# Freshwater ecology: semi-quantitative macroinvertebrate sampling in hard-bottomed streams

Version 1.0

This specification was prepared by Duncan Gray in 2013.

## Contents




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**Inventory and monitoring toolbox: freshwater ecology**

**DOCDM-722563**

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 for sampling macroinvertebrate communities in hard-bottomed streams.

This protocol is designed for riffles, although it may be used less effectively in deeper habitats, or sand through to boulder bedrock substrates. It can produce qualitative or semi-quantitative data by applying coded abundance or fixed count processing methods. Data is suitable for inventory, general ecosystem condition monitoring and threatened species monitoring where quantitative data are not considered necessary. If you require greater detail on the species present (inventory or threatened species monitoring) consider also sampling stream habitats other than stony riffles, e.g. macrophyte beds, overhung banks or woody debris. However, inclusion of extra habitats complicates comparisons between sites, and samples of additional habitat ought to remain separate during sorting (for a full discussion of the options for objectives and methodologies, see 'Introduction to macroinvertebrate monitoring in freshwater ecosystems'—docdm-724991). It should also be noted that in the case of rare species, monitoring each taxa may have certain specific habitat requirements which dictate sampling methods and location. For example, freshwater crayfish, or kōura, are often well hidden beneath banks during the day and are more readily located during night sampling. Sampling targeted at a particular species should be informed by available information about its natural history and behaviour. This data may be used to calculate a variety of species richness and relative abundance metrics as well as perform multivariate analyses. The principle advantage of this method is that it is cheap and straightforward to apply. However, always consult a biometrician or experienced freshwater ecologist before you start sampling to ensure that your design and methods are suitable to meet your objectives.

To maintain comparability of samples between sites and/or studies it is important to standardise sampling effort. Stark et al. (2001) recommend a pre-defined area approach where a single kick-net contains invertebrates collected over 0.6 to 1.0 $m^2$ of stream bed across the range of water velocity present in the riffle(s). It is acceptable to sample a single riffle provided the area of stream bed available falls between 0.6 to 1.0 $m^2$. Should any single riffle not comprise an adequate area, adjacent riffles may be sampled until the desired area has been covered. Kick-nets have a mesh size of 0.5 mm and are 30 to 40 cm wide at the base. Samples are taken by agitating the stream bed immediately (< 0.5 m) upstream of the net. The foot-kick method is recommended (Fig. 1) and intensity and duration should be standardised where possible, e.g. a single kick-net sample is performed by the same field observer for 3 minutes over 0.6 to 1.0 $m^2$ of stream bed. Separate kick-net samples may be pooled to prevent clogging of the net. Within-site replication of sampling is not required for this semi-quantitative technique.

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

Figure 1. Kick-net sampling. Note the substrate is disturbed directly upstream of the net which is held firmly against the stream bed. Photo: Tanya Blakely.

Each sample should have preservative (usually 70% ethanol) added as soon as possible after it is extracted from the net. A unique identifying code must be clearly marked on the lid and on a slip of waterproof paper inside the pottle (Fig. 2). Pottles should also be marked with the location, date and the field observer's name.

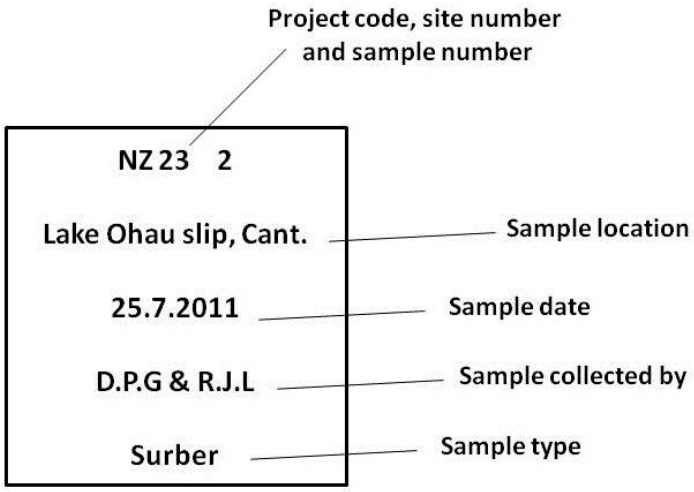


Figure 2. An example wet label with the required information to identify each sample should be written in pencil on waterproof paper.

