



Ministry of  
Environment

# **GUIDELINES FOR SAMPLING BENTHIC INVERTEBRATES IN BRITISH COLUMBIA STREAMS**

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APPENDIX A List of Ministry of Environment Contacts

## ACKNOWLEDGEMENTS

This document is an update of the 1991 internal Ministry of Environment document entitled *Guidelines for Sampling Benthic Invertebrates in Streams* written by Les McDonald and peer/expert reviewed by R.D. Kathman, Joseph M. Culp and Mr. Bill Duncan. The author wishes to acknowledge the efforts of Mr. McDonald as a co-author of this document, for his summary of the benthic invertebrate workshop held December 1989 in Prince George, BC, lead by Dr. R.D. Kathman, which formed the basis for the original 1991 benthic invertebrate sampling guideline document.

The 2003 version of this report was based somewhat on the original 1991 document with the addition of several other sections summarizing various sampling and analysis approaches that have come into use over the last decade. In January 2006, Chris Perrin further edited and incorporated new information to update the report for publication.

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## 1.0 INTRODUCTION

Benthic invertebrates are commonly used as indicators in the evaluation of impacts to stream ecology and entire watersheds from a variety of point and non-point pollution sources (Barbour *et al.* 1999, Karr and Chu 1997, Lenat and Crawford 1994, Plafkin *et al.* 1989). Benthic invertebrates have been favoured in environmental effects monitoring because they are sessile or limited in their range of movement and therefore cannot avoid pollution (Gaufin 1973). They are generally abundant and can be found year round so are easily sampled. Since many aquatic species have a life span in water of approximately a year, they provide an indication of water quality conditions over that period. In other words, their life span is long relative to most short-term environmental impacts that occur. As primary consumers in aquatic ecosystems, benthic invertebrates are an extremely important food source for many aquatic organisms including fish and are mediators in nutrient cycling in aquatic systems by the breakdown and utilization of suspended or attached organic material. As well, benthic invertebrate monitoring data provides a link between the effects of human activities on the physical and chemical properties of water and aquatic ecosystem health (Norris and Hawkins 2000).

The heterogeneous or “patchy” distribution of benthic invertebrates in aquatic environments makes the task of study design, sampling and data interpretation somewhat difficult. The effects of a pollutant on a benthic community, measured by upstream/downstream or time-series sampling, often cannot be distinguished from natural within-site sampling variation. However, carefully defining the purpose (i.e. what is the question you are trying to answer), then selecting the appropriate study design and analytical tools for your benthic invertebrate monitoring program can overcome these limitations.

There is a wealth of printed literature as well as information accessible on the internet from agencies and universities, which can provide good advice on sampling devices, analytical and statistical methods to produce more reliable and reproducible information. These guidelines are intended for a wide audience as opposed to benthic ecology experts. However, the guidelines are an attempt to assist government and non-government investigators, proponents of land development or industry, by providing pertinent references as well as recommendations for standardizing sampling methods acceptable to the ministry to enhance the comparability and validity of benthic invertebrate monitoring data in B.C. Appendix 1 has a list of contacts in Victoria and in each of the ministry’s regional offices, who may be contacted for more information and/or advice.

## 2.0 STUDY DESIGN

### 2.1 Overview

The study design is the most critical phase of implementing a benthic invertebrate monitoring program. The first important step in developing a study design is defining the problem and your study question. Depending on your level of expertise and professional background you may wish to establish testable hypotheses, develop lines of evidence, conduct a sediment quality triad (Chapman 2000) study, develop your “problem formulation” risk assessment model to guide the study design, or you may wish to blend several approaches. However, a clear definition of the purpose of your program in the form of a specific question or series of questions is always needed to ensure that appropriate approaches are used to collect and examine the data.

As well, the investigator should also be aware of and consider, at the planning stage, how the results of monitoring will be used to make decisions about impacts and mitigation or remediation of those impacts. Although this is often thought to be a consideration only for “managers”, it is an important step in any impact evaluation approach. For example, what are your ecological management objectives and have you defined your decision thresholds to quantify what is “acceptable” change? How will you present the results in a way that conveys the important findings and leads the decision makers to actions that may be necessary for improving environmental quality? These are some examples of questions that should be addressed through the course of your planning and in your study design.

Once you have defined the study objectives, there are many different approaches to monitoring benthic invertebrates each with their own set of assumptions, requirements and data assessment tools. Finding the right “fit” for your monitoring program can be time consuming but it is time well spent. Several good references exist to assist the investigator in this phase of study planning (Cavanagh *et al.* 1998a, Cavanagh *et al.* 1998b, Environment Canada 1997, Green 1984, Green 1987, Underwood 1997, Zar 1996, Krebs 1999, Bailey *et al.* 2004). Karr and Chu (1999) provide some very good guidance for understanding biological, physical and chemical attributes which affect the response of benthic invertebrate communities and selecting the appropriate “signals” to monitor for diagnosing environmental degradation.

