The Canadian Aquatic Biomonitoring Network

Field Manual

Prepared by
Ministry of Environment
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Watershed and Aquifer Science Section
for the Resources Information Standards Committee

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Preface

The bioassessment of stream condition using benthic macroinvertebrates has been conducted in British Columbia over the years using a variety of differing assessment protocols and data assessment techniques. Without methods standardization producing comparable data, the results have been of limited value at a provincial level. To address this issue, the British Columbia Ministry of Environment has partnered with Environment Canada to implement the Canadian Aquatic Biomonitoring Network (CABIN), a national bioassessment framework, at the provincial level. This represents a significant step forward in providing a consistent approach to biological assessment in British Columbia, and in creating a structure whereby several of the commitments of British Columbia's Living Water Smart plan can be achieved.

CABIN is a collaborative program for the collection and assessment of biological data to determine the health status of aquatic ecosystems. Maintained by Environment Canada, CABIN provides participants with a nationally standardized protocol for the collection of benthic macroinvertebrate and stream information that is shared across the network using online tools. Collaborating with other agencies and organizations (both public and private) allows all participants to evaluate aquatic ecosystem health in a scientifically defensible manner at a fraction of the cost of doing so individually.

Mention is made throughout this document to CABIN online training modules. CABIN provides this training program through the University of New Brunswick (http://www.unb.ca/cri/cabin_criweb.html) and completion of this training is required of all partners to access the resources of the CABIN network. The reader is referred to the above mentioned website for more information regarding enrollment in this program.

Completion of the CABIN online training modules and adherence to CABIN protocols (both in this document and through the online training) represents the minimum information requirements for provincially approved aquatic bio-monitoring in British Columbia. The reader is directed to the Resources and Information Standards Committee aquatic index page (http://www.ilmb.gov.bc.ca/risc/pubs/aquatic/index.htm) for current data collection requirements and protocols in British Columbia.

Abstract

The Canadian Aquatic Biomonitoring Network (CABIN) is the national biomonitoring program developed by Environment Canada and accepted by the Province of British Columbia to provide standardized sampling protocols and a recommended assessment approach called the Reference Condition Approach (RCA) for assessing aquatic ecosystem condition in British Columbia.

The collection of benthic macroinvertebrates for biological assessments of streams and the data on the presence, absence, and relative abundance of this group of organisms gives a scientifically defensible indication of site specific and cumulative impacts of anthropogenic stress on stream ecosystems. Additionally, as benthic macroinvertebrates are ubiquitous in streams, their collection for stream ecosystem condition assessment is cost effective. CABIN represents the best available science and provides the tools necessary to conduct consistent, comparable, and scientifically credible biological assessments of streams in British Columbia.

This field manual gives an overview of the standardized protocols for the collection of macroinvertebrate biological and habitat data from British Columbia for entry into the national CABIN database, information on safety considerations while in the field, as well as procedures to follow to ensure quality assurance and quality control both in data collection and in taxonomic identification procedures. The manual is designed to complement the online CABIN training program offered through the University of New Brunswick (http://www.unb.ca/cri/cabin_criweb.html).

Acknowledgments

The Government of British Columbia provides funding of the Resources Information Standards Committee work, including the preparation of this document. The Resources Information Standards Committee supports the effective, timely and integrated use of land and resource information for planning and decision making by developing and delivering focused, cost-effective, common provincial standards and procedures for information collection, management and analysis. Representatives to the Committee and its Task Forces are drawn from the ministries and agencies of the Canadian and the British Columbia governments, including academic, industry and First Nations involvement.

The Resources Information Standards Committee evolved from the Resources Inventory Committee which received funding from the Canada-British Columbia Partnership Agreement of Forest Resource Development (FRDA II), the Corporate Resource Inventory Initiative (CRII) and by Forest Renewal BC (FRBC), and addressed concerns of the 1991 Forest Resources Commission.

> For further information about the Resources Information Standards Committee, please access the RISC website at: http://www.ilmb.gov.bc.ca/risc/index.htm.