Samples should be transported to a laboratory for storage prior to processing. Processing methods are detailed in Stark et al. (2001) and 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 (a record of species presence) or semi-quantitative (an index of relative abundance).

## Advantages

- Kick-netting requires no specialised equipment or resources.
- Kick-netting 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 hard-bottomed stream semi-quantitative sampling protocol is particularly suitable for inventory as the method captures the majority of macroinvertebrates found in New Zealand streams, is cheap to undertake and process and requires minimal specialised training or equipment. The method can be tailored to focus specifically on inventory objectives by including further habitats in a reach (e.g. woody debris, banks and boulders) in an attempt to capture a wider range of species. However, such sampling effort bias prevents the use of data for semi-quantitative comparisons.

## Suitability for monitoring

Kick-netting is suitable for monitoring when semi-quantitative data is considered adequate (e.g. where very large changes are expected or where there is low risk associated with failure to detect change). Sampling effort and habitat types must be standardised as much as possible. Where comparable habitats or reference streams (non-treatment or un-managed sites) are not available biological data must be supported by physical habitat data to qualify any conclusions made about differences in communities.

## 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 hard-bottomed streams may be carried out by a single field observer. However, in the interests of safety it is recommended that sampling is done by teams of at least two.

Standard field equipment includes:

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

Specialist equipment required:

- Kick-net (0.5 mm mesh) (Fig. 3)
- White tray or 10 litre bucket
- Sieve or sieve bucket
- Plastic sample containers or ‘pottles’ (usually 500–1000 ml volume)
- Preservative (usually 70% ethanol)

Figure 3. The kick-net (bottom) compared to a Surber sampler. Note triangular net with reinforced outer rim to prevent undue wear during sampling.

# 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 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 and are used to inform biological data. 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

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

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 a member of the field team, 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.

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 semi-quantitative hard-bottomed stream sampling 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. An evaluation of benthic macroinvertebrate community metrics in relation to ecological integrity is provided by Schallenberg et al. (2011). 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 (MCI).

Semi-quantitative abundance data may be used to calculate all of the above indices as well as the Semi-Quantitative Macroinvertebrate Community Index (SQMCI) (Stark 1998) 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 during inventory and ecosystem condition 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 generalised linear modelling approach may be used whereby the richness of taxa at each site is compared to the value for an environmental condition at that site. The latter 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 for 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

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. However, despite criticisms, it has proven to be an effective measure of the effects of a number of different impacts on stream invertebrate communities and is regularly used for ecosystem condition monitoring. Each taxa is assigned a score (1–10) which represents its tolerance to pollution. The MCI score for a sample is calculated thus:

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

Where $a_i$ is the MCI 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 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, QMCI and SQMCI 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 |

## Semi-quantitative MCI

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 (SQMCI). This index may be used primarily for ecosystem condition monitoring. Coded abundance protocols were originally developed to add value to presence/absence data but more recently has been viewed as the most cost-effective alternative to quantitative methods. Taxa are assigned to a category (Table 2) based on rough tallies recorded by an experienced taxonomist. Alternatively there are fixed count protocols which are less time consuming than fully quantitative methods, but depending on the nature of samples, the fixed count used (100, 200 or 300), and the experience of the laboratory personnel, may or may not be more cost-effective than coded abundances. The fixed count protocol recommended by Stark et al. (2001) involves counting all individuals from a sub-sample up to and including the 200th individual and optionally a scan of the entire sample for rare taxa. Fixed count data allow percentage community compositions to be calculated but not comparisons of density when used to process kick-net samples. If coded abundance data are received in alpha code form, i.e. r, c, a, va, vva, they may be converted to numerical form according to Table 2. Like the MCI, SQMCI is designed to be calculated from kick-net samples collected over a standardised area (0.3–0.6 $m^2$), but unlike the MCI, SQMCI scores range from 0–10. The SQMCI 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 $\mathrm{M}$ 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 scores, but not MCI. 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 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 |

## Community composition

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 although with less reliability than quantitative data. 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'. Another basic descriptive technique for comparing invertebrate communities between sites/occasions would be to list the five most abundant taxa.

