Freshwater ecology: quantitative macroinvertebrate sampling in hard-bottomed streams

Version 1.0

This specification was prepared by Duncan Gray in 2013.

Contents

Inventory and monitoring toolbox: freshwater ecology

DOCDM-724830

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. Quantitative sampling of hard-bottomed, wadeable New Zealand streams is designed to produce high-precision estimates of the population density of macroinvertebrates at a sampling location. Consequently, this data requires greater cost, time and resources to acquire and is only justified in situations such as threatened species or restoration monitoring and research where density effects are of interest. There are no limits on the metrics and analyses which can be performed on quantitative count data. 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.

Numerous techniques are available for quantitative sampling of riffles in stony streams; however, the Surber sampler is recommended by Stark et al. (2001) for sampling hard-bottomed streams. The Surber sampler (Surber 1937) is a net of given mesh size fastened around a square frame which permits the user to isolate a known area of stream bed for sampling. The Surber sampler framing directs the current and organism into a collecting 'sock'.

Figure 1. A Surber sampler (top) and kick-net. Note framing around the base of the Surber sampler to isolate a known area of stream bed, side walls to direct current, and receptacle to concentrate sample. Photo: Tanya Blakely.

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

A Surber sampler, usually 0.1 m^2 with mesh size 0.5 mm, is placed on an undisturbed patch of stream bed facing directly into the current, preferable in water shallower than the sampler frame. Deeper water can be sampled but care must be taken not to lose organisms over the net or through backwash. It is important that the area upstream of the sample location is also undisturbed as the Surber sampler will also collect drifting individuals. The sampler is not effective in low velocity areas although a current can be created by hand. The operator stands downstream of the sampler and after establishing good seal between the sampler frame and the substrate begins to agitate the substrate within the sampler frame. Large substrates should be lifted and brushed to remove individuals whilst smaller substrates may be disturbed by digging and 'winnowing' in the current. The effort and duration of agitation should be same for all samples. Except in clay or bedrock substrates, sampling penetrates down into the stream bed. As far as possible this depth should be standardised amongst samples. The Surber sampler is limited to use amongst substrates that fit within the frame. In particularly uneven stream beds the seal can be improved by using towels or a rubber flap.

Figure 2. Collecting the Surber sample. Note the operative stands adjacent to or downstream of the sampler. Photo: Duncan Gray.

The high spatial and temporal variability of macroinvertebrate communities in streams means it is difficult to recommend a universally appropriate number of replicate Surber samples to sufficiently estimate population densities. However, Boothroyd & Stark (2000) suggest that between three and six replicates from the habitat type of focus should be adequate to characterise most macroinvertebrate communities.

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. 3). Pottles should also be marked with the location, date and the field operative names.

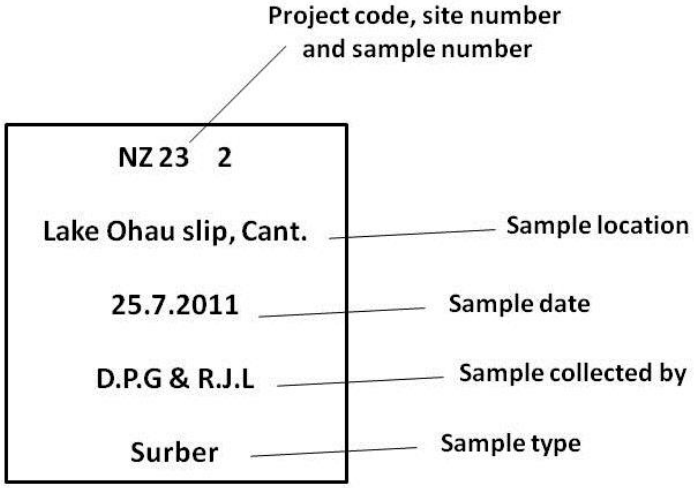

Figure 3. An example wet label with the required information to identify each sample should be written in pencil on waterproof paper and added to the sample.

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.
- Sampling collects individuals located within the sampling frame, not those that enter by drifting from upstream.

Advantages

- Surber sampling provides high-precision information about the richness and composition of macroinvertebrate communities.
- Allows the user to isolate an known area of stream bed for sampling.
- The Surber sample is one of the most commonly used devices for quantitative sampling of hard-bottomed streams in New Zealand and overseas. Hence, results are comparable with many other studies and the methodology is unlikely to be criticised if applied correctly.