This report and the standardized methods contained within were developed by Environment Canada's Water Science and Technology Directorate under the Canadian Aquatic Biomonitoring Network (CABIN) program. The report has been modified to conform to RISC publication standards.

Changes to individual methods included in this report as well as any changes to the appearance of the original Environment Canada report were prepared by Leon Gaber of the BC Ministry of Environment.

We would like to thank Stephanie Strachan and Sheena Pappas of the Pacific & Yukon Water Quality Monitoring Office of Environment Canada for their help in expediting the publication of this document, and their continued collaboration with the government of British Columbia's provincial biomonitoring program.

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Introduction

The Canadian Aquatic Biomonitoring Network (CABIN) is the national biomonitoring program developed by Environment Canada that provides a standardized sampling protocol and a recommended assessment approach called the 'Reference Condition Approach (RCA)' for assessing aquatic ecosystem condition. CABIN provides the tools necessary to conduct consistent, comparable, and scientifically credible biological assessments of streams.

The aim of this field manual (and associated online training program) is to learn the nationally standardized CABIN protocol for the collection of benthic macroinvertebrate samples and associated stream information in wadeable streams. This manual also discusses the importance of following standardized laboratory protocols for CABIN, such as sub-sampling techniques, which are equally important for data comparability and sharing. This field manual (and corresponding online training module 3 "Field Sampling Using Standard CABIN Protocols") will enable the reader to:

- Know when to sample;
- Understand the importance of safety first;
- Understand the importance of using a standardized field sampling protocol;
- Know how to collect required non-field data before sampling;
- Know when and how to collect a standardized CABIN sample and related habitat data in the field;
- Know what equipment is required to sample and collect habitat data;
- Know the standard protocols that are needed for CABIN sample processing in the laboratory.

1.1 Why Benthic Macroinvertebrates

Traditionally, water quality networks have been mainly based on chemical and physical measures where water quality assessments are done through comparison to guidelines established to protect specific uses, such as the Canadian Water Quality Guidelines for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment, 1999, updated 2006). Monitoring using these measures provides a 'snap-shot' of the status of water quality.

Recent approaches to river health assessment acknowledge the importance of physical, chemical and biological interactions. Adding the biological component to lotic and/or lentic monitoring complements the more traditional approach by providing the 'effect' measurement to the assessment (that is, the effect of a stressor on the biota). Stressors that may have an impact on aquatic biota that cannot be measured by taking water samples alone include changes in water quantity, presence of invasive species, habitat degradation, and synergistic and cumulative impacts.

For water quality assessment, measurements of aquatic biota summarize the preceding river conditions for weeks or months before their collection. For example, an episodic pollution event, such as a chemical spill, may go undetected by periodic water sampling regimes but damage to aquatic biota can be detected long after the cause of the impact has passed.

Benthic macroinvertebrates are common inhabitants of lakes and streams where they are important in moving energy through food webs. The term "benthic" means "bottom-living", so these organisms usually inhabit bottom substrates for at least part of their life cycle; the prefix "macro" indicates that these organisms are retained by mesh sizes of ~200-500 µm.

The most diverse group of freshwater benthic macroinvertebrates is the aquatic insects, which account for ~70% of known species of major groups of aquatic macroinvertebrates in North America. The remaining ~30% are non-insects such as oligochaetes, nematodes, and mites. More than 4,000 species of aquatic insects and water mites alone have been reported in Canada. Thus, as a highly diverse group, benthic macroinvertebrates are excellent candidates for studies of changes in biodiversity.

Benthic macroinvertebrates are the most commonly used biological indicators for freshwater resources because they are:

- Sedentary = integrate site-specific impacts
- Ubiquitous and generally abundant = can be easily collected everywhere
- Long-lived (1-3 years) = reflect cumulative impacts
- Diverse = respond to a wide range of stressors
- Key part of the food web = ecologically important

1.2 When to Sample

For bioassessments, it is important to sample each site in the same season of each year to ensure comparability of the data. Typically, CABIN sampling is done in the fall or late summer when the largest portion of the taxa in the stream are likely to be present in an aquatic life stage, flow conditions are optimal for safe sampling, and the stream that you are sampling has been wet for most of the year.. The best time of year may vary depending on your location in Canada. For example, the best time in Nova Scotia would be in September-October whereas in the Iqaluit area of Nunavut it may be in mid to late August. Note that sampling must not be undertaken if streams are in flood.