Other factors which can dramatically affect study design can include: a lack of adequate funds, limitations in access to stream reaches potentially impacted, flow regulation for flood or hydroelectric generation, multiple other recreational and industrial uses as well as consumptive uses (water extraction), and in-stream groundwater recharge areas

which may significantly alter water quality. All of these “logistical” factors must be considered and accommodated in the planning and study design phase. Care must be taken to ensure careful execution in carrying out the program, recording the information and paying attention to small details or a study can fail to produce and record the results that will address the original question(s).

## 2.2 Study Timing

Depending on the question of interest, sampling is often conducted just before the majority of emergence of adult insects, during periods of maximum growth or prior to annual physical events such as floods (Rosenberg and Resh 1992). For interior streams, the ideal program would incorporate sampling in both spring before freshet and in the fall. However, if only one sampling per year can be performed, late summer to early fall (August through October, depending upon climate) is the optimal time for peak biomass and benthic community diversity. In coastal streams, peak biomass can occur in late spring, much earlier than in interior streams. Depending on the purpose of the study, year-to-year comparisons can be useful in benthic invertebrate assessments. If spring sampling is anticipated, ensure that sampling is timed to occur following ice breakup but prior to freshet so vulnerable populations are not reduced by the high flows. As a general rule for all areas, late summer and fall is the best time for sampling, mainly because the invertebrates are abundant and stream flows are lowest of the year, which allows for easy sample collection.

## 2.3 Site Selection

Site selection is another important consideration when planning a benthic invertebrate monitoring program (Cavanagh *et al.* 1998a, Environment Canada 1997, Barbour *et al.* 1999). Some general things to incorporate into your program are:

- Once established, monitoring sites should be considered “permanent” so they can be re-sampled in the future. Visibly mark the site on the stream bank with flagging and a stamped or “write-on” aluminum marker. Identify the site location using a global positioning system (GPS), describe it in relation to permanent land forms, and record the information on waterproof sampling forms. Make detailed notes about the location, site characteristics, weather and other field measurements. Photo-documentation is a very good method of “describing” site characteristics and can be very useful means of comparing sites both spatially and over time.
- Site selection should be driven by the objectives of the study, the number of habitat types represented in the system under investigation and somewhat by logistics. However, sites which are to be compared to each other should be “normalized” as much as possible, i.e. select areas that are representative of the dominant habitat type(s) of the system and ensure each site is similar with respect to as many characteristics as possible such as flow velocity, canopy cover,



depth of overlying water as well as substrate size and type. Pool, riffle and run composition of the site should also be standardized if possible.

- Sites to avoid include those that may be strongly influenced by other factors, including sites near tributary confluences, bridges, culverts or channelized areas, unless of course, this is part of your study design. Similarly, avoid backwater areas or eddies immediately upstream or downstream of large boulders or debris jams.
- Where your study design calls for it, establish **at least two control** (reference) sites, which, particularly in the case of a baseline studies, will remain controls after the disturbance or discharge has occurred. This type of study design relies on adequate control sites to establish whether or not effects are occurring, so adequate numbers of control sites are essential. The study should have sufficient data to demonstrate that control or reference sites are representative of a reference condition, or it may not be possible to detect the impact of some disturbance (see section on cluster analysis). For impact assessment purposes, the “upstream/ downstream” approach is typically used to evaluate the degree and magnitude of impact. This approach is also called the “control/impact” or “reference /exposure” study design (Environment Canada 1997, Green 1999), which incorporates impact sites along a downstream gradient of effect. However, regardless of which approach is taken the most important criteria for selecting the appropriate number of sites is the ability to describe inherent variability within and between different sites.
- In a traditional upstream/downstream environmental impact assessment (EIA), control sites should be located far enough upstream to be out of the influence of the disturbance or discharge being studied but close enough to be under the same range of natural influences as the impact site(s).
- Where possible, controls and impact stations should be sampled prior to start-up of the disturbance or discharge being studied, thus facilitating a layout known as a before-after/control-impact (BACI) design (Steward-Oaten *et al.* 1986). In this approach replicated differences between some measure (e.g. counts or biomass) at an upstream control site (or sites) and a downstream treatment site among years before treatment, are compared to the differences after treatment is applied using analysis of variance (ANOVA). In its simplest form, years are replicated and the paired differences between an impacted and control site over years are analyzed by one way ANOVA. In a stronger case involving two or more control sites, asymmetric analysis of variance (Underwood 1994) is used to test for a

difference between mean abundance (or biomass) in the impacted location and that in the control locations before compared to after a disturbance began. Each of these cases involve univariate analytical approaches that require sampling over years, ideally using a specific time window of interest within any given year. They require the “correct” selection of an endpoint among many taxa that are usually found in streams. To avoid missing taxa of potential importance, total abundance or biomass may be analysed or a series of metrics (e.g. abundance of the combination of mayflies, stoneflies, caddisflies; abundance of chironomids; etc.) may be selected for independent analysis. A BACI design that avoids pseudoreplication can require many years of sampling before a treatment effect can be statistically tested.

- This design relies on comparison of invertebrate abundances between control and impact sites, before and after commencement of the discharge (Stewart-Oaten and Murdoch 1986, Green 1999).
- Sample each site from downstream to upstream so that the act of sampling one location does not disturb the next. This is especially important for upstream sites that may, by disturbance of sampling, generate “drift” to downstream sites confounding results.

## 2.4 Alternative Approaches

A difficulty with some univariate designs, including BACI, is they can take months to years to answer questions and in so doing they can be very expensive and impractical to complete. In addition, basic assumptions of the statistical analyses may be violated perhaps due to insufficient funding to collect enough samples at any site, logistics that prevent repeated sampling, or other factors that constrain an ideal layout of sample collection.

These are some of the reasons why rapid bioassessment protocols have been developed. They involve comparisons between the invertebrate biota that are observed at a given site and the biota that would be expected to be present at that site in the absence of a disturbance (e.g. Kearns and Karr 1994). Rapid bioassessment based on the use of multimetric indices to define an Index of Biotic Integrity (IBI) has largely been adopted in the United States as a standard protocol (Barbour et al. 1999). Alternatively, rapid impact assessment based on multivariate statistical analysis and modeling (Bailey et al. 2004) is gaining increasing interest and widespread use in the UK (Wright *et al.* 2000),

Australia (Parsons and Norris 1996), and Canada (Bailey *et al.* 2004, Sylvestre *et al.* 2005, Reynoldson *et al.* 1997, Reynoldson *et al.* 2001).

In British Columbia, the multivariate approach is developing rapidly as the basis for what is known as the Reference Condition Approach (RCA) to bioassessment (Bailey *et al.* 2004). For a particular watershed of interest, a model is developed to explain variability in the benthic invertebrate communities among reference sites using environmental attributes at those sites. The model then predicts what biota should be present at a “test” site for a given set of environmental attributes found at that test site. If the biota found at the test site is similar to that predicted to be present, the site can be considered in a “reference condition”. If, however, the biota found at the test site is different from that predicted to be present, the site is considered disturbed. The use of the RCA is dependent on the availability of an RCA model that describes the reference condition for a particular watershed of interest. Up until recent years, these models have not been developed for watersheds in British Columbia, but this is rapidly changing. RCA models have now been developed for the Fraser River Basin (Reynoldson *et al.* 2001), the Georgia Basin (Sylvestre *et al.* 2005) and they are presently being developed for the Skeena and other northern basins (Sharpe *et al.* 2005). Once the northern models are developed, the only area in British Columbia without an RCA model will be the Kootenay – Columbia region. Hence, the RCA approach can now be applied or soon will be applicable to much of the Province. To find out whether your area or site of interest is covered by the RCA, it is best to ask the regional environmental assessment biologist for your region (Appendix A).

All procedures that are required to actually run an RCA analysis are provided on the CABIN (Canadian Aquatic Biomonitoring Network) website (<http://cabin.cciw.ca/cabin/asp/english/welcome.asp>). The protocols used in CABIN do not require sophisticated or expensive equipment or advanced knowledge of multivariate statistical analysis. All the statistics run behind the scenes on the CABIN website once pertinent data are entered using instructions on the website. The approach is very rapid in comparison to conventional BACI designs. In its simplest application, one or a few benthic invertebrate samples are collected using kick net protocols from a “test site”, they are enumerated to the family level and with some environmental information that is defined on the CABIN website, the data are entered and the analysis is run. The output provides information on whether a test site is within or outside of a reference condition. In most cases, samples might also be collected from undisturbed sites within the watershed of interest or over a gradient downstream of a disturbed site. Analysis of all these samples through CABIN would provide a check that an undisturbed site is indeed typical of a reference condition and examination of the downstream samples would support an analysis of the recovery of a test stream from

disturbance over the downstream gradient. A collection of samples over time would support an interpretation of time course recovery of a site or group of test sites from disturbance.

Results from an analysis in CABIN indicate if a test site is or is not in a reference condition. If a test site is found to be outside of the reference condition, examination of additional environmental data or further experimentation or monitoring is required to determine the specific *cause* of that condition. Hence, the RCA can be considered a screening tool, albeit a very powerful one for assessing environmental impact in streams.

RCA field techniques and methods can be taught easily to a wide range of individuals, including community and volunteer groups who wish to participate in the network. Training by experts at Environment Canada is offered, and is intended to prepare individuals from an organization to:

- Locate practical sampling locations
- Use field equipment properly
- Store and prepare samples in the field for analysis
- Identify benthic taxa to the Family level using keys provided
- Use data interpretation software

Several consulting organizations and government personnel in British Columbia are presently qualified in running RCA analyses through the CABIN website and some of these groups are involved the development of the RCA throughout the Province (e.g. Sharpe *et al.* 2005). A list of qualified personnel can be obtained by contacting the regional biologist for your area of interest (Appendix A) or follow the links to contact information on the CABIN website.

### 3.0 SAMPLE COLLECTION AND QUALITY CONTROL (FIELD)

There are many references an investigator can access to assist in developing a monitoring protocol that will address specific study needs. Some things to consider are:

- Selection of sampling equipment is dependent on the purpose and type of study being conducted. For instance, in a contaminants assessment the investigator may wish to focus on erosional (gravelly or cobble stream substrate) or depositional (soft muddy substrate) zones in the stream or river, depending on the fate and transport of the contaminant(s) of interest. It is important to note that the equipment for monitoring erosional stream habitats is much different than that used for depositional habitats. These details should be worked out at the study design and planning stage of program implementation. If RCA protocols are being followed, field methods are described on the CABIN website (<http://cabin.cciw.ca/cabin/asp/english/welcome.asp>).

#### 3.1 Erosional Stream Habitats

- For a qualitative, semi-quantitative or a “pre-study” preliminary stream assessment, a Surber sampler, kicknet or even a common kitchen sieve can be acceptable. Although kicknets have been thought to provide semi-quantitative data, both EPA’s Rapid Bioassessment Protocols (RBP) and Environment Canada’s Reference Condition Approach (RCA) study designs employ kicknets for quantitative assessment in erosional habitats. The investigator should keep in mind that qualitative assessment has many limitations to interpretation. For more rigorous quantitative evaluation of stream invertebrates using natural substrates, the sampler of choice is typically a cylindrical Hess, Waters-Knapp or Neill sampler (Clark 1996, Cavanagh *et al.* 1994a, Barbour *et al.* 1999). For the cylindrical-type samplers, the maximum rock size should be 15 cm. in diameter with the apparatus deployed to a depth of at least 10 cm into the substrate. However, other natural substrate samplers such as a Surber are as effective, providing the water depth and velocity are not too great.
- When sampling gravel substrates, position the sampler firmly in the substrate or sampling area, and use hands (gloves are optional) to gently stir the substrate within the sampler. Care should be applied in using a trowel as it may damage the organisms making identification and enumeration impossible. It may be necessary to use a small (hand size) garden rake to loosen embedded substrate. For stream monitoring, use the same “routine” and length of time for disturbing and “washing” the gravels. Time the sample collection process (2 to 5 minutes,

depending on size of sampler) at each site and for each replicate so equal sampling effort is exerted for every sample. Employ the same person to collect all the samples in the study so as not to introduce possible error in the data as a result of inconsistent techniques that may be employed by different samplers.

- A rubber or neoprene collar attached to the lower rim of the cylindrical sampler may be helpful where the substrate is compacted and will not allow penetration of the rim.

### 3.2 Depositional Habitats

- For sampling in depositional stream reaches, lakes or marine environments, there is a variety of sampling equipment that is suitable including Eckman or Peterson dredges, ponar or petit ponar dredges as well as a range of simpler grab samplers, scoops or bucket apparatus (Ecological Services for Planning Ltd. 1993).
- Whether in depositional or erosional habitats, the investigator must still adhere to recommendations with respect to study timing, site selection, quality assurance and other requirements of the selected monitoring approach.

### 3.3 Mesh Size

- The selection of sampler mesh size is dependent on the questions that are being asked in a specific project. For quantitative assessments that are not linked to regional or national protocols and important endpoints are abundance and biodiversity, a mesh size of 253  $\mu\text{m}$  is a typical standard; anything larger could miss up to 90% of the organisms (R.D. Kathman personal communication). However, the USEPA (Barbour et al. 1999) recommends a mesh size of 500  $\mu\text{m}$  in their national biomonitoring programs to reduce processing time, costs and offer some national “standardization”. The RCA that is being developed nationally in Canada (see CABIN website) and the Australian national biomonitoring program that is known as AUSRIVAS (the Australian River Assessment System, Simpson and Norris 2000) use a sampler mesh size of 400  $\mu\text{m}$ . While it is known that a larger numbers of animals can pass through a 400 or 500  $\mu\text{m}$  mesh net than through a 200  $\mu\text{m}$  mesh net, the diversity of animals that are retained has been found to be no different (Rosenberg et al. 1999). For the purposes of the multivariate assessment methods in RCA and AUSRIVAS, the no net loss of diversity is important but overall abundance is not, thus making the larger mesh net acceptable for standard practice. A major benefit of the larger mesh is that the time to process samples in the lab is a fraction of what it is when a smaller mesh net is used. In contrast, if the study being conducted requires more detailed data or relies on presence/ absence of indicator species, using a finer mesh size is critical (Rosenberg and Resh 1992, Taylor 1997). Hence it is up to the principle investigator to determine what mesh size to use because it is dependent on study objectives. If in doubt, it is preferable to use a mesh size of approximately 250  $\mu\text{m}$ .
- Consideration of mesh size used in previous historical studies should also be a strong motivation for selection of appropriate mesh size. Where possible, it is

best to at least attempt to repeat the same methods to allow comparison and interpretation with historical data.

### 3.4 Artificial or Introduced Substrates

- To overcome logistical problems in the field, which limit the use of natural substrates, some consideration may be given to the use of “artificial” or “introduced” substrates such as ceramic tile plates, leaf packs or rock baskets (Merritt *et al.* 1996). There is much controversy around the use of introduced substrates, so caution should be used when relying on this method of study (Peckarsky and Penton 1990, Modde and Drewes 1990, Foissner *et al.* 1992, Boothoyd and Dickie 1989). Artificial substrates select for a given range of particle sizes. For example, gravel or small cobble is typically placed in baskets, which means that they should only be used in projects where particle size is not of interest in the questions being asked. The selection of a given range of particles reduces the variance in invertebrate metrics associated with particle size. This can be an advantage when questions associated with flow or chemical contamination or any factor other than particle size is of interest because the artificial substrates eliminate unwanted variance. In addition, artificial substrates can generally be placed in a greater range of water depths, thus enabling sampling over a greater range of physical conditions than are possible when using standard wading samplers (e.g. Hess or Surber samplers). Repeated measurements of physical and chemical attributes at the site where an artificial sampler is placed, allows for recording of environmental conditions throughout the period of invertebrate community development. This attribute can be very useful in studies where there is interest to examine factors that are most important in determining abundance or diversity, where an *a priori* decision is made that the effect of substrate particle size distribution is not of interest.
- There is risk in using artificial substrates. In flashy streams, a shallow site where a sampler may be placed can become dewatered when water levels recede thus making that sample useless. At extreme storm flows, samplers can become dislodged and they may be washed away. Careful placement can avoid these problems but they do impose some risk. Because repeated visits to a site are required when using artificial substrates, the cost of using them is much higher than that associated with single visits using a Hess or Surber sampler. Finally, the samplers must be left at a site long enough for a community to become established that is representative of the surrounding community at that site. Typically that period is six to eight weeks. Overall, artificial samplers should be regarded as specialized to specific types of impact assessments and should not be



considered for everyday use. In most cases, it is preferable to sample natural substrates. Where introduced substrates must be used, extreme care should be taken to ensure there is a high degree of confidence the resultant community which is established will adequately represent benthic impacts.

### 3.5 Number of Samples Per Site – Replication

- If the study design requires multiple samples at one site (termed replicates), the number of samples at each site or for each treatment should be driven by statistical considerations and analysis (Green 1999, Peterman 1990). However, based on recommendations from R.D. Kathman (personal communication), the ministry has used a minimum of four (preferably five) replicates collected per site, being careful to ensure the substrate and environmental conditions of the replicates are as similar as possible. Three replicates per site are acceptable if time and cost are a problem. If cost is an issue, it is suggested that five replicates be collected, but a minimum of three be processed and analyzed and the rest archived. If properly preserved, the two archived replicates can be stored indefinitely until additional resources become available or if high variability of the three analyzed replicates necessitate analysis of the archived replicates. Keep the number of replicates constant between sites and throughout the study. Replicate sample locations should also be part of site selection to ensure that replicates are as similar as possible, particularly in the choice of one of a riffle, pool, or run area.
- There are now techniques and study designs such as Environment Canada's Reference Condition Approach (RCA), which require only one "composite" sample per site, greatly reducing time, cost and still allowing for statistical assessment (Rosenberg *et al.* 1999). The US EPA employs the well-known Rapid Bioassessment Protocol (RBP), which has a one-sample per site methodology (Barbour *et al.* 1999). Both these one-sample techniques rely on the sampler gathering one continuous sample for a fixed duration, or a fixed number of intermittent sub-samples across a 100 metre stream reach. Rather than collecting discrete samples, which are then combined to form a composite, the sampler using these methods is actually compositing the sample as it is collected.
- For one sample per site composite methodologies, it is recommended that duplicate samples be collected randomly at roughly 10% of the total number of sites for quality assurance purposes. At least one duplicate sample per study should be collected and analyzed as the other samples and the data reported

separately as part of the quality control/quality assurance (QA/QC) carried out for the study (Barbour *et al.* 1999).

### 3.6 Other Field Measurements

- For stream environments, flow velocity should be measured at each sampling site or replicate, at the standard 0.4 of the total depth from surface to substrate and recorded on field data sheets or in field notes. Acceptable variation in velocity between repeated measures is approximately 25 %.
- A thorough visual assessment of the physical characteristics of the site should be conducted and recorded in field notes. Important characteristics to record are: channel width; height of bank, slope, and stability of the stream bank (obvious erosion occurring); vegetation; % riparian overstory; % forest canopy cover; presence of large organic debris (LOD); leaf packs; large boulders; riffle, pool or run; substrate type and variability; upstream sources of pollution; other obvious characteristics that could potentially influence the benthic community.
- Field notes that should be taken at each sample location or replicate include: date, time, sampler(s) as well as stream characteristics such as dissolved oxygen, temperature, pH, conductivity, depth of overlying water and stream flow velocity noting if the sample was taken in a backeddy (i.e. reverse flow).
- Depending on the type of study, concurrent water and sediment quality analyses (nutrients, general ions, metals, organic pollutants) as well as toxicological assessment may be necessary and should be conducted at each site or at least at key locations where changes are expected. As with any other study, approximately 10% of the samples for water or sediment quality should be for QA/QC (i.e. duplicates, travel blanks)
- Again, depending on the purpose of the study, samples of the substrate (usually two per site) are recommended for particle size analysis using the Wentworth scale (Murdoch and McKnight 1991) as part of the concurrent information needed for assessment. Samples can be taken using a grab, core or dredge type sampler depending on the site characteristics and goal of the study. There are many references to guide the selection of appropriate sampling equipment (Ecological Services for Planning Ltd. 1993, Cavanagh *et al.* 1994b). Although some researchers may have specific needs or preferences, the ministry has recommended a minimum depth of 10 cm of substrate be collected to conduct particle size analysis, with care taken to not wash out the fine sediment.

- As well, the visual stream substrate conditions should be described for every sample collected. An acceptable method of describing the substrate conditions is outlined in Platts *et al.* (1983), or Murdoch and McKnight (1991).

### 3.7 Sample Handling and Preservation

- Samples from the sampling device can be transferred into a sample bottle taking care not to crush tiny soft-bodied organisms and ensuring no insects are left clinging to the net. A poly wash bottle can be used for a final rinse of the net and collection cup. As a preliminary sort, very large stones or debris can be removed but only after they are carefully examined, washed and “picked” clean for clinging insects, which are then saved. The sampler may wish to remove some excess water but in doing so should be very careful not to lose any insects. Sieving of the sample to remove excess water and fines may be done using the same or finer sieve size as was used to collect the sample.
- Make sure containers are well labelled. Always place a paper label inside the container, written with a pencil or indelible ink, as well as on the outside of the container using a permanent marker. .
- It is recommended that all samples be preserved with buffered (neutralized) 10 % formalin, **immediately** after collection and a preliminary sort. Buffered formalin can be purchased at lab supply retail outlets. While alcohol (denatured ethanol or ethyl alcohol) is less noxious to the sampler, it does not penetrate tissues as well. If sufficient quantities of preservative are not used, it may result in deterioration of invertebrate tissue and inability to identify invertebrates (loss of data). For sample processing, it is acceptable to transfer samples to 60 % alcohol after three days in buffered formalin.
- A small amount of Rose Bengal or other tissue-staining compound can be added to the buffered formalin or alcohol to stain body parts and make identification easier. Remember that long term and permanent storage of samples requires at least a 10% buffered formalin solution. If there is a lot of water in your initial, unpreserved sample then ensure the buffered formalin you add will not be diluted to less than 10% if you started with a 10% solution. Either remove nearly all the water, or carry stronger buffered formalin solutions.
- For longer-term storage (up to 1 year) samples should be placed in either 10% buffered formalin or 90-95% ethyl alcohol. In case there is breakage, leaking or

evaporation, sometimes 5% glycerin is added since glycerin will keep the sample/specimen moist (Pennak 1953).

- After all animals are in the sample bottle, make sure lids are securely fastened. Breakable glass containers are not recommended.

## 4.0 SAMPLE ANALYSIS AND QUALITY CONTROL (LABORATORY)

### 4.1 Sorting and Quality Control

- Sorting the samples to pick out every individual invertebrate should be done by an experienced professional. Preferably the same individual sorts all samples in the study to reduce potential error, using good light and a white sorting tray for optimal recognition and identification of insects. Using soft tweezers, place the invertebrates into vials containing 95% ethanol, sorting by taxa if possible or necessary.
- Sort using at least a 6X to 10X dissecting scope for major taxa, stronger magnification (60X zoom) may be necessary for smaller invertebrates, for example those captured using a 250 µm or 210 µm mesh net. Some investigators are sieving whole samples into two fractions using a 1 mm sieve resulting in one fraction containing the macrobenthos (taxa greater than 1 mm in size) and the fraction < 1 mm, between 1 mm and the mesh size of the sampler used.
- Pay attention to the more rare species as they have been shown to be important in assessing environmental variables.
- Acceptable enumeration methods include total counts, a predetermined volume sub-sampling of a well mixed sample, using a sub-sampling device and procedure such as a Marchant box (Marchant 1989), Folsom-type sample splitter, or inverted Imhoff cone (Wrona *et al.* 1982). Regardless of what method is selected, the technique and rationale for selection must be reported. It is generally accepted that a minimum of 300 individual organisms per sample be enumerated (Perrin *et al.* 2005). If a sample has been sieved into two size fractions, all individuals in the macrobenthos fraction should be identified and enumerated, and a minimum of 300 individuals identified and enumerated from the <1mm sub-samples. If the minimum number is met within a given sub-sample, the entire sub-sample should be still be enumerated.
- 20 % of the total number of samples should be randomly selected and resorted. Resorted counts should be within 10 % of the original count.
- Reports should discuss all details of the sorting, identification and enumeration methods and all QA/QC aspects of the program including the qualifications of the individuals involved in the various aspects of the project. If “lay-samplers” are used to collect benthic samples, all pre-monitoring training provided as well

as auditing and additional QA/QC of the sample collection process must be reported.

#### 4.2 Taxonomic Identification and Quality Control

- Taxonomic identification should be conducted only by qualified professionals considered to be experts in the identification of aquatic invertebrates.
- Identifications should be taken to the lowest possible taxonomic level and for each taxonomic group should be to the same level for all sites being compared (Oregon DEQ 1998). For insects this can usually be done to genus, for planarids to family, leeches to genus and nematodes to class. The table below, from the Oregon Department of Environmental Quality, provides additional guidance from their "Sampling Protocol" (Oregon DEQ 1998).

Taxon	Level of Identification				
	Order	Family	Sub-family	Genus	Species
Amphipoda				X	X
Arachnida	X				
Coleoptera (most)		X			
Elmidae				X	X
Diptera (most)				X	
Chironomidae			X		
Ephemeroptera				X	X
Gastropoda		some		X	
Hemiptera				X	
Lepidoptera				X	
Megaloptera				X	
Odonata		some		X	
Oligochaeta	X				
Ostracoda	X				
Pelecypoda	X				
Plecoptera		some		X	X
Trichoptera				X	
Turbellaria	X				

From: <http://www.cqs.washington.edu/salmonweb/oregon/Macro.html>

- The taxonomic references used must be documented and reported with results. Some popular references include Merritt and Cummins (1996), Wiggins (1996), Oliver and Roussel (1983), Needham (1996) and McCafferty (1981).
- If a taxonomist has any question about the certainty of an identification (anomalous identifications), the specimen(s) should be sent to another qualified expert for verification, preferably one that has a specialized ability in that particular taxon. For junior or less experienced taxonomists, it is recommended that a project reference collection be assembled. At least 10% of the project reference collection should be submitted for review by an expert taxonomist, focusing on the more difficult taxa or uncertain identifications. The misidentifications should be no greater than 10%, determined by dividing the number of misidentifications by the total number of specimens submitted for verification by an expert entomologist, multiplied by 100 to get a percentage.
- A reference collection of species from each major taxon found in each region is strongly encouraged by the ministry. In some cases, reference collections exist or have been started and data collected could be used to verify and/or build on those existing collections. Please contact your local Ministry of Water, Land and Air Protection office for further information and access to ministry-held reference collections (see Appendix 1).
- Finally, any specimens entered into the reference or “voucher” collection must be properly prepared and verified by an expert entomologist for accurate identification. Guidance for proper preparation of specimens can be found in section 8 of the Resources Information Standards Committee (RISC) document entitled, Voucher Specimen Collection, Preparation, Identification and Storage Protocol: Animals (RISC 1999).

### 4.3 Data and Statistical Analyses

Before the study design is finalized and carried out, the method for data analysis must be selected and incorporated into the monitoring program. There has been much discussion among professional biologists regarding the preferred method of analyzing data and many new methods developed since the 1991 edition of this protocol document was written. A good summary of benthic invertebrate monitoring design, protocols and methods as well as the pros and cons, exist in Underwood (1997), the Aquatic Effects Technical Evaluation report on benthic invertebrate monitoring (Taylor and Bailey 1997, Green 1999) and in the EEM Recommendations from Cycle 1 Review,

Benthic Invertebrate Community Expert Working Group report (Environment Canada 1997). As well, the ministry has several published guidance documents funded by the Resources Information Standards Committee (formerly the Resources Inventory Commission) which are also informative (Cavanagh *et al.* 1994a, 1994b, 1998a and 1998b). If the RCA methods are being used, all analytical tools are available on the CABIN website that is managed by Environment Canada (<http://cabin.cciw.ca/cabin/asp/english/welcome.asp> ).

The Ministry Environment prefers a “weight of evidence” approach which means using multiple analytical tools for evaluating impact. Some of these data evaluation methods include grouping by functional feeding group, using community metrics or multimetric analysis (Karr and Chu 1997, Karr and Chu 1999, Barbour *et al.* 1999), population fitness parameters (Feltmate and Fraser 1999), identifying species tolerances or presence-absence, using univariate (t-tests or ANOVA) and multivariate (MANOVA, principal components analysis) statistical analyses (Green 1984, Green 1999, Zar 1996) as well as the “reference condition” multivariate approach (Rosenberg *et al.* 1999).

Whatever analytical tools are used, the investigator must report the rationale and supporting literature for selection of specific analytical tools used in their study. It is always prudent, if in doubt, to discuss the study design and analytical tools with a qualified biostatistician well in advance of finalizing the study design and carrying out the monitoring. Below are some general recommendations for benthic invertebrate monitoring studies:

- Regardless of what analytical approach and statistical tests are applied to the data, the report must present all the raw data collected with replicate data listed separately. Submission of an electronic copy of the report and raw data tables is recommended. As well, tables of raw data should have a complete list of taxa found in the study to facilitate inter-site comparison.
- Tolerance categories used for Hilsenhoff’s Biotic Index (Hilsenhoff 1977) or other such index, must be assigned with care. Each major invertebrate taxon contains species exhibiting a wide range of tolerance to various types of pollution, which underscores the importance of identifying taxa to the lowest level possible. Tolerance values can be obtained from Appendix B in Barbour *et al.* (1999)
- For proper assessment of data, it is not recommended that use of community metrics or other “rapid assessment” techniques be done in isolation of statistical data assessment techniques. An entire community cannot adequately be described with one number. Rigorous evaluation of benthic invertebrate data



and a “weight of evidence” approach should always incorporate simple descriptive statistics and where appropriate, univariate or multivariate statistical analysis.

- Descriptive multivariate methods such as cluster analysis, ordination (principal axis correlation) and discriminant function analysis may be useful to reduce the complexity of the data set or reveal major patterns, but may not be sufficient in themselves to determine impact (Taylor and Bailey 1997, Rosenberg *et al.* 1999). Exceptions are the multivariate tools that are the basis of the reference condition approach that are explained on the CABIN website (<http://cabin.cciw.ca/cabin/asp/english/welcome.asp> ). Other simple examples of multivariate tools are the Jaccard Cluster Analysis for presence/absence, and the Bray Curtis measure that is the basis for various clustering routines and algorithms used in multidimensional scaling analyses. Software programs include Sigtree, Comtree and multivariate analyses found in SAS and Systat. Dedicated multivariate software such as PRIMER (Clarke and Gorley 2001) is more powerful and easier to use than SAS or Systat.
- Even if metric or multimetric analyses are used, cluster analyses should always be run on the data to determine the similarity (variability) between replicates as well as, or between sites to make inter-site comparisons more valid. As well, relatively simple methods are available to determine the statistical significance of similarity coefficients between communities (sites) from random (replicate) variance (Nemec and Brinkhurst, 1988).
- For studies with small numbers of sampling sites, there are randomization techniques (e.g. bootstrapping), which could be used as a data assessment tool to conduct statistical analysis.

Careful selection of data analysis tools ensure the data can be used for: providing supplemental data in watershed assessments, defining fisheries habitat potential, determining impact to benthic communities as a result of changes in land use or industrial effluents, and providing critical information for making management decisions. It is important that all aspects of a benthic monitoring study be integrated with the overall approach and program implementation to ensure that sampling design, methodology, identification and interpretation are sound, since important management decisions could rely on study results.

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## **Appendix A**

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## Ministry of Environment Contacts in British Columbia

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