Disadvantages

- Generates a greater number of replicate samples than a semi-quantitative method. There will be a significant increase in time and cost of processing.

- Unsuitable for deep or fast flowing streams.
- Unsuitable where substrates are larger than the area of the sampler frame.

Suitability for inventory

This technique is not suitable for inventory which does not require quantitative estimates of population size. Collection and processing of Surber samples for inventory would be a considerable waste of time and resources and alternatively a more efficient semi-quantitative sampling or processing method could be considered.

Suitability for monitoring

This method is suitable for monitoring where the density of individuals is considered to be of interest. If high-precision estimates of population density are not important, consider using a more efficient semi-quantitative sampling or processing method.

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

Quantitative sampling of hard-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:

- Surber sampler (0.1 m^2, 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 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 to inform biological data. Some 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 and/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.

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

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.

Forward copies of completed survey sheets to the survey administrator, or enter data into an appropriate spreadsheet as soon as possible. Collate, consolidate and store survey information securely, also as soon as possible, and preferably immediately on return from the field. The key steps here are data entry, storage and maintenance for later analysis, followed by copying and data backup for security.

Summarise the results in a spreadsheet or equivalent. Arrange data as ‘column variables’—i.e. arrange data from each field on the data sheet (date, time, location, plot designation, number seen, identity, etc.) in columns, with each row representing the occasion on which a given survey plot was sampled.

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.

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 Surber sampling are either semi-quantitative fixed counts or more commonly full counts of all individuals. They are high-precision estimates of population size. There is no limit to the indices or analyses that can be produced with this data. Common basic indices calculated from this data are:

- Taxa richness
- Richness of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa or % EPT abundance
- Macroinvertebrate Community suite of indices, especially the Quantitative Macroinvertebrate Community Index (QMCI) (Stark 1985)

Taxa richness

Taxa richness is simply the number of taxa that were found at a site and is most commonly used for inventory or ecosystem condition monitoring. 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. Many of the species within these groups require undisturbed habitats and so this metric may be more sensitive to impacts than taxa richness alone. EPT richness may be presented as a proportion of total richness, e.g. % EPT, and is commonly calculated for ecosystem condition monitoring.

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 in 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

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), also used in ecosystem conditioning monitoring. If coded abundance data are received in alpha code form 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 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

The real value of full count data is in allowing the calculation of the QMCI which is the quantitative variant of the MCI and the preferred metric for threatened species or restoration monitoring and research. The QMCI is calculated thus:

$$= \sum (n_i \, a_i) / \mathrm{N}$$

Where n_i is the number of individuals in the i^{th} taxon and N is the total number of individuals. Scores may be interpreted in terms of water quality according to Table 1 and are directly comparable with SQMCI scores, but not MCI.

Community composition

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 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 (e.g. 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 and summary plots, the user will require specific training and 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: the influence of aquatic plants on macroinvertebrate communities in spring-fed streams

Synopsis

This case study features data from an experiment to investigate the influence of aquatic plants on the macroinvertebrate communities within spring-fed streams (D. Gray, unpubl. data). Although this is a randomised, manipulative experiment the methods of comparing communities used are applicable to Before–After Control Impact (BACI) designs and broader surveys across different land use types. The case study illustrates the application, analysis and reporting of quantitative Surber sampler data from a hard-bottomed stream as well as quantitative sampling of macrophytes.

The removal of macrophytes altered the resource base and invertebrate community structure in spring streams. Some of the differences between communities were subtle; however, quantitative full count data allowed these differences to be detected with a high degree of confidence and defensibility.

Objectives

- To test the influence of aquatic macrophytes on the resource base and community structure of macroinvertebrate communities in spring streams.

Sampling design and methods

Between 5th January and 9th February 2005 a macrophyte manipulation experiment was conducted at One Tree Swamp, Arthur's Pass National Park. On 5th January, within each spring-source, eight 1 m^2 quadrats were selected (Fig. 4). Half of these quadrats, selected at random, were cleared of all macrophyte and bryophyte material, whilst aquatic plant material in the others was left untouched, as controls. Some bryophytes were difficult to remove from the larger substrates, so the substrates were removed. To avoid a substrate size effect on benthic communities, aquatic plant-free substrates of equivalent size taken from within the springs were placed within the quadrats. In all quadrats, benthic invertebrate densities, macrophyte biomass and inorganic benthic substrate chlorophyll-*a* were measured on the 9th February 2005.