2.0 Procedures Before Field Sampling

There are some key tasks you need to accomplish before you do your actual field sampling. These are all necessary for successful data collection in the field.

2.1 Study Designing and Site Selection

Once a study design has been developed and the objective has been defined using the tools provided in online Module 2, sites must be selected to address the question that has been formulated. Protocols for selecting a sampling location will differ depending on the type of site visited; reference or test site.

2.1.1 Reference sites

Module 2 "Study Design, Site Selection, and Predictive Models" provided a more detailed summary of designing your study and how to select reference sites. A brief summary of how to select a reference site is provided below:

2.1.2 Selecting a reference site

A reference site should have no point sources of pollution and also have natural habitat features similar to those of exposure areas (Figure 1).

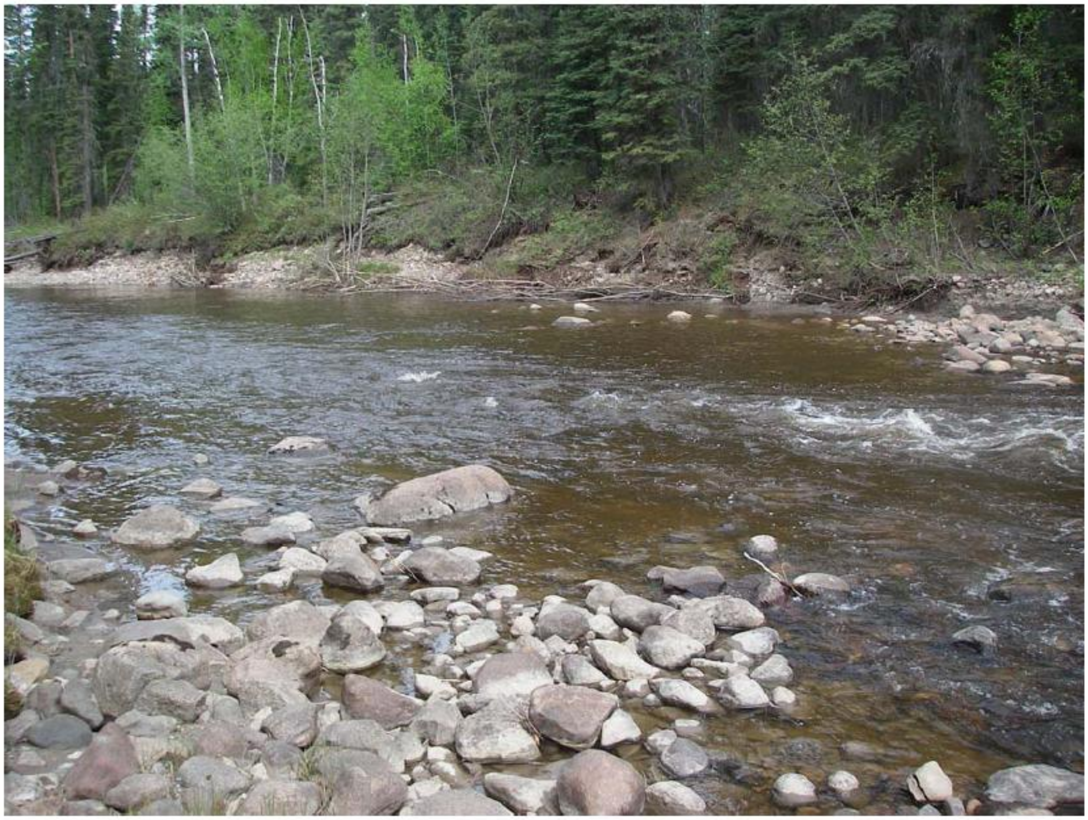


Figure 1 - Example of a potential reference site

- Reference areas may not represent pristine (or pre-European settlement) conditions, but areas in which impacts are lowest or disturbance is minimal (Simon 1991, Omernik 1995).
- For example, Omernik (1995) identifies references sites in drainage basins that lack municipal and industrial influence even within the polluted Huron-Erie corridor of the Great Lakes.
- A second example can be found on the prairies where minimally impacted reference areas may be located in drainage basins that lack point source effluents or logging activities but, unavoidably, have a high degree of agricultural activity.
- Therefore, the characteristics of these “least impacted” areas will, necessarily, differ across the country, however, the approach to defining and identifying reference areas should be consistent.

