portions above the water surface), submerged, or floating.
- Macroinvertebrate: An aquatic invertebrate (e.g. insect, snail, worm, crustacean) living in a stream or other waterbody that is retained by a 0.5 mm sieve.
- Macroinvertebrate Community Index (MCI): A biotic index used to determine stream or river health based on the presence (or absence) of different macroinvertebrate taxa. Originally developed for use in riffles of stony-bottomed wadeable streams it has since been modified for use in soft-bottom streams. Each taxon is assigned a value or score based on its tolerance to pollution or organic enrichment. These values are dependent on whether the sample was collected from a hard-bottomed (HB) or soft-bottomed (SB) stream and are used to calculate a single MCI value for a site (denoted MCI_{HB} and MCI_{SB}, respectively).
- Macroinvertebrate taxonomist: Individual with expertise/training in the identification of a particular group or groups of aquatic macroinvertebrates.
- Mesohabitat: Instream habitats (e.g. run, riffle, pool), typically a few metres to 10 metres in length.
- Microhabitat: A small instream habitat, such as a rock, leaf or twig.
- Missed (rare) taxa scan: A visual inspection by naked eye of the portion of the sample that was not sorted (sample residue) for any macroinvertebrate taxa not already recorded from the sorted residue. Applies to macroinvertebrate samples processed using the fixed-count method, as specified under the NEMS *Macroinvertebrates.* Missed taxa replaces the commonly described 'rare taxa' scan to avoid ambiguity with taxa assigned a conservation threat classification.
- Perennial stream: A stream or river that always flows in some reaches all year round (during years of normal rainfall).

- Pool: An area of slow-flowing or standing water with a smooth water surface; usually found where a stream or river widens and/or deepens.
- Quantitative Macroinvertebrate Community Index (QMCI): A quantitative variant of the MCI based on both the number and relative abundance of different taxa present in a macroinvertebrate sample. Different pollution or organic enrichment tolerance values are used for each taxon to calculate the QMCI depending on whether the sample was collected from a hard-bottomed (HB) or soft-bottomed (SB) stream. These values are denoted as $QMCI_{HB}$ and $QMCI_{SB}$, respectively. As for MCI, a single QMCI value is calculated for each site.
- Reach: A section of a stream or river selected for monitoring. In the NEMS *Macroinvertebrates*, a reach is between 20 and 100 m in length.
- 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 in size than that found in a run.
- Run: An area of stream or river intermediate in character between a riffle and a pool, with low to moderate water depth, slow to moderate water velocity, uniform to slightly variable current, and water surface smooth to rippled and unbroken.
- Sample processing: The preparation and sorting of a macroinvertebrate sample, followed by identification and enumeration of individual organisms in the sample. Sample processing methods include fixed count and full count.
- Sample residue: The unsorted (or 'unpicked') component of the macroinvertebrate sample that will undergo a scan for missed (rare) taxa after a representative subsample has been removed and processed.
- Semi-Quantitative Macroinvertebrate Community Index (SQMCI): A semi-quantitative variant of the MCI based on the relative abundance of different taxa present in a macroinvertebrate sample.
- Soft-bottomed stream: A stream or river in which the bed substrate comprises more than 50% sand/silt/mud/clay. These streams are typically low-gradient, slow-flowing and often dominated by macrophytes in unshaded reaches and woody debris in shady forested reaches.
- Sorted residue: A representative subsample from which all macroinvertebrates must be removed ('picked'). This residue often contains detritus and other material (e.g. gravel) and is retained for quality control purposes to assess sorting efficiency.
- Stream habitat: The place or environment where aquatic organisms live. Typically assessed by physical features such as substrate size and composition, water depth and velocity, bank stability, riparian vegetation and shade.
- Surber sampler: A device with a square-shaped mesh net that is generally used for quantitative sampling of aquatic macroinvertebrates in wadeable (and typically hard-bottomed) streams or rivers. The NEMS *Macroinvertebrates* specifies a mesh size of 0.5 mm.

- Stand-down period: In the NEMS *Macroinvertebrates,* the period of time after a high flow event before macroinvertebrate sample collection can proceed. Various flow magnitudes and stand-down periods exist; see the NEMS *Macroinvertebrates.*
- Taxa richness: The total number of macroinvertebrate types (taxa) present in a sample; the most common measure of biodiversity.
- Tolerance value: A value or score between 1 and 10 assigned to macroinvertebrate taxa (usually at the genus level, or higher) for the calculation of the Macroinvertebrate Community Index (MCI). The value relates to the tolerance or sensitivity of a particular taxon to organic enrichment (and often sedimentation), where 1 = very high tolerance to enrichment and 10 = very low tolerance (i.e. more sensitive).
- Unit effort: Applies in the NEMS *Macroinvertebrates* to a unit of sampling effort of approximately 0.1–0.15 m^2. Each sampling effort represents a single subsample that contributes to a composite macroinvertebrate sample.
- Visit metadata: Field observations and other information captured when visiting a sampling site (e.g. date, flow state, water colouration, and weather conditions).
- Wadeable (stream or stream reach): A river or stream reach in which someone can safely wade and reach down to the streambed at a sufficient number of suitable locations to collect a representative macroinvertebrate 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 *Safe collection of environmental data: Guidelines for safe working when undertaking environmental monitoring*
- NEMS *Discrete Water Quality – Part 2 (Rivers)*
- NEMS *Periphyton*
- NEMS *Water Level,* and
- NEMS *Open Channel Flow.*

About this Standard

Introduction

Monitoring of aquatic macroinvertebrate communities is carried out in rivers and streams across New Zealand for many purposes. While traditionally macroinvertebrate data have been used as an indicator of water quality 'condition', today they are used more as a fundamental measurement of aquatic ecosystem health by regional councils and other agencies. Monitoring benthic macroinvertebrates for this purpose is now mandatory under the National Policy Statement for Freshwater Management (NPS-FM).

Benthic macroinvertebrates are good indicators of ecosystem health. They are integral components of the stream's food web and are affected by physical, chemical and biological conditions of the stream, responding to changes in water quality, flow, stream habitat and invasive species. Unlike traditional water quality assessments, macroinvertebrates may show the impacts of habitat loss and the cumulative impacts of pollution and other stressors present within the upstream catchment.

Assembling a valuable long-term freshwater macroinvertebrate data record is underpinned by a consistent approach to sample collection, sample processing and data management. This is because information yielded from macroinvertebrate sampling is influenced by both sampling technique and effort, as well as the performance of laboratory staff who sort, identify and count the taxa present.

This Standard has been prepared principally for field technicians, programme managers and environmental scientists or consultants who collect, quality check or report on freshwater macroinvertebrate data. The primary focus is on data acquisition (sampling and processing), quality assurance (QA), and archiving associated with long-term or ongoing State of the Environment (SOE) monitoring programmes. The principal purpose of SOE macroinvertebrate monitoring in streams is to assess state (condition) and trends in ecosystem health through time.

Macroinvertebrate sampling is carried out by many agencies for purposes other than long-term monitoring for SOE reporting, such as investigating the potential effect of proposed activities in or adjacent to streams, monitoring the effects of existing consented activities (e.g. wastewater discharges, water abstraction), and characterising biodiversity. While sampling for these additional purposes is not addressed in detail in this document, some guidance is provided and the underlying principles of sample collection and processing are relevant. This Standard, therefore, provides an up-to-date normative reference for most macroinvertebrate sampling carried out in rivers and streams across New Zealand.

Objective

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

Scope

The Standard covers macroinvertebrate sampling in the freshwater reaches of rivers and streams (henceforth referred to as streams only). The focus is primarily on wadeable reaches of perennially and intermittently flowing streams but brief guidance is also provided on approaches to sampling deep, swifter (i.e. non-wadeable) streams.

This Standard focuses on long-term monitoring programmes (e.g. SOE monitoring). However, for completeness, brief guidance is also provided on macroinvertebrate monitoring to satisfy Assessment of Environmental Effects (AEE), resource consent compliance and biodiversity monitoring.

The Standard addresses:

- fieldwork preparation
- sampling point (stream reach) selection
- sampling equipment preparation and use, including decontamination
- field observations (visit metadata) and associated measurements
- sample collection methods
- sample handling, preservation, storage and transport
- laboratory processing, taxonomic resolution and reporting
- metric calculations
- quality assurance (QA) and quality control procedures
- data quality coding, and
- data archiving.

Macroinvertebrate samples are collected for a defined monitoring objective(s). This objective will influence the selection of sampling site(s), the timing and frequency of sampling, and the sample collection and processing methods. It is beyond the scope of this document to address 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 freshwater macroinvertebrate data record.

The following suite of macroinvertebrate metrics – or indices – are addressed in this Standard. Although other metrics exist, this suite represents the metrics typically calculated on data obtained on a routine and ongoing basis as part of long-term SOE programmes for perennial streams. Note that the selection of which metric(s) to use depends on the monitoring application. Generally, more than one metric should be used to evaluate stream health. Expert opinion may be required to inform selection and application of these and/or other suitable metrics, especially for biodiversity-related assessments. For example, existing Macroinvertebrate Community Index (MCI) tolerance values were developed for wadeable streams and, as such, many of the tolerance values may be of questionable value in larger rivers. It is also important to note that the MCI has not been calibrated or evaluated to assess the health of lake margins or wetlands, or waterways influenced by geothermal inputs.

Metric/index	Abbreviation
Taxa richness	
Macroinvertebrate Community Index - hard-bottomed streams - soft-bottomed streams	MCI MCI_{HB} MCI_{SB}
Semi-Quantitative Macroinvertebrate Community Index [1]	SQMCI
Quantitative Macroinvertebrate Community Index - hard-bottomed streams - soft-bottomed streams	QMCI $QMCI_{HB}$ $QMCI_{SB}$
Number of Ephemeroptera, Plecoptera and Trichoptera taxa	EPT_{-HA} taxa richness[2]
Percentage of Ephemeroptera, Plecoptera and Trichoptera taxa	$\%EPT_{-HA}$ taxa richness[2]
Percentage abundance of Ephemeroptera, Plecoptera and Trichoptera	$\%EPT_{-HA}$ abundance [2]
Macroinvertebrate Average Score Per Metric	ASPM

[1] This metric is referenced here due to its historic use by some monitoring agencies. However, this Standard does not recommend the use of the coded-abundance sample processing method on which the SQMCI is derived.

[2] -HA denotes the exclusion of the hydroptilid caddisflies *Oxyethira* and *Paroxyethira*

Relationship with Existing Guideline Documents

For nearly 20 years, *Protocols for sampling macroinvertebrates in wadeable streams* (Stark et al., 2001) has provided the foundation guidance for macroinvertebrate sampling in New Zealand. This Standard updates and supersedes the Stark et al. (2001) guidance and the subsequent MCI user guide (Stark and Maxted, 2007).

This Standard also:

- updates and supersedes initial recommendations for national SOE macroinvertebrate sampling 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, and
- takes into account the macroinvertebrate community metrics set out in the NPS-FM 2020 (New Zealand Government, 2020).

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 – Macroinvertebrates

Note: The following requirements apply to macroinvertebrate samples collected for State of the Environment-type monitoring purposes. Some, but not all, of the requirements can also be applied to macroinvertebrate samples collected for other purposes.

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

<table>
<tr><th colspan="3">Field measurements and sample collection</th></tr>
<tr><td>Certification
Section 1</td><td colspan="2">Macroinvertebrate samples and associated field measurements and 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.</td></tr>
<tr><td>Quality Assurance
Section 1.3</td><td colspan="2">As a minimum, macroinvertebrate sample collection and pre-treatment practices shall be checked in the field:
internally, by experienced personnel within the collection agency holding a training day prior to the start of each summer sampling season, and
externally, every three years using suitably qualified and experienced personnel from an external agency.</td></tr>
<tr><td rowspan="2">Equipment Specifications</td><td>Kick-net
Section 1.5.1</td><td>A D-framed hand or kick-net (D-net) or similar net (i.e. triangle net) with a mesh size of 0.5 mm, net length of at least 50 cm, and a width along its base of 30–40 cm shall be used for macroinvertebrate sampling.
Note: A net of narrower width may be necessary for sampling some stream reaches or mesohabitats.</td></tr>
<tr><td>Sample preservative
Section 1.6.2</td><td>Isopropyl alcohol (isopropanol) or ethanol shall be used to preserve macroinvertebrate samples.</td></tr>
<tr><td>Equipment Maintenance</td><td>Sampling nets
Section 1.5</td><td>All sampling nets shall be cleaned between sites and inspected for holes or damage before each use, with any holes repaired before use or a backup net used.</td></tr>
<tr><td rowspan="2">Site Location and Stationarity
Sections 2.1 & 2.2</td><td colspan="2">The site shall be a reach of stream with habitat characteristics representative of the broader stream. The length of the reach shall be about 20 times the average channel width, with a minimum length of 20 m and a maximum length of 100 m.</td></tr>
<tr><td colspan="2">The site location for sample collection shall be clearly identifiable and retained through time as long as possible.</td></tr>
<tr><td>Site Metadata
Section 2.3</td><td colspan="2">Site metadata, including site name, location, altitude, photographs, and access details, shall be recorded.</td></tr>
</table>

Site Visit Metadata	Field Record Form *Section 1.4*	A Field Record Form shall be completed indicating the location and date of macroinvertebrate sample collection, the sampling equipment used, the proportions of each stream mesohabitat sampled, and the environmental conditions (e.g. flow state) under which samples and any associated field measurements (e.g. periphyton cover) were collected.
	Photographs *Section 3.3.4*	Photographs of the site in both upstream and downstream directions shall be taken at least annually.
	Habitat assessment *Section 4.1*	A rapid or basic habitat assessment (at minimum) shall be performed at the time of macroinvertebrate sample collection targeting stream channel and bank characteristics, instream characteristics and riparian and catchment characteristics.
Time Records *Section 3.3.3*		The time of 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 these measurements.*
Sample Collection	Time of year *Section 3.2.1*	Macroinvertebrate sample collection shall be undertaken between the months of November and April inclusive.
	Antecedent flow conditions *Section 3.2.2*	Macroinvertebrate samples shall not be collected within 14 days following a stream flow that results in: • bed movement in a hard-bottomed stream, or • removal/scouring of macrophytes, woody debris or other stable mesohabitat in soft-bottomed streams, or • movement of fine bottom sediments (e.g. mud, pumice) greater than what is typical at baseflow. The exception is hard-bottomed streams in high rainfall or heavily urbanised catchments subject to frequent and significant bed disturbance; in these streams, macroinvertebrate samples shall not be collected within 7 days of a stream flow exceeding three times the median. *Note: Where information on substrate mobilisation is lacking, macroinvertebrate samples shall not be collected within 14 days following a stream flow exceeding three times the long-term median.*
	Stream habitats *Section 2.1.1*	Sampling shall target the most common wadeable mesohabitats (e.g. riffles, runs) within the sampling reach that are likely to support the greatest range of macroinvertebrates.

Sample Collection *cont.*	Sampling technique *Section 3*	Sample collection shall move progressively from downstream to upstream. *Hard-bottomed stream habitats* A kick-net shall be placed on the streambed facing directly into the direction of flow. The surface substrate in an area not exceeding 0.4 m from the mouth of the net shall be disturbed with the feet (and/or hands) to dislodge macroinvertebrates and detritus on and under the substrate. *Soft-bottomed stream habitats* A kick-net shall be placed into the stream directly into the direction of flow and a jabbing motion used to dislodge macroinvertebrates associated with macrophytes and bankside root substrate, followed by 2–3 cleaning sweeps to collect organisms in the water column. Woody debris shall be hand-brushed, either into the net (large logs) or into a bucket.
	Sampling effort *Section 3*	A single composite sample shall be collected comprising 4–8 unit efforts (subsamples), with effort split based on the proportional contribution of each mesohabitat in the sampling reach. Each unit effort shall be approximately 0.1–0.15 m^2 in area and the total sampling area shall be 0.6–0.9 m^2. *Note 1: Additional sampling effort may be required in some streams dominated by pumice substrate or soft sediment substrate that are devoid of macrophytes and woody debris, contributing to lower densities of invertebrates. Additional sampling effort shall seek to obtain at least 200 individuals but shall not exceed 10 minutes in duration.* *Note 2: Avoid sampling of mobile sediments as well as mud and other soft substrate within deeper anoxic layers.*
Sample Sorting and Preservation	Sorting *Section 3*	Large debris (e.g. stones, sticks and leaves) and, as far as practicable, fine sediment shall be rinsed and removed from the sample prior to transfer to the sample container. *Note: Elutriation may be needed where samples contain a large amount of fine gravel.*
		After sample transfer, the kick-net shall be inspected for macroinvertebrates embedded in the mesh; as far as practicable any found shall be removed and added to the sample container.
	Preservative *Section 3.3.7*	Preservative shall be added to the sample container to achieve a 70–80% concentration (allowing for the stream water already present), with an approximately 1 cm air gap left for safe transport.

Sample Traceability and Integrity	Labelling *Section 3.3.6*	Each macroinvertebrate sample shall be clearly and permanently labelled on the outside of the sample container and by placing a waterproof label inside the container with, as a minimum: • site name and number • sampling date • sampler initials, and • container number, if applicable. The site number shall be referenced on both the Field Record Form and the Chain of Custody Form.
	Chain of Custody Form *Section 3.5.1*	Macroinvertebrate samples shall be accompanied by a completed Chain of Custody Form that provides sample traceability from the field to the laboratory.
Sample Storage *Section 3.5*	Macroinvertebrate samples shall be stored in cool (preferably < 15°C) conditions within a hazardous substances storage facility prior to dispatch to the laboratory for processing. Sample container seals shall be checked prior to storage and prior to sample dispatch to ensure preservative is not lost, with preservative levels topped up if needed. *Note: Sample storage and transport must comply with the requirements of the Hazardous Substances and New Organisms Act 1996.*	
Laboratory processing		
Certification *Section 5.1*	Macroinvertebrate samples shall be sorted and processed by staff trained in taxonomic identification with evidence of the following: • adherence to either formal standard protocols (i.e. Stark et al., 2001) or subsequently implemented protocols (i.e. this Standard), including internal and external QA and quality control practices, and • regular (at least 3-yearly) external quality control of macroinvertebrate taxa identification accuracy and sorting efficiency.	
Sample Arrival at the Laboratory *Section 5.2*	Laboratory staff shall record the date of receipt of macroinvertebrate samples on the accompanying Chain of Custody Form, along with any anomalies in sample container condition with the potential to impact processing (e.g. split or leaking containers). Samples shall then be stored within a secure hazardous storage facility until processing.	
Sample Condition *Section 5.3.1*	Laboratory staff shall open and inspect each macroinvertebrate sample immediately prior to pre-treatment and record any anomalies in its condition with the potential to impact processing (e.g. odour indicating poor preservation and subsequent biodegradation of sample).	
Sample Pre-treatment *Section 5.4*	Macroinvertebrate samples shall be thoroughly rinsed in a clean 0.5 mm sieve to remove preservative, fine sediment and any remaining unwanted organic material (e.g. whole leaves, twigs). *Note: Pre-rinsing in a coarser sieve(s) may be required.*	

Processing Method *Annex E*	Macroinvertebrate samples shall be processed using the 200+ Fixed Count with Scan for Missed Taxa method outlined in Annex E, with: • a random, representative subsample removed from the full sample for sorting and identification under a binocular microscope, and • a visual scan by eye undertaken of the entire remaining sample for any 'missed' taxa not present in the subsample, with missed taxa picked out and retained separately for identification using a binocular microscope. *Note 1: This method is modified from Protocol P2 (200 Fixed Count + Scan for Rare Taxa) described by Stark et al. (2001).* *Note 2: Although not the primary recommendation of this Standard, macroinvertebrate samples may also be processed using the Full Count with Subsampling Option, modified from Protocol P3 of Stark et al. (2001), as outlined in Annex F.*	
Taxonomic Resolution *Annex D*	Macroinvertebrate taxonomic resolution shall be at least to a level consistent with that outlined under the MCI, as provided in Annex D.	
Sorted Residue *Section 5.4.6.1*	The 200+ individuals identified and counted shall be retained in a vial, with the associated 'sorted residue' retained in a separate container, each labelled with the same details as the original sample container and an identifier code for the laboratory staff member processing the sample.	
Sample Residue *Section 5.4.6.1*	The unsorted remainder of the macroinvertebrate sample shall be retained in the original sample container after it has been scanned for missed taxa (with these taxa retained in a labelled 'Missed Taxa' vial).	
Quality Control *Section 5.5*	Frequency	At least once every three years the monitoring agency shall engage an independent laboratory to re-examine 10% of their annual routine macroinvertebrate samples to check the processing laboratory's accuracy of taxa identifications, sorting and enumeration. The results shall be documented in a Quality Control report.
	Taxonomic accuracy	The accuracy of the taxonomic identifications of the 200+ individuals in the subsample shall be checked. For each subsample checked, the percentage Taxonomic Disagreement (%TD) shall be calculated following the procedure outlined in Annex E. For an individual sample to pass the quality control check the %TD shall be ≤ 10%. For a batch of quality control samples to pass this component of quality control, the mean %TD across all quality samples ($\%BE_{TD}$) shall be ≤ 10%.