Physico-chemical conditions including temperature, dissolved oxygen levels, pH and discharge were measured in each spring to account for differences between springs in water chemistry.

After 30 days a Surber sampler (0.1 m^2, mesh size 250 µm) was placed randomly within each 1 m^2 quadrat to collect benthic invertebrates. In control quadrats (macrophytes present) all macrophytes and bryophytes within the Surber sampler frame were placed in a bucket and washed thoroughly to remove all invertebrates. Aquatic plant biomass was returned to the laboratory, oven dried (at 45°) and weighed (to 0.01 g). Invertebrate samples were preserved in 70% ethanol in the field and sorted in the laboratory under 40× magnification. Identifications were made from keys by Winterboun (1973), Chapman & Lewis (1976), Cowley (1978), McLellan (1998), Winterbourn et al. (2000), and Smith (2001). Identification was carried out to the lowest taxonomic level possible, except for Oligochaeta which were not differentiated below order and Chironomidae which were not separated below tribe.

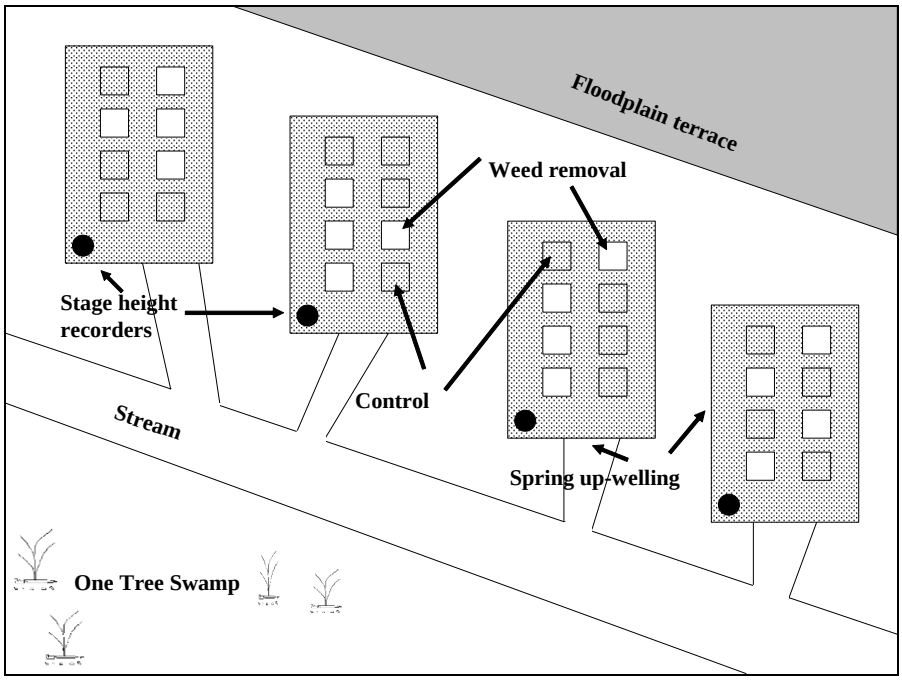

Figure 4. Schematic diagram of the macrophyte manipulation experiment. Quadrats in springs were placed randomly and spring-sources drained into the associated spring brook.

At the end of the experiment periphyton biomass (organic layers on the stone surfaces), within control and treatment quadrats, was estimated by measuring chlorophyll-*a*. Three stones devoid of macrophytes or bryophytes were randomly collected from each quadrat, within each treatment, and were placed in 100 ml of 90% ethanol for 24 hours at 4°C in the dark. Periphyton biomass was estimated using standard techniques.

The statistical techniques used to analyse this data are specific to the design and objectives of this study and are not detailed here. Experimental design and analysis is a highly specialised process and an experienced biometrician or ecologist should be consulted both before and after field work is carried out.

Results

Temperature and discharge in the four springs were very similar (Fig. 5) suggesting that any differences between springs or treatments were unlikely to be due to physical differences between sites. This is an important control and illustrates the importance of collecting basic habitat data to inform biological results.