Careful consideration is required, not only for the consistent application of the selection criteria for reference areas, but also in determining how many reference areas or stations will be examined for all study designs. The following specific points should be considered during the selection of reference sites for a benthic invertebrate assessment study.

- As reference areas are to be selected to match the habitat features of the exposure area(s), identification of the types of test sites that will be assessed and their habitat features should precede the selection of reference sites.
- If non-point or other point source inputs occur upstream of the test sites, select reference site(s) in the nearest comparable drainage basin with minimal development that occurs within the same ecoregion.
- If additional sources of disturbance to the river valley are associated with the test sites, stressor effects may be confounded by the disturbance. Accordingly, additional physically matched reference areas should be selected.

2.1.3 Selecting test sites

The test sample site will be determined prior to going into the field according to the study parameters and desired outcomes.

- The test site will be selected based on a point of interest such as point source pollution or other study objectives (Figure 2).
- The actual sample reach (site) cannot be determined until one physically visits the site and performs some basic reconnaissance. However, the general location should be determined before going into the field.

Figure 2 - Example of a potential test site

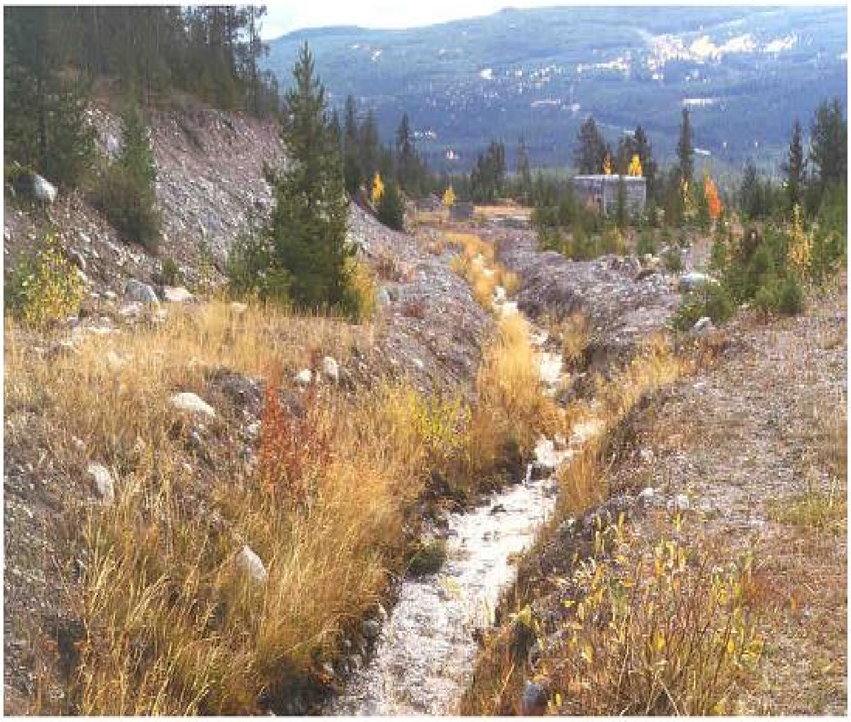


Figure 3 - Example of a potential test site

2.2 Creating site identifiers and unique codes

A site nomenclature should be established before you conduct your field sampling. Sites will be maintained in a database as unique entries therefore sites should be assigned a unique site code that identifies them in both space and time. Site codes can be assigned by the project leader and should reflect qualities of the site that help to define its location and other identifiers.