Quality Control *cont.*	Sorting accuracy (missed taxa) *Section 5.4*	For each individual sample, the sample residue shall be scanned for additional missed taxa. The percentage Missed Taxa error (%MT) shall be calculated following the procedure outlined in Annex E. For an individual sample to pass the quality control check, the %MT shall be ≤ 20%. For a batch of quality control samples to pass this component of quality control, the mean %MT across all quality control samples ($\%BE_{MT}$) shall be ≤ 20%.
	Sorting accuracy (missed individuals) *Section 5.4*	The sorted residue of each sample shall be scanned for missed individuals. The percentage Missed Individuals error (%MI) shall be calculated following the procedure outlined in Annex E. For an individual sample to pass the quality control check, %MI shall be ≤ 10%. For a batch of quality control samples to pass this component of quality control, the mean %MI across all quality control samples ($\%BE_{MI}$) shall be ≤ 10%.
	Quality control pass/fail evaluation *Section 5.4*	Each of the three batch quality control components ($\%BE_{TD}$, $\%BE_{MT}$ and $\%BE_{MI}$) shall pass to pass quality control overall. Where one or more components fail, this shall be reflected in a lower quality code. Where any individual quality control sample fails on %TD, the original taxa list for the sample shall be corrected to align with the quality control results and a corrective action report shall be prepared by the laboratory that processed the original samples. *Note: No adjustments shall be made to the original dataset where quality control is deemed to have passed.*
Macroinvertebrate Index Calculation and Reporting *Section 5.6*	Taxa richness	Taxa richness shall be calculated according to the taxonomic resolution specified in Annex D. Where subsampling occurs, taxa richness shall be based on both the taxa in the subsample plus additional taxa found in the scan of the unsorted sample residue.
	MCI and QMCI	The MCI_{HB}, MCI_{SB}, $QMCI_{HB}$ and $QMCI_{SB}$ shall be determined using the taxonomic resolution and tolerance values specified in Annex D[1,2], and calculated in accordance with Table 3 in Section 5 of this Standard. *Note: Missed taxa shall be included in the calculation of MCI_{HB} and MCI_{SB}.*

Macroinvertebrate Index Calculation and Reporting *cont.*	EPT-HA taxa richness, %EPT-HA taxa richness and %EPT-HA abundance	EPT-HA taxa richness, %EPT-HA taxa richness and %EPT-HA abundance shall be calculated according to the taxonomic resolution specified in Annex D, with both *Oxyethira* and *Paroxyethira* excluded from the metric calculations.
	ASPM	ASPM shall be calculated in accordance with Table 3 (subsection 5.6.1) from the MCI_{HB} or MCI_{SB}, EPT-HA taxa richness and %EPT-HA metrics.
Data Records *Section 5*	The laboratory shall record and report: • the date(s) of sample collection, and both sample receipt and sample processing at the laboratory • the collection and processing method, including details of the amount of the sample processed • a unique identifier code for the laboratory staff member that processed each sample, and • any anomalies with the condition of the sample upon receipt and commencement of processing.	
Quality Coding *Section 6*	All data shall be quality coded as per the Quality Codes flowchart and associated matrix.	

[1] The substrate classifications in the report from which Annex D is sourced (Clapcott et al., 2017) are based on version 1 of the River Environment Classification (REC) and REC is known to be inaccurate for characterisation of some stream reaches across New Zealand. Stream substrate should be verified in the field.

[2] Some taxa do not appear in Annex D. Recommended MCI tolerance values for some additional taxa are provided in Annex H.

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

Field Documentation	Visit metadata and any associated field measurements are recorded electronically in the field. Where paper forms are unavoidable, waterproof paper is used.
Site Photographs	A camera or mobile phone with geo-referencing capability is used.
	A photograph of the streambed is taken on each sampling visit, preferably with the use of a polarising filter.
Supporting Variables	An underwater viewer (bathyscope) and/or Wolman sampler are used to assess streambed habitat characteristics.

Sample Labels	The following additional information is included on sample labels: • a unique sample identification number • sample type (e.g. kick-net), and • substrate type (e.g. hard-bottomed, soft-bottomed). The external sample label is checked for legibility prior to sample dispatch. *Note: Do not write over existing labels. Replace damaged or incorrect labels with a new one.*
Taxonomic Resolution *Annex D*	Taxa are identified to the lowest taxonomic level achievable with a dissecting microscope. ' *Note If taxa are identified to a finer resolution (lower taxonomic level) than that listed in Annex D, these should be listed alongside, but separate from, identifications made in accordance with Annex D.'*
Sample Retention	Laboratories store macroinvertebrate samples for at least nine months after initial processing to permit reassessment where required and appropriate.
Reporting and Data Checks	Data reported by the laboratory are checked by the collection agency against past sampling results within four weeks of receipt.
Voucher Specimens	Laboratories retain a voucher macroinvertebrate reference collection to facilitate consistent taxonomic identification. Vouchers for each taxon should ideally comprise at least two specimens in good condition and be: • verified by a second taxonomist • accompanied with a label written in pencil or a pen with alcohol-proof ink on waterproof paper that lists the collection and identification details (taxa name, date and location of collection, and the names of the collector, taxonomist and verification personnel) • checked regularly to ensure levels of preservative are adequate, and • updated as new taxa are found.
	When voucher specimens are removed from a sample this should be recorded with the sample data to ensure that the removed specimens are accounted for during sample processing quality control.
Data Archiving	File, archive indefinitely, and back up regularly in a database: • site and field visit metadata • date and condition of samples received at the laboratory, and • any sample anomalies, 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 container requirements and preservatives/pre-treatment, sample handling, and quality assurance.

1.1 Office and Field Manual

It is important that everyone involved in a macroinvertebrate 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

Collection of macroinvertebrate samples from streams involves some elements of danger that shall be considered in a Health and Safety Plan, prepared in accordance with the monitoring agency's organisational processes.

Safe access to monitoring sites is particularly important. Macroinvertebrate sampling necessarily involves wading and can be carried out when waters up to 0.6 m deep can be waded safely. Special care is needed to avoid slipping on substrate covered by periphyton and when sampling during swift and/or turbid stream flow. 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. Field personnel may wish to wear gloves when sampling in streams likely to present a significant health and safety risk (e.g. due to known sewage contamination, presence of glass).

For further guidance on safety precautions when collecting macroinvertebrate samples refer to the NEMS *Code of Practice Safe Collection of Environmental Data – Guidelines for safe working when undertaking environmental monitoring.*

1.3 Quality Assurance

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

The QA procedure should comprise all the steps described in this Standard to ensure that valid data are produced. This includes documented evidence that sampling personnel are trained and competent, sampling equipment is well maintained, and appropriate sample collection, preservation and handling methods are employed. In particular, sampling personnel should be given training in identification of different macroinvertebrate habitats, sample collection techniques, in-field sample processing and correct completion of Field Record Forms (either by an experienced member of staff or through a suitable external agency). This is best done through a combination of annual internal training exercises and periodic (e.g. three-yearly) joint sampling exercises with another experienced monitoring agency, as outlined in subsection 1.3.1.

QA (and quality control) programmes are mandatory components of laboratory practice to ensure measurements are accurate. Refer to subsections 5.4.6 and 5.5 for details.

1.3.1 External 'Field Audit'

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

1. collect and deliver to the laboratory samples that are representative of the macroinvertebrate community at the site and are adequately preserved, and
2. obtain accurate measurements of supporting variables.

The QA field exercise should target specific practices around:

- selecting the different mesohabitats to sample and how to sample these in proportion to their presence across the sampling reach
- collecting and handling macroinvertebrate samples, including elutriation and preservation of samples, and labelling of sample containers
- completing a stream habitat assessment, and
- completing the Field Record Form, including site visit metadata.

The exercise should involve two or more staff simultaneously, but independently (at least initially), completing the tasks above.

1.4 Field Record Form

A standard Field Record Form shall be used to record field visit metadata (both observations and any field measurements that may be collected). This form provides a vital record that verifies the location, timing, methods and conditions under which sampling was carried out, along with other factors that may influence the data being collected (for example, the collapse of a stream bank or blanketing of gravels with fine silt). This record is also essential for later reconciliation with macroinvertebrate data received from the laboratory (Section 6).

Electronic Field Record Forms are becoming increasingly common and have the advantage of offering more efficient data capture and the ability to build in automated calculations and

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

Metadata that shall be recorded when collecting macroinvertebrate samples are outlined in subsection 3.3.4. 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, habitat types and weather conditions, will reduce time spent filling out the form on site and provide for consistent record keeping.

1.5 Monitoring Equipment

The core monitoring equipment that will generally be required includes:

- waders, gumboots or dive booties
- a D-framed hand (or kick) net or other similar net (see subsection 1.5.1)
- gloves (if working in potentially contaminated waters or sites with rubbish, glass, etc.)
- a white tray and/or bucket
- a sieve or sieve bucket (0.5 mm mesh)
- tweezers for picking off invertebrates caught on the net
- a long-handled, soft brush for removing invertebrates from substrate
- a water bottle for rinsing material from the sieve and sampling net
- plastic screw-top sample containers of 500–1,000 mL volume (see subsection 1.6)
- isopropyl alcohol (isopropanol) or ethanol for preservation of macroinvertebrate samples (see subsection 1.6.2)
- waterproof sample container labels (see subsection 1.6.1)
- a waterproof marker pen and pencil
  Note: A pencil is essential as it will not be influenced by preservative.
- a metal ruler
- measuring tapes – one up to 100 m for measurement of sampling reach and one of 30 m length for any transect-specific data (e.g. wetted widths)
- Field Record Forms to record visit metadata and any field measurements
- habitat assessment forms and associated equipment, including an underwater viewer (bathyscope) and/or gravelometer (or metric rule or equivalent) for assessment of streambed characteristics
- a GPS receiver
- a camera
- disinfectant (for sampling equipment and for working in faecal contaminated waters), and
- a large chilly bin, sturdy plastic container or polystyrene bin for the safe storage of macroinvertebrate samples.

A calibrated multivariable hand-held field meter may also be useful for spot measurements of water quality variables such as water temperature, dissolved oxygen, specific conductivity, pH and/or turbidity.

It is essential that all equipment and devices are properly maintained and cleaned/disinfected to avoid transfer of organisms, particularly pest organisms, between sites.

Note: A Surber sampler or Hess sampler with 0.5 mm mesh may replace a kick-net where the sampling is for an Assessment of Environmental Effects or resource consent monitoring. Refer to subsection 3.3.5.

1.5.1 Net and Sampler Specifications

A range of nets and devices exist for macroinvertebrate sample collection. Those most commonly used in New Zealand are outlined here.

1.5.1.1 Mesh size

Although different mesh sizes are available, this Standard prescribes the use of a 0.5 mm mesh in all nets and samplers used to collect macroinvertebrates. This is consistent with the term 'macroinvertebrates' which, by convention, refers to invertebrates large enough to see without the aid of a microscope.

1.5.1.2 Hand nets

This Standard requires the use of hand nets, more commonly referred to as kick-nets (Figure 1) because they are suitable for sampling the wide range of stream habitats that exist in New Zealand. These nets come in a variety of shapes, including D-shaped (sometimes called a D-net), rectangular, triangular or circular. For consistency, a net with the following specifications (Stark et al., 2001) is required:

- a width along its base of 30–40 cm to ensure macroinvertebrates and debris dislodged during streambed 'foot-kicking', brushing or hand rubbing are captured in the net
- a net length of at least 50 cm to minimise clogging and backflow around its mouth, and
- attachment of the mesh to the frame by a sleeve of calico or plastic to prolong the life of the net.

Note: A net of narrower width may be necessary for sampling some stream reaches or mesohabitats. It is also possible to modify the sampling net or device so that a sample container can be screwed directly onto the end of the net (Figure 1). This modification can reduce time spent transferring samples contents from the net to a sample container.

Figure 1 – Example of a kick-net (a D-net, left) and a net modified so that a sample bottle can be attached (right)

Photos: www.iwla.co.nz (left) and EOS Ecology

1.5.1.3 Surber samplers

Surber samplers are useful for quantitative benthic macroinvertebrate sampling; their design ensures that a known fixed area of the streambed is sampled (Figure 2). However, these samplers are generally limited to use in wadeable streams of moderate current with hard (and small) substrate and low embeddedness (i.e. they are not suitable for use in deep or soft-bottomed streams).

Figure 2– A Surber sampler

Source: www.envco.co.nz

1.5.1.4 Hess samplers

Hess samplers are similar to Surber samplers, being best suited to wadeable gravel and cobble bottom streams, but do not capture invertebrates drifting in the water column. These samplers are cylindrical with enclosed sides and an open top (Figure 3); two strong, reversible handles are used to forcibly push and rotate the cylinder into the streambed.

Figure 3– A Hess sampler with capacity to attach a sample container to the end of the net
Source: www.envco.co.nz

1.6 Sample Containers

Sturdy screw-top plastic containers of 500 mL to 1,000 mL volume are preferred for macroinvertebrate samples (Figure 4). Filling instructions are summarised in subsection 3.3.5.

Figure 4 – Examples of commonly used containers for storage of macroinvertebrate samples
Photo: EOS Ecology

1.6.1 Labels

All sample containers shall be labelled as outlined in subsection 3.3.6, with a label on waterproof paper placed inside the container in addition to a waterproof label on the outside. On-site labelling is recommended to prevent accidental switching of pre-labelled containers.

Note: Use of customised sample bottle labels with pre-printed site names and numbers can reduce labelling time in the field but site details should be checked prior to sample collection).

A hazardous substances sticker is also required on the outside of each sample container stating the product, concentration and type of hazard (i.e. flammable).

1.6.2 Preservative

A range of preservatives can be used for the preservation of macroinvertebrate samples, with ethyl alcohol (ethanol)-based preservatives the most common. For consistency, this Standard prescribes the use of either isopropyl alcohol (isopropanol) or ethanol only. Details on preservative use are provided in subsection 3.3.7.

Note 1: Isopropanol and ethanol are highly flammable and must be stored and transported in accordance with the requirements of the Hazardous Substances and New Organisms Act 1996.

Note 2: Formalin is a suspected carcinogen and should not be used in the field to preserve samples.

1.7 Sample Storage and Transport

Preserved macroinvertebrate samples shall be stored and transported in accordance with the requirements of the Hazardous Substances and New Organisms Act 1996. A courier approved for shipment of hazardous samples shall be used to transport the samples to the laboratory for processing. Sample storage, handling and transport are discussed further in subsection 3.5.

1.8 Chain of Custody Form

A completed Chain of Custody (CoC) Form (Annex C) shall accompany macroinvertebrate samples to provide an audit trail from sample dispatch (subsection 3.5.1) to sample arrival at the laboratory and a record of sample condition upon receipt at the laboratory (subsection 5.2). The CoC information shall be used to provide a quality assurance check on sample integrity (subsection 6.2.1).

1.9 Managing Changes in Methods

Long-term macroinvertebrate 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 as methods evolve over time. This highlights the need for good documentation of field and laboratory methods.

Subsection 5.7 addresses changes in taxonomy and laboratory methods.

2 The Monitoring Site

In This Section

This section focuses on monitoring site considerations: locating the reach where macroinvertebrate sample collection and any associated field measurements will occur, site stationarity, and site metadata requirements.

2.1 Site (Reach) Location

Monitoring benthic macroinvertebrate communities requires the collection of samples at a *reach* scale. This Standard requires a monitoring reach length of around 20 times the average channel width (with a minimum length of 20 m and a maximum length of 100 m).

The monitoring reach should be upstream of any nearby bridges and river crossings (either vehicle or stock) and not include tributary junctions. Upstream confluences with point-source discharges, tributary streams or drains should be far enough upstream that their waters will be completely mixed in the stream channel in the monitoring reach.

The monitoring reach should be easily identifiable, preferably with a permanent location marker (e.g. waratah or external staff gauge) to ensure safe access and consistency in 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).

Different stream characteristics influence the location (as well as method) of sample collection, including substrate and water depth.

2.1.1 Hard-bottomed Streams vs Soft-bottomed Streams

Hard-bottomed streams comprise around 60–80% of New Zealand's streams and are characterised by a bed substrate comprising at least 50% particles of 2 mm or greater in size (i.e. dominated by gravel, cobble, boulder and bedrock substrates). Riffle and run mesohabitat are common in these streams (Figure 5), reflecting a steeper stream gradient. Pools, rapids, chutes, backwaters and eddies can also be present (Figure 6). Other mesohabitats may include undercut banks and root mats.

Soft-bottomed streams have a substrate comprising more than 50% sand/silt/mud/clay, with the underlying geology, stream slope and land use all contributing factors to the sediment composition. These streams are typically low-gradient runs and may be dominated by macrophytes or woody debris (Figure 7). Other mesohabitats may also be present, such as riffles, chutes, undercut banks and root mats.

In reality, some stream reaches will comprise a mix of hard-bottomed and soft-bottomed substrates and macroinvertebrates will need to be collected from both types to obtain a representative sample.

Note: Some hard-bottomed stream reaches may be considered soft-bottomed because of inundation with fine sediment. Consideration of whether this inundation is anthropogenically or

naturally derived will be required before selecting whether to use hard-bottomed or soft-bottomed taxa tolerance values (Annex D) in the calculation of MCI and QMCI metrics from macroinvertebrate data collected from these reaches. The tolerance values used to calculate these metrics shall be determined by the dominant natural substrate type present across the sampling reach (e.g. hard-bottomed tolerance values would apply where at least 50% of the substrate comprises one or more of gravel, cobble, boulder and bedrock, and also where the substrate is 80% inundated with fine sediment but was naturally hard-bottomed).

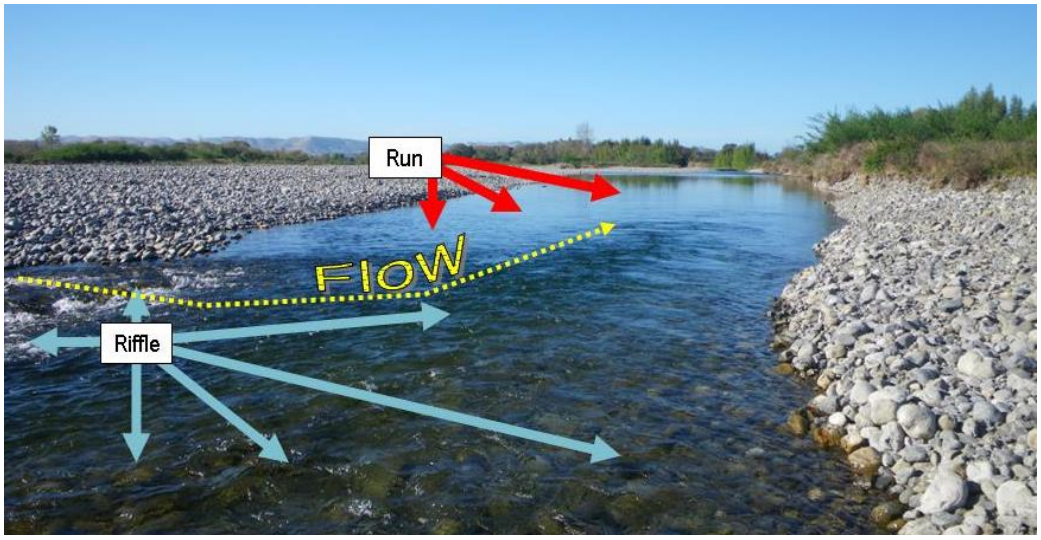

Figure 5 – The Waingawa River near Masterton, an example of a typical hard-bottom stream dominated by run and riffle mesohabitat

Photo: Shyam Morar

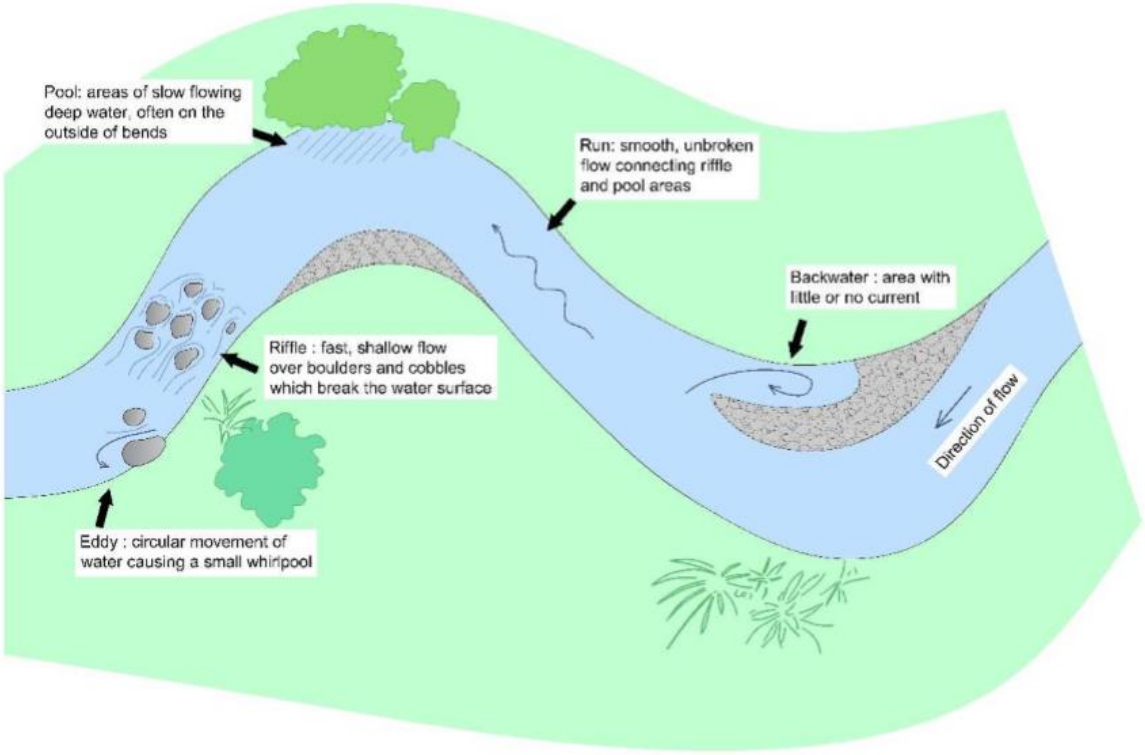

Figure 6 – Schematic of a hard-bottomed stream showing run, riffle, pool and other mesohabitats

Figure: Quentin Gilkinson

Figure 7 – Examples of soft-bottomed streams in Auckland and Waikato
Photos: Bioresearches Group (left) and NIWA

2.1.2 Wadeable vs Non-wadeable Streams

This Standard focuses on the sampling of stream reaches between 0.1 and 0.6 m deep that can be safely accessed by wading. However, given deeper, non-wadeable streams are present across many parts of New Zealand, brief guidance is provided on sampling these in subsection 3.3.5.4. Water velocity, substrate type and sampler confidence are other factors that affect wadeability.