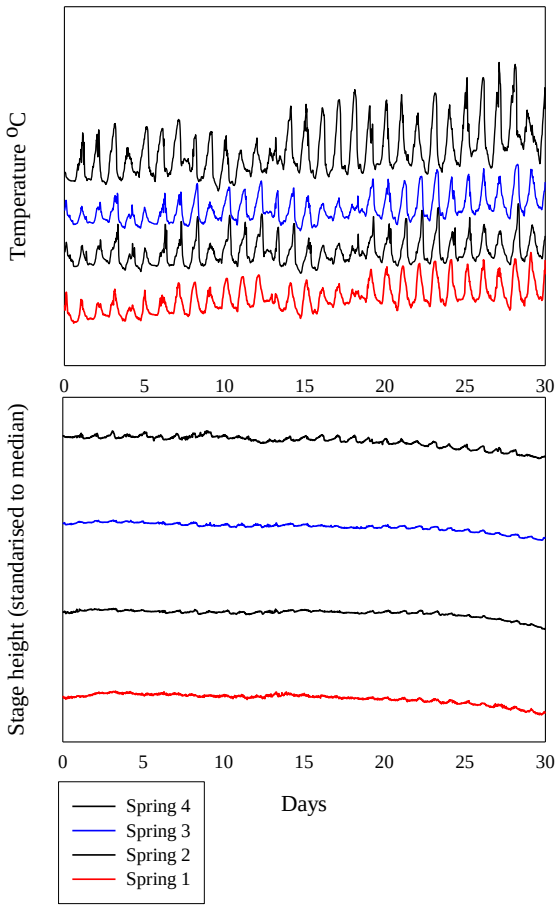

Figure 5. Water temperature and standardised stage height within the four springs over the 30 days of the experiment.

Periphyton biomass

The substrate and habitat is the stage upon which the ecology of stream communities is performed. Periphyton (or biofilm) is the organic layer coating stones on the stream bed, assumed to be primarily autotrophic algae, and providing the basal food resource for most of the stream community. After removal of aquatic macrophytes there was a significant increase in the biomass of periphtyon on cobbles (treatment effect, $F = 42.55_{1, 86,}$ $p = 0.007$) (Fig. 6).

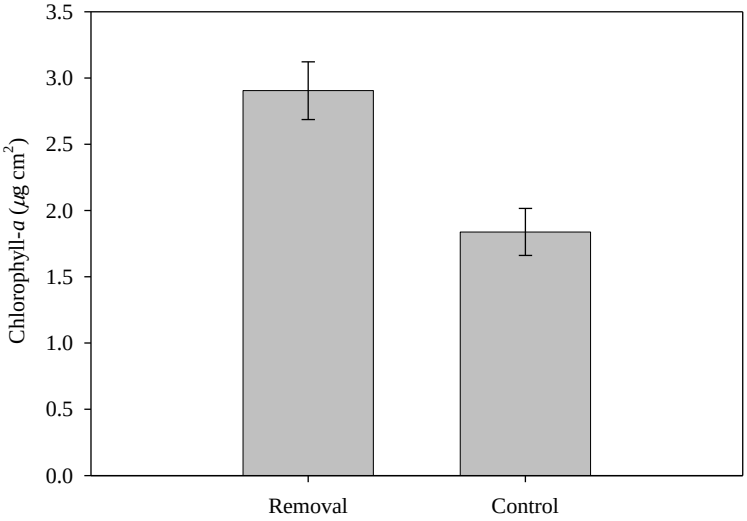

Figure 6. Mean chlorophyll-*a* levels (µg cm^2) on stones taken from removal and control (macrophytes present) quadrats (± 1 SE, calculated from all stones in all quadrats combined).

Benthic macroinvertebrates

Overall, spring communities contained 12 dipteran taxa, 8 caddisfly taxa, 1 mayfly *(Deleatidium*), and 2 stonefly taxa (*Austroperla cyrene* and *Zelandobius pilosus)*. The common snail, *Potamopyrgus antipodarum* was the only mollusc collected in these springs. The flatworms were represented by the triclad *Neppia montana*, and the allocoel *Prorhynchus putealis,* a phreatic flatworm which has previously been found in springs and trout redds in the beds of up-welling reaches of Canterbury and Southland spring-fed rivers, as well as springs in the Cass Basin (Percival 1945). Also present were the aquifer-dwelling amphipods *Paraleptamphopus* sp. and *Phreatogammarus fragilis*, both of which are known from springs, up-welling river reaches and groundwater samples (Chapman & Lewis 1976). On average, springs were numerically dominated by *Potamopyrgus antipodarum,* orthoclad chironomids, *Pycnocentrodes*, diamesinae chironomids and the mayfly *Deleatidium.* However, the relative abundances of taxa were altered by the removal of macrophytes.

Taxonomic richness was greater in the control quadrats than the removal quadrats, $F = 15.140$, $p =$ **0.030** (Fig. 7a). However, rarefied taxonomic richness, a statistical technique which controls for the number of individuals present in each sample, indicated no difference between treatments, $F = 8.4800$, $p = 0.061$ (Fig. 7b). Significantly more individuals were found in the control treatments than

in the removal quadrats, F = 136.70, *p* = **0.001** (Fig. 7c). Concomitant with a highly significant increase in abundance, a fall in evenness was observed in the control treatments compared to the removal treatments, F = 136.70, *p* = **0.001** (Fig. 7d).

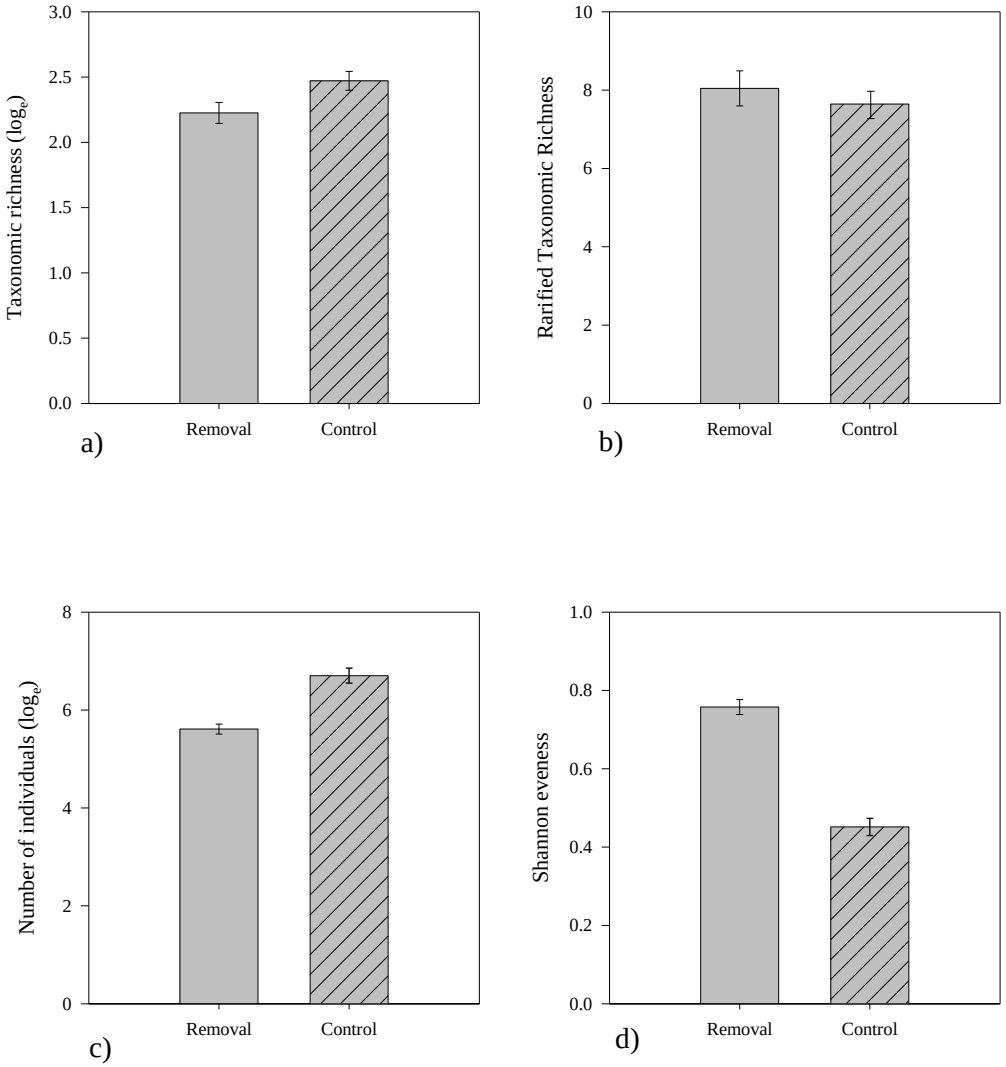

Figure 7. Comparison of invertebrate community indices in removal and control treatments (mean ± 1 SE calculated from all quadrats combined).

The relative abundance of mayflies was much higher in the removal quadrats than the control, F = 505.90, *p* < **0.001** (Fig. 8a). Similarly the relative abundance of caddisflies was higher in the removal treatment, F = 98.400, *p* = **0.002** (Fig. 8b).

Mayflies and caddisflies were replaced by dipterans, which showed a significant proportional decrease within the cleared treatment (Fig. 8c). ANOVA indicated no treatment effect for dipteral, F = 1.3830, *p* = 0.320, but a significant interaction effect, F = 6.130, *p* = **0.003**. This was due to one spring in which dipterans were proportionally more abundant in the removal treatment. However, the overall trend of strong Diptera dominance in controls is clear.

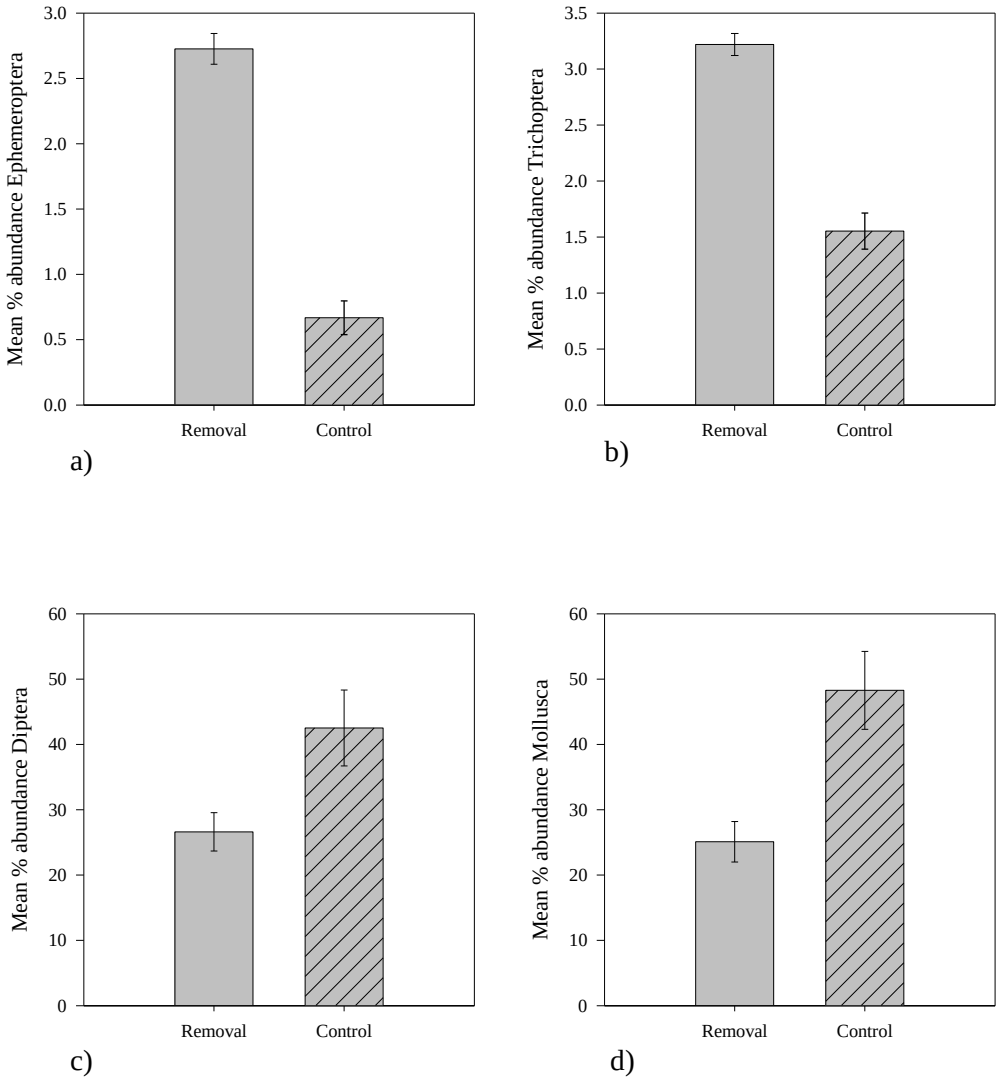

Figure 8. Mean % abundance of Ephemeroptera, Trichoptera, Diptera and Mollusca in removal and control treatments (mean ± 1 SE).

The mean abundance of molluscs, which were entirely *Potamopyrgus,* was greater in the control treatments than the removal treatment (Fig. 8d). No significant treatment effect was found due to high variability between springs, F = 4.7580, p = 0.117; however, the significant interaction term, F = 5.741, p = **0.004,** and inspection of an interaction plot indicated that a treatment effect does occur and the pattern was consistent across springs.

Multivariate ordination

An alternative way to examine differences between invertebrate communities at different sites is to use multivariate ordination. This analysis locates sites on a graph plot according to the similarity or otherwise of their communities. Sites that contain similar invertebrate communities will be located close to each other, whereas those with different communities will be further apart (Fig. 9).

Non-metric multidimensional scaling (NMDS) ordination of sites using $\log_e$ abundance data was performed to assess similarities in terms of community composition of macrophyte removal and control treatment communities (Fig. 9). The ordination shows that the communities in the treatments are quite different with very little overlap between them.

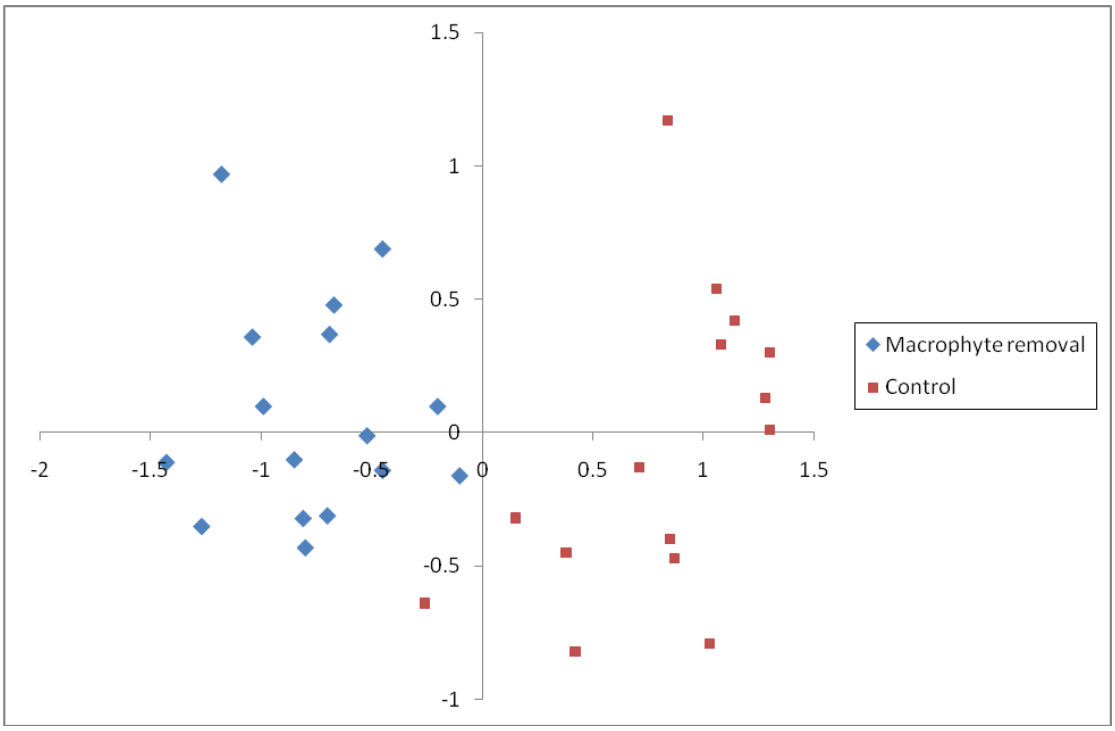

Figure 9. NMDS ordination of invertebrate communities in macrophyte removal and control sites using log abundance data. Stress = 0.11 (*n* = 79) (D. Gray, unpubl. data).