2.2.1 CABIN recommended site coding system

The structure of Environment Canada's site nomenclature system depends in part on the spatial and temporal frequency of sampling. Site nomenclature uses:

- 3 letters for a basin/sub-basin code;
- 2 numbers for a site number code;
- 2 numbers or letters for another level of sampling within a site (e.g. replication).

For example, GLD07 would be the site code for site #7 on the Gold River. If there is a replication element, another identifier can be used with the site code. For example, the first sample could be designated with A (GLD07A), second sample with a B (GLD07B) and the third sample designated with C (GLD07C).

Note: Other attributes that identify the sampling site like the date of the sampling, study/project name, the watershed according to the "Know Your Watershed" classification (http://map.ns.ec.gc.ca/kyw/) the basin name, specific watercourse name and watershed code if one exists, should also be recorded on the field sheet and entered into the CABIN database.

2.3 Site attributes determined on a map

There are a number of site attributes that can be determined prior to going into the field, using topographic maps, for example. These are all simple to acquire and useful in characterizing sites and assisting in site selection. Some adjustments may be necessary once the site is actually visited.

2.3.1 Ecoregions and Ecodistricts

An objective of site selection is to initially match reference and test sites, therefore the identification of ecoregions and ecodistricts may be useful. Ecoregions and ecodistricts are levels of ecological land classification. This classification is a process of delineating and categorizing ecologically distinctive areas. Each area can be viewed as a discrete system which has resulted from the mesh and interplay of the geologic, landform, soil, vegetative, climatic, wildlife, water and human factors which may be present. The dominance of any one or more of these factors varies with the given ecological land unit. This holistic approach to land classification can be applied incrementally on a scale-related basis from site-specific ecosystems to very broad ecosystems. This is a hierarchical classification (i.e. ecozones, ecoprovinces, ecoregions, ecodistricts, etc.) where areas gain their identity through spatial differences in a combination of landscape characteristics. The factors that are important vary from one place to another (at all spatial scales).

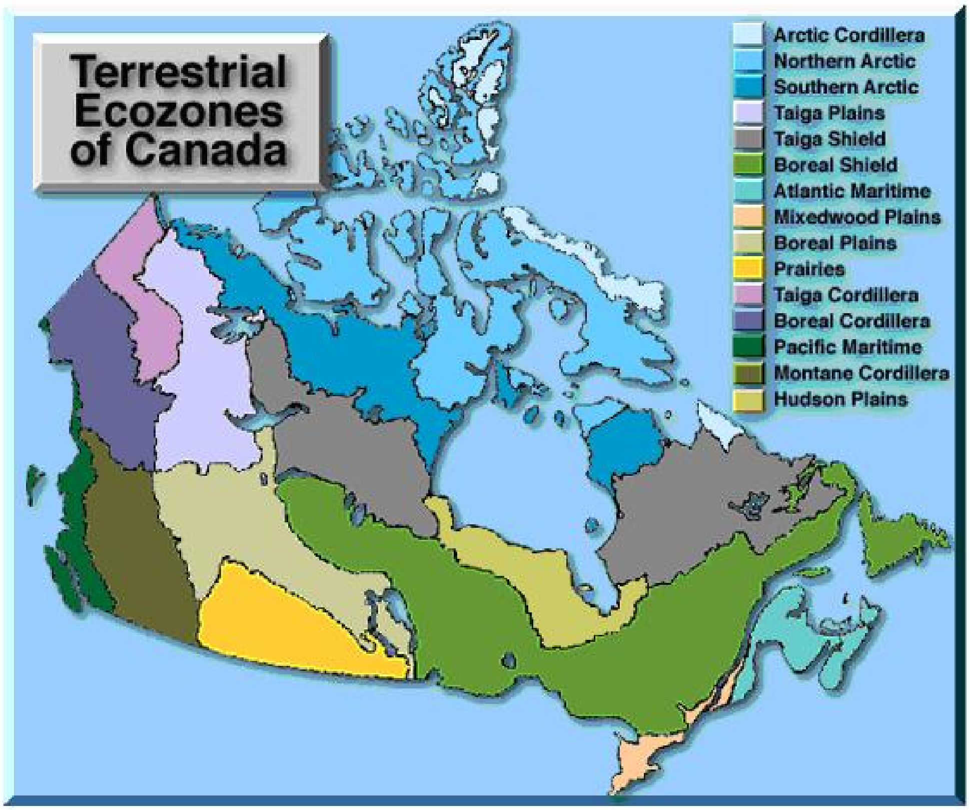

Terrestrial Ecozones of Canada
Arctic Cordillera
Northern Arctic
Southern Arctic
Taiga Plains
Taiga Shield
Boreal Shield
Atlantic Maritime
Mixedwood Plains
Boreal Plains
Prairies
Taiga Cordillera
Boreal Cordillera
Pacific Maritime
Montane Cordillera
Hudson Plains

Figure 4 - Terrestrial Ecozones of Canada

Method: Ecoregion information can be obtained from the following website, and can be entered onto the field sheet. http://www.ec.gc.ca/soer-ree/English/Framework/Nardesc/default.cfm

2.3.2 Stream order

A seminal theory in riverine ecology was the River Continuum Concept (Vannote et al. 1980). Although it is no longer widely regarded, it still contains important principles of how streams function and the relative abundances of functional groups of organisms with changing energy sources along the stream length; external sources, such as leaf litter (allochthonous) in headwater areas to internal sources, such as algal and plant production (autochthonous) in lower reaches. Stream order is the hierarchical ordering of streams based on the degree of branching (AFS Aquatic Habitat Inventory). It is a simple quantitative method of assigning a number to stream segments, which indicates the relative position (i.e. size) of the segment within the drainage basin. All references to stream order for a project should be done on the same scale map. For large basins, 1:250,000 scale topographical maps are recommended, however, the national standard is to use 1:50,000. Test sites must be characterized by the same map scale as the reference data.

Method: Stream order is determined prior to arriving at a sampling location as follows:

a) The entire basin for the river in question is mapped out using a 1:50,000 scale map.

b) First order streams are defined as those segments having no tributaries. First order streams are assigned a value of 1.

c) Subsequent stream orders are assigned values according to the method established by Strahler (1957, p914).

d) Where stream segments of the same order come together, the resulting segment is assigned the next highest order (e.g. where first order streams join, the resulting stream segment is elevated to the second order).

e) Where segments of differing orders come together, the resulting segment retains the order of the highest contributing tributary.

f) Where a stream enters a lake, the lake is treated as part of the stream. If more than one stream enters a lake, the outflow of the lake retains the order of the highest contributing tributary segment (Figure 4). If two streams of the same order run into a lake, the outflow increases to the next stream order.

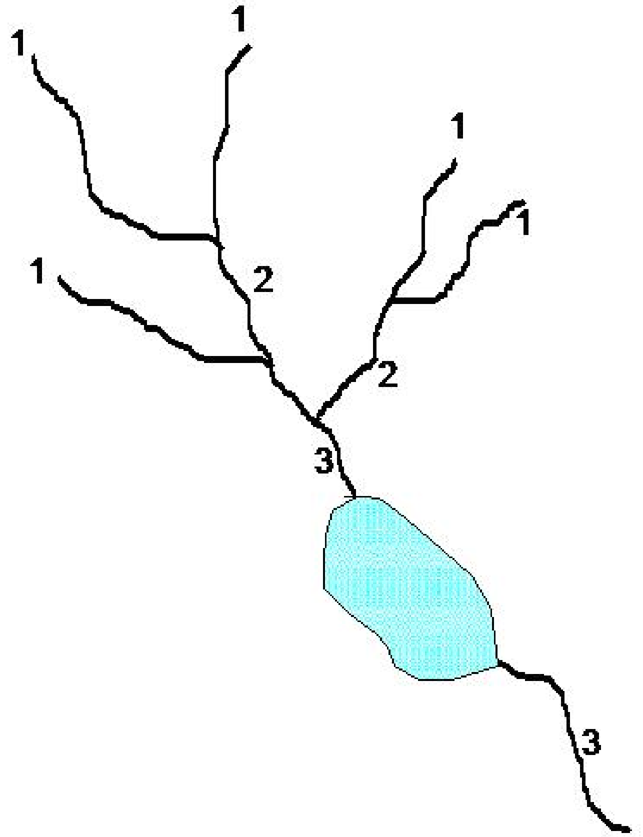


Figure 5 - Example of Strahler's stream order classification

2.3.3 Location

CABIN uses latitude and longitude as location coordinates. UTMs must be converted before entry into CABIN. The location coordinates can be approximated on a topographic map before going out into the field. The GPS coordinates and the geographic description may have to be altered when you arrive at the site if conditions are not suitable for sampling or access is not possible. Coordinates should be recorded as degrees, minutes, seconds or decimal degrees. Degrees, minute, seconds will automatically be converted to decimal degrees when entered into the CABIN database. A geographic description of the site is also useful (e.g. 100 m upstream of the bridge crossing) so that a different field crew is able to return to the exact sampling location if subsequent visits are required.

Method: Latitude and longitude are readily measured by a GPS or from a topographic map and can be entered onto the field sheet ahead of time. The coordinates can be adjusted once in the field. Coordinates should be recorded as degrees, minutes, seconds or decimal degrees. It is also a good idea to note directions to the site, especially if it is somewhat obscure to get to!

2.3.4 Distance from Source

Distance from source is the distance between the headwaters and the site being sampled. It is a measure of the position of the site along the stream and is an alternative indicator to stream order that conveys a different type of information related to the River Continuum Concept (Vannote et al. 1980).

Method: The distance is easily calculated directly from topographic maps using a consistent map scale.

2.3.5 Catchment area

The size of the catchment area (drainage area above a certain point) and particularly land uses in the catchment area are important in determining the quality of the stream. Catchment area is readily calculated using a topographic map. Land use is not as readily obtained, but if available, local drainage should be acquired as it will assist in determining the suitability of reference sites.

Method: Standard approaches with topographic maps or from GIS systems if available.

2.3.6 Altitude

Altitude is important as it locally affects the temperature regime at a site. It is the indirect correlation of climatic factors to the benthic community that is important thus altitude is always recorded.

Method: Altitude is readily measured from topographic maps and using GPS. Some handheld GPS units can be very inaccurate with altitudes. Be sure to verify these values with your topographic maps using contour lines.

2.3.7 Summary

These six pieces of information can be collected prior to going in the field:

1) Ecoregion
2) Stream Order
3) Location
4) Distance from source
5) Catchment area

6) Altitude

2.4 Field Safety - Your Number One Priority

Before you head out to the field the most important thing you must remember is your own, personal safety and the safety of all crew members. No sample is worth risking your health or your life.

2.4.1 Things to Remember

If for any reason you believe that collecting a sample will be unsafe – e.g. due to higher or swifter water than usual, weather conditions, dangerous wildlife in the area, construction etc. – do not collect a sample.

Always inform someone where you are going and when you expect to return, and never sample alone. Always have a call-in procedure in place before you go so that someone is aware of your safe return, or that you may be in need of assistance if you do not return at the expected time.

Wear your lifejacket whenever you are working near or in water, no matter how safe the conditions appear to be.

Each person is required to follow any safety procedures outlined by their own organizations' regulations. Environment Canada is not responsible for any safety training, however, Environment Canada field sampling requirements and training for Environment Canada employees are listed here as an example of the requirements you may be instructed to follow by your organization:

- Swiftwater Rescue Training Course (Rescue Canