Freshwater ecology: semi-quantitative macroinvertebrate sampling in soft-bottomed streams

Version 1.0

This specification was prepared by Duncan Gray in 2013.

Contents

Synopsis 2
Assumptions 3
Advantages 3
Disadvantages 4
Suitability for inventory 4
Suitability for monitoring 4
Skills 4
Resources 5
Minimum attributes 5
Data storage 6
Analysis, interpretation and reporting 7
Case study A 11
Full details of technique and best practice 17
References and further reading 17
Appendix A 19

Department of Conservation
Te Papa Atawhai

Synopsis

The protocol described here is based upon that described by Stark et al. (2001)[1] as being an appropriate minimum requirement. This protocol is designed to collect presence/absence or semi-quantitative data from habitats within soft-bottomed New Zealand streams. Consequently, it is appropriate for use with coded abundance and fixed count processing methodologies and provides data suitable for general ecosystem condition and threatened species monitoring where quantitative data are not considered necessary. This data may be used to calculate a variety of species richness and relative abundance metrics as well as perform multivariate analyses. Always consult a biometrician or experienced freshwater ecologist to ensure your design and methods meet your objectives.

The fine, silty beds of soft-bottomed streams do not tend to harbour large numbers of invertebrates. Rather, sampling should focus on available habitats that provide a more suitable instream habitat structure. Unfortunately, there is no single substrate in soft-bottomed streams that is consistently present. Woody debris is considered the soft-bottomed stream equivalent to a riffle, but may be absent in streams where riparian vegetation has been cleared. Alternatively, in open streams, aquatic macrophytes may be the dominant habitat for invertebrates. Bank margin habitats are important in both stream types. Stark et al. (2001) recommend that a single sample is collected from an area of approximately 3 m^2 and that habitats are sampled in proportion to their occurrence. Within-site replication of sampling is not required for this semi-quantitative technique.

Sampling is undertaken using a kick-net. Whilst moving progressively upstream so that disturbed silt does not obscure the stream bed, each habitat type is sampled according to its occurrence and this proportion is recorded on the field sheet. Hard substrates are avoided or sampled separately to permit data comparability between soft-bottomed sites.

The soft-bottomed stream protocol samples a greater area than the hard-bottomed equivalent because the abundance of invertebrates tends to be lower. A single sample will comprise 10 unit efforts of approximately 0.3 m^2 area each. Each unit effort is transferred individually to a bucket or sieve bucket to avoid clogging of the net.

Different habitat types require a different sampling technique. Bank margins are vigorously jabbed with the net or a boot before making 2–3 sweeps of the net to collect dislodged organisms. Woody debris is often placed over the mouth of the net or bucket and brushed by hand (this requires two operatives). Debris should be inspected visually to locate any remaining organisms and care taken at all times not to damage individuals, e.g. with a stiff brush or forceps. Large logs may be sampled *in situ* provided there is sufficient velocity to wash disturbed organisms into the net. Each meter of woody debris represents 0.3 m^2 of habitat sampled. Finally, aquatic macrophyte beds are sampled by jabbing the net into submerged plants for a distance of 1 m followed by 2–3 sweeps of the net. Plants may also be shaken and brushed by hand to dislodge individuals. In all cases it is wise to avoid contact with the bed of the stream as fine sediments may cause clogging of the net.

[1] http://www.cawthron.org.nz/coastal-freshwater-resources/downloads/protocols-full-manual.pdf

Each sample should have preservative (usually 70% ethanol) added as soon as possible. A unique identifying code must be clearly marked on the lid and on a slip of waterproof paper inside the pottle (Fig. 1). Pottles should also be marked with the location, date and the field operative names.

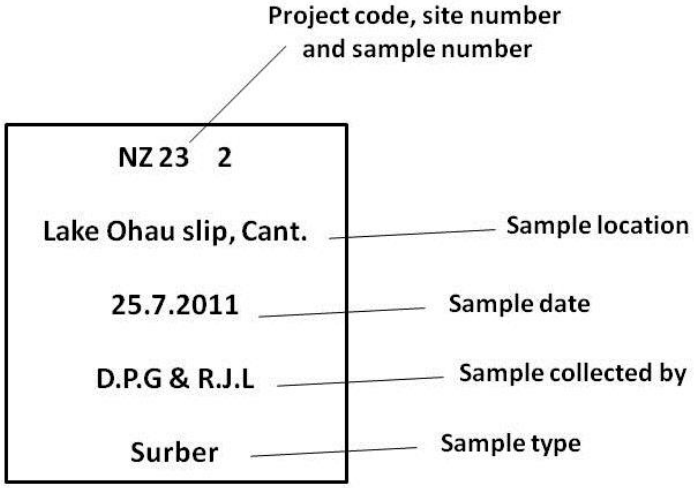

Figure 1. An example wet label with the required information to identify each sample should be written in pencil on waterproof paper.

Samples should be transported to a laboratory for storage prior to processing. Processing methods are detailed in Stark et al. (2001), but require experienced taxonomists to oversee the process.

Assumptions

- The sample is representative of the wider macroinvertebrate population.
- Sampling effort and duration is standardised across all sample sites.
- Data derived is qualitative (species lists) or semi-quantitative (an index of relative abundance).

Advantages

- Semi-quantitative sampling requires no specialised equipment or resources.
- Semi-quantitative sampling requires minimum time and effort to implement.
- Kick-netting provides robust basic information about the richness and composition of macroinvertebrate communities.
- Presence/absence data can provide baseline inventory data efficiently and for minimal cost (particularly for rare species) providing assumptions and inherent biases are understood.
- Presence/absence data can be used as a surrogate for abundance *providing* the monitoring objective is more interested in measuring the proportion of sites occupied (spatial distribution) and the probability of failing to detect target species within surveyed areas is estimated.
- Resource selection relationships can be addressed (if the appropriate habitat information is collected) and sites of conservation significance identified.

- Able to examine distribution changes over very large spatial scales.

Disadvantages

- Semi-quantitative sampling does not provide abundance data adequate to detect subtle shifts in community composition.
- Ensuring the assumptions are met is dependent on observer effort.
- Observer effort is unlikely to be consistent. This can significantly bias the number of species counted and habitats surveyed within a sample unit—particularly as scale increases.
- Presence/absence data and distribution data unadjusted for detectability can only confirm presence of a species, not the certainty of absence of a species.
- Population trends in density/abundance are unlikely to be detected.
- Methodology (particularly scale) must be standardised to ensure comparability over time.
- Use of a kick-net in streams with large quantities of organic matter or silt may result in rapid clogging of the net and loss of individuals through backwash. This can be avoided by emptying the net into a bucket prior to clogging.

Suitability for inventory

This soft-bottomed stream semi-quantitative sampling protocol is particularly suitable for inventory as the method samples a variety of habitat-types, captures the majority of macroinvertebrates found in New Zealand streams, is cheap to undertake and process, and requires minimal specialised training or equipment.

Suitability for monitoring

Soft-bottomed stream semi-quantitative sampling is suitable for monitoring when semi-quantitative data is considered adequate. Sampling effort and habitat types must be standardised as much as possible. Where comparable habitats or reference streams are not available, biological data must be supported by physical habitat data to qualify any conclusions made about differences in communities. The method is cheaper to apply than quantitative protocols.

Skills

Field observers will require:

- Basic training in stream macroinvertebrate and habitat sampling
- Basic outdoor and river-crossing skills
- A reasonable level of fitness

Study design, sample processing and quality control are specialised processes that require input from a freshwater specialist.

Resources

Semi-quantitative sampling of soft-bottomed streams may be carried out by a single field operative. However, in the interests of safety it is recommended that sampling is done by teams of at least two people.

Standard equipment includes:

- Waterproof notebook or field data sheets
- Pencil
- Permanent marker pen
- Wet labels
- Waders or gumboots, dependent on stream depth
- GPS and map

Specialist equipment required:

- Kick-net (0.5 mm mesh)
- White tray or 10 litre bucket
- Sieve or sieve bucket
- Plastic sample containers (usually 500–1000 ml volume)
- Preservative (usually 70% ethanol)

Minimum attributes

Consistent measurement and recording of these attributes is critical for the implementation of the method. Other attributes may be optional depending on your objective. For more information refer to ‘Full details of technique and best practice'.

DOC staff must complete a ‘Standard inventory and monitoring project plan’ (docdm-146272).

The more information that is collected at each site, the more thorough and complete will be any interpretation of the biological data collected. However, some basic information should be recorded with each sample collected:

- Substrate composition
- Riparian vegetation
- Stream width
- Stream depth
- Stream velocity

- Periphyton and macrophyte community composition

It is also commonplace to collect basic water chemistry information where possible. Temperature (°C), electrical conductivity (µS), pH and dissolved oxygen may all be measured by handheld meters. Some basic habitat and sites notes are also worthwhile, e.g. the occurrence of stock at the site or evidence of recent flooding. The 'Stream habitat assessment field sheet' (docdm-761873) is a good guide to the basic information that can be collected without recourse to specialised equipment or processing in a laboratory. Basic training in the use of this habitat sheet or a thorough perusal of Harding et al. (2009) is required before use of this habitat assessment sheet.[2] As with all visual and qualitative assessments it is important to standardise collection protocols within a group of field observers or within a particular project. There is considerable opportunity for user bias with this method of habitat assessment.

Data storage

If data storage is designed well at the outset, it will make the job of analysis and interpretation much easier. Before storing data, check for missing information and errors, and ensure metadata are recorded. Forward copies of completed field survey sheets to the survey administrator, or enter data into an appropriate spreadsheet as soon as possible. The key steps are data entry, storage and data checking/quality assurance for later analysis, followed by copying and data backup for security.

It is quite likely that biological sample processing will be outsourced to an accredited laboratory. During sample processing, data is conventionally recorded on a hardcopy data sheet prior to transfer to an electronic format. Hardcopy sheets will be clearly marked with the details of the project and identity of samples. The format of hardcopy data sheets is normally columns representing samples and rows for each species or taxa group. Data should be entered into an electronic media in the same format to avoid confusion (see 'Stream invertebrate data sheet example'—docdm-761858). Electronic data sheets should contain all the information required to identify each sample, and any habitat or water chemistry data that was collected simultaneously may be appended on a separate worksheet within the electronic file (usually Excel).

It is important that habitat and water chemistry data are entered in a comparable format to biological data, i.e. columns as sites, and this should be done as soon as possible by the field operative so that details are fresh. All hardcopies of habitat data and notes should be labelled and stored in a project file and retained.

All electronic files should have a notes sheet which details any relevant information for future users. In particular each user, beginning with the field operative who enters the data, should record details of any changes to the data, including when and why they were made. It is also recommended to retain a single version of the data which has undergone quality control and may not be altered. All analysis is performed on copies of this master sheet.

[2] http://www.cawthron.org.nz/coastal-freshwater-resources/downloads/stream-habitat-assessment-protocols.pdf

Storage tools can be either manual or electronic systems (or both, preferably). They will usually be summary sheets, other physical filing systems, or electronic spreadsheets and databases. Use appropriate file formats such as .xls, .txt, .dbf or specific analysis software formats. Copy and/or backup all data, whether electronic, data sheets, metadata or site access descriptions, preferably offline if the primary storage location is part of a networked system. Store the copy at a separate location for security purposes.

Analysis, interpretation and reporting

Seek statistical advice from a biometrician or suitably experienced person prior to undertaking any analysis.

The invertebrate data derived from the semi-quantitative soft-bottomed stream sampling protocol may take two forms: 1) presence/absence, or 2) semi-quantitative abundance derived from coded abundance or fixed count processing protocols. Presence/absence data is essentially a list of the species which are present and may be used to generate a number of invertebrate community metrics. The most common indices calculated from this data are:

- Taxa richness
- Richness of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa or % EPT abundance
- Macroinvertebrate Community Index for soft-bottomed streams (MCI-sb).

Semi-quantitative abundance data may be used to calculate all of the above indices with the addition of the Semi-Quantitative Macroinvertebrate Community Index for soft-bottomed streams (SQMCI-sb) (Stark & Maxted 2007) and the proportions of dominant taxa within and between sites.

Taxa richness

Taxa richness is simply the number of taxa that were found at a site and is commonly used in inventory and ecosystem condition monitoring studies. Sites may be compared in terms of taxa richness provided the sampling effort and taxonomic resolution at each site is standardised. If groups of sites are to be compared, e.g. forest streams versus grassland streams, then it is important that equal numbers of each site type have been sampled. If this assumption is violated the degree of difference must be noted or comparisons will require rarefaction and a biometrician should be consulted (Magurran 2004). If sample numbers and effort are balanced, i.e. equal, then basic Analyses of Variance (ANOVA) or *t*-tests can be used to compare between the mean values for habitat types. Alternatively, instead of comparing richness between groups, a gradient approach may be used whereby the richness of taxa at each site is compared to the value for an environmental condition at that site. Such a correlative approach is more appropriate when sites do not fit into meaningful groupings.

EPT richness

EPT richness is the number of taxa which are members of the Ephemeroptera (mayfly), Trichoptera (caddis fly) and Plecoptera (stonefly) orders and is commonly used in ecosystem condition monitoring. Many of the species within these groups require undisturbed habitats and so this metric may be more sensitive to impacts than taxa richness alone. Accordingly, EPT richness may be presented as a proportion of total richness, e.g. % EPT.

MCI-sb

The Macroinvertebrate Community Index (MCI) was initially proposed by Stark (1985) to assess organic enrichment in the stony riffles of New Zealand streams and rivers and is regularly used in ecosystem condition monitoring. A variant index for soft-bottomed streams (MCI-sb) was created by Stark & Maxted (2007) which uses the same formula as the MCI but different tolerance values for individual taxa. Each taxa is assigned a score (1–10) which represents its tolerance to pollution. The MCI-sb score for a sample is calculated thus:

$$= 20 \sum a_i / \mathrm{S}$$

Where a_i is the MCI-sb tolerance score for the i^{th} *taxon* and S is the total number of taxa. Taxon tolerance scores can be found in Table 3.

MCI-sb values range from 0–200, which may be interpreted in terms of water quality according to Table 1. The same analyses and assumptions apply as for taxa richness and EPT richness. All comparisons should be made with reference to habitat data.

Table 1. Interpretation of MCI-sb, QMCI-sb and SQMCI-sb values from stony riffles (after Boothroyd & Stark 2000).

Interpretation	**MCI**	**QMCI & SQMCI**
Clean water	> 120	> 6.00
Doubtful quality of possible mild pollution	100–119	5.00–5.99
Probable moderate pollution	80–99	4.00–4.99
Probable severe pollution	< 80	< 4.00

Coded abundance and fixed count data provide rough estimates of the relative numbers of the different taxa and so provide the ability to calculate an additional index—the Semi-Quantitative Macroinvertebrate Community Index for soft-bottomed streams (SQMCI-sb). If coded abundance data are received in alpha code form they may be converted to numerical form according to Table 2. Like the MCI-sb, SQMCI-sb is designed to be calculated from kick-net samples collected over a standardised area (0.3–0.6 m^2), but unlike the MCI-sb, SQMCI-sb scores range from 0–10. The SQMCI-sb is calculated thus:

$= \sum (c_i \, a_i) / \mathrm{M}$

Where c_i is the coded abundance of individuals in the i^{th} taxon and M is the coded abundance total number of individuals. Scores may be interpreted in terms of water quality according to Table 1 and are directly comparable with QMCI-sb scores, but not MCI-sb. The same analyses and assumptions apply as for taxa richness and EPT richness. All comparisons should be made with reference to habitat data.

Table 2. Abundance classes, count ranges and coded abundance used for the calculation of SQMCI-sb scores. Abundance class may be converted to coded abundance for the purposes of analysis. (Reproduced from Stark 1998.)

Abundance class	Counts	Coded abundance
R—rare	1–4	1
C—common	5–19	5
A—abundant	20–99	20
VA—very abundant	100–499	100
VVA—very very abundant	500+	500

Semi-quantitative macroinvertebrate data may also be used to compare the abundance of groups of taxa between sites or examine changes in the dominant taxa at a site. Relative or absolute abundance of different taxa groups are commonly displayed as a stacked bar graph where each column represents a location or sampling event and the column is divided vertically according to the proportional or absolute abundance of major taxa groups. Taxa groupings can be defined according to the objectives of the study, but conventionally approximate the major orders, such as Ephemeroptera, Trichoptera, Mollusca and other. An example of a stacked bar graph is shown in 'Case study A'. A further basic descriptive technique for comparing invertebrate communities between sites/occasions would be to list the five most abundant taxa.

It is commonplace to provide a number of these summary statistics, such as richness and coded abundance of taxa along with habitat summary data, prior to any more complicated analyses in order to 'set the scene' for the reader.

There are numerous indices and statistical techniques used for describing richness and diversity (a function of the number of both taxa and individuals) which are available. However, an experienced biometrician / freshwater ecologist should be consulted before applying these techniques. The best overview of available statistical measures of diversity may be found in Magurran (2004). Further, 'multivariate' techniques, such as NMDS, DCA or RDA, are also available for investigating differences in entire communities often in relation to accompanying habitat data; however, these techniques require an experienced practitioner.

The majority of collation and calculation described here can be performed in a basic spreadsheet package such as Excel, although there are a variety of commercial and freeware packages available to calculate summary statistics and perform more in-depth analyses. However, beyond the

basic descriptive statistics, such as richness, MCI-sb, and summary plots, the user will require specific training or experience.

Table 3. Recommended minimum level of macroinvertebrate identification (based on Stark 1998; Winterbourn et al. 2000) with associated MCI, SQMCI and QMCI tolerance values.

INSECTA		**Neuroptera**		**Trichoptera** (Cont.)	
Ephemeroptera		*Kempynus*	5	*Hydrobiosella*	9
Acanthophlebia	7	**Diptera**		*Hydrobiosis*	5
Ameletopsis	10	*Aphrophila*	5	*Hydrochorema*	9
Arachnocolus	8	*Austrosimulium*	3	*Kokiria*	9
Atalophlebioides	9	*Calopsectra*	4	*Neurochorema*	6
Austroclima	9	Ceratopogonidae	3	*Oeconesidae*	9
Coloburiscus	9	*Chironomus*	1	*Olinga*	9
Deleatidium	8	*Corynoneura*	2	*Orthopsyche*	9
Ichthybotus	8	*Cryptochironomus*	3	*Oxyethira*	2
Isothraulus	8	*Culex*	3	*Paroxyethira*	2
Mauiulus	5	Culicidae	3	*Philorheithrus*	8
Neozephlebia	7	Dolichopodidae	3	*Plectrocnemia*	8
Nesameletus	9	Empididae	3	*Polyplectropus*	8
Oniscigaster	10	Ephydridae	4	*Psilochorema*	8
Rallidens	9	*Eriopterini*	9	*Pycnocentrella*	9
Siphlaenigma	9	*Harrisius*	6	*Pycnocentria*	7
Zephlebia	7	*Hexatomini*	5	*Pycnocentrodes*	5
Plecoptera		*Limonia*	6	*Rakiura*	10
Acroperla	5	*Lobodiamesa*	5	*Tiphobiosis*	6
Austroperla	9	*Maoridiamesa*	3	*Triplectides*	5
Cristaperla	8	*Mischoderus*	4	*Triplectidina*	5
Halticoperla	8	*Molophilus*	5	*Zelolessica*	10
Megaleptoperla	9	Muscidae	3	**Lepidoptera**	
Nesoperla	5	*Nannochorista*	7	*Hygraula*	4
Spaniocerca	8	*Neocurupira*	7	**Collembola**	6
Spaniocercoides	8	*Neoscatella*	7	ACARINA	5
Stenoperla	10	*Nothodixa*	5	**CRUSTACEA**	
Taraperla	5	*Orthocladiinae*	2	Amphipoda	5
Zelandobius	5	*Parochlus*	8	Copepoda	5
Zelandoperla	10	*Paradixa*	4	Cladocera	5
Megaloptera		*Paralimnophila*	6	Isopoda	5
Archichauliodes	7	*Paucispinigera*	6	Ostracoda	3
Odonata		Pelecorhynchidae	9	*Paranephrops*	5
Aeshna	5	*Peritheates*	7	*Paratya*	5
Antipodochlora	6	Podonominae	8	*Tanaidacea*	4
Austrolestes	6	*Polypedilum*	3	**MOLLUSCA**	
Hemicordulia	5	Psychodidae	1	*Ferrissia/Grunlachia*	3
Xanthocnemis	5	Sciomyzidae	3	*Gyraulus*	3
Procordulia	6	Stratiomyidae	5	*Hyridella*	3

Hemiptera		Syrphidae	1	*Latia*	3
Anisops	5	Tabanidae	3	*Lymnaea/ Austropeplia*	3
Diaprepocoris	5	Tanypodinae	5	*Melanopsis*	3
Microvelia	5	*Tanytarsini*	3	*Physa*	3
Sigara	5	*Tanytarsus*	3	*Physastra*	5
Coleoptera		Thaumaleidae	9	*Potamopyrgus*	4
Antiporus	5	*Zelandotipula*	6	Sphaeriidae	3
Berosus	5	**Trichoptera**		OLIGOCHAETA	1
Dytiscidae	5	*Alloecentrella*	9	HIRUDINEA	3
Elmidae	6	*Aoteapsyche*	4	PLATYHELMINTHES	3
Homeodytes	5	*Beraeoptera*	8	NEMATODA	3
Hydraenidae	8	*Confluens*	5	NEMATOMORPHA	3
Hydrophilidae	5	*Conuxia*	8	NEMERTEA	3
Liodessus	5	*Costachorema*	7	**COELENTERATA**	
Ptilodactylidae	8	*Edpercivalia*	9	Hydra	3
Rhantus	5	*Ecnomidae/Zelandotipula*	8		
Scirtidae	8	*Helicopsyche*	10		
Staphylinidae	5	*Hudsonema*	6		

Case study A

Case study A: a comparison of invertebrate communities in soft-bottomed streams within differing substrate types

Synopsis

This study used quantitative sampling, but analysis methods would suit semi-quantitative data except where noted. Data used with permission of Kevin Collier (Collier et al. 1998). If your sampling is quantitative, then more emphasis may be placed on estimates of abundance and your study will be able to detect subtle shifts in community composition. In a semi-quantitative context it is relative, or proportional, abundance and indices of richness which are of primary interest.

Lowland streams in agricultural areas often appear degraded because of high turbidity, extensive bank erosion, high macrophyte abundance and silt-laden beds. However, these soft-bottomed streams do contain macroinvertebrate populations which can be sampled in a quantitative or semi-quantitative manner. Collier et al. (1998) compared inorganic substrates, macrophyte and invertebrate communities between four different soft-bottomed streams. The format of data presented here is descriptive and highlights the major differences between streams. More in-depth analysis can be found in the original paper. However, the primary findings of this study are that soft-bottomed streams can be highly variable in terms of macrophyte species present and cover, and in the invertebrate communities that occupy both the macrophytes and inorganic sediment. Accordingly, in a soft-bottomed stream, whether your sampling is quantitative or semi-quantitative, it is important to estimate or measure the cover of macrophytes and sample the different macrophytes species and other habitat types in any stream under consideration. Collier et al.

(1998) also showed that woody debris was an important substrate in soft-bottomed streams, particularly for sensitive EPT taxa. Therefore, when present, woody debris should be sampled using the methods outlined in the protocol.

Objectives

- To compare the inorganic substrates, macrophyte and invertebrate communities in four soft-bottom streams in the Waikato.

Sampling design and methods

Study area

Streams in the lowlands of Waikato drain some of the most extensively used landscapes in New Zealand. Soils in this region are predominately yellow-brown loams and yellow-brown pumice overlying sedimentary rocks with volcanics and some peat basins. The combination of erodible soils and intensive land use has resulted in a considerable sedimentation of many streams which, due to their low gradient, do not flush sediments during high flow events.

Habitat assessments

Ten evenly spaced transects were established across reaches. At each transect, size and composition of bed substrates were estimated by classifying 10 particles recorded at evenly spaced points across the transects. Classifications were “small” wood (< 10 cm diameter), “large” wood (≥ 10 cm diameter), roots, or inorganic particle size classes: < 2 mm, sand; 2–4 mm, fine gravel; 4–8 mm, small gravel; 8–16 mm, small–medium gravel; 16–32 mm, medium–large gravel; 32–64 mm, large gravel; 64–128 mm, small cobble; 128–256 mm, large cobble; > 256 mm, boulder.

Invertebrate and macrophyte sampling

Five invertebrate samples from macrophytes and inorganic substrates were collected at randomly chosen locations in each study reach using a 0.25 mm mesh kick-net. For each inorganic substrate sample an area equivalent to 0.1 m^2 was washed into the net. Grab samples of macrophytes were made into a net held downstream. Each macrophyte sample was washed in a bucket of water to dislodge invertebrates, shaken to remove excess water and divided according to species (of macrophyte), and weighed with a spring balance. All invertebrate samples were picked live on a white tray. When taxa were particularly common and easy to identify, a randomly selected quarter was counted and counts were adjusted for total area (of tray). All invertebrates were then stored in 70% isopropyl alcohol and identified under a binocular microscope.

Results

The Waitoa stream bed was entirely composed of sand and silt, while the Kāniwhaniwha and Waihou stream beds had approximately 50% coverage of silts and sand (Fig. 2). In the

Kāniwhaniwha Stream the rest of the bed was covered with woody debris, while the Waihou Stream had woody debris, bedrock and some gravels. The Ōhinemuri Stream had the most diverse bed substrates including woody debris, bedrock, cobbles, gravel and about 15% silt and sand.

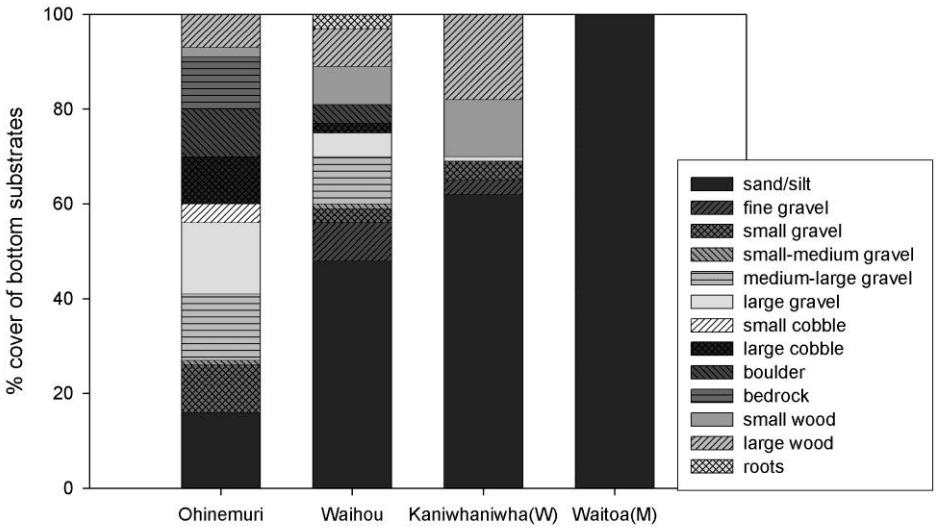

Figure 2. Percentage of bottom substrates classified according to 10 size groups for inorganic material, and large wood (≥ 10 cm diameter), small wood (< 10 cm diameter), or roots.

Macrophyte communities in the streams were quite variable. *Potamogeton* sp. was present at all sites except the Kāniwhaniwha Stream. The Ōhinemuri Stream had the most diverse macrophyte community, possibly due to the diversity of inorganic substrate types found in this stream.

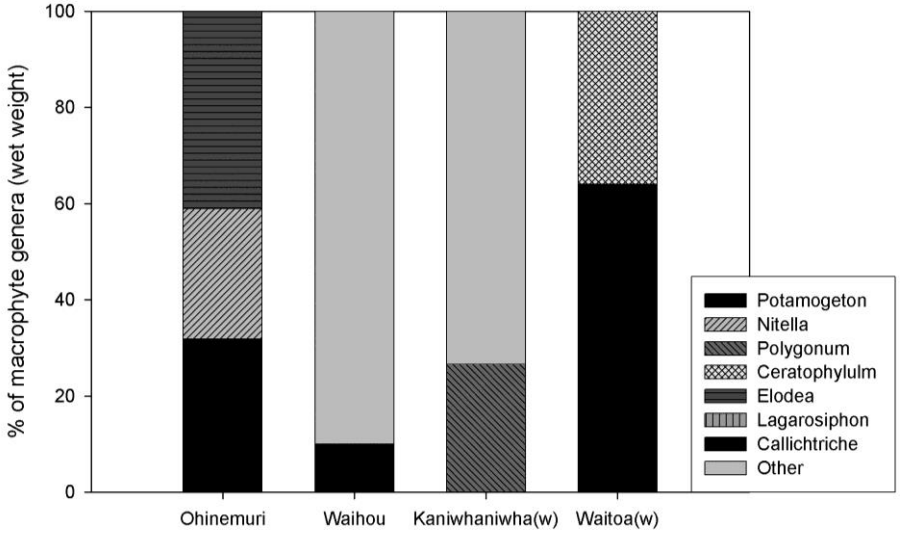

Figure 3. Percentage of different macrophyte genera (wet weight) collected from five locations at four lowland streams in the Waikato.

A total of 106 invertebrate taxa were recorded during this survey. Most were Trichoptera (32 taxa), Diptera (22) and Ephemeroptera (16). Plecoptera, Mollusca and Coleoptera made up 5–7 taxa each. Only the dipteran *Austrosimulium* sp. and the mollusc *Potamopyrgus antipodarum* were found at all sites. An average of 27 taxa were found at each site.

Macroinvertebrate communities on inorganic substrates in the Ōhinemuri, Kāniwhaniwha and Waitoa streams were proportionally dominated by the mollusc *Potamopyrgus antipodarum* (Fig. 4). The Waihou Stream contained *P. antipodarum* but was proportionally dominated by Dipteran, Coleopteran and mayfly taxa. However, in general, Ephemeroptera, Trichoptera and Plecotpera (EPT taxa) were relatively uncommon on inorganic substrates except in the Waihou Stream.

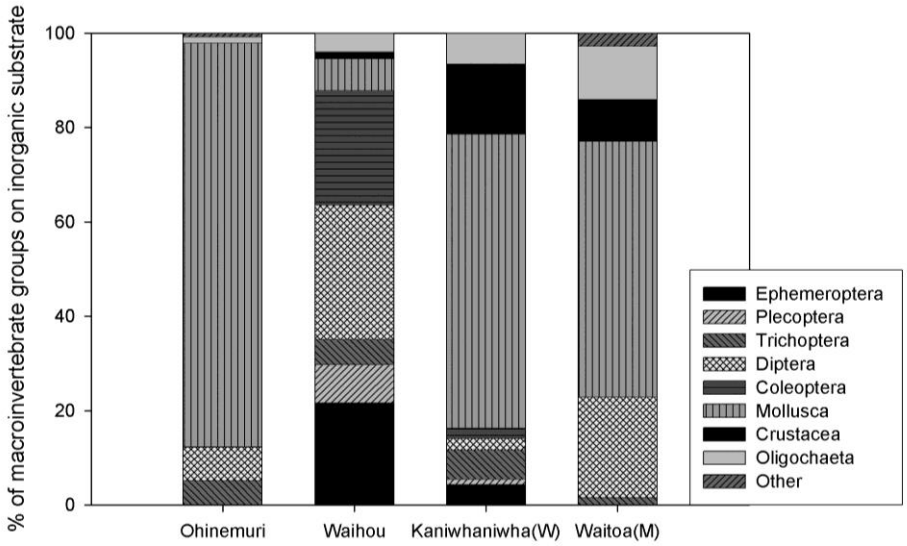

Figure 4. Percentage of different macroinvertebrate groups collected from inorganic substrates at four lowland stream sites.

On macrophytes there were some similarities between invertebrate communities but also some marked differences (Fig. 5). The Ōhinemuri and Kāniwhaniwha stream macrophyte dwelling invertebrate communities were similarly dominated by *P. antipodarum*. However, in the Waitoa Stream, Diptera replaced *P. antipodarum* as the most common taxa. In the Waihou Stream, invertebrates on macrophytes were composed of > 50% EPT taxa, some Diptera, and the ubiquitous snail *P. antipodarum*.

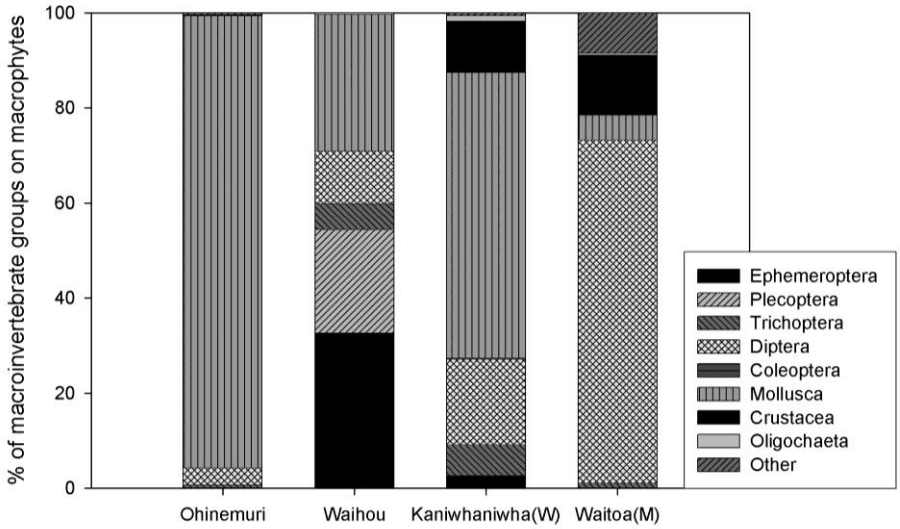

Figure 5. Percentage of different macroinvertebrate groups collected from macrophytes at four lowland stream sites.

Note: the following section ideally requires full count, quantitative data. It would not be ideal to display abundance based on semi-quantitative data. Between streams the total abundance of invertebrates on macrophytes and inorganic sediments followed a very similar pattern (Fig. 6). Abundances were greatest in the Ōhinemuri Stream and lowest in the Waihou Stream. It is not appropriate to compare the abundances between macrophytes and inorganic substrates because the sampling methods and units are different.

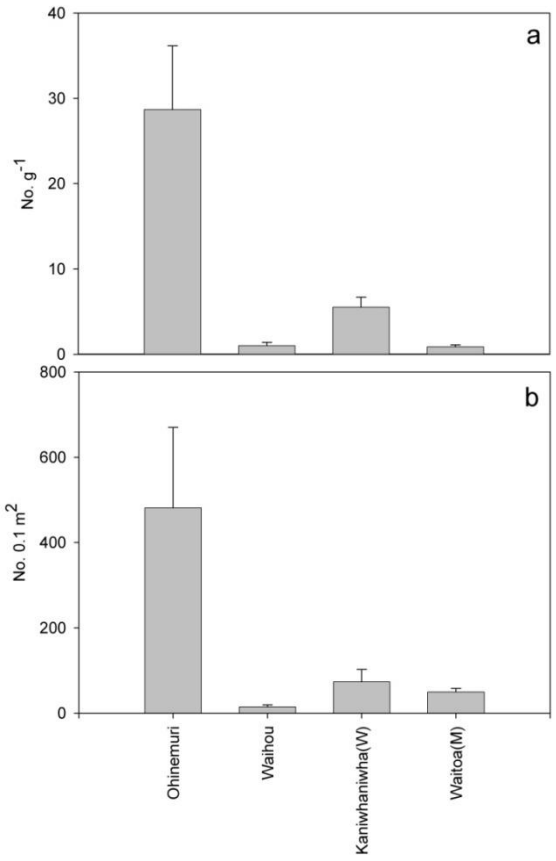

Figure 6. Mean (+ 1 SE, *n* = 5) abundance of total invertebrates from **a**) macrophytes samples (per g wet weight) and **b**) inorganic sediment samples (0.1 m^2).

Limitations and points to consider

This case study presents a method and protocol for sampling both inorganic substrates and macrophytes in soft-bottomed streams that are very similar to ones described in this Toolbox module. All of the graph and presentation techniques described are applicable to both quantitative and semi-quantitative data, except where noted. However, it is important to acknowledge the limitations of your data. If your sampling is quantitative then more emphasis may be placed on estimates of abundance/density and your study will be able to detect subtle shifts in community composition. In a semi-quantitative context it is relative, or proportional, abundance and indices of richness which are of primary interest.

References for case study A

Collier, K.J.; Wilcock, R.J.; Meredith, A.S. 1998: Influence of substrate type and physic-chemical conditions on macroinvertebrate faunas and biotic indices of some lowland Waikato, New Zealand, streams. *New Zealand Journal of Marine and Freshwater Research 32*: 1–19.

Full details of technique and best practice

A complete and detailed guide to this technique can be found in Stark et al. (2001).

Protocol:

1. Ensure that the sampling net is clean.
2. Approach sample site by moving upstream through the waterway. Determine plant species to be sampled. Consistency in plant species is important for comparisons between sizes, although not always possible. Standardise the depth/velocity conditions of sampling points, where possible.
3. Collect replicate samples ($n \geq 4$) of submerged macrophyte tips (approx. 100 g wet weight of top 20–30 cm of plant, which is equivalent to 1.5–2 L of weed) 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 approx. 1 L of clean water to each bucket and firmly attach lid. Shake bucket vigorously (20×) 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. With the aid of a wash bottle, transfer material retained on the sieve to a plastic container.
7. Add preservative. Aim for a preservative concentration in the sample container of 70–80% (i.e. allow for the water already present). Be generous.
8. Place a sticky label on the side of the sample container and record the site code/name, date, and replicate number (if applicable) using a permanent marker. Write on the label when it is dry and do not rely on a label on the pottle lid! Place a waterproof label inside the container. Screw the lid on tightly.
9. Note the sample type, collector's name and preservative used on the field data sheet.
10. Drain the plant material of excess water (leave to stand in sieve for 2 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 (70°C for at least 24 hrs) and weighing.
11. Record wet weight of macrophyte material associated with each replicate sample. Also record the species and condition (i.e. senescent, flowering, covered in epiphytes) for the macrophyte bed from which the sample was taken.

References and further reading

Boothroyd, I.K.G.; Stark, J.D. 2000: Use of invertebrates in monitoring. Pp. 344–373 in Collier, K.J.; Winterbourn, M.J. (Eds): New Zealand stream invertebrates: ecology and implications for management. New Zealand Limnological Society, Christchurch.

Harding, J.S.; Clapcott, J.; Quinn, J.; Hayes, J.; Joy, M.; Storey, R.; Greig, H.; Hay, J.; James, T.; Beech, M.; Ozane, R.; Meredith, A.; Boothroyd, I. 2009: Stream habitat assessment protocols for wadeable rivers and streams of New Zealand. School of Biological Sciences, University of Canterbury, Christchurch. http://www.cawthron.org.nz/coastal-freshwater-resources/downloads/stream-habitat-assessment-protocols.pdf

Magurran, A.E. 2004: Measuring biological diversity. Wiley-Blackwell, London. 260 p.

Stark, J.D. 1985: A macroinvertebrate community index of water quality for stony streams. *Water & Soil Miscellaneous Publication 87*. National Water and Soil Conservation Authority, Wellington.

Stark, J.D. 1998. SQMCI: a biotic index for freshwater macroinvertebrate coded abundance data. *New Zealand Journal of Marine and Freshwater Research 32*: 55–66.

Stark, J.D.; Boothroyd, I.K.G.; Harding, J.S.; Maxted, J.R.; Scarsbrook, M.R. 2001: Protocols for sampling macroinvertebrates in wadeable streams. Prepared for the Ministry for the Environment, Sustainable Management Fund Project No. 5103. http://www.cawthron.org.nz/coastal-freshwater-resources/downloads/protocols-full-manual.pdf

Stark, J.D.; Maxted, J.R. 2007: A biotic index for New Zealand's soft-bottomed streams. *New Zealand Journal of Marine and Freshwater Research 41*: 43–61.

Winterbourn, M.J.; Gregson, K.L.D.; Dolphin, C.H. 2000: Guide to the aquatic insects of New Zealand. *Bulletin of the Entomological Society of New Zealand 13*.

Appendix A

The following Department of Conservation documents are referred to in this method:

docdm-959842	Freshwater habitat assessment
docdm-146272	Standard inventory and monitoring project plan
docdm-761873	Stream habitat assessment field sheet
docdm-761858	Stream invertebrate data sheet example