Ordination analyses require a number of influential choices to be made during analyses, have several caveats about data types or the interpretation of results and there is controversy about which specific type of ordination to use. Consequently, an experienced biometrician or freshwater ecologist should be consulted before analysis is attempted.

Discussion

Raw taxa numbers indicated that aquatic plants in spring-source habitats supported a higher taxonomic richness than stony substrates. This finding is in accordance with results of several overseas studies suggesting that aquatic plant invertebrate communities are highly diverse. However, after rarefaction to control for the number of individuals in each sample, the difference between taxa numbers within the two substrates was no longer significant. It is possible that because aquatic plants support much higher densities of benthic invertebrates, taxonomic richness is higher by chance alone.

These results concur with findings in New Zealand and overseas, that macrophytes support higher abundances of benthic invertebrates than inorganic substrates. A number of mechanisms could explain this phenomenon. First, macrophytes provide complex 3-dimensional living space to benthic invertebrates. Although cobble/gravel substrates do provide a third dimension of living space, the

hyporheic zone is often not sampled by conventional techniques, e.g. a Surber sampler. Macrophyte beds extend available habitat up into the water column and therefore equate to a larger volume of habitat than is sampled by conventional techniques. Secondly, macrophyte and bryophyte beds provide protection from de-faunating flow velocities. This allows the density of invertebrates able to live on aquatic plants to reach high levels. Furthermore, aquatic plant communities have been shown to be depauperate in predatory taxa. Finally, the high abundance of benthic invertebrates within aquatic plant beds were probably supported by elevated levels of food resources. Living macrophytes and bryophytes rarely provide a direct source of food for New Zealand benthic invertebrates, but epilithon and detritus that collects on them are a constant source of food. Thus, it is likely that invertebrate abundance on aquatic plants was enhanced by the increased living space they provide, benign flow conditions, relatively low levels of predation and high food resource availability.

The shift in community composition and dominance of quadrats from chironomids and molluscs in control quadrats to mayflies and caddis in removal quadrats is consistent with our understanding of the ecology of these taxa. *Deleatidium* and the cased caddisflies *Pycnocentrodes* and *Pycnocentria* are predominantly stone-surface grazers, which ingest algal periphyton, and detritus that becomes entrained within the algae on stone surfaces. The lack of light below aquatic plant beds significantly reduced levels of periphyton on stones, and therefore, the algal food resources of mayflies and caddisflies. Conversely, the protection from high flows and predation, plus the possibility of enhanced levels of epilithon and organic matter retention on the aquatic plants themselves, provide conditions more suitable for mollusc and chironomid taxa that may be more capable of negotiating the complex architecture of plants.

Limitations and points to consider

- Surber sampling allowed subtle changes in the composition of macroinvertebrate communities to be examined. This would not have been possible using semi-quantitative data.
- Due in part to the intensity of sampling, the spatial spread of this comparison is reduced. Consequently caution must be exercised when extrapolating these results to other stream systems. To test generalities it is not unusual to link a broadscale semi-quantitative survey with more focused small-scale quantitative sampling.
- As in all situations the objectives dictate the methods used. Revisit the ‘Decision tree’ in the ‘Introduction to macroinvertebrate monitoring in freshwater ecosystems’ (docdm-724991) to confirm you have chosen the correct path.

References for case study A

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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 is clean.
2. Select a suitable sample reach and habitat (e.g. riffle). Sample beginning at the downstream end of the reach and proceeding across and upstream.
3. Place the sampler on the streambed ensuring a good fit around the perimeter. The sampler should be positioned so that the water current washes dislodged material into the net.
4. 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 quadrate. Place clean cobbles outside of the sampler quadrat. Disturb the finer substrate remaining within the quadrate to a depth of 5–10 cm. Beware of broken glass and other sharp objects.
5. 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 sample from collection net either by inverting net into a suitable container, or by removing container attached to end of collection net. Elutriation may also be required (i.e. repeated rinsing of sample to separate organic and inorganic fractions).
6. Let the sample settle for a few minutes and decant off excess water via the sieve. Return any macroinvertebrates that are washed out with the water to the sample container. (Tweezers may be useful here.)
7. 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, moss or periphyton).

8. Place a sticky label on the side of the sample container and record the side 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 (e.g. Surber 0.1 m^2), collector's name and preservative used on the field data sheet.

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.

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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