It is common 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, to move beyond the basic descriptive statistics, such as richness, MCI and summary plots, we recommend you seek advice from a freshwater specialist.

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: invertebrate diversity in braided rivers**

## Synopsis

Large braided rivers are a distinctive feature of the landscape in several regions of New Zealand. The invertebrate communities of braided rivers have been described as taxonomically depauperate, but recent research suggests otherwise. This case study details results from a field survey of 11 braided rivers, collecting benthic invertebrates from six reaches dispersed down each river, and sampling up to five habitats per reach. Gray & Harding (2009) compared the habitat characteristics and taxonomic richness between individual braided rivers and between the different floodplain habitats within those rivers.

## Objectives

- To compare invertebrate communities between different rivers and different habitats within those rivers.

The authors begin by describing the design and methodology of their survey. They then characterise the different rivers in terms of their physical characteristics which provides context for biological information. Finally, various aspects of the diversity of invertebrate communities are presented and discussed.

## Sampling design and methods

Eleven rivers were sampled, three in the North Island and eight in the South Island, distributed in proportion to the abundance of braided rivers within New Zealand (Fig. 4a). Six reaches, approximately 1 km long, were selected at intervals along each river (Fig. 4b). The uppermost reach was in the steeper headwaters, above the point where a distinct floodplain first appears on a

1:50 000 topographical map. In these reaches the river was generally 3rd–4th order (Strahler 1957). The lowest reach was close to the river mouth, beyond estuarine and brackish water zones and above tidal influence. Intermediate reaches were distributed evenly between the uppermost and lowermost reaches. Where present, a gorge reach was included. Anthropogenic impacts generally increased downstream although there was considerable variation among rivers.

A single transect located at the approximate mid-point of each reach was established across the entire floodplain. Transects were approximately straight and perpendicular to the main channel. All habitats visible either upstream or downstream from the transect line were assessed and the most successionally mature example of each of five habitat types, when present, was sampled (Fig. 4b). Habitat types were: the main channel, a side braid or secondary channel (with upstream and downstream connection to the main channel), a floodplain pond, a spring source and a spring creek located at least 50 m downstream from the source of another spring-fed stream.

The physical characteristics of each river were assessed using broadscale databases and GIS layers. This is analogous to the desktop assessment described by Harding et al. (2009) and is appropriate for inventory studies (see 'Introduction to macroinvertebrate monitoring in freshwater ecosystems'—docdm-724991; and 'Stream habitat assessment field sheet'—docdm-761873).

Biological samples were collected during baseflow conditions between December 2006 and April 2007 and consisted of a single extensive kick-net (mesh size 250 µm) sample (using the protocol described in Stark et al. 2001). Kick-netting was performed for 5 minutes over an approximately 3 $m^2$ area within each habitat.

Samples were preserved in 70% ethanol in the field, concentrated on a 250 µm mesh sieve in the laboratory and sorted under 40 × magnification. Identifications were made to the lowest taxonomic level possible, except for Oligochaeta, which were not differentiated below order and Chironomidae, which were not separated below tribe. Identifications were made using the keys and guides of Winterbourn (1973), Chapman & Lewis (1976), Cowley (1978), McLellan (1991, 1998), Winterbourn et al. (2000), Smith (2001), Scarsbrook et al. (2003) and a description by Percival (1945). Taxa were counted using a 200-individual fixed count protocol and scan for rare taxa (Stark et al. 2001)

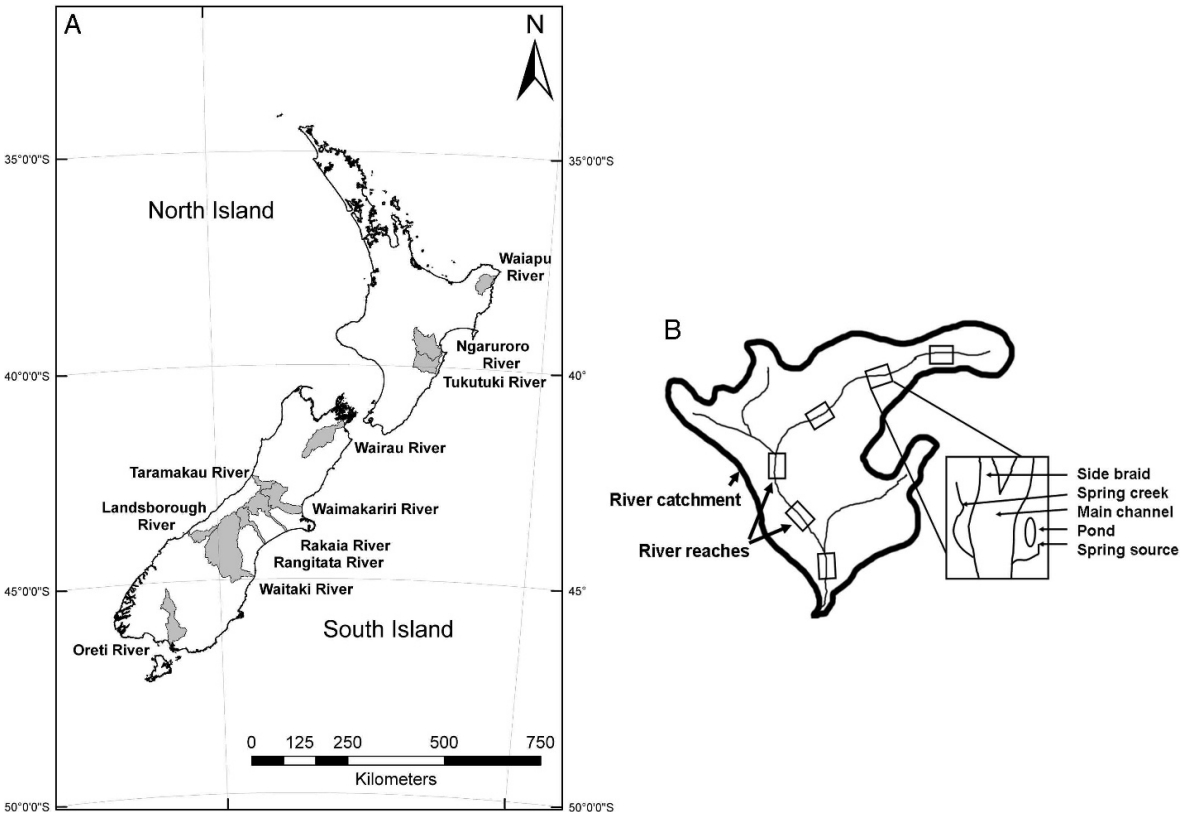


Figure 4. A) Eleven braided river catchments included in the survey, three in the North Island and eight in the South Island. B) Up to six reaches were sampled within each catchment and five floodplain habitats in each reach.

# Results

## Physical characteristics

The rivers ranged in mean discharge from 44 $m^3/s$ (Waiapu River) to 370 $m^3/s$ (Waitaki River), and catchment area ranged from 998 $km^2$ (Taramakau River) to 11 887 $km^2$ (Waitaki River). Rivers were further characterised according to hydrology, climate, and vegetation cover categories, which were derived from the Freshwater Environments of New Zealand (FWENZ) database[3] (Wild et al. 2005) and the River Environment Classification (Snelder et al. 2005). The Waitaki River is highly modified in its lower reaches by a series of impoundments. Therefore, for the sake of characterisation, the river was considered to consist of separate entities above and below the dams (Table 4). Rivers were characterised hydrologically using the average number of floods/y that were three times the median flow of the river (FRE3), a criterion that is ecologically relevant to stream biota (Clausen & Biggs 1997). Values ranged from an average of 24 events/y in the Landsborough River to 0.6 in the regulated lower Waitaki River. FRE3 values were generally lower for North Island rivers than South Island rivers, which have more alpine catchments. Rain days per year was highest in rivers on the west coast of the South Island, e.g., the Landsborough and Taramakau rivers, and lowest in the Ōreti River in the south of the South Island. In general, South Island rivers experienced more rain days per year than did North Island rivers. Predominant catchment vegetation cover in the North

[3] http://www.doc.govt.nz/conservation/land-and-freshwater/freshwater/freshwater-ecosystems-of-new-zealand/

Island was pasture. However, in the South Island, catchment vegetation in the northern and western regions was dominated by indigenous forest, whereas catchment vegetation in east coast rivers was mainly scrub and bare ground. These differences are predominantly due to climatic and topographic differences between the North and South islands. The alpine spine of the South Island in particular produces extreme levels of orographic rain and dictates river hydrology and vegetation patterns.

Table 4. Catchment characteristics of the 11 braided rivers considered in the study. Rivers are ordered north to south, the first three being on the North Island.

| Catchment | Region | Catchment area ($km^2$) | River order[a] | Mean discharge ($m^3s$-1)[b] | FRE 3 excedence | Days rain/year[b] | Catchment vegetation[c] |
|---|---|---|---|---|---|---|---|
| Waiapu | East Cape | 1574 | 6 | 82 | 7.1 | 19.3 | Pasture |
| Ngaruroro | Hawkes Bay | 2009 | 6 | 46 | 10.4 | 10 | Pasture |
| Tukituki | Hawkes Bay | 2495 | 6 | 44 | 10 | 7.98 | Pasture |
| Wairau | Nelson-Marlborough | 3574 | 7 | 99 | 11.5 | 13.3 | Indigenous forest |
| Taramakau | West Coast | 998 | 6 | 150 | 22.6 | 64.7 | Indigenous forest |
| Waimakariri | Canterbury | 3541 | 7 | 128 | 15.3 | 17.2 | Scrub/tussock |
| Rakaia | Canterbury | 2830 | 7 | 175 | 14.3 | 24.1 | Bare ground |
| Rangitātā | Canterbury | 1809 | 6 | 109 | 10.9 | 26.2 | Bare ground |
| Landsborough | West Coast | 1341 | 6 | 277 | 24 | 81.7 | Indigenous forest |
| Waitaki (upper) | Canterbury | 11 887 | 7 | 370 | 9.4 | 52.1 | Bare ground |
| Waitaki (lower) | Canterbury | | | | 0.6 | 14.1 | Scrub/tussock |
| Ōreti | Southland | 3513 | 7 | 62 | 13.4 | 4.9 | Pasture |

[a] River Order (Strahler 1957).

[b] Variables derived from FWENZ (Wild et al. 2005) correspond to the lowest segment of each river system.

[c] Catchment vegetation assigns rivers to seven categories representing the predominant land-cover of the catchment (from REC, Snelder et al. 2005).

The FRE3 value represents the annual frequency of flows exceeding three times the median flow (Clausen & Biggs 1997).

## Invertebrate communities

We identified 145 taxa from a total of 203 sites using the combined quantitative and qualitative data set; 61 were common taxa and 84 were rare. The majority of individuals (63%) belonged to 5 taxa, of which the leptophlebiid mayfly, *Deleatidium* and Orthocladiinae (Chironomidae) comprised 44% of all individuals. Chironominae, the gastropod *Potamopyrgus antipodarum* and Elmidae (Coleoptera) complete the list of five most common taxa overall. Of the 145 taxa, 37 (25%) were unique to the South Island and 9 (6%) were only found in the North Island. Twenty-six taxa were

represented by 5 or fewer individuals, including several represented by single individuals. Singleton taxa were Trichoptera (3), Plecoptera (1), Odonata (1), Diptera (2) and Coleoptera (1).

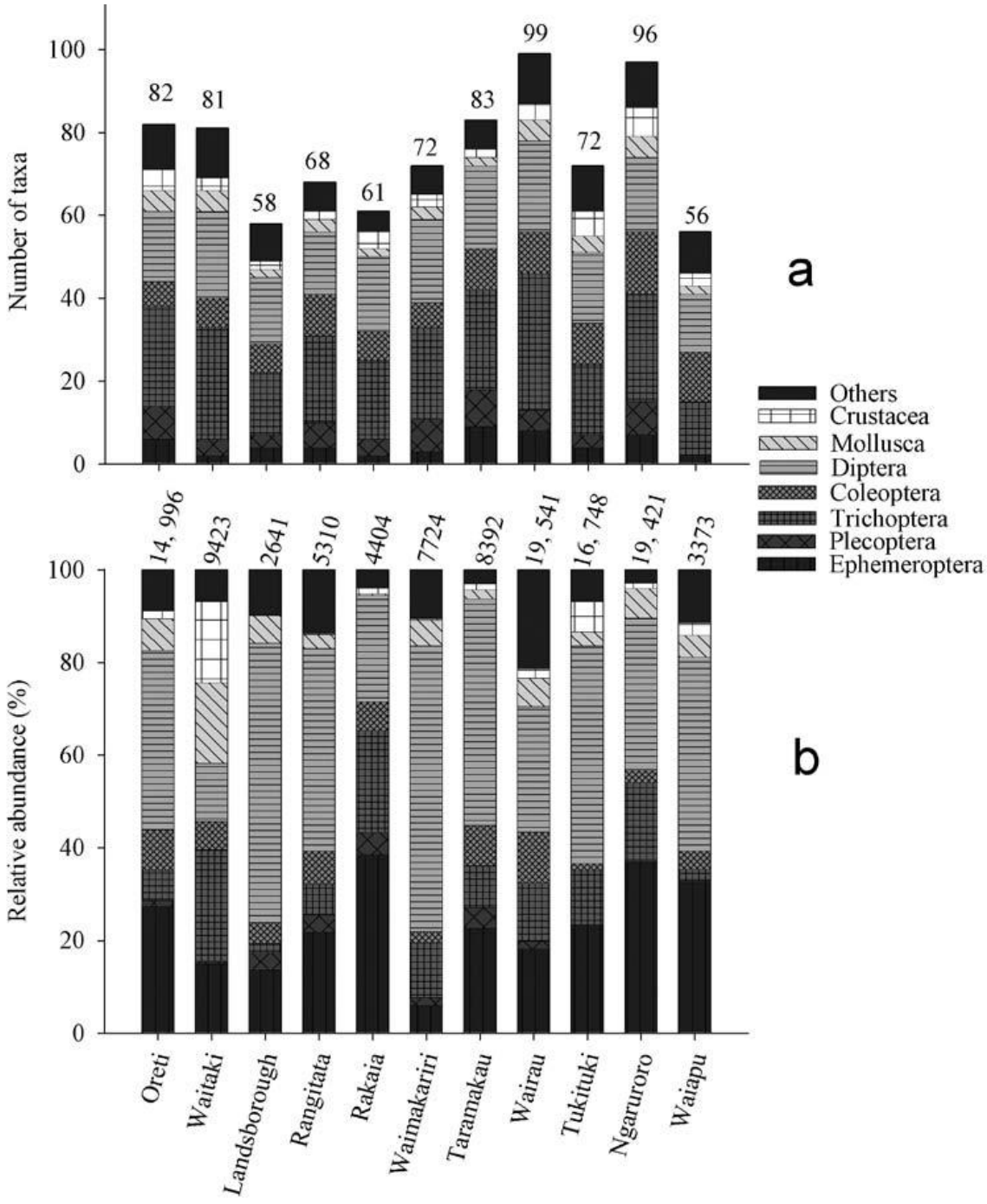


Figure 5. A) Taxonomic richness of ordinal (or higher) groups for rivers sampled during austral summer between December 2006 and April 2007. Total taxonomic richness is shown above each bar. B) Relative coded abundance of ordinal (or higher) groups collected in each river. Total number of individuals is shown above each bar.

Taxonomic richness ranged from 56 taxa in the Waiapu River to 99 taxa in the Wairau River, and represented 38% and 68% of the entire taxa pool, respectively (Fig. 5a). The 5 rivers with the highest taxonomic richness were in 5 separate geographic regions. All ordinal groups were

represented in each river system with the exception of Plecoptera, which were absent from the Waiapu River. Most rivers were dominated by trichopteran taxa, except the Landsborough and Waiapu rivers, which contained a greater number of dipteran taxa. The proportions of ordinal groups were similar among rivers despite considerable variation in overall richness. Total number of individuals ranged from approximately 19 500 in the Wairau and Ngaruroro rivers, to fewer than 3500 individuals in the Landsborough and Waiapu rivers (Fig. 5b). In terms of relative coded abundance of individuals, all rivers were dominated by Diptera, except the Ngaruroro, Rakaia and Waitaki, which contained proportionally more mayflies.

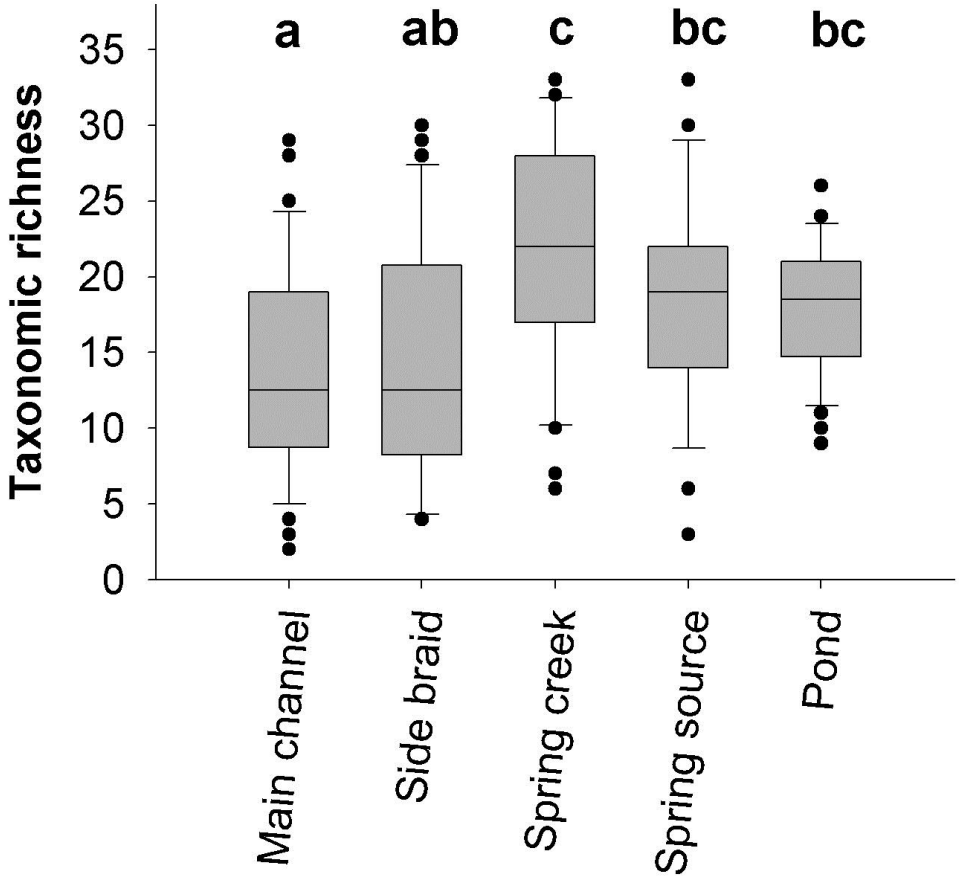


Figure 6. Floodplain habitat-scale analysis of taxonomic richness (median, 25th and 75th percentiles, and outliers) in five habitat types sampled across six reaches of 11 braided rivers. Values with the same letter above the plot are not significantly different (Bonferroni post-hoc test, $P > 0.05$). Taxonomic richness, $d.f._{4, 196}$, $F = 9.743$, $p < 0.001$.

Taxonomic richness varied significantly among habitat types (Fig. 6). Thus, spring sources, spring creeks and ponds had greater richness than main channels, whereas side braids had intermediate diversity. Spring creeks had the highest mean taxonomic richness. Ponds and spring creeks had significantly higher densities than main channels and side braids. This result is due to the variation in physical disturbance by flooding. Main channels are regularly disturbed while springs and ponds tend to be more stable allowing more taxa to colonise.

## Limitations and points to consider

This case study taken from Gray & Harding (2009) illustrates some of the issues that are likely to occur in a survey of benthic macroinvertebrates and options for the analysis and presentation of results. The kick-net method is ideal to assess the taxonomic richness of habitats and rivers (Fig. 5a) however, semi-quantitative abundance data is less informative for comparisons of invertebrate abundance (Fig. 5b). Labour-intensive high-precision population size estimates were exchanged for greater spatial coverage. This was appropriate to the study objective of a broadscale inventory of braided rivers across New Zealand, but would not be appropriate for a detailed examination of community composition at a few sites.

### Sampling design and methods

Both the rationale for design and methodology for sampling are carefully described. This is very important as it allows the reader to judge the validity of results and the degree of comparability to other studies. It should be possible to completely replicate the study carried out by using descriptions in the methods section. A number of choices were made during the design of this survey in order to best meet the objectives within resource constraints. The primary aim was to objectively assess patterns in invertebrate diversity at multiple spatial scales. Had the focus been on environmental drivers of communities it might have been desirable to collect local scale environmental data. If the focus had been on how communities change their composition between different habitats, fully quantitative invertebrate data would have been required. At a larger spatial scale it might have been possible to assess the effects of variation in catchment vegetation by choosing sites according to their vegetation characteristics. Bigger rivers have a greater area and therefore it might be justified to sample them at proportionally more locations. However, an unbalanced design such as this makes statistical analysis difficult and potentially increases the resource requirements of the project. The study design used was a compromise between resources and data quality.

The precise location of sampling sites is unimportant because the assumption of this survey is that the sites investigated are representative of the habitats/rivers in question and so the same patterns will be found by following the methodology at different locations.

### Results

The scene is set by describing the physical characteristics of each river and noting major groupings of river types and any unusual systems. Summary data is presented in a table as well as text. This allows the reader to begin developing a mental picture of the types of habitats and rivers under consideration.

Invertebrate communities are initially described at a coarse level, giving the number of taxa found, those which were rare and those which were common and any taxa unique to broadscale groupings of river systems. These overviews provide a great deal of information and provide context for the more specific analyses.

The graphs in Fig. 5 provide river-specific information. Figure 5a shows the overall taxonomic richness of invertebrate communities in each river and breaks richness down into the number of taxa within each order or higher grouping. Figure 5b shows the proportional coded abundance of taxa in those same rivers as well as the number of individuals found overall. Although in Fig. 5 the data are combined across multiple habitats within each river, such graphs are also appropriate for comparing single samples at different sites, or the same site on different occasions.

Figure 6 shows the results of an Analysis of Variance (ANOVA) comparing the richness of invertebrate communities in different habitat types on braided river floodplains. Box-plots are a useful way to present this, as they show means, percentiles and outliers. Significant differences derived from ANOVA are shown in the same graph.

The graphs presented in this study are designed to provide as much information as possible and address the objectives of the study.

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# 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 and bucket/sieve are clean.
2. Select the appropriate habitat (e.g. riffle).
3. Sample beginning at the downstream end of the reach and proceed across and upstream.
4. Select an area of substrate (0.1–0.2 $m^2$) to sample with a natural flow that will direct organisms into the net. Place the net on the streambed and step into the sampling area immediately upstream of the net, disturb the substrate under your feet by kicking to dislodge the upper layer of cobbles or gravel and to scrape the underlying bed. The area disturbed should extend no further than 0.5 meters upstream from the net. Remove the material from the net into the tray, bucket or sieve bucket if the net begins to get clogged.

5. Repeat Step 4 at several different locations within a 50 m stream reach and covering a variety of velocity regimes until a total area of 0.6–1.0 $m^2$ of riffle habitat has been sampled. Transfer this material to a white tray or bucket approximately half full of water, or to a sieve bucket. Wash or pick all animals off the net.
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.
7. Transfer the sample to the sample container 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. (Tweezers may be useful.)
8. Add preservative. Aim for a preservative concentration in the sample container of 70–80% (i.e. allowing for the water already present). Be generous with preservative for samples containing plant material (leaves, sticks, macrophytes, or moss).
9. 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. Make notes on the field data sheet describing the substrates sampled (cobble size, periphyton, embeddedness, etc.), the collector's name, sample type (e.g. D-net, 0.5 mm), and preservative used.

# References and further reading

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# Appendix A

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

| | |
|---|---|
| docdm-724991 | Introduction to macroinvertebrate monitoring in freshwater ecosystems |
| docdm-146272 | Standard inventory and monitoring project plan |
| docdm-761873 | Stream habitat assessment field sheet |
| docdm-761858 | Stream invertebrate data sheet example |