Initial site selection should ideally be carried out during summer baseflow conditions and should include consideration of the extent of the site that will be wadeable under a range of baseflow conditions. Essentially, While access down to the streambed across the entire reach area is not required (and may not be possible in larger streams at baseflows), it should be safe to wade along the entire length of the reach to access suitable mesohabitats for sample collection.

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 macroinvertebrate monitoring may become unsuitable. Other disturbances may affect suitability for monitoring, such as gravel extraction or changes in public access, or site access may change over time (e.g. changes to access to private property, erosion that makes tracks too dangerous to negotiate). In all these cases, a macroinvertebrate monitoring reach may need to be relocated to one that remains representative of those in the stream in that part of the catchment.

A stream habitat assessment (see subsection 4.1) should be carried out to identify a new reach that is still representative of the broader stream reach and, ideally, matches the key mesohabitat and flow characteristics of the previous site (e.g. when selecting a new channel in a braided river system, select a channel with a comparable thalweg for sample collection). Ideally, the new location will be close to the original site, such as within the same NZREACH (River Environment Classification (REC) 1), or NZSegment (REC2.4).

Features such as water depth and velocity, substrate type and sizes, stream slope, degree of braiding, and shading are important characteristics that determine the 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:

- river/stream name
- site name and number
- site location; for example, GPS units or latitude/longitude coordinates expressed to a minimum of six decimal places
- site altitude
- related sites and records, including the nearest weather station and flow/level recorder
- a summary of key stream riparian, bank, channel and instream characteristics
- relevant catchment features – immediate land use / riparian margin features (e.g. fenced/unfenced, vegetation, eroded banks) and presence of structures (e.g. stormwater or irrigation pipes)
- start date of monitoring
- photographs of the site taken looking both upstream and downstream
- landowner contact details and, where relevant, procedures for accessing the site, and
- specific health and safety considerations.

A Field Record Form for visit metadata (see subsection 3.3.4) 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 Section 6).

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.

Note: Photos from each end and the midpoint of the reach, as well as a photo of the streambed substrate, may also be useful. A camera with a polarising filter will reduce reflection and improve capture of instream habitat features (e.g. substrate and periphyton cover).

3 Field Sampling

In This Section

This section focuses on the collection of macroinvertebrate samples. It outlines sample collection methods and strategies, the timing of sample collection, visit metadata (field records), sampling procedures, sample labelling, in-situ sample preservation, and sample transport and handling.

3.1 Sample Collection Methods and Strategies

There are multiple methods for macroinvertebrate sample collection. The choice of method and the number and type of instream habitats to sample form a critical part of monitoring programme design and must be informed by a clear purpose. Other important considerations are the timing of sample collection and whether or not replicate samples should be collected.

The Stark et al. (2001) macroinvertebrate sampling protocols standardised four sample collection methods for use in wadeable New Zealand streams:

- two semi-quantitative protocols, for hard-bottomed (Protocols C1) and soft-bottomed streams (Protocol C2), and
- two quantitative protocols, for hard-bottomed (Protocols C3) and soft-bottomed streams (Protocol C4).

These protocols have been revisited and modified for use in this Standard, as outlined in the following subsections.

3.1.1 State of the Environment Monitoring

For the purposes of SOE and other similar monitoring that seeks to assess overall stream ecosystem health, this Standard requires the use of a kick-net to sample mesohabitats likely to support a good diversity of macroinvertebrates. In most hard-bottomed streams, this will be a combination of runs and riffles.

A single composite sample shall be collected comprising between four and eight unit efforts from the different mesohabitats in proportion to their abundance across the monitoring reach. Each unit effort shall be approximately 0.1–0.15 m^2 in area and the total sampling area shall be 0.6–0.9 m^2.

Note 1: This is a modification of both Protocol C1, where sampling was limited to riffle mesohabitat and a fixed area of between 0.6 and 1 m^2, and Protocol C2, which recommended collection from 3 m^2 of stream habitat (Stark et al., 2001). Laboratory sample processing specialists have identified that the Protocol C2 sampling effort typically results in very large volumes of sample material, with only a small proportion fully processed as a subsample.

Note 2: Protocol C1, as described in Stark et al. (2001), promoted the sampling of riffles to minimise variability and improve the validity of between-site and temporal comparisons. However, riffles are often not abundant in hard-bottomed streams (especially in many lowland stream systems) and sampling across the most common mesohabitats in proportion to their abundance provides a more representative assessment of stream condition at a site. As reach-scale habitat

may change through time, a habitat assessment is also a mandatory requirement under this Standard (see subsection 4.1).

Note 3: Through standardising and recording the area of substrate/mesohabitat sampled with a kick-net (see subsection 3.3.5), the subsequent composite sample is deemed suitable for the determination of abundance-based metrics such as QMCI provided laboratory sample processing is carried out in accordance with the requirements of Section 5.

Additional sampling effort may be required in some streams dominated by pumice or soft sediment substrate that are devoid of macrophytes and woody debris, contributing to lower densities of invertebrates. Examination of historical data will be useful to assess if additional sampling effort may be required, as will examining the number of individuals captured during sample collection. Additional sampling effort shall seek to obtain at least 100 (and preferably 200) individual macroinvertebrates but, for practical reasons, shall not exceed 10 minutes in duration.

Note: Although invertebrates may be living in a variety of mesohabitats across the sampling reach, the aim is to sample across the more common mesohabitat types rather than to seek out a relatively 'rare' mesohabitat; however, if sampling for biodiversity purposes, all available mesohabitats and microhabitats, however small in area, might be of interest. Bedrock, mud/silt, and clay substrates should not be avoided if present but it may be appropriate to 'down-weight' time spent sampling these so that the pool of macroinvertebrate taxa collected reflects aspects of stream health other than solely substrate.

3.1.2 Other Monitoring

Macroinvertebrate samples are collected for many purposes other than SOE monitoring. Although not the primary focus of this Standard, some brief guidance on sample collection for these purposes is provided here and in subsection 3.3.5 to promote national consistency.

3.1.2.1 Assessment of Environmental Effects (AEE) and Resource Consent Monitoring

This Standard recommends the use of quantitative protocols C3 and C4 described by Stark et al. (2001) to establish a robust baseline of stream condition to inform an AEE for a proposed activity (e.g. stream realignment or piping) or to assess the effects of consented activities (e.g. wastewater or sediment discharges to water, water abstraction, gravel extraction).

Monitoring the effects of activities such as point-source discharges or water abstraction requires similar physical habitat(s) to be sampled upstream and downstream of the activity to minimise confounding effects on macroinvertebrate communities. Riffles, where present, are often a good mesohabitat to target because they are typically highly productive, support the greatest number of taxa sensitive to organic enrichment and often support larger specimens making identification easier (Stark et al., 2001). A quantitative habitat assessment is needed to support interpretation of the macroinvertebrate samples.

Note: Runs are often the dominant habitat in larger hard-bottomed streams in lowland areas where some point-source discharges are still present.

The nature (e.g. intermittent or continuous), scale and significance of the activity need to be considered when determining:

- the timing (season) of sampling

- the number of sites sampled (e.g. whether there is a single downstream site or additional sites to measure the extent of impact)
- the number of replicate samples collected per site
- the frequency of sampling, and
- the scope of the accompanying quantitative habitat assessment and any associated physico-chemical water or sediment quality measurements.

It is generally best practice to have more than one downstream impact and upstream reach to quantify between-reach variability. Replicate samples are also generally recommended so that estimates of variance in macroinvertebrate densities, metrics and statistical techniques (e.g. ANOVA) can be calculated.

Note: Due to their small fixed area, quantitative sampling using a Surber or Hess sampler will also require sample replication to obtain a representative taxa list for a site (Stark et al., 2001).

All sites should be sampled on the same day and samples processed using quantitative protocols (see subsection 5.4.1).

3.1.2.2 Biodiversity Assessments

This Standard recommends the use of a kick-net for assessments of biodiversity. Except where the purpose of the assessment is focussed on characterising a particular mesohabitat, the aim is to sample as many microhabitats and mesohabitats as possible across the sampling site (reach) to capture full range of species present. The need for fixed transects and replicate samples will depend on the specific monitoring objective(s).

Note: A kick-net will capture larval stages, but for the most accurate and complete biodiversity assessment adult invertebrate stages should also be sampled, preferably using a combination of methods (e.g. light traps, Malaise traps).

3.2 Timing of Sample Collection

3.2.1 Time of Year

Macroinvertebrate sampling shall be undertaken between the months of December and March inclusive, wherever practical, but may be extended to between November and April inclusive. In many, but not all, parts of New Zealand, the most stable streamflow conditions occur during summer and early autumn (December to March inclusive).

Note: The December to March period is consistent with the requirements of the National Policy Statement for Freshwater Management (New Zealand Government, 2020). However, this timeframe is considered overly restrictive. Under this Standard macroinvertebrate data obtained from sample collection in November or April will still be eligible for the highest quality rating of QC 600.

3.2.2 Antecedent Flow Conditions

Antecedent flow conditions are one of the primary determinants of macroinvertebrate abundance and biomass. Floods, particularly those that mobilise bed material, can cause large

mortality and displacement of macroinvertebrates; several weeks of stable flows may be required for communities to regain characteristics observed prior to the flood.

A flow of three times the long-term median has traditionally been used to trigger the default 'stand-down' period before sample collection can proceed. However, streams vary in their hydrological regime and knowledge of the 'flashiness' of a particular system, including the flow at which bed material is likely to be mobilised, can inform decisions on a more appropriate flow to trigger a 'stand down'.

Under this Standard, macroinvertebrate samples shall not be collected within 14 days following a stream flow that results in:

- significant (e.g. >25% of sampling habitat) bed movement in a hard-bottomed stream, or
- extensive (e.g. >50%) removal/scour of macrophytes, woody debris or other stable mesohabitat in a soft-bottomed stream, or
- extensive (e.g. >50%) movement of fine bottom sediments (e.g. mud, pumice) greater than what is typical at baseflow.

The sole exception to the above shall be for hard-bottomed streams within high rainfall or heavily urbanised catchments subject to frequent and significant bed disturbance; in these streams, macroinvertebrate samples shall not be collected within 7 days of a stream flow exceeding three times the median.

Note: Streams that are frequently exposed to high rainfall or disturbance events (e.g. many streams on the West Coast of New Zealand and urban streams that receive significant stormwater runoff from impervious surfaces) will support macroinvertebrate communities that have adapted to frequent hydrological disturbance and a shorter 'stand-down' period is considered appropriate.

Where a monitoring agency lacks information on the 'flashiness' of a particular system, including the flow at which the dominant substrate or bed material is likely to be mobilised, then the default position under this Standard shall be to avoid collection of macroinvertebrate samples within 14 days of a stream flow that exceeds three time the median.

Note: There are empirical calculations for substrate stability that might assist for determining stand-down periods for specific sites (e.g. Duncan et al., 1999). Flow information from the nearest gauged catchment can also be used to estimate a stand-down period.

The antecedent flow conditions and any 'stand-down' period observed shall be recorded. In some cases, the antecedent flow conditions may have led to a change in the streambed or drying and re-wetting of certain parts of the channel.

3.3 Sample Collection

3.3.1 On-site Risk Assessment

Field personnel should carry out a rapid personal risk assessment upon arrival at the site. This assessment should be used to determine if:

- it is safe to carry out sampling and/or field measurements in the usual manner, or
- sampling and/or field measurements only can be attempted with suitable modifications.

If conditions are considered unsafe, sampling should not be attempted. Record any health and safety issues (e.g. on the Field Record Form and/or in an appropriate database) and update the Health and Safety Plan (subsection 1.2) as appropriate.

3.3.2 Site and Visit Identifiers

Each macroinvertebrate sample shall be referenced against existing unique identifiers for the monitoring site and the sampling occasion (e.g. WRC_2020/01) and recorded as visit metadata. This ensures that the relevant metadata and laboratory processing data, as well as any associated field measurements, can be linked as a single visit record.

3.3.3 Time Records

The sample collection time shall be recorded to:

- the nearest 1 hour in New Zealand Standard Time (NZST) from commencement of sampling, or
- where sample collection coincides with the collection of spot in-situ water quality measurements (e.g. dissolved oxygen) or samples, to the nearest minute of taking the measurements, consistent with the NEMS *Water Quality*.

Note: Accurate time recording can be assured by using a device that is regularly synchronised with reference time, for example, a smartphone or smartwatch.

3.3.4 Visit Metadata

A standard Field Record Form shall be used to record field metadata (observations) on each visit to the site to collect macroinvertebrate samples. This form shall include:

- the name of the stream
- the name of the site location
- reach length
- any unique sample identification number(s) assigned
- the name(s) of the field personnel
- date and time (in NZST) of sample collection and any associated field measurements
- the weather conditions at the time of measurements
- flow state (rated, gauged or estimated – at least as 'low', 'moderate', 'high')
- the sampling protocol/equipment used, including any deviations from the protocol (e.g. samples collected from a depth > 0.6 m or only from a specific type of mesohabitat), or if there were any difficulties with sample collection
- general water colouration (clear/colourless, turbid or brown) and if the water had any unusual smell, scums, foams or floatables
- the proportion of the sample taken from each type of mesohabitat (e.g. 70% run, 30% riffle if a hard-bottomed site or 50% wood, 25% banks, 25% macrophytes if a soft-bottomed site), including the number of sample units collected from each, and
- notes of significant changes in reach characteristics (where known), for example, main channel now running along the true right bank, fodder crop grazed to bank, bulldozer in the river, bank erosion or slumping that may result in sediment entering the water.

An example Field Record Form is provided in Annex B. Photographs of the site looking both upstream and downstream shall also be taken at least annually. A camera with geo-referencing capability is recommended and a polarising filter is also useful.

3.3.5 Macroinvertebrate Collection

The mesohabitats sampled shall be representative of those present across the site (defined in subsection 2.1 as a reach between 20 and 100 m in length). Sampling shall start at the downstream end of the reach and move progressively upstream to ensure sampling areas are not disturbed by material dislodged from upstream. All nets, sieves and other equipment shall be clean before commencing sampling.

Note: All sampling devices shall have a mesh size of 0.5 mm. Refer to subsection 1.5 for equipment specifications.

Additional guidance may be required for sampling of intermittently flowing streams (e.g. Morias et al., 2004) and braided rivers. The general approach for SOE-related sampling in a braided river is to return to the same general area to sample (e.g. the same general riffles and runs in the main channel).

3.3.5.1 Semi-quantitative kick-net sampling

Note: This Standard requires macroinvertebrate samples for SOE monitoring purposes to be collected in accordance with this method.

Sampling shall target all suitable macroinvertebrate mesohabitats (e.g. riffle, run, bank margins/edge vegetation, submerged woody debris, aquatic macrophytes) in proportions equal to their presence across the monitoring site (reach). The sample collection procedure is illustrated in Figure 8 for hard-bottomed substrate and Figure 9 for soft-bottomed substrate and involves the following steps (modified from Protocols C1 and C2 of Stark et al., 2001, respectively):

1. Select a set area (0.1–0.15 m^2) of mesohabitat to sample (ideally in flowing water) using the following procedures.

    - Cobbles and gravels (in runs and riffles) – place the kick-net on the streambed facing directly into the direction of flow (to direct organisms into the net) and disturb the surface substrate immediately in front of the net by foot-kicking (and by hand where necessary) to dislodge macroinvertebrates and detritus on and under the substrate and to scrape the underlying bed. Turn over and examine large cobbles/small boulders for any tightly adhering individuals (e.g. fixed retreats or pupal cases), and remove these with a brush or your fingers.

      Note: For a kick-net 30 cm wide, disturbance of the streambed to a distance of approximately 30 cm in front of the mouth of the net represents an area of ~0.1 m^2. The disturbed distance in front of the mouth of the net should not exceed ~0.4 m to avoid organisms drifting outside of the net. Avoid scraping more than 10 cm below the bed and adjust kicking effort according to the degree of substrate embeddedness (e.g. loose gravel requires significantly less effort to turn over than large cobbles).

    - Bedrock – place the kick-net on the bed facing directly into the direction of flow to direct organisms into the net and disturb the surface of the bedrock immediately in

front of the net by foot-kicking as well as by hand to dislodge macroinvertebrates and detritus. Focus on habitable areas of the bedrock (e.g. if bryophytes such as mosses are present, place the net downstream of them, and rub them vigorously to dislodge the macroinvertebrates amongst them, taking care not to dislodge the bryophyte clump if possible).

- Woody debris – select a single, small point with submerged and partially decayed woody debris (>50 mm diameter preferred). Place the wood over the mouth of the bucket, sieve bucket or net. Pour water over the wood while brushing it gently by hand to remove organisms. Larger pieces of wood may be sampled in situ by brushing the log while holding the net directly behind it.
- Bank margins/edge vegetation – locate a single (~0.5 m^2) area of bank with good structure and aggressively jab the net into the bank to dislodge organisms, followed by two cleaning sweeps to collect organisms in the water column.
- Macrophytes – jab at, or sweep the net through, a single point within a macrophyte bed on the flowing water side to dislodge organisms, followed by two cleaning sweeps to collect organisms in the water column. Alternatively, place the net downstream of the macrophytes and shake them by hand to dislodge the macroinvertebrates.
- Soft mud/sand/leaf litter – carefully and lightly disturb the top oxygenated layer of substrate immediately in front of the net with your hand or foot.

  Note: Avoid deeply dredging the net through anoxic mud or sampling stagnant backwaters.

2. Transfer the material from the net into a white tray, bucket or sieve bucket. Large fragments of macrophyte caught in the net can be picked out first and washed within the net to catch any macroinvertebrates.

   Note: Transfer of net contents is only required when the net begins to get clogged with periphyton, leaf litter or other material.

3. Repeat steps 1 and 2 above until a total area of 0.6 to 0.9 m^2 has been sampled (i.e. typically between four and eight unit efforts depending on the net dimensions).

   Note: For a hard-bottomed monitoring site comprising 80% runs and 20% riffles, the number of unit efforts collected for a 40 cm wide kick-net should be 3–4 in runs and 1 in riffle mesohabitat. Similarly, for a soft-bottomed stream comprising 50% macrophytes, 25% woody debris and 25% overhanging vegetation, collect 4 unit efforts from the macrophytes, and 2 each from the woody debris and overhanging vegetation.

4. Holding the net in the water face up, remove any larger stones and vegetation, ensuring any macroinvertebrates are first washed off into the net.

5. Transfer all remaining collected material to a white tray or bucket approximately half full of water, or to a sieve bucket. Wash or pick all macroinvertebrates off the net.

   Note: Take care not to damage any macroinvertebrates. Tweezers may be useful.

6. Rinse and remove any unwanted large debris items (e.g. stones, sticks, leaves) that may not fit into the sample container or will absorb and diminish the effectiveness of the preservative. Excessive gravel can be elutriated by tipping the sample into a bucket, stirring the contents and floating off organic matter back into the net. This process may

need to be repeated a few times. As far as practicable, rinse and remove any fine sediment prior to transferring to the sample container.

7. Transfer the material to a pre-labelled sample container(s) via a 0.5 mm sieve if a sieve bucket is not used. Inspect the sieve or sieve bucket and return any macroinvertebrates to the sample container. The sample container should not be more than half full of material and should contain minimal amounts of water to prevent excessive dilution of preservative; use another container(s) for any remaining sample material.
8. Add an internal label and isopropanol or ethanol preservative to the sample container in accordance with the instructions in subsection 3.3.6 and subsection 3.3.7, respectively. Ensure that the container lid is screwed on tightly.
9. Complete sampling details on the Field Record Form outlined in subsection 3.3.4, including records of the reach length, the types and proportions of mesohabitat sampled (e.g. 80% run and 20% riffle or 50% macrophytes, 25% banks and 25% woody debris) and the collector's name.
10. Ensure that all sampling nets and other equipment are clean and free of macroinvertebrates, periphyton, macrophytes and detritus before you leave the site.

Note: Steps 2 to 6 will differ if the end of the kick-net has been modified to enable a sample container to be attached (subsection 1.5.1). At the completion of the sampling process, the net is held up vertically so that any remaining material caught in the net is washed into the container. Excess water is drained from the container by inverting it against the mesh net, and then any trapped material is washed back into the sample container. If possible, any large debris should be removed before preserving the sample.

Figure 8 – Kick-net sampling in a hard-bottomed stream

This net has a modified end enabling a sample container to be added. Filling the container with stream water prior to sample collection will ensure that the rear end of the net doesn't float up.

Photo: EOS Ecology

Figure 9 – Sampling edge vegetation (left) and brushing invertebrates from a piece of wood into a net (right) in soft-bottomed streams

Photos: ausrivas.ewater.org.au (left) and NIWA

3.3.5.2 Quantitative sampling of hard-bottomed streams using a Surber or Hess sampler

Note: The following guidance applies to samples collected for purposes other than SOE monitoring, notably AEE and resource consent monitoring. It assumes collection of five replicate samples, with each sample comprising a single unit effort.

A Surber or Hess sampler is only suitable for use in:

- shallow streams (nominally < 0.5 m depth),
- on uniform areas of substrate (generally comprising gravel and small cobbles) where an effective seal can be formed between the areas of streambed to be sampled and the bottom of the sampler's frame, and
- where there is sufficient current to carry macroinvertebrates into the net without risk of loss from backflow (i.e. generally in riffles and runs).

The sample collection procedure is illustrated in Figure 10 and involves the following steps (modified from Protocol C3 of Stark et al., 2001):

1. Select an area of suitable substrate and habitat to sample at the downstream end of the site (reach) and place the sampler on the streambed ensuring a good fit around the perimeter with the sampler positioned so that the water current washes dislodged material into the net. If using a Hess sampler, ensure that the base of the sampler is firmly rotated into position to form a good seal on the streambed.

2. Brush material from the upper surface of all cobbles contained within the sample quadrat. Pick up each cobble and, holding it immediately in front of the net mouth, brush all sides of the cobble clean. Repeat for all of the larger substrate elements within the sampler quadrat. Place clean cobbles outside of the sampler quadrat. Disturb the finer substrate remaining within the quadrat to a depth of 5–10 cm.

3. Remove the sampler from the water, rinse the net several times to concentrate the sample in the bottom of the net (take care not to lose material during this process), and return to the stream bank. Remove and discard large substrate elements that may have entered the net, taking care to remove adhering invertebrates before disposal. Remove

the sample either by inverting the net into a container or by removing the container attached to the end of the collection net. Elutriation (i.e. repeated rinsing of sample) may also be required to separate organic and inorganic fractions.

Note: This is only required when the net begins to get clogged.

4. Let the sample settle for a few minutes and decant off excess water via a sieve. Return any macroinvertebrates washed out with the water to the sample container.

   Note: Take care not to damage any macroinvertebrates. Tweezers may be useful.

5. The sample container should not be more than half full of material; fill another container(s) with any remaining sample material. Add an internal label and isopropanol or ethanol preservative to the sample container in accordance with the instructions in subsection 3.3.6 and subsection 3.3.7, respectively. Ensure that the container lid is screwed on tightly.

6. Repeat steps 1 and 5 above four times but progressively shifting further upstream with each sampling effort. The last sample should be collected from the upstream end of the reach.

7. Complete sampling details on the Field Record Form outlined in subsection 3.3.4, including the Surber sample area (e.g. 0.1 m^2), the number of replicates collected and the collector's name.

8. Ensure that all samplers and other equipment are clean and free of macroinvertebrates, periphyton, macrophytes and detritus before you leave the site.

Figure 10 – Using a Surber sampler in shallow hard-bottomed streams – note the use of a heavy chain to form an effective seal between the base of sampler frame and the streambed (left)

Photos: EOS Ecology

3.3.5.3 Quantitative sampling of soft-bottomed streams

Note: The following guidance applies to samples collected for purposes other than SOE monitoring, notably AEE and resource consent monitoring. It is written with a focus on comparing differences in water quality between sites. For a quantitative assessment focused more broadly on ecosystem health, consistent with subsection 3.3.5.1, sampling should be in proportion to the different mesohabitats present.

Sampling of macroinvertebrates associated with macrophytes is the easiest way to quantitatively sample macroinvertebrates in open soft-bottomed streams. Consistency in plant species and the parts of plants targeted is important for comparisons between sites if the aim is to investigate differences in water quality, although not always possible. The sample collection procedure involves the following steps from Protocol C4 of Stark et al. (2001):

1. Determine the dominant plant species to be sampled.
2. Standardise the depth/velocity conditions of sampling points, where possible.
3. Collect at least four replicate samples of submerged macrophyte tips (approximately 100 g wet weight of top 20–30 cm of macrophyte, which is equivalent to 1.5 – 2 L of plant material) in flowing water by moving net upstream into macrophyte bed and breaking off required portion of plant material. Place each replicate sample in a separate bucket. Rinse net thoroughly between replicates.
4. Add approximately 1 L of clean water to each bucket and firmly attach lid. Shake bucket vigorously (20 times) to detach invertebrates from macrophyte material.
5. Pour dislodged macroinvertebrates and detritus through a 0.5 mm sieve. Rinse each sample twice more in a similar manner.
6. Use a wash bottle to transfer material retained on the sieve to a sample container. Add an internal label and isopropanol or ethanol preservative in accordance with the instructions in subsection 3.3.6 and subsection 3.3.7, respectively. Ensure that the container lid is screwed on tightly.
7. Complete sampling details on the Field Record Form outlined in subsection 3.3.4, including sample type, number of replicates collected, and the collector's name.
8. Drain the plant material of excess water (leave to stand in sieve for two minutes) and then weigh to the nearest 5 g using a spring balance. If greater precision is required, place plant samples in labelled plastic bags and return to laboratory for drying (at 70°C for at least 24 hours) and weighing.
9. Record the wet weight of macrophyte material associated with each replicate sample. Also record the species and condition (e.g. senescent, flowering, covered in epiphytes) of the macrophyte bed from which the sample was taken.
10. Ensure that all sampling nets and other equipment are clean and free of invertebrates, macrophytes and detritus before you leave the site.

Note: Where macrophytes are absent, quantitative sampling of woody debris is recommended. Artificial substrates may also be useful for reducing inherent variability of sampling different habitats (although no formal guidance is currently available).

3.3.5.4 Sampling of non-wadeable streams

Standardised national methods do not exist for sampling macroinvertebrates in non-wadeable streams in New Zealand and the development of such methods is outside of the scope of this Standard. For those wanting more information, Clapcott et al. (2012) reviewed the main methods employed overseas and within New Zealand and a more recent quantitative method is described by Di Sabatino et al. (2016). A considerable amount of guidance, including standardised sample collection procedures, has been documented by the United States Environmental Protection Authority (USEPA) (e.g. Flotemersch et al., 2006; USEPA, 2013). These procedures include both passive and active sampling methods (Table 1). Figure 11 illustrates sample collection using a kayak and Ponar grab sampler. In New Zealand, artificial leaf packs have been used successfully to sample macroinvertebrates in various streams, including streams with a substrate dominated by willow roots and streams polluted by coal mining (e.g. Barnden and Harding, 2005). Collier and Hamer (2014) describe both benthic and littoral sampling methods used to monitor macroinvertebrates in the lower Waikato River.

Figure 11 – Sampling a non-wadeable stream from a kayak (top) using a Polman grab sampler

Photos: EOS Ecology

Table 1: Overview of methods for sampling macroinvertebrates in non-wadeable streams

Based on material summarised from Flotemersch et al. (2006).

Method	Description	Comments
Passive methods		
Artificial substrate samplers 	Devices made of natural or artificial materials of various composition and configuration that are placed in the water for a predetermined period (typically 4–6 weeks) of exposure and depth for colonisation by biofilm and subsequent macroinvertebrate fauna. Usually deployed at a depth of 1 to 3 m. Common examples are rock basket samplers (typically consist of plastic or wire baskets filled with local rock or gravel) and variations of the Hesty-Dendy (H-D) multi-plate sampler. A H-D sampler is typically made of Masonite board or porcelain with spacers placed in between and bolted together to form stacks (see image left) that are attached horizontally to a brick or cinder block and placed on the streamed; spacing between plates is typically varied to provide different refuge sizes and flow regimes within the stacks.	Can be used to obtain qualitative and quantitative macroinvertebrate samples. Recommended for use in deep or turbid waters and in areas with muddy, sandy, or otherwise unstable bottoms. Although easy to use in shallow and deep water and able to effectively reflect water quality: • individuals can be lost when retrieving the sampler, potentially biasing results • they can indicate water quality, but not sediment or other habitat quality • exact placement of individual sampler units can skew results (e.g. high vs low velocity), and • damage or loss of artificial substrates can occur due to vandalism, high flows, shifting channels, etc.
Active methods		
Deep water	**Main channel sampling:** Deep habitats of large rivers can be sampled from a boat using various dredge or bottom grab sampling devices (e.g. Peterson, Ponar (see Figure 11), Ekman, van Veen samplers). Grab samplers are lowered to the bottom and penetrate the sediments under their own weight. Jaws of the samplers are forced shut by weights, levers, springs or cables to retrieve samples from a known surface area.	These samplers are specifically designed for sampling deep and less stable substrates (e.g. sand, silt) usually found in depositional areas but some samplers can be adapted to shallow waters by rigging samplers on poles or by physically pushing samplers into the substrate. Organisms are often lost in 'washout' as devices are lifted onto the boat and removed from water. The 'jaws' of many samplers can also be easily blocked by debris.
	SCUBA diving: Diver-operated devices for sampling benthos, including suction samplers, grab samplers and corers.	This quantitative method can be used to sample a variety of deep-water habitats, including coarse substrates. However, the method is expensive.

Shallow water	**Shoreline sampling:** Typically involves wading in shallow near-shore areas of larger rivers, which can be a productive and diverse zone for macroinvertebrates. Conventional D-frame or other kick-nets are most commonly used at the wadeable margins and sampling involves kicks, dips, jabs, or sweeps in one or more habitat types. Surber and Hess samplers can also be used but require greater flow velocity. Grab samplers, corers, and suction samplers can also be used to sample fine sediments along the shoreline. When sampling larger substrate types that can be easily handled (e.g. rocks, woody debris/snags, macrophytes), macroinvertebrates may be removed by scrubbing the substrate with a soft brush or picking them individually with forceps.	Active sampling methods along the shoreline include a variety of qualitative, semi-quantitative, and quantitative techniques. While these methods have the advantage of not requiring the use of a boat and enabling sampling of a range of stable and unstable habitats: • samples can be variable due to diversity of habitat types and the patchy distribution of organisms, potentially requiring more replicate samples to reduce this variability • sorting macroinvertebrates from the debris of shoreline samples increases sample processing time and costs, and • sampling is difficult/impossible where there are steep drop-offs or cliffs at the river's edge.
	Snag sampling: Sampling woody debris or 'snags' (usually > 10 cm in diameter) which are known to be some of the most productive macroinvertebrate habitats of large, soft-bottomed rivers. Snags are most frequently sampled by placing a hand net on the downstream side and gently scrubbing the snag surface with a soft brush, allowing the current to carry dislodged material into the net. Specialised 'snag nets' and 'snag bags' also exist.	Can also be used in the deep waters of a main river channel, from a boat. Snags have an advantage over artificial substrates because, in addition to providing stable habitats, they are natural substrates and the decomposing wood and associated biofilms serve as a food resource for macroinvertebrates. However, irregular size and shape often make it difficult to standardise the area sampled. The length of time the snag has been in the water, or the period of colonisation, is also typically unknown.

3.3.6 Sample Labelling

All sample containers collected shall be clearly labelled both inside and out with the following information:

- site name (including stream name) and number
- sampling date
- sampler initials, and
- container (and replicate) number if applicable (e.g. '1 of 2', '2 of 2').

Although not essential, it is best practice to also include a sample code (i.e. a unique number for each sample that is not the site code), the sampling method (e.g. kick-net) and the dominant substrate type sampled (hard-bottomed or soft-bottomed).

A sticky label on the side of the sample container is recommended (Figure 12), with the information written using a permanent marker (i.e. waterproof and alcohol-resistant felt pen or pencil) prior to sample collection to ensure the label is dry. The internal label must comprise waterproof paper, either pre-printed or handwritten in the field **in pencil**.

Note: It is not sufficient to label container lids only – lids can easily be interchanged. Take care if using pre-printed labels to ensure that correct, matching sample labels are placed on the outside and inside of container. If labels are handwritten and a mistake is made, rewrite the label from scratch for clarity.

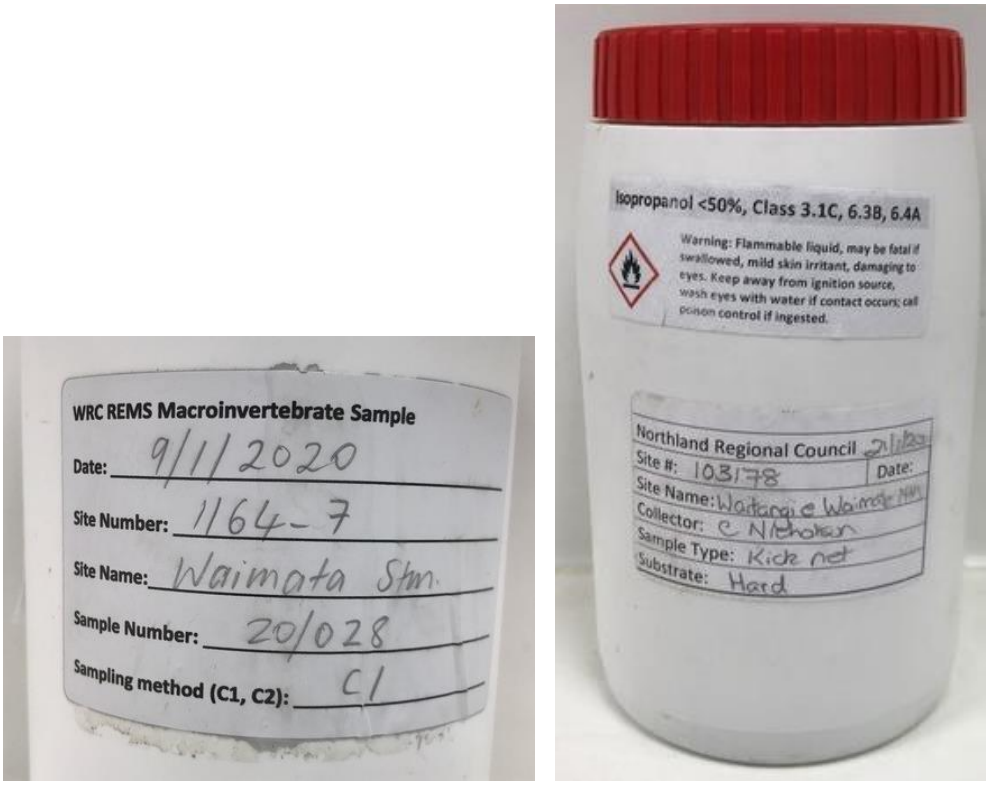

Figure 12– Examples of suitable sample labels.
Photos: EOS Ecology

3.3.7 Sample Preservation

Either isopropanol or ethanol preservative shall be added to the sample container immediately upon completion of sample collection. A 70–80% concentration (allowing for the stream water already present and any expected leaching of water from organic material) is required, with an air gap of approximately 1 cm left for safe transport (Figure 13). Ensure the container lid is tightly sealed before gently inverting the container to mix the preservative throughout the sample.

Note: The container should not be more than half full of sample material and the amount of stream water present should be minimised to ensure that the preservative is effective. If a large amount of fresh organic matter (e.g. macrophytes) is present in the sample, it may be necessary to change the preservative after 24-48 hours to ensure the desired concentration is maintained prior to storage and dispatch.

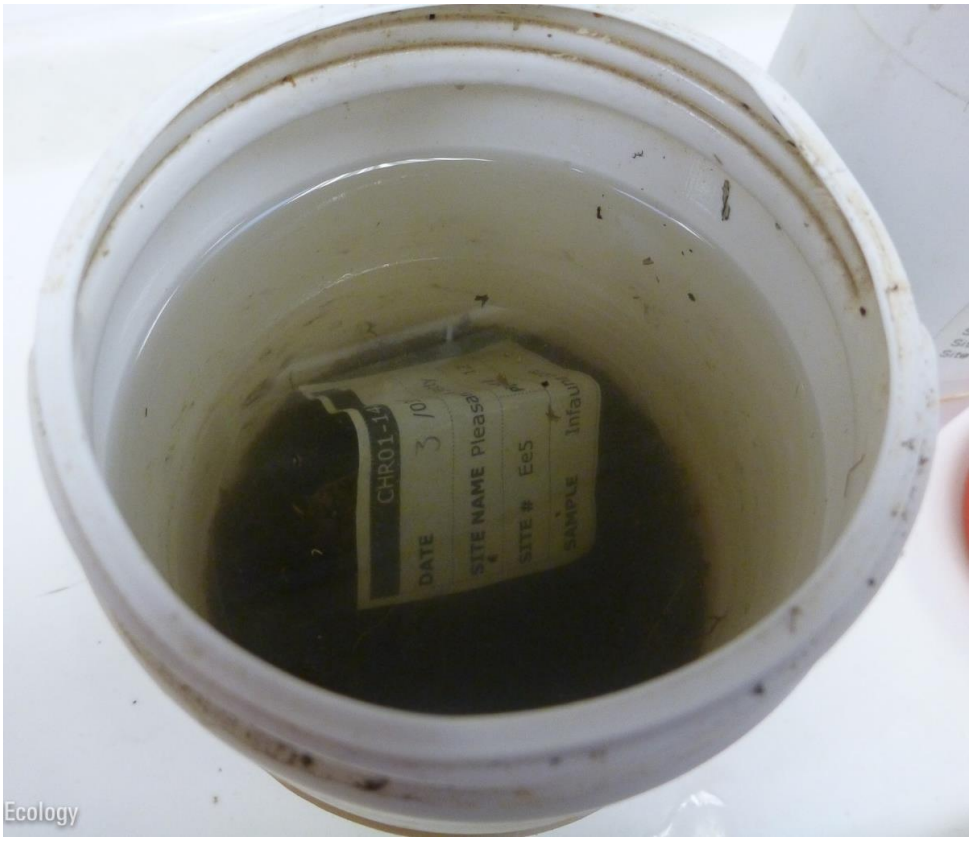

Figure 13 – The sample after preservative and a label has been added to the sample container – note the large ratio of preservative relative to sample

Photo: EOS Ecology

3.4 Decontamination

All field equipment and sampling devices should be properly cleaned to avoid transfer of biological pests (e.g. didymo *(Didymosphenia geminata)* and fragments of invasive aquatic weeds such as hornwort (*Ceratophyllum demersum*)) and contaminants between monitoring sites. This is particularly important where multiple streams or catchments are visited on the same day.

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

Note: Complete drying of equipment is the best form of decontamination.

3.5 Sample Transport, Storage and Handling

Preserved macroinvertebrate samples shall be stored in cool (preferably < 15°C) conditions within a hazardous storage facility prior to dispatch to the laboratory for processing. The container seals should be checked prior to storage to ensure preservative has not been lost.

It is recommended that samples are dispatched (in batches) to the processing laboratory in a sealed, sturdy plastic or polystyrene bin promptly after collection where dedicated storage conditions are available. Ensure the lids of all sample containers are tightly sealed and carefully pack the containers stored upright (vertical) to avoid leakage during transit. Each container should have a hazardous substances sticker on the outside.

Note: Transport in cardboard boxes is not recommended. Likewise, plastic bins must be very sturdy and well-sealed to support the weight of sample containers without leakage.

A courier approved for shipment of hazardous samples shall be used to transport the samples to the laboratory for processing. The courier ticket number(s) should be recorded on the Field Record Form to assist with prompt tracing of any bins lost or delayed in transit to the laboratory.

3.5.1 Chain of Custody

A Chain of Custody (CoC) Form (Annex C) shall be completed and inserted (within a sealed, waterproof bag) inside the bin for the reasons outlined in subsection 1.8. As a minimum, the CoC Form shall include:

- a list of each sample in the shipment, including the sample site name (exactly as written on the container label), site number, the dominant substrate sampled, the date and method of collection, and the number of containers used
- anything unusual about the samples, if relevant (e.g. sample contains a 'non-standard' amount of organic material), and
- the name of the person dispatching the samples and the date of dispatch.

If more than one 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 (e.g. 'Bin 2 of 2 – see CoC in Bin 1').

Note: It is recommended that the CoC Form is filled in electronically and a copy is sent to the laboratory, along with the printed copy. The electronic version will save the laboratory time with data entry and reduce the risk of transcription errors.

4 Supporting Field Measurements

In This Section

This section focuses on the collection of measurements that will support interpretation of the macroinvertebrate sample data: a mandatory stream habitat assessment and optional stream flow and water quality measurements.

4.1 Stream Habitat

Stream habitat, including riparian cover, and substrate and flow composition and variability strongly influence macroinvertebrate communities, including taxonomic richness and abundance. Maintaining a record of stream habitat features is therefore a critical component of SOE monitoring for tracking changes in macroinvertebrate communities and stream condition through time – and for understanding differences between sites.

In order to be eligible for a rating of QC 600 under this Standard, a rapid or basic habitat assessment (as a minimum) shall be performed at the time of macroinvertebrate sample collection. This assessment shall address the following characteristics along the length of the sampling reach:

- channel and bank characteristics – e.g. wetted channel width, vegetated bank width, channel shape, flow conditions, flow types (e.g. laminar, turbulent), lower and upper bank height, bank stability, the presence of undercut banks, and bank cover composition
- instream characteristics – e.g. streambed substrate, bed stability, the presence and type of macrophytes, shading, the presence of overhanging vegetation, and the abundance of periphyton, wood, moss and leaves
- riparian and catchment characteristics – e.g. the width of each riparian margin, stock access, stock damage, pest plants, riparian cover composition, adjacent land use and catchment land use.

It is beyond the scope of this Standard to specify the method of habitat assessment that shall be used. The reader is referred to *Stream Habitat Assessment Protocols for Wadable Rivers and Streams of New Zealand* (Harding et al., 2009) for guidance. These protocols include a qualitative Rapid Habitat Assessment (RHA) as well as more comprehensive semi-quantitative and quantitative habitat assessment protocols.

Note: Although the RHA is designed to be conducted from the stream bank, some monitoring agencies may prefer to assess periphyton and substrate cover in situ using an underwater viewer (e.g. bathyscope – Figure 14). The Wolman Pebble Count Procedure, performed walking the stream reach using a gravelometer (or ruler or equivalent), is a common technique to characterise the size of streambed substrates. Various protocols also exist to assess deposited fine sediment cover in streams (see Clapcott et al., 2011).

Clapcott (2015) revised the RHA protocol to enable a total 'habitat quality score' (HQS) to be determined for a site. The HQS is generated by assigning a score between 1 and 10 to each of the following habitat variables:

- deposited sediment

- invertebrate habitat diversity
- invertebrate habitat abundance
- fish cover diversity
- fish cover abundance
- hydraulic heterogeneity
- bank erosion
- bank vegetation
- riparian width, and
- riparian shade.

Figure 14 – Assessing streambed substrate cover using an underwater viewer (left) and substrate size using a gravelometer (right)

Photos: EOS Ecology (left) and Jo Hoyle

4.1.1 Non-SOE Monitoring

Semi-quantitative or quantitative stream habitat assessment protocols are recommended where macroinvertebrate samples have been collected for AEE or resource consent monitoring using protocols C3 and C4 of Stark et al. (2001). Refer to Harding et al. (2009) for details of suitable stream habitat assessment methods.

4.2 Stream flow

Stream flow conditions in the weeks leading up to and including the time of sampling will influence macroinvertebrate richness and abundance. Differences in the periods of time that have elapsed between the last significant 'flood' (or channel drying and re-wetting) and macroinvertebrate sample collection may be of interest when interpreting macroinvertebrate data between years.

Stream flow (or discharge) at the time of sampling may be determined from a nearby continuous flow recorder site, a site gauging at the time of sampling, or from reading water level at a staff gauge close to the monitoring site that has an established stage–discharge rating to convert water levels to approximate flows.

Further guidance on flow measurements is provided in:

- NEMS *Water Level*, and
- NEMS *Open Channel Flow*.

4.3 In-situ Water Quality

Water quality can influence macroinvertebrate richness and abundance. Although spot in-situ physico-chemical measurements are not overly informative (because macroinvertebrate communities generally reflect the time-integrated impacts of water quality), water temperature, dissolved oxygen, pH, conductivity and/or turbidity are easily measured in the field using a multi-probe meter to provide information on water quality conditions at the time of sample collection. Guidance on in-situ water quality measurements, together with water sample collection for nutrient analysis, is provided in the NEMS *Water Quality (Part 2 – Rivers).*

Note: Although not a requirement of this Standard, collection of conductivity and turbidity measurements can provide useful supporting information.

5 Laboratory Processing and Reporting

In This Section

This section focuses on activities undertaken by the processing laboratory. It addresses laboratory credentials, receipt of macroinvertebrate samples at the laboratory, sample preparation (sieving and sorting), sample processing (taxonomic identification and enumeration), quality control audits, macroinvertebrate metric calculation and reporting, and managing changes in taxonomic guides and laboratory providers.

5.1 Laboratory Credentials

There is currently no laboratory accreditation process in New Zealand that applies to macroinvertebrate sample processing. Under this Standard, macroinvertebrate samples shall be processed at a laboratory that can provide evidence of the following:

- adherence to formal standard protocols (i.e. Stark et al., 2001) or subsequently implemented protocols (i.e. this NEMS) for the processing of macroinvertebrate samples. This may include, but is not necessarily limited to, documenting all stages of processing, including internal and external quality assurance and quality control practices
- staff suitably qualified/trained in the sorting and identification of macroinvertebrate taxa to at least the taxonomic resolution specified in subsection 5.4.4 and Annex D, and
- regular (at least three-yearly) external quality control audits of macroinvertebrate taxa sorting efficiency and identification accuracy.

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.

5.2 Sample Receipt

Macroinvertebrate 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, if possible) return of this to the monitoring agency with a laboratory reference or job number.

5.2.1 Date of Arrival

Laboratory staff shall record the date of sample arrival at the laboratory on the CoC Form.

5.2.2 Sample Container Integrity on Arrival

Laboratory staff shall inspect the condition of sample containers on arrival at the laboratory. Any externally visible anomalies with the potential to impact processing (e.g. split or leaking containers) shall be recorded on the CoC Form and in their sample notes for inclusion in reporting of macroinvertebrate data (see subsection 5.5).

5.3 Sample Preparation

5.3.1 Sample Condition

Laboratory staff shall open and inspect each macroinvertebrate sample immediately prior to preparation for processing and record any anomalies in its condition with the potential to impact processing (e.g. odour indicating poor preservation and subsequent biodegradation of sample).

5.3.2 Sample Label

Each internal sample label should be checked to ensure that it contains the correct information (refer to subsection 3.3.6). This label should be cross-referenced to the external container label and information supplied on the CoC Form.

Note: Where an external and internal label differ, the details on the internal label should become the primary reference unless the CoC Form or monitoring agency indicate otherwise. The discrepancy should be noted in laboratory reporting.

5.3.3 Sieving

The macroinvertebrate sample container contents should be emptied onto a thoroughly rinsed, clean 0.5 mm sieve to remove preservative, fine sediment and any remaining unwanted organic material (e.g. whole leaves, twigs). Avoid using rinse water at high pressure which can break up oligochaetes and other fragile animals. Any used preservative should be contained for safe disposal or topped up with fresh preservative if it is to be reused later.

For sorting efficiency, a series of stacked sieves of different sizes (e.g. 4 mm, 2 mm and 0.5 mm) are commonly used to split the sample into manageable fractions for removal of macroinvertebrates or excessive detrital material (Figure 15). All macroinvertebrates removed from each fraction need to be re-combined with the main sample prior to processing.

Figure 15 – A series of nested sieves for sorting sample contents prior to processing samples, with Bogorov trays containing sorted samples for identification under a microscope

Photos: EOS Ecology

5.4 Sample Processing

5.4.1 Method

There are three common methods for processing macroinvertebrate samples in New Zealand, as described in Stark et al. (2001) and referred to in that document as Protocols P1, P2 and P3. This Standard requires macroinvertebrate samples collected for SOE monitoring-related purposes to be processed using a modified version of Stark et al.'s (2001) Protocol P2, referred to in this Standard as the **'200+ Fixed Count with Scan for Missed Taxa'** method. Broadly, this method involves randomly removing a representative fixed-fraction subsample and identifying all individual macroinvertebrates present, ensuring that a sufficient amount of subsample is removed to identify at least 200 individuals. This is then followed by a scan of the remaining sample residue for any additional taxa not present in the subsample (traditionally termed 'rare taxa' but, to avoid confusion with the threatened species classification of 'Rare', renamed in this Standard as 'missed taxa').

Note: Different subsample sizes (e.g. 100, 200 or 300 individual macroinvertebrates) have sometimes been used, with evidence that 100 individuals may be sufficient for robust assessments of most indices. However, for consistency and to reflect the dominant current practice across New Zealand, this Standard recommends a fixed count of 200+ individuals. A maximum quality rating of QC 400 shall apply where assessment of fewer than 100 individuals is made.

The 200+ Fixed Count with Scan for Missed Taxa method, along with Protocol P3 (Full Count with Subsampling Option) of Stark et al. (2001), is also suitable for the processing of macroinvertebrate samples collected using quantitative methods (e.g. Surber sampler) for AEE and consent monitoring purposes.

Note 1: Where employed, Protocol P3 should be modified from Stark et al. (2001) in accordance with Annex F. Although considered unnecessary, this protocol may also be applied to SOE samples.

Note 2: Protocol P1 (Coded Abundance) of Stark et al. (2001) is no longer recommended for use in routine SOE macroinvertebrate monitoring, nor AEE and consent monitoring. It remains a useful method for indicative screening assessments and community monitoring initiatives.

The processing method required for this Standard is described in Annex E and is equivalent to Protocol P2 described by Stark et al. (2001) but with some additional detail provided to clarify and standardise practice across laboratories. In particular:

- sorting and identification of the subsample shall be completed under a binocular (dissecting) microscope to reduce the number of missed individuals, and
- the scan for missed taxa shall be made by naked eye, with any missed taxa picked out then identified under a binocular microscope.

A Bogorov tray is useful for identification and enumeration of taxa in the subsample (Figure 16). Good lighting is also essential (Figure 17). A series of tally-counters can be a useful way to keep track of the counts of individual taxa, so that is easy to determine when 200 individuals have been counted.

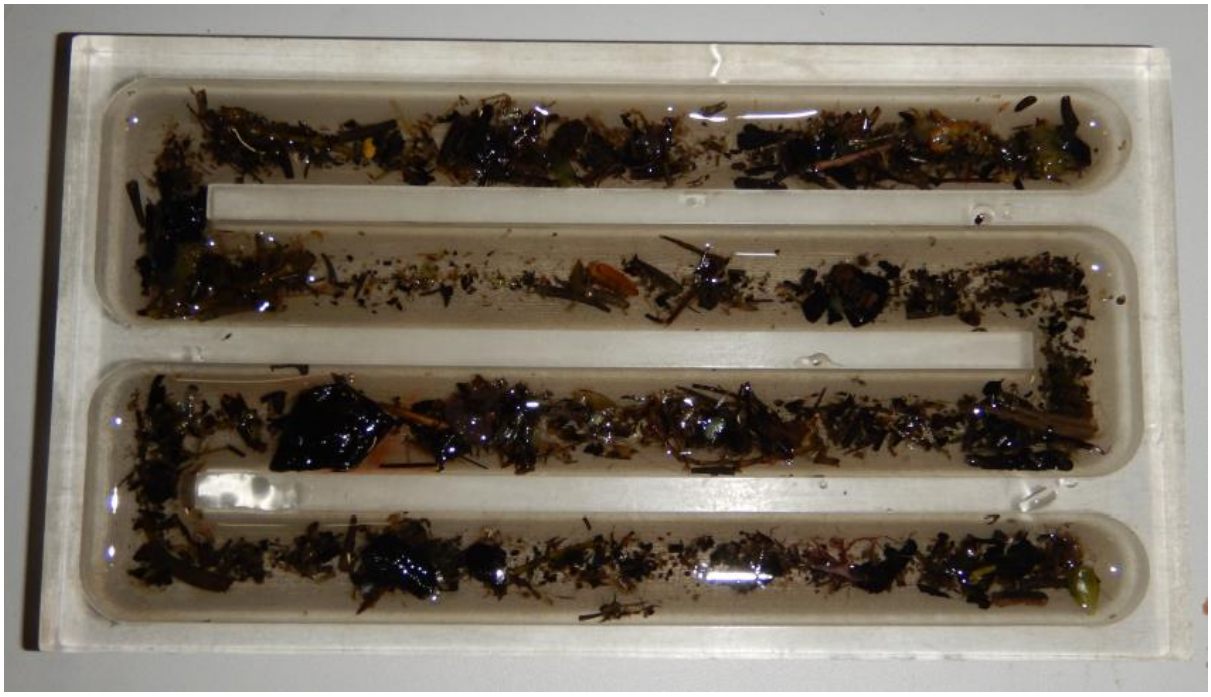

Figure 16 – Bogorov tray comprising a portion of a macroinvertebrate sample for sorting
Photo: Environment Canterbury

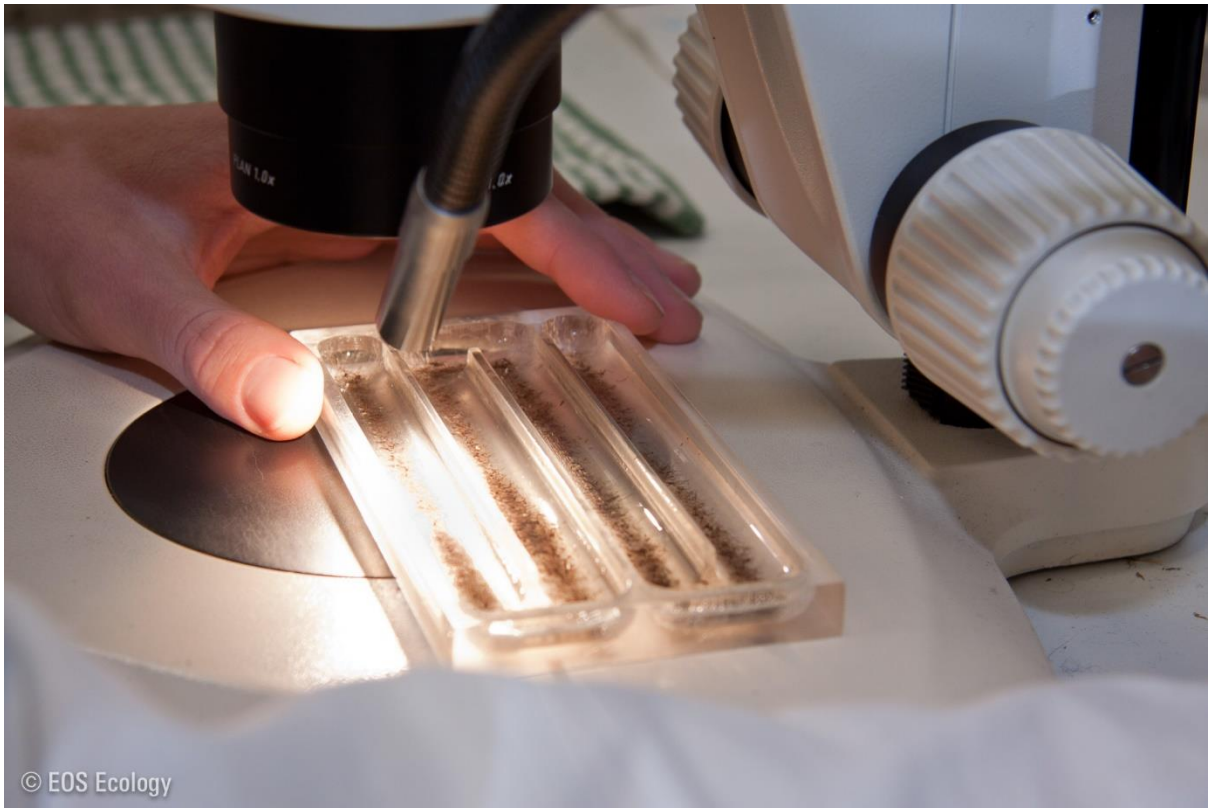

Figure 17 – Identification of macroinvertebrates in a subsample under a binocular (dissecting) microscope with additional lighting
Photo: EOS Ecology

5.4.2 Subsampling

Obtaining a representative and known amount of subsample is a critical part of the fixed-count processing method. This Standard recommends sample contents are equally distributed across a shallow white plastic tray(s) and a robust 'cookie cutter' or 'grid' device is used to isolate a randomly selected fixed portion(s) of the sample for processing (Figure 18). Care is needed to ensure that macroinvertebrates are not cut or otherwise damaged while using these devices.

Note: It is difficult to accurately remove a full portion of the contents of a grid(s) where grid lines have only been marked on the plastic tray.

A barrel sample splitter is an acceptable alternative method for subsampling (Figure 18, Annex E). It is essential that the sample contents in the barrel remain well mixed to ensure an even split of the sample contents. Multiple splits may be required to achieve a workable volume of sample.

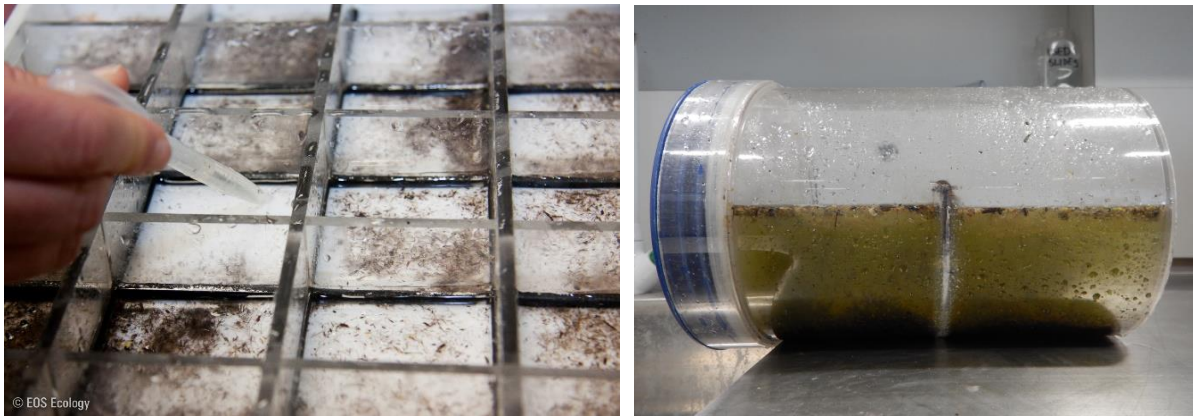

Figure 18 – Gridded sorting tray (left) and barrel sampler splitter
Photos: EOS Ecology (left) and Environment Canterbury

All contents of the subsample shall be identified and counted, regardless of whether 200 individuals have already been obtained before the subsample is completely processed. This is necessary to ensure that the portion of the total sample processed can be quantified (e.g. 3 of 12 gridded squares or one quarter of the sample split using a barrel sampler splitter) and reported, enabling counts of individuals within each subsample taxa to be scaled up for calculation of macroinvertebrate metrics on the full sample. This Standard requires the amount of the sample processed to be recorded.

Note: Do not re-mix the original sample between extraction of subsamples as this will dilute the unsorted fraction with that fraction from which macroinvertebrates have already been removed.

5.4.3 Taxonomic Identification

Recommended taxonomic guides for use in the identification of macroinvertebrate taxa include:

- *Guide to the Aquatic Insects of New Zealand* (Winterbourn et al., 2006)
- *Guide to the Freshwater Crustacea of New Zealand* (Chapman et al., 2011), and
- NIWA Macroinvertebrate identification guides (NIWA, n.d.).

Note 1: These guides are not complete and taxa names can change over time. See subsection 5.7.

Note 2: There is potential in the future for genetic techniques, such as eDNA, to be employed for taxa identification. Such techniques are very developmental at the present time but should be investigated in future reviews of this Standard.

It is a best practice recommendation under this Standard that laboratory providers maintain voucher specimens as part of a reference collection that can be used to verify taxonomic identifications.

5.4.4 Taxonomic Resolution

As a minimum requirement to be eligible for QC 600 under this Standard, macroinvertebrate taxonomic resolution shall be consistent with that outlined in Clapcott et al. (2017) and reproduced in Annex D. Every taxon present in a macroinvertebrate sample that has an assigned tolerance value in Annex D should appear in the taxa list for a site. Any taxon found that is not listed in Annex D shall be identified to the lowest practicable taxonomic level.

As an additional best practice measure, identification of macroinvertebrates to the lowest practical or defensible taxonomic level achievable (with a dissecting microscope) is recommended where possible; this additional information may be valuable for other purposes (e.g. biodiversity inventories, modelling, future index development).

Note: Identification of macroinvertebrates to the lowest practical or defensible taxonomic level will result in higher laboratory processing costs due to the added time involved. As outlined in subsection 5.6, macroinvertebrate metric calculations shall be standardised to the taxonomic resolution reproduced in Annex D.

A conservative approach to identification is appropriate; if a taxon cannot be positively verified based on the available information then a higher level of identification should be used. This is the case for early instar individuals, individuals that are damaged and missing essential identifying features, or taxa that cannot be identified by morphology alone, are not described, or belong to a species-complex. For example, the common snail *Potamopyrgus* is often identified to species level, but based on morphology alone (i.e. without genetic analysis) identification to species level is, at best, an educated guess. More caddisfly species have been described at the adult than the larval stage so identification of larvae to species level is often impossible or unreliable.

Note: Although pupae are not considered in sorting protocols (see subsection 5.4.5), they can act as a surrogate for adults and, therefore, provide a substantially greater confidence in taxonomic validation than the juvenile stage alone.

5.4.5 Taxa Enumeration

Empty shells (e.g. molluscs), empty cases (e.g. caddisflies), and pupae (e.g. Diptera and caddisflies) shall not be counted (but may be used to confirm or increase identification confidence). The following requirements apply when counting damaged/incomplete animals:

- the head of an individual shall only be counted if it can be clearly identified, and
- only the rounded portions (i.e. the ends) of fragmented worms shall be counted, with the total divided by two to obtain the number of worms present.

Note: While every effort should be made during sample collection and processing preparation not to damage any macroinvertebrates, it is inevitable that many individuals found in a sample may be missing body parts.

5.4.6 Quality Checks

Quality checks specific to the 200+ Fixed Count with Scan for Missed Taxa method are outlined here. General internal and external quality control measures that apply to macroinvertebrate sample processing are outlined in subsection 5.5.

Note: Quality control is normally denoted QC and forms a component of QA. To avoid confusion with NEMS quality codes (e.g. QC 400), quality control is written in full in this subsection (and elsewhere in this document).

5.4.6.1 Sorted and sample residue

On completion of sample processing there should be four vials retained for quality control purposes: '200+ individuals', 'missed taxa', 'sorted residue' and 'sample residue' – as outlined in Annex E and below:

- 'sorted residue' – the subsample from which the 200 (or more) individual macroinvertebrates have been removed (and retained in a separate vial), containing detritus, organic matter and, to a lesser extent, inorganic material such as sand or small gravels. This residue is retained in a separate container for the quality control check of sorting efficiency, and
- 'sample residue' – the unsorted remainder of the macroinvertebrate sample (retained in the original sample container) that has been scanned for missed taxa by the processing laboratory (with these taxa retained in a 'missed taxa' vial). This residue is scanned by the quality control auditor for any additional taxa missed by the processing laboratory.

Each vial and container shall be clearly labelled with its component name, site name/code, unique laboratory processor identifier and sample collection date.

5.4.6.2 External Quality Control

Under this Standard, at least once every three years the monitoring agency shall engage an independent laboratory to re-examine 10% of their annual SOE macroinvertebrate samples to check the processing laboratory's accuracy of taxa identification, sorting and enumeration. The samples to be re-examined shall be selected by the monitoring agency after samples have been processed and the results of this independent laboratory examination shall be documented in a quality control report that includes a unique processor identifier number (e.g. LP1, LP2, LP3, etc.). This enables traceability of individual processors with specific samples and can expediate remedial processes if performance issues arise, if required. An example quality control report is provided in Annex G.

Taxonomic Identification

The accuracy of taxonomic identification is the primary (but not sole) focus of the quality control check. The 'picked' animals in the '200+ individuals' vial and the 'missed taxa' vial shall be examined to determine the number of individuals incorrectly identified. This quality control check shall be reported as percentage Taxonomic Disagreement (%TD) in accordance with Table 2, where:

- an individual sample passes quality control if %TD is ≤ 10%; and
- this component of quality control passes overall if the average %TD across the batch of quality control samples (the batch error, $\%BE_{TD}$) is ≤ 10%.

Note: These calculations of quality control pass/fail represent a change from Protocol P2 described by Stark et al. (2001). Annex E outlines the quality control requirements in detail.

Where quality control of taxonomic identification fails, whether for a single sample or as a batch of samples, the original taxa list shall be corrected to align with the quality control results. The original processing laboratory shall also investigate and report on the causes for the quality control failure and proposed amendments to internal quality control processes to improve future performance. One action may be to examine a further 10% of samples for taxonomic accuracy.

No adjustments shall be made to the original dataset where quality control is deemed to have passed, irrespective of whether or not some minor discrepancies were identified.

Note: If the correct taxonomic identification of a macroinvertebrate is disputed, then a specimen should be checked by an agreed expert (i.e. third party).

Sorting Accuracy

Two different elements of sorting accuracy shall form a secondary focus of quality control:

1. Missed taxa – the unsorted 'sample residue' shall be checked for additional taxa not recorded by the laboratory processor. The residue shall be scanned visually by naked eye for up to 30 minutes, with any new missed taxa identified under a binocular microscope.
2. Missed individuals – the 'sorted residue' shall be checked to identify whether any individual macroinvertebrates (or additional taxa) were missed during the removal of the 200+ individual macroinvertebrates. The residue shall be emptied into a Bogorov tray and systematically scanned under a microscope to search for missed individuals.

Note: Quality control of sorting accuracy can be very time consuming and results depend on effort. An assessment by Smith (2020) found that the majority of missed taxa are recovered in the first 10 minutes of searching. Allowing a maximum of 30 minutes will standardise effort across quality control providers whist ensuring that most missed taxa are recovered.

The respective errors for sorting accuracy quality control checks across sample residue and sorted residue are outlined in Table 2. As with %TD, the checks shall be reported as percentage errors (percentage Missed Taxa, %MT, and percentage Missed Individuals, %MI), where quality control passes overall if the mean error across the batch of quality control samples is ≤ 20% for %MT and ≤ 10% for %MI.

Note: An individual from an unrecorded taxon found in the sorted residue should count as both a missed taxon under Sorting Accuracy 1 (%MT) and a single individual missed under Sorting Accuracy 2 (%MI). The higher error threshold of 20% applied to the MT check reflects the fact that these taxa need to be 'picked' from (often significant volumes of) the unsorted sample residue and are generally low in abundance.

Where one or more components of quality control of sorting accuracy fail, the processing laboratory shall investigate the causes(s) of the failure and, where necessary, amend its internal quality control processes to improve future performance.

Overall pass/fail

Each of the three batch quality control components ($\%BE_{TD}$, $\%BE_{MT}$ and $\%BE_{MI}$) shall pass for external quality control to pass overall. Where one or more components fail, this shall be reflected in a lower quality code that is assigned to all samples (subsection 6.2.1), unless the original processing laboratory is able to rectify the causative errors through corrective action and verify this through the results of further external quality control of this batch of samples.

Table 2: Summary of quality control metrics under this Standard

Note: The pass requirements apply to an individual quality control sample. To pass a quality control metric overall, the mean error across the batch of quality control samples shall be ≤ 10% for %TD and %MI and ≤ 20% for %MT. Failure of one or more of the quality control metrics at the batch level may impact the quality rating assigned to the monitoring agency's data (see subsection 6.2.1).

Quality control component	**Quality control metric**	**Calculation**	**Pass requirement**
Taxonomic accuracy	% Taxonomic Disagreement (%TD)	$\frac{No.\,misidentified\ individuals}{Total\ no.\,individuals} \times 100$ Where *No. misidentified individuals* is the total number of individuals misidentified by the laboratory in the '200+ individuals' and 'missed taxa' vials , *and Total no. individuals* is the total number of individuals in the '200+ individuals' and 'missed taxa' vials, as counted by the quality control processor	%TD shall be ≤ 10%
Sorting accuracy of 'sample residue' (for missed taxa)	% Missed Taxa (%MT)	$\frac{No.\,additional\ missed\ taxa}{Total\ no.\,taxa} \times 100$ Where *No. additional missed taxa* is the number of missed taxa identified in the sample residue by the quality control processor that were not identified by the laboratory, and *Total no. taxa* is the total number of all taxa identified (including those in the '200+ individuals' vial, the 'sorted residue', and the 'sample residue') by the quality control processor	%MT shall be ≤ 20%
Sorting accuracy of 'sorted residue' (for missed individuals)	% Missed Individuals (%MI)	$\frac{No.\,missed\ individuals\ in\ sorted\ residue}{Total\ no.\,individuals} \times 100$ Where *Total no. individuals* is the total number of individuals in the 'sorted residue' and '200+ individuals' vial combined, as counted by the quality control processor	%MI shall be ≤ 10%

5.5 Quality Control

Both internal and external quality control measures shall be implemented to provide confidence in laboratory performance. Internal measures should address the full range of activities associated with sample processing and include training and upskilling requirements. Correct taxonomic identification underpins good quality macroinvertebrate data and shall be a key focus for both internal and external quality control checks. The quality control checks need to be performed at the level of individual processors within laboratories.

5.5.1 Internal Quality Assurance and Quality Control

Laboratory internal QA and quality control practices fall outside of the scope of this Standard. However, as a minimum, the following practices would be expected:

- an internal check of 5–10% of processed samples, conducted on a regular, ongoing basis to enable rapid detection of processing errors (i.e. missed taxa and missed individuals)
- two-yearly reviews of the processing performance of individual laboratory staff, and
- maintenance of a voucher specimen reference collection.

5.5.2 External Quality Control

External quality control shall be managed by the monitoring agency on the basis of having external quality control of macroinvertebrate samples processing undertaken at least once every three years by an independent laboratory as described in subsection 5.4.6.2. The results shall be documented in a Quality Control Report.

5.6 Laboratory Reporting

The processing laboratory shall report the macroinvertebrate sample results for each site together with:

- the date(s) of sample collection, and both sample receipt and sample processing at the laboratory
- the collection and processing method, including the amount of the sample processed
- a unique identifier code for the laboratory staff member who processed each sample, and
- any anomalies in the condition of the sample upon receipt or commencement of processing (e.g. odour arising from inadequate sample preservation).

5.6.1 Macroinvertebrate Metrics

Macroinvertebrate metrics may be calculated and reported by the laboratory or by the monitoring agency after receipt of the laboratory data. The metrics for SOE and similar monitoring shall be calculated at the taxonomic resolution outlined in Annex D, in accordance with the methods and description in Table 3.

Note: It is recognised that the Macroinvertebrate Community Index (MCI) 'pollution' tolerance values were developed more than 30 years ago and some macroinvertebrate taxa lack a tolerance value. Greenwood et al. (2015) presented a combined set of revised or new MCI tolerance values for 234 taxa found in hard-bottomed streams across New Zealand. However, at the present time, these

values are not widely in use. Until such time as an updated national dataset is formally introduced, the tolerance values presented in Annex D shall apply under this Standard.

Under this Standard, any other metrics calculated on macroinvertebrate data collected for SOE and similar monitoring purposes shall also be calculated at the taxonomic resolution outlined in Annex D.

Table 3: Requirements for calculation of macroinvertebrate metrics under this Standard

Note: This table is based on collection of macroinvertebrate samples using a kick-net (in accordance with subsection 3.3.5) and subsequent processing using the 200+ Fixed Count with Scan for Missed Taxa method. The table should be read in conjunction with Annex D. Calculations are from Stark and Maxted (2007) unless indicated otherwise.

Metric	Units	Requirements/Notes	Calculation
Taxa richness	N/A	The taxonomic resolution shall be consistent with that provided in Annex D. Any taxa not on this list shall be identified to the lowest practicable or defensible taxonomic level.	Calculated based on presence data by summing the total number of different taxa present in the macroinvertebrate sample. *Note: Under the fixed-count method, where subsampling is usually required, taxa richness is based on the taxa found in both the subsample and the sample residue (i.e. missed taxa).*
MCI_{HB} and MCI_{SB}		The Macroinvertebrate Community Index (MCI) comprises a set of macroinvertebrate pollution tolerance values developed for hard-bottomed taxa (mostly genus level) (MCI_{HB}) and a separate set of values derived for taxa in soft-bottomed streams (denoted MCI_{SB}). The MCI_{HB} and MCI_{SB} shall be determined using the taxonomic resolution and tolerance values provided in Annex D. *Note: The soft sediment substrate classifications used in the report from which Annex D is sourced (Clapcott et al., 2017) are based on version 1 of the River Environment Classification (REC), which does not accurately characterise some stream reaches in New Zealand. Substrate should be field-verified to confirm the appropriate classification.*	Calculated based on presence data as follows: $MCI = \frac{\sum_{i=1}^{i=S} a_i}{S} \times 20$ where S = the total number of scoring taxa in the sample, and a_i = the tolerance score for the ith taxon. *Note: Under the fixed-count method, where subsampling is usually required, the MCI shall be determined based on the taxa found in both the subsample and the sample residue (i.e. missed taxa).*

Metric	Units	Requirements/Notes	Calculation
$QMCI_{HB}$ and $QMCI_{SB}$		The Quantitative Macroinvertebrate Community Index (QMCI) shall be determined for hard-bottomed ($QMCI_{HB}$) and soft-bottomed ($QMCI_{SB}$) streams using the taxonomic resolution and tolerance values outlined for MCI above. *Note: The QMCI shall be determined based on the taxa found in both the subsample and the sample residue (i.e. during the scan for missed taxa). An abundance of 0.5 shall be applied to each taxon picked up in the missed taxa scan.*	Calculated based on count (abundance) data as follows: $QMCI = \sum_{i=1}^{i=S} \frac{(n_i \times a_i)}{N}$ where S = the total number of taxa in the sample, n_i = the abundance for the ith scoring taxon, a_i = the tolerance score for the ith taxon and N = the total abundance of the scoring taxa for the entire sample.
EPT-HA taxa richness		Two genera belonging to the family Hydroptilidae, *Oxyethira* and *Paroxyethira*, shall be excluded from metric calculations. *Aoteapsyche* and *Orthopsyche* shall be recorded separately as *Hydropsyche-Aoteapsyche* and *Hydropsyche-Orthopsyche,* respectively. *Note: Strictly speaking, Oxyethira and Paroxyethira should be included in the EPT taxa richness metric but, for consistency in application and common interpretation of EPT metrics under this Standard, these taxa shall not be included (as denoted by -HA).*	Calculated from presence/absence data as the number of taxa belonging to the orders Ephemeroptera, Plecoptera or Trichoptera.
% EPT-HA taxa richness	%		Calculated from presence/absence data as the percentage of taxa belonging to the orders Ephemeroptera, Plecoptera or Trichoptera.
% EPT-HA abundance	%		Calculated from relative abundance data as the percentage of individual macroinvertebrates that belong to the orders Ephemeroptera, Plecoptera or Trichoptera.
ASPM		The Average Score Per Metric (ASPM) is a multi-metric index developed by Collier (2008). *Oxyethira* and *Paroxyethira* shall be excluded from the EPT-related metric calculations.	Calculated from three metrics – the MCI, EPT-HA taxa richness and % EPT-HA abundance (each calculated as outlined above) – by taking the mean of the three metrics. Each metric is firstly scaled (normalised) by: $X' = [X - X_{min})] / [X_{max} - X_{min}]$ where X' is the scaled site score, X is the raw site score and X_{min} and X_{max} are the minimum and maximum site scores of the entire dataset. When normalising scores for the ASPM, use the following minima and maxima: %EPT-HA abundance (0–100), EPT-HA taxa richness (0–29), MCI (0–200).

5.7 Managing Changes in Taxonomy and Laboratory

Because the objective of most long-term macroinvertebrate monitoring programmes is to be able to detect changes through time, long-term monitoring programmes would ideally retain the same taxonomy and processing laboratory to avoid the potential for inconsistencies that can arise when a change is made to either of these. However, this is unrealistic; taxonomy changes through time, as will laboratory contracts. The effects of these changes can be minimised through ensuring that:

- only laboratories with the credentials outlined in subsection 5.1 are utilised, and
- good documentation is maintained of sample processing details, including consistent use of taxonomic names in accordance with Annex D.

6 Data Processing and Quality Checking

In This Section

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

6.1 Data Types

Macroinvertebrate data can be split into several data types:

- 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 3.3.4), which are specific observations recorded on a Field Record Form about where and when macroinvertebrate samples and associated field measurements were collected, and
- macroinvertebrate data, which generally consist of taxa lists and calculated metrics.

All three data types shall be stored in a database.

6.1.1 Site Metadata

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

6.1.2 Visit Metadata

Adequate mechanisms shall be put in place to store all relevant visit-related metadata listed in subsection 3.3.4, together with the macroinvertebrate and associated data (subsection 6.1.3).

6.1.3 Macroinvertebrate Data and Associated Measurements

Each macroinvertebrate dataset comprising the taxa list(s), calculated metrics and habitat assessment data shall be stored with:

- the measurement date and time
- all relevant visit-related metadata (subsection 3.3.4), including the name(s) of personnel who conducted the sampling and collected any associated field measurements
- processing laboratory name and location
- processing method and laboratory staff processing identification number
- any relevant comments from the laboratory report (subsection 5.6), and
- an associated quality code (assigned in accordance with subsection 6.2.1).

Note: Depending on the level of taxonomic resolution requested of the processing laboratory, there may be two sets of macroinvertebrate taxa to store: a set at the taxonomic resolution required for calculation and reporting of metrics under this Standard and a second set to a finer taxonomic resolution.

6.2 Data Processing

Processing of macroinvertebrate data primarily involves the monitoring agency assigning quality codes through consideration of field practices, sample preservation efficacy, documentation and laboratory practices. Any adjustments to data should be documented.

6.2.1 Quality Coding

6.2.1.1 Performance

All macroinvertebrate data shall be quality coded in accordance with the NEMS *Quality Code Schema* (Figure 19) and matrix (Table 4) that forms part of this Standard. A quality code shall be assigned to each individual metric generated from the list of taxa identified and enumerated from a discrete macroinvertebrate sample.

The schema comprises seven 'parent' codes and permits valid comparisons within and across multiple data series. In most cases, macroinvertebrate metrics determined from long-term (e.g. SOE) monitoring will be assigned QC 400, QC 500 or QC 600. Table 4 provides a framework for calculating 'demerit' points associated with each individual macroinvertebrate metric, the total of which is then used to assign a quality code, where:

- QC 600: < 3 points
- QC 500: 3–11 points *
- QC 400: 12+ points

***However, if 3 or more criteria are in the '3 points' column of Table 4, the data are deemed to be a maximum of QC 400.**

Note: 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*.*

6.2.1.2 Considerations

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

- office and Field Manual (subsection 1.1)
- field Record Form (subsection 3.3.4 and Annex B)
- completed Chain of Custody Form (subsections 3.5.1 and 5.2, and Annex C), and
- laboratory report (subsection 5.6).

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

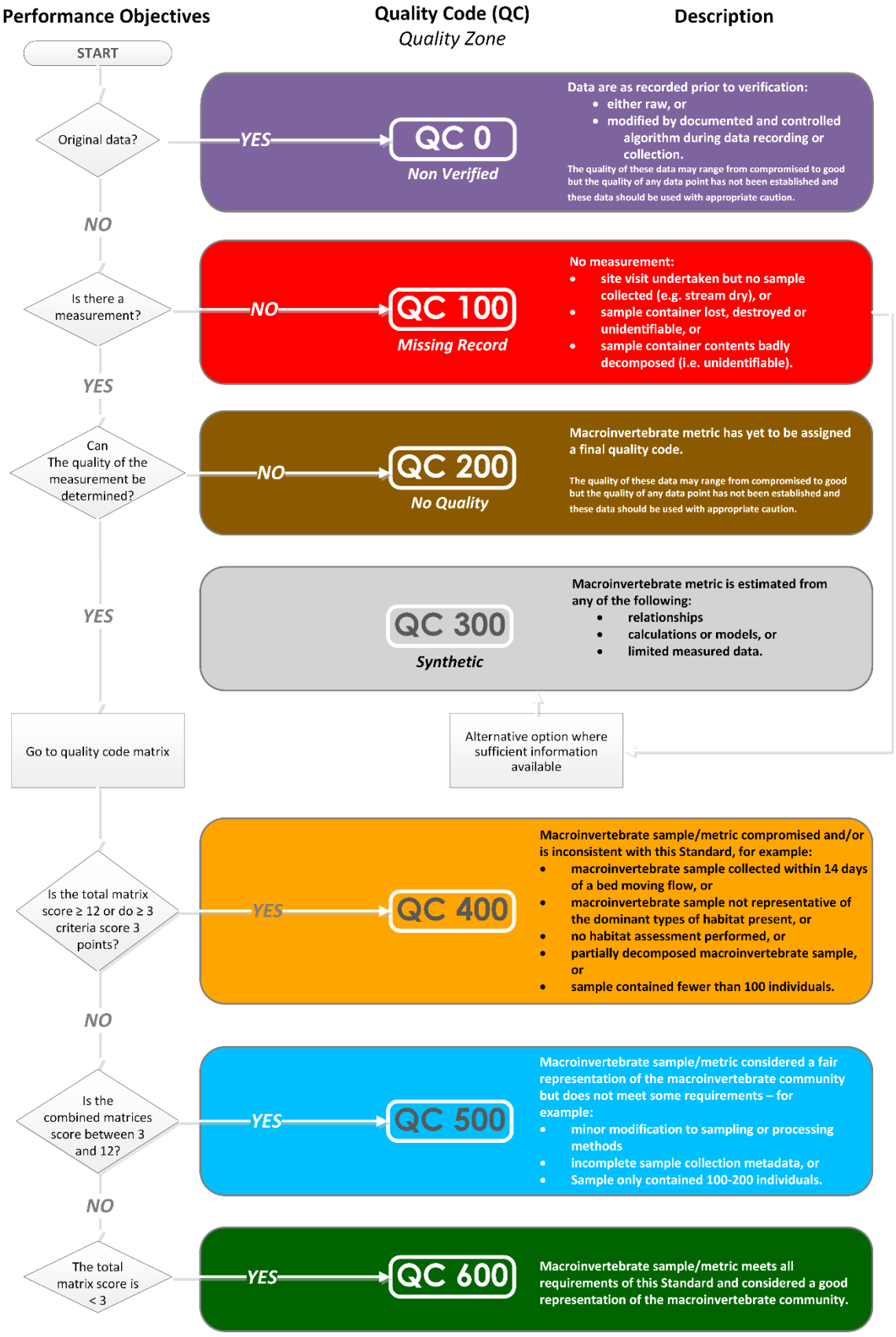

Figure 19 – NEMS *Macroinvertebrates* Quality Coding Schema

Use the flowchart as a framework to assign quality codes to macroinvertebrate data (metrics) using the associated matrix (Table 4).

Table 4: Criteria for assigning 'demerit' points to macroinvertebrate metrics under this Standard. The total number of points is then used in the flowchart (Figure 19) to assign a quality code to macroinvertebrate metrics.

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

Criteria	12 points	3 points	1 point	0 points
Field and office				
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 since the last sampling occasion
Site Stationarity *(subsection 2.2)*		Different sample location that is not truly representative of the original site		Sample location consistency maintained *Note: Where a site has to be relocated (e.g. because of a flood or change in access), the new site shall be representative of the broader stream reach and close to the original site.*
Equipment Specifications *(subsection 1.5)*	A D-net or equivalent hand-net with a mesh size > 0.5 mm was used		A D-net or equivalent hand-net with a mesh size < 0.5 mm was used	A D-net or similar hand-net with 0.5 mm mesh size was used

Criteria	12 points	3 Points	1 Point	0 Points
Visit Metadata *(subsection 3.3.4)*	No metadata recorded other than the site and date sample collection	Visit metadata generally recorded in accordance with the Standard but lacks a site photo or details of the habitats sampled	All visit metadata recorded except time of sample collection	Visit metadata captured on a Field Record Form in accordance with the Standard, including: • the location, date and time of sample collection • the sampling equipment (including net width) and method used • the proportions of each streambed meso-habitat sampled and the number of sample unit efforts ('kicks'), and • the environmental conditions under which the sample and any associated field measurements were collected
Sample Representativeness *(subsection 3.1.1)*	Sample not representative of the site (e.g. limited to riffle mesohabitat when run and other mesohabitats were present in high abundance)	Multiple mesohabitats sampled but not in proportion to their relative abundance		All common wadeable mesohabitats likely to support macroinvertebrates sampled in proportion to their abundance across the sampling site (reach)
Sampling Effort *(subsection 3.1.1)*	A single composite sample was collected comprising fewer than four unit efforts and/or less than 100 individuals			A single composite sample was collected comprising between four and eight unit efforts across a total area of 0.6–0.9 m^2 *Note: Additional sample effort is acceptable for reaches characterised by low densities of invertebrates in order to obtain at least 200 individuals.*
Sample Collection Timing *(subsection 3.2.1)*	Sample collected outside of November to April inclusive			Sample collected between November and April inclusive

Criteria	12 points	3 Points	1 Point	0 Points
Antecedent Flow Conditions *(subsection 3.2.2)*	Sample collection occurred within 14 days of a bed-moving stream flow (or within 7 days, if sampling was of a hard-bottomed stream in a high rainfall or heavily urbanised catchment)	Sample collection occurred within 14 days of a stream flow exceeding three times the median that may have resulted in bed movement (or within 7 days, if sampling was of a hard-bottomed stream in a high rainfall or heavily urbanised catchment)		Sample collection did not occur within 14 days of either a stream flow exceeding three times the median or, where known, the bed movement conditions specified under this Standard OR Sample collection was from a hard-bottomed stream in a high rainfall or heavily urbanised catchment and macroinvertebrate sample collection did not occur within 7 days of a stream flow exceeding three times the median
Stream Habitat Assessment *(subsection 4.1)*	No habitat assessment performed with macroinvertebrate sample collection	A stream habitat assessment was performed at the time of macroinvertebrate sample collection but it did not encompass all three of the characteristics listed under the Standard		A basic stream habitat assessment (at minimum) was performed at the time of macroinvertebrate sample collection addressing stream channel and bank characteristics, instream characteristics and riparian and catchment characteristics
Laboratory				
Laboratory Credentials *(subsection 5.1)*	Sample sorted and/or processed by staff not sufficiently trained in taxonomic identification and/or the procedures required under this Standard, including a lack of evidence of internal QA procedures	Sample sorted and processed by staff trained in taxonomic identification following a documented procedure that is consistent with the supporting information contained in this Standard but no evidence of ongoing internal QA procedures		Sample sorted and processed by staff trained in taxonomic identification following a documented procedure that is consistent with the supporting information contained in this Standard and includes evidence of internal and external QA and quality control practices

Criteria	12 points	3 Points	1 Point	0 Points
Sample Traceability *(subsections 3.5.1, 5.2 and 5.3.2)*	Sample received with illegible identification but site location and date can be inferred from other samples or information	Sample received with Chain of Custody and legible labels but lacks details other than a unique sample identifier and collection date	Sample received with Chain of Custody and legible labels comprising all minimum details required under this Standard except one of either the sampler initials, site name or site number	Sample received with Chain of Custody and legible labels comprising all minimum details required under this Standard, including: • site name and number • sampling date • sampler initials, and • container number (if applicable)
Sample Condition *(subsections 5.2.2 and 5.3.1)*	There were signs to indicate that soft tissues of taxa were decomposed to the point that the majority of individuals of some taxa could not be identified or enumerated	Some invertebrates in the sample were noted as being degraded such that, whilst identification was undertaken, identification surety was compromised for the majority of individuals of some taxa	Minor signs of sample leakage but sample integrity unaffected	Sample condition was as expected for a well-preserved sample and identification and enumeration of taxa was possible
Sample Processing Method *(subsection 5.4.1)*	A total of less than 100 individuals were enumerated from the sample OR Sample was processed using a different method to the 200+ Fixed Count with Scan for Missed Taxa method or Full Count with Subsampling Option method	Sample was processed using a modification of the 200+ Fixed Count with Scan for Missed Taxa method set out in this Standard (e.g. identification of taxa by eye)	Sample was processed using the 200+ Fixed Count with Scan for Missed Taxa method in accordance with the requirements of this Standard but the total number of individuals enumerated in the sample was between 100 and 200 OR Sample was processed using the Full Count with Subsampling Option method	Sample was processed using the 200+ Fixed Count with Scan for Missed Taxa method in accordance with the requirements of this Standard

Criteria	12 points	3 Points	1 Point	0 Points
Taxonomic Identification and Resolution *(subsections 5.4.3 and 5.4.4)*	Taxonomic identification and resolution consistently did not meet the minimum requirements outlined in Annex D*			Taxonomic identification and resolution were, as a minimum, consistent with that outlined in Annex D*
Metric Calculation and Reporting *(subsection 5.6)*	Macroinvertebrate metrics not calculated and reported in accordance with the macroinvertebrate taxa list and tolerance values in Annex D	Macroinvertebrate metrics calculated and reported using the macroinvertebrate taxa tolerance values in Annex D, but the amount of the sample processed as a fixed count was not provided		Macroinvertebrate metrics calculated and reported using the macroinvertebrate taxa list and tolerance values in Annex D, along with a unique identifier number for the sample processor, the amount of sample processed as a fixed count, and any anomalies in sample condition

External Quality Control (QC) *(subsection 5.4.6)*	No evidence of external quality control being conducted for the monitoring agency within the last three years as required under this Standard OR External quality control requirements under this Standard carried out within the last three years for the monitoring agency, but the quality control component for taxonomic identification (as average %TD) failed and the errors were not able to be rectified through corrective action	Evidence of external quality control being conducted for the monitoring agency in the last three years but one or both measures of sorting accuracy (%MT and/or %MI) across the quality control sample batch failed to meet the requirements of this Standard	Evidence provided of conducting external quality control for the monitoring agency in the last three years but the quality control batch comprised only 5–10% of the SOE sample batch and/or the % Missed Individuals metric batch error was between 10 and 20% OR Evidence of external quality control being conducted for the monitoring agency in the last three years and one or more of the quality control components for taxonomic identification (as average %TD), missed taxa (as average %MT) and/or missed individuals (as average %MI) failed, but evidence provided that the errors were rectified through corrective action	Evidence provided of conducting and passing, within the last three years, the external quality control requirements under this Standard for macroinvertebrate taxa identification and sorting accuracy, as applied to a subset of 10% of the monitoring agency's SOE samples
Total Score				

**Notwithstanding identification issues for taxa at different life stages or where condition is compromised*

6.2.1.3 Batch Coding

Undertaking 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.

6.2.1.4 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.

6.3 Data Preservation, Storage and Audits

National requirements for preserving, storing (including archiving) and auditing environmental data are currently under establishment as part of the wider NEMS initiative. In the interim, macroinvertebrate data, habitat assessment data and visit metadata shall be stored together in a data system, linked with a single date. 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.

Any data that have 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,
- correction of a taxon identification following failure in an external quality control check, and
- correction of a macroinvertebrate metric (e.g. MCI).

Annex A – List of Referenced Documents

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Annex B – Example Field Record Form

Field Record Form: Macroinvertebrates

Programme Name _____

Stream Name _____

Site Name _____

Site Code _____ **Sample No.** _____

Reach Length _____ m

GPS Location (midpoint of reach) Easting _____ Northing _____

Date _____ **Time (NZST)** _____

Field Personnel _____

Visit Metadata

Weather ○ Fine ○ Overcast ○ Drizzle ○ Rain Rain in last 24 hours? ○ Y ○ N

Stream Level ○ Low ○ Normal ○ High ○ Other _____ m Odour ○ Y ○ N

Comments *(e.g. local bank erosion, change in riparian cover, stream discoloured, new hazards, etc.)*

Collection Details

Method ○ Kick-net ○ Other (describe) _____

Net Width (base) _____ cm Mesh size ○ 0.5 mm ○ Other _____ mm

Substrate(s) and Habitats Sampled _____
(e.g. riffle 30%, run 30%; macrophytes 50%, bank margins 25% and woody vegetation 25%)

All Habitats Sampled in Proporton to Abundance? ○ Yes ○ No

Streambed Area Sampled _____ m^2 No. of kicks _____

Preservative ○ Ethanol ○ Isopropanol ○ Other (describe) _____

Supporting Measurements

Habitat Assessment? ○ Yes Method: _____ ○ No

In-situ Water Quality Measurements? ○ Yes Variables: _____ ○ No

Flow Measurement? ○ Yes _____ L/s ○ No

Other _____

Other Notes *(e.g. deviation from protocols, QA/QC)*

Annex C – Example Chain of Custody Form

Chain of Custody Form for Macroinvertebrate Samples

Client Name _____ **Client Contact** _____

Client Reference _____ **Email*** _____

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

Client to complete

Sample Dispatch Date _____

Name _____ Signature _____

Additional Notes _____

_____

_____

Laboratory to complete

Sample Arrival Date _____

Name _____ Signature _____

Overall Comments _____

(sample condition) _____

_____

_____

Job. No. _____

Sample Details *(Client to complete)*

Collection method _____

Type of preservative used? _____

Sample No./ID	Sample Site Name & Code	Collection Date	Sample Collector	No. of Containers	Sample Condition Comments* *(Lab to complete)*	Lab No.
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

** G =Good/satisfactory, D = Damaged, L = leaking, I = Inppropriate bottle used, E = external label missing/illegible*

Annex D – Macroinvertebrate Taxon Resolution and Tolerance Values

Taxon tolerance values or scores used for Macroinvertebrate Community Index (MCI) calculations for hard-bottomed (HB) and soft-bottomed streams (SB) are listed in the following table. These scores have been reproduced from Table A1.1 of Clapcott et al. (2017) and update those provided by Stark and Maxted (2007). Use of these scores aligns with the requirements of the NPS-FM 2020.

Note 1: It is recognised that these tolerance scores need to be reviewed (refer subsection 5.6.1) and the taxa list extended. Refer to Annex H for recommended tolerance scores to assign to taxa not listed here.

Note 2: As noted in subsection 2.1.1, some hard-bottomed stream reaches may be considered soft-bottomed because of inundation with fine sediment. Consideration of whether this inundation is anthropogenically or naturally derived will be required before selecting whether to use HB or SB taxa tolerance scores in the calculation of MCI and QMCI metrics from macroinvertebrate data collected from these reaches. The tolerance scores used to calculate these metrics shall be determined by the dominant natural substrate type across the sampling reach (e.g. HB tolerance scores would apply both where at least 50% of the substrate comprises one or more of gravel, cobble, boulder and bedrock and where the substrate is 80% inundated with fine sediment but was naturally hard-bottomed).

Taxon	HB	SB	Taxon	HB	SB	Taxon	HB	SB
COELENTERATA			**Odonata *(cont.)***			**Diptera *(cont.)***		
Hydra	3	1.6	*Procordulia*	6	3.8	*Stictocladius*	9	-
PLATYHELMINTHES	3	0.9	*Uropetala*	5	0.4	Stratiomyidae	5	4.2
RHABDOCOELA	-	0.9	*Xanthocnemis*	5	1.2	Syrphidae	1	1.6
BRYOZOA	-	4.0	**Hemiptera**			Tabanidae	3	6.8
NEMATODA	3	3.1	*Anisops*	5	2.2	Tanypodinae	5	6.5
NEMATOMORPHA	3	4.3	*Diaprepocoris*	5	4.7	Tanytarsini	3	4.5
NEMERTEA	3	1.8	*Microvelia*	5	4.6	*Tanytarsus*	3	-
OLIGOCHAETA	1	3.8	Saldidae	5	3.9	Thaumaleidae	9	8.8
POLYCHAETA	-	6.7	*Sigara*	5	2.4	Tipulidae	5	3.4
HIRUDINEA	3	1.2	**Coleoptera**			*Zelandotipula*	6	3.6
TARDIGRADA	-	4.5	*Antiporus*	5	3.5	**Trichoptera**		
CRUSTACEA			*Berosus*	5	-	*Alloecentrella*	9	-
Amphipoda	5	5.5	*Copelatus*	5	3.7	*Beraeoptera*	8	7.0
Cladocera	5	0.7	Dytiscidae	5	0.4	*Confluens*	5	7.2
Copepoda	5	2.4	Elmidae	6	7.2	*Conuxia*	8	-
Halicarcinus	-	5.1	*Enochrus*	5	2.6	*Costachorema*	7	7.2
Helice	-	6.6	Hydraenidae	8	6.7	*Cryptobiosella*	9	-
Isopoda	5	4.5	Hydrophilidae	5	8.0	*Diplectrona*	9	-
Mysidae	-	6.4	*Liodessus*	5	4.9	Ecnominidae	8	-
Ostracoda	3	1.9	*Onychohydrus*	5	-	*Ecnomina*	8	9.6
Paracalliope	5	-	*Podaena*	8	-	*Edpercivalia*	9	6.3
Paraleptamphopus	5	-	Ptilodactylidae	8	7.1	*Helicopsyche*	10	8.6
Paranephrops	5	8.4	*Rhantus*	5	1.0	*Hudsonema*	6	6.5
Paranthura	-	4.9	Scirtidae	8	6.4	*Hydrobiosella*	9	7.6
Paratya	5	3.6	Staphylinidae	5	6.2	*Hydrobiosis*	5	6.7
Tanaidacea	4	6.8	**Neuroptera**			*Hydrochorema*	9	-
INSECTA			*Kempynus*	5	-	*Hydropsyche - Aoteapsyche*	4	6.0
Ephemeroptera			**Diptera**			*Hydropsyche - Orthopsyche*	9	7.5
Acanthophlebia	7	9.6	*Aphrophila*	5	5.6	*Kokiria*	9	-
Ameletopsis	10	10.0	*Austrosimulium*	3	3.9	*Neurochorema*	6	6.0
Arachnocolus	8	8.1	*Calopsectra*	4	-	*Oecetis*	6	6.8
Atalophlebioides	9	4.4	Ceratopogonidae	3	6.2	Oeconesidae	9	6.4
Austroclima	9	6.5	Chironomidae	2	3.8	*Olinga*	9	7.9
Austronella	7	4.7	*Chironomus*	1	3.4	*Oxyethira*	2	1.2
Coloburiscus	9	8.1	*Corynoneura*	2	1.7	*Paroxyethira*	2	3.7
Deleatidium	8	5.6	*Cryptochironomus*	3	-	*Philorheithrus*	8	5.3
Ichthybotus	8	9.2	*Culex*	3	-	*Plectrocnemia*	8	6.6
Isothraulus	8	7.1	Culicidae	3	1.2	*Polyplectropus*	8	8.1
Mauiulus	5	4.1	Diptera indet.	3	2.9	*Psilochorema*	8	7.8
Neozephlebia	7	7.6	Dixidae	4	7.1	*Pycnocentrella*	9	-
Nesameletus	9	8.6	Dolichopodidae	3	8.6	*Pycnocentria*	7	6.8
Oniscigaster	10	5.1	Empididae	3	5.4	*Pycnocentrodes*	5	3.8
Rallidens	9	3.9	Ephydridae	4	1.4	*Rakiura*	10	-
Siphlaenigma	9	-	Eriopterini	9	7.5	*Synchorema*	9	-
Tepakia	8	7.6	*Harrisius*	6	4.7	*Tiphobiosis*	6	9.3
Zephlebia	7	8.8	Hexatomini	5	6.7	*Triplectides*	5	5.7
Plecoptera			*Limnophora*	3	4.5	*Triplectidina*	5	-
Acroperla	5	5.1	*Limonia*	6	6.3	*Zelandoptila*	8	7.0
Austroperla	9	8.4	*Lobodiamesa*	5	7.7	*Zelolessica*	10	6.5
Cristaperla	8	-	*Maoridiamesa*	3	4.9	**Lepidoptera**		
Halticoperla	8	-	*Mischoderus*	4	5.9	*Hygraula*	4	1.3
Megaleptoperla	9	7.3	*Molophilus*	5	6.3	Collembola	6	5.3
Nesoperla	5	5.7	Muscidae	3	1.6	**ACARINA**	5	5.2
Spaniocerca	8	8.8	*Nannochorista*	7	-	**ARACHNIDA**		
Spaniocercoides	8	-	*Neocurupira*	7	-	*Dolomedes*	5	6.2
Stenoperla	10	9.1	*Neolimnia*	3	5.1	**MOLLUSCA**		
Taraperla	7	8.3	*Nothodixa*	4	9.3	Ampullariidae	3	1.6
Zelandobius	5	7.4	Orthocladiinae	2	3.2	*Glyptophysa = Physastra*	5	0.3
Zelandoperla	10	8.9	*Parochlus*	8	-	*Gundlachia = Ferrissia*	3	2.4
Megaloptera			*Paradixa*	4	8.5	*Gyraulus*	3	1.7
Archichauliodes	7	7.3	*Paralimnophila*	6	7.4	*Hyridella = Echyridella*	3	6.7
Odonata			*Paucispinigera*	6	7.7	*Latia*	3	6.1
Aeshna	5	1.4	Pelecorhyncidae	9	-	Lymnaeidae	3	1.2
Anisoptera	5	6.0	*Peritheates*	7	-	*Melanopsis = Zemelanopsis*	3	1.9
Antipodochlora	6	6.3	Podonominae	8	6.4	*Physa = Physella*	3	0.1
Austrolestes	6	0.7	*Polypedilum*	3	8.0	*Potamopyrgus*	4	2.1
Hemianax	-	1.1	Psychodidae	1	6.1	Sphaeriidae	3	2.9
Hemicordulia	5	0.4	*Scatella*	7	-			
Ischnura	-	3.1	Sciomyzidae	3	3.0			

Annex E – 200+ Fixed Count with Scan for Missed Taxa Macroinvertebrate Processing Method

The following macroinvertebrate sample processing protocol is a modification of Protocol P2 of Stark et al. (2001) and includes clarification and alteration of some details to promote greater national consistency in application. The Quality Control procedure that follows contains more significant revisions and was informed by both a workshop with laboratory processing experts and findings from a review of macroinvertebrate processing quality control procedures (Smith, 2020).

Processing protocol

1. Inspect the sample upon opening and record any anomalies in its condition with the potential to impact processing (e.g. odour indicating biodegradation of sample, evident overfilling of the container or lack of preservative).

2. Tip the sample material through a clean 500 µm (0.5 mm) mesh sieve with a catch tray underneath to retain the preservative. If the retained preservative is of sufficient concentration (i.e. there was no indication of degradation of the sample), it can be returned to the sample container for re-preservation of the processed sample.

3. Using a wash bottle or tap hose rinse out anything that may be stuck to the inside of the sample container into the sieve. It is important not to use too much force when washing samples as macroinvertebrates are delicate (particularly after a period of preservation in alcohol) and prone to damage, which will compromise their identification.

4. Gently rinse the sieve contents to remove any remaining preservative, and continue until the wash water runs clear and the sample is thoroughly homogenised (i.e. break down lumps of algae, etc). There are several washing techniques that may be useful depending on sample contents and volume. Larger and more complex samples will likely require additional washing and preparation prior to sorting (see step 5). For small sample volumes, the entire sample may be transferred directly from the sieve to a white tray for sorting (proceed to step 6).

5. Use a wash bottle or tap hose to carefully wash material from the sieve into a bowl or small pail (e.g. 4 litre) half to two-thirds-filled with clean water. If large amounts of stones/gravel are present, swirl the pail to put the lighter macroinvertebrates and organic material into suspension. Elutriate the sample by decanting off the floating organic material from the pail into the sieve. This process may be repeated several times, ensuring that all lighter macroinvertebrates have been separated from the stones/gravel and returned to the sieve. In samples with lots of heavier macroinvertebrates, such as molluscs or stony cased caddisflies, it may be necessary for some gravel to remain in the sample to ensure that no macroinvertebrates are lost. Many caddisflies produce protective stony pupal chambers that may also be present at the bottom of the pail. These should be retained to assist with validation of identifications as required. Other washing techniques that may be useful depending on sample contents are as follows:

- large organic material (whole leaves, twigs, algal or macrophyte mats, etc.) not removed in the field should be rinsed, visually inspected for organisms, and discarded.
- rinsing through a series of nested sieves (e.g. 2 mm, 1 mm) may be useful for homogenising the sample and rinsing fine sediment out of algal mats, etc.
- samples with large amounts of macrophytes or filamentous algae can be floated in deep white trays to make it easier to dislodge macroinvertebrates and remove the vegetation (any aquatic plants and clumps of algae may also need to be pulled apart and thoroughly washed).
- a coarse sieve (e.g. 4 mm) can be helpful for removing larger pieces of unwanted organic material so long as all macroinvertebrates are picked out and placed into the 500 µm sieve.

6. After washing, transfer contents of sieve to a white sorting tray with clearly demarcated grids of equal size. Alternatively, a tray with removable plastic divider grids may be used. Visually check the sieve to ensure that no macroinvertebrates are left behind. Using the wash bottle or a low-pressure hose, spread the sample evenly across the tray. There should be enough water to just cover all material. If the samples have been preserved in alcohol some organisms (particularly ostracods and early instar insects) may float on the surface. If this occurs, add a drop of washing detergent and stir gently. Evenly distribute any filamentous algae that is present – this may require teasing the algae apart using forceps.

7. Use a random numbers table or other means of randomisation to select a starting grid square within the tray for the purposes of identifying a subsample. A fixed-grid device (refer to Figure 18, subsection 5.4.2) or cookie cutter the same size as the demarcated grid squares should be used to delineate the chosen grid square, with care taken to ensure macroinvertebrates are not cut or otherwise damaged. Remove all material from the selected square using forceps or a suction device (e.g. plastic pipette). Place sample material either directly into Bogorov trays or (if there is a large amount of material) into a fine mesh sieve to be transferred into Bogorov trays. It is important to ensure that Bogorov trays are not overfilled, as this makes it difficult to see macroinvertebrates present; it is best to spread sample material across multiple Bogorov trays to reduce the risk of missing any individuals. Check that all material from the selected square is removed, and no macroinvertebrates remain.

   Note 1: Any organism that is lying over a line separating two grids is considered to be in the square containing its head. In those instances where it may not be possible to determine the location of the head (e.g. worms), the organism is considered to be in the square containing most of its body.

   Note 2: An alternative subsampling method is to use a barrel sampler splitter, particularly if it is likely that a large portion of the sample will need to be processed to achieve a count of at least 200 macroinvertebrates (see below).*

8. Under a dissecting microscope, work systematically through each Bogorov tray to identify, count, and record all macroinvertebrates. Once recorded, pick each macroinvertebrate into a vial containing preservative. This vial should include a label indicating that it contains the '200+ individuals', and also the sample information as per the original sample label.

   The minimum level of identification required is that specified in Annex D, and all individual macroinvertebrates in the vial shall be identified under a dissecting microscope. Do not include aerial adult insects, pupae, terrestrial invertebrates, empty snail shells, caddisfly cases or exuviae. Note also that the following requirements apply when counting damaged macroinvertebrates:

- enumeration of individuals should follow the principle of counting heads – a body without a head should not be counted, but a head without a body may be counted if sufficient features are available on the head to provide a positive identification. If only a single specimen of a given taxa is present, only the back end is present, and the back end is sufficient to identify the individual, it should be recorded – this is the one exception to counting heads.
- as segmented worms are often fragmented in samples, and it is difficult to determine which end is the head, only the rounded portions of segmented worms are counted, with the total divided by two to obtain the number of worms present. If an uneven number of rounded portions are counted, this should be rounded up by one to provide a whole number count of worms present. However, in cases where the prostomium is clearly distinguishable, it is acceptable to count head ends alone as a single worm., and
- a conservative approach to identification is appropriate; if a taxon cannot be positively verified based on the available information then a higher level of identification or most defensible taxonomic resolution should be used. This is the case for early instar individuals, individuals that are damaged and missing essential identifying features, or taxa that cannot be identified by morphology alone, are not fully described, or belong to species-complexes. When an individual cannot be identified with certainty to the level specified in Annex D, it should be identified to the most defensible level of identification.

9. Continue until the Bogorov trays have been sorted. Sorted material (the 'sorted residue') should be transferred to a container with preservative (or to a fine mesh sieve to be transferred to such a container at the end of processing the 'sorted residue'). This container should have a waterproof internal label that identifies it as the 'sorted residue', as well as recording sample information as per the original sample label. If there is any remaining unsorted material from the selected subsample, transfer this to the empty Bogorov trays and continue identifying and counting until the selected subsample is complete.

10. If a total of at least 200 macroinvertebrates has been obtained, sample sorting ceases. However, once a square has been started it must be finished, even if the number of individuals has exceeded 200. If less than 200 macroinvertebrates have been enumerated, select another random subsample without disturbing the rest of the sample and repeat steps 5–7. Once at least 200 macroinvertebrates have been recorded, and the current subsample portion has been fully processed, the total number of grid squares sampled should be recorded. The total number of individuals that have been identified and enumerated should also be recorded.

11. Complete the taxa list by a visual scan of the 'sample residue' (the remaining sample) for 'missed taxa' (i.e. taxa not present in the subsample). This is carried out by the naked eye with the sample spread thinly in white sorting trays. Larger samples will need to be distributed across multiple trays, such that only a small amount of material (often only a couple of tablespoons) is present when scanning each tray. Working systematically across each tray, pick out individuals that may belong to 'missed taxa'. Once all sample material has been scanned, the individuals that have been picked out should be examined under the dissecting microscope. Any missed taxa that are identified should be recorded as a count of '1', and a single representative individual of the taxa should be placed in a vial containing preservative. This vial should have a label indicating that it contains the 'missed taxa', and also recording sample information as per the original sample label. The missed taxa scan is also an opportunity to remove larger (e.g. late instar) or better conditioned individuals of taxa already encountered to assist in identification. If these individuals are placed in the

'200+ individuals' vial, it should be clearly recorded in the data that they are there to assist identification but are not to be added to the count of the 200+ individuals.

12. Return to the 'sample residue' any leftover macroinvertebrates picked out during the missed taxa scan. Return the sample residue to its container with the original labels.

13. On completion of sample processing there should be: (1) A labelled container holding the sample residue (already scanned for missed taxa); (2) A labelled container holding the sorted residue (from which the 200+ individuals have been removed); (3) A labelled vial containing the 200+ individuals removed from the sorted residue; and (4) A labelled vial containing the missed taxa removed from the sample residue.

14. Thoroughly wash all sample processing equipment to prevent cross-contamination between samples. Check data records for completeness. Store sample containers/vials in an appropriate location so that they are accessible for quality assurance and quality control processes.

*<u>Subsampling using a barrel sampler splitter</u>

- transfer the rinsed sample material to the barrel sample splitter, ensuring that no material remains on the sieve.

- fill the barrel splitter with water to just underneath the shelf/divider. Replace the lid, making sure it is on tightly. Turn the barrel on its side, with the shelf/divider on top, and carefully agitate or tilt the barrel splitter from side to side to evenly distribute the sample along the barrel. Check that sample material is evenly distributed horizontally along the barrel splitter (both floating material and heavier material that settles to the bottom). If a sample contains algal mats, these should be teased apart using forceps at the washing stage so that they can be evenly distributed and split during subsampling.

  Note: Adding a few drops of dishwashing detergent will lower the surface tension of the water and ensure even the lightest organic material sinks.

- holding the splitter horizontally or resting it against a bench, invert/rotate the barrel, so that the shelf is at the bottom of the splitter, and the sample has now been split in half (Figure E2).

- carefully remove the lid and pour off half of the sample into a clean 0.5 mm (or finer mesh) sieve; the shelf/divider of the barrel splitter will retain the other half. Rinse out any material that remains in the open half of the barrel splitter or stuck to the lid into the same sieve. You now have your first 'split' portion of the sample, containing half of the sample material.

- if required, repeat the above steps, until the portion of the sample to be processed or sorted (the part left in the barrel splitter) is made up of enough material to obtain approximately 200 individuals. Keep each split portion in a separate labelled sieve, such that you can return to a known fraction (e.g. 1/8, 1/4, 1/2) of the sample should you need to process an additional portion.

- distribute the subsample portion into Bogorov trays and proceed with identification and enumeration as in step 6 above. If necessary, process additional fractions of the sample. Any time the sample is split using the barrel splitter, it is essential to note what fraction is being processed. As with the gridded tray subsampling method, once a fraction is begun it must be completed (even if 200 individuals have been counted). It is, therefore,

important to subsample carefully to avoid the need to count significantly more than 200 individuals.

- once at least 200 macroinvertebrates are obtained and the current subsample portion is completed, record the total proportion of the sample that was processed (e.g. 1/8, 3/4, etc.). If the whole sample has not been processed to obtain the 200+ individuals, proceed with the missed taxa scan of the sample residue as in step 11.

Figure E1 – Barrel splitter (left) and split sample contents captured in a sieve

Photos: Environment Canterbury

Quality Control protocol

1. For quality control, at least 10% of a batch of macroinvertebrate samples, selected by the monitoring agency, are to be re-examined by another taxonomist familiar with sorting procedures and the full range of macroinvertebrate taxa from running waters in New Zealand. This taxonomist will be provided by the monitoring agency with the results from the first taxonomist, along with any accompanying notes from the first taxonomist that may be of use during quality control (e.g. notes on sample condition, invertebrate condition, etc.).

2. Component 1: For each sample, the quality control processor will first check the taxonomic accuracy of the individual macroinvertebrates in the '200+ individuals' vial, followed by the taxonomic accuracy of the individuals in the 'missed taxa' vial. The total number of individuals incorrectly identified as a percentage of the total number of individuals found by the quality control processor should be calculated. This value shall be reported as percentage Taxonomic Disagreement (%TD) in accordance with Table E1. A sample passes quality control for Taxonomic Disagreement if this value is ≤ 10%.

   Note: If the correct taxonomic identification of a macroinvertebrate is disputed, then a specimen should be checked by an agreed third-party expert.

3. Component 2: The 'sample residue' should be checked for additional missed taxa not recorded by the laboratory processor. For this check, lay the sample residue out in trays and scan by naked eye for a maximum of 30 minutes. Identify any new taxa that were missed under a dissecting microscope. Calculate the number of new taxa found (i.e. additional missed taxa) as a percentage of the total number of missed taxa (as recorded by the quality control processor). This value shall be reported as percentage Missed Taxa (%MT) in accordance with Table E1. A sample passes quality control for Missed Taxa if this value is ≤ 20%.

   Note: In some circumstances, the original processor may misidentify a taxa in the '200+ individuals' vial, such that they believe they have already recorded this taxa and thus do not pick it out of the sample residue and record it during the scan for missed taxa. In such circumstances, this will count as a misidentification and will contribute to % Taxonomic Disagreement but will not count as a missed taxon and will not contribute to % Missed Taxa.

4. Component 3: For each sample, the 'sorted residue' should be checked to identify whether any individual macroinvertebrates were missed during the removal of the 200+ individuals. For this check, distribute the residue in Bogorov trays and systematically scan under a microscope. Pick out and record any missed individuals and calculate the number of missed individuals as a percentage of total individuals counted in the 200+ individuals (as counted by the quality control processor). This value shall be reported as percentage Missed Individuals (%MI) in accordance with Table E1. A sample passes quality control for Missed Individuals if this value is ≤ 10%.

   Note: An individual from an unrecorded taxon found in the sorted residue should be counted as both a missed taxon under %MT and a missed individual under %MI.

5. After a sample has been completely sorted, all sieves, trays and equipment should be thoroughly cleaned and picked free of macroinvertebrates and debris before the next sample is begun.
6. Once all samples have undergone quality control, the batch error can be calculated to determine whether the batch of quality control samples as a whole has passed each quality control component. For each of %TD, %MT and %MI, the mean across the batch of quality control samples shall be ≤ 10% for quality control to pass.
7. The quality control results should be documented in a report comprising tables in a format similar to that presented in Annex G. This report is to be provided to the monitoring agency, who should then also provide a copy to the original processing laboratory to assist with ongoing quality assurance and quality control.
8. Where quality control fails, a monitoring agency may request that the processing laboratory undertake targeted corrective actions specific to the criterion on which they failed (i.e. re-checking samples containing large numbers of routinely misidentified taxa if they failed on %TD). Once the laboratory is satisfied with the corrective action, there is an opportunity for a further 10% quality control to be done (specifically on the failed criterion).
9. If quality control fails for an entire batch of samples, and no further steps are taken to correct systemic errors and/or commission further quality control, the original taxa list should be corrected to align with the quality control results. No adjustments are needed to the original dataset where quality control is deemed to have passed.

Table E1: Summary of Quality Control metrics

Note: The pass requirements apply to an individual quality control sample. To pass a quality control component overall, the mean error across the batch of quality control samples shall be ≤ 10% for %TD and %MI and ≤ 20% for %MT.

Quality control component	Quality control metric	Calculation	Pass requirement
Taxonomic accuracy	% Taxonomic Disagreement (%TD)	$\frac{No.\ misidentified\ individuals}{Total\ no.\ individuals} \times 100$ Where *No. misidentified individuals* is the total number of individuals misidentified by the laboratory in the '200+ individuals' and 'missed taxa' vials , *and Total no. individuals* is the total number of individuals in the '200+	%TD shall be ≤ 10%

		individuals' and 'missed taxa' vials, as counted by the quality control processor	
Sorting accuracy of 'sample residue' (missed taxa)	% Missed Taxa (%MT)	$\frac{No.additional\ missed\ taxa}{Total\ no.taxa} \times 100$ Where *No. additional missed taxa* is the number of missed taxa identified in the 'sample residue' by the quality control processor that were missed by the laboratory, and *Total no. taxa* is the total number of all taxa all identified (including those in the '200+ individuals' vial, the 'sorted residue', and the 'sample residue') by the quality control processor	%MT shall be ≤ 20%
Sorting accuracy of 'sorted residue' (missed individuals)	% Missed Individuals (%MI)	$\frac{No.\ missed\ individuals\ in\ sorted\ residue}{Total\ no.individuals} \times 100$ Where *Total no. individuals* is that counted by the quality control processor in the 'sorted residue' and '200+ individuals' vial combined	%MI shall be ≤ 10%

Annex F – Full Count with Subsampling Option Macroinvertebrate Processing Method

The following macroinvertebrate sample processing protocol represents a modification of Protocol P3 of Stark et al. (2001). It involves a full count of macroinvertebrates present in the sample at a number of different sieve sizes. Depending on the size of the sample, there is an option to subsample by splitting up the sample at each sieve size to reduce the processing time. If subsampling, the remainder of the unsorted sample is scanned for 'missed taxa' in the same way as for the scan associated with the 200+ Fixed Count with Scan for Missed Taxa method (Annex E).

Note: The method described here assumes the use of Bogorov trays.

Protocol

1. Pour the sample through a 63 or 125 µm (0.063 or 0.125 mm) sieve into a white tray to catch the preservative. Wash the sample from the sieve and the remainder of sample material from the pottle through a stack of nested sieves. Stack these with 125 µm (or smaller) on the bottom as a catch sieve (to prevent silt going down the drain; the material in the 125 µm sieve is NOT processed), then 500 µm (0.5 mm), 1 mm, and 2 mm placed consecutively on top (i.e. 2 mm is the top sieve). Pour the preservative from the tray back into the container (note that if preservation is poor, discard that preservative and re-preserve with new preservative).

    - if there are large stones or sticks in the sample, a 10 mm sieve can be used on top of the 2 mm sieve to remove any large material but ensure that you check for any macroinvertebrates (including stick-cased caddisfly larvae). Do not use too much water pressure when washing as macroinvertebrates could be damaged. Mayflies are very fragile so be especially careful when washing samples containing these animals. Pick out any large stones and sticks from the 2 mm or 10 mm sieve and wash these by hand over the sieve to remove any macroinvertebrates.

    - if there is a large amount of material that floats (macrophytes, leaves etc.), tip the contents of the 2 mm or 10 mm sieve into a large white tray and fill with water. Carefully pick out the pieces of floating material with forceps and discard, checking to make sure macroinvertebrates are not stuck to them, then return the contents of the tray to the sieve.

2. Wash the sample until most of the small material has passed through the 2 mm sieve. If the sample is to be subsampled, wash the material from the sieve into the sample splitter. If no subsampling is needed, either elutriate the material or wash the contents of the sieve into a smaller (unused) micron sieve (i.e. 500 µm or smaller). This will make it easier to transfer the material to a Bogorov tray and help prevent the loss of small macroinvertebrates through the sieve during the rest of the process. Process the material from the 2 mm sieve. Process the 1 mm and 500 µm sieves in the same way.

3. If subsampling is to be performed, wash the contents of the sieve into the sample splitter and fill with water to just below the divider. Place the lid on firmly and gently rock and rotate the splitter around to evenly disperse the material before bringing it to a horizontal orientation with the middle divider at the top. Once the water has settled and is level, rotate

the sample splitter so that the middle divider 'splits' the sample in two. Snails and caddisflies with stony cases will sink so look at the underside of the splitter to check that the heavy material has also divided evenly. Place the splitter on the bench so the divider is sitting straight on the bottom half of the splitter and the end with the opening is over the sink. Place a sieve under the lid and slowly take the lid off, opening it from the bottom first, and allow the water to drain out. Use the hose to wash out any remaining material from the lid half of the splitter (do not tip it, as material from the other half may spill over the divider). If subsampling further, refill the splitter to halfway and repeat the process, noting how many times the sample was split. Retain the subsampled material that is not to be processed under the microscope to be checked for missed taxa.

Notes:

- *subsample only where necessary and as little as possible. One-eighth is the recommended smallest subsample level. However, it is preferable to subsample to no less than one-quarter.*
- *it is not always easy to judge how much to subsample. It is a good idea to keep the subsampled portion that was not to be processed separate in case you need to process it as well. For example, if you have subsampled to one-quarter, keep the unprocessed quarter separate from other material in case one-half was a more appropriate subsample level.*
- *remember to record the subsample level for each sieve on the data sheet.*

If there is a large amount of heavy non-organic material (e.g. sand, gravels) present, processing is made easier by decanting the 'floatables' out to separate this material from the heavy particles. This can be achieved by tipping the material from the sieve into a white tray, filling the tray with water and then carefully pouring the water and floating material back into the sieve. This may need to be repeated to separate all of the floatables from the heavier material. Elutriation is less effective in samples with molluscs and other heavy macroinvertebrates that may also sink. Before discarding the heavy material, check thoroughly to ensure that no macroinvertebrates are being discarded with it.

Note: Adding a few drops of dishwashing detergent will lower the surface tension of the water and ensure even the lightest organic material sinks.

To process the sample, put a small amount of material into each Bogorov tray and fill with enough water to just cover the material. Evenly spread the material along the grooves. Correctly filling the Bogorov trays ensures that macroinvertebrates will be visible when viewed under the microscope.

Process under the dissecting microscope, identifying and counting macroinvertebrates as you go. Five representative individuals of each taxa should be removed to a vial labelled 'representative taxa.' Preserve these and label clearly with site information and processor name.

<u>Missed taxa scan (if sample was subsampled)</u>

Once all sieves have been processed, any material from subsampling that was not processed under the microscope needs to be checked for 'missed taxa' (taxa not yet recorded in the sample). To begin a missed taxa scan, place 1–2 tablespoons of unpicked residual in a shallow white tray. Fill with water until all corners of the tray are just covered, and evenly distribute material across the tray.

Scan the tray by eye, and pick out any macroinvertebrates that you think might be missed taxa into a Bogorov tray. Once the tray has been scanned thoroughly, empty the tray into a sieve and repeat the process with the next 1–2 tablespoons of unpicked residual. Continue this way for the entire sample, ensuring that you keep the scanned and un-scanned portions of your sample separate. Once all the unsorted/unpicked residue has been scanned, check the identification of the macroinvertebrates in your Bogorov tray under the microscope. If any missed taxa are found, add the counts in the 'missed' column of the data sheet. For missed taxa scans it is only necessary to count a single individual for each missed taxon (i.e. a count of '1' is recorded in the 'missed' column). All taxa recorded in the 'missed' column of the data sheet should be placed in a vial of preservative and labelled 'missed taxa'. Also label clearly with site information and processor name. The remaining individuals not placed in the 'missed taxa' vial should be returned to the unsorted residue.

Preserve the organic and non-organic residue from the sorted or picked portion (i.e. the portion processed under the microscope) in a container and label this 'sorted residue'.

Return the remainder of the sample to the original container with the original label, re-preserve and label 'sample residue'.

Quality Control protocol

The quality control check is of both the taxonomic accuracy and the missed taxa scan (of the unprocessed portion) to see if any taxa have been missed. Refer to Annex E for quality control metric calculations.

Annex G – Macroinvertebrate Laboratory QC Tables

The following tables present the recommended format for reporting of macroinvertebrate quality control results.

Table G1: Summary of percent Taxonomic Disagreement (%TD), Sorting Accuracy 1 (Missed Taxa, %MT) and Sorting Accuracy 2 (Missed Individuals, %MI)

Note: This table should list every sample processed under Quality Control. A bold value indicates a sample that has failed Quality Control. The mean % value determines the overall pass/fail for each Quality Control metric.

Sample name	Processing lab ID	Monitoring agency site no.	%TD	%MT	%MI
Tai R @ Tai Rd Br	I190105	BRC52	0	2.5	0.05
Wai R @ Wai School	I190110	BRC61	5.8	**21.7**	1.43
…					
Mean % disagreement			*2.9*	*12.1*	*0.74*

Table G2: Example summary of 'taxonomic disagreement' (TD) in '200+ individuals' and 'missed taxa' vials [M]

Sample name	Processing lab ID	Monitoring agency site no.	Processing lab identification	QC lab identification	No. mis-identified
Tai R @ Tai Rd Br	I190105	BRC52	• *Zephlebia* • *Zelandobius* [M]	• *Austroclima* • *Acroperla* [M]	3 1
Wai R @ Wai School	I190110	BRC61	• *Pycnocentria*	• *Olinga*	2

Table G3: Example summary of additional missed taxa recovered from the unsorted sample residue (Sorting Accuracy 1, 'missed taxa', MT). * < 90% agreement with Quality Control provider.

Sample name	Processing lab ID	Monitoring agency site no.	Additional taxa recovered
Tai R @ Tai Rd Br	I190105	BRC52	Eriopterini
Wai R @ Wai School	I190110	BRC61*	*Austroperla, Austrosimulium, Berosus*

Table G4: Example summary of taxa and number of individuals recovered from the picked sorted residue (Sorting Accuracy 2, 'missed individuals', MI). * not recorded by the processing laboratory

Sample name	Processing lab ID	Monitoring agency site no.	Taxa	No. individuals recovered
Tai R @ Tai Rd Br	I190105	BRC52	• *Pycnocentrodes* • Acari • Orthocladiinae • *Potamopyrgus*	1 1 3 1
Wai R @ Wai School	I190110	BRC61	• *Olinga* • Tanytarsini*	1 1

Annex H – Additional Macroinvertebrate Taxon Tolerance Values

The following table lists recommended tolerance values (scores) for macroinvertebrate taxa that do not appear in Annex D (derived from the score assigned to the nearest taxon presented in Annex D). These values (presented in bold black font) have been collated through Working Group member input and have not, as yet, been formally endorsed for use in national reporting. As noted in subsection 5.6.1, the list of macroinvertebrate tolerance scores in Annex D needs to be revised and expanded to include scores for taxa such as those listed here.

Taxon	HB	SB	Taxon	HB	SB
RHABDOCOELA	**1**	0.9	**Trichoptera**		
BRYOZOA	**4**	4.0	*Alloecentrella*	9	**9.0**
Polychaeta	**7**	6.7	*Connuxia*	8	**8.0**
TARDIGRADA	**5**	4.5	*Cryptobiosella*	9	**9.0**
CRUSTACEA			*Diplectrona*	9	**9.0**
Mysidae	**5**	6.4	Economidae	8	**8.0**
Paracalliope	5	**5.5**	Hydrobiosidae	**7**	**7.4**
Paraleptamphopus	5	**5.5**	*Hydrochorema*	9	**9.0**
Paranthura	**5**	4.9	Hydropsychidae	**6**	**7.0**
INSECTA			Hydroptilidae	**2**	**2.3**
Diptera			*Kokiria*	9	**9.0**
Ephydrella	**4**	**1.4**	Leptoceridae	**6**	**6.2**
Calopsectra	4	**3.8**	Polycentropodidae	**8**	**8.0**
Cryptochironomus	3	**3.8**	*Pycnocentrella*	9	**9.0**
Culex	3	**1.2**	*Rakiura*	10	**10.0**
Nannochorista	7	**7.0**	Synchorema	9	**9.0**
Neocurupira	7	**7.0**	*Triplectidina*	5	**5.7**
Parochlus	8	**3.8**	**Hemiptera**		
Pelecorhynchidae	**9**	**9.0**	Mesoveliidae	**5**	**4.6**
Peritheates	7	**7.0**	**Coleoptera**		
Scatella	7	**1.4**	*Berosus*	5	**8.0**
Stictocladius	9	3.2	*Hydora*	**6**	**7.2**
Tanytarsus	3	**4.5**	*Onychohydrus*	**5**	**0.4**
Ephemeroptera			*Podaena*	8	**6.7**
Siphlaenigma	9	**9.0**	**Neuroptera**		
Plecoptera			*Kempynus*	5	**5.0**
Gripopterygidae	**8**	**7.8**			
Cristaperla	8	**8.0**	**Odonata**		
Halticoperla	8	**8.0**	*Hemianax*	**1**	1.1
Spaniocercoides	8	**8.8**	*Ischnura*	**3**	3.1

HB = hard-bottomed stream reach
SB = soft-bottomed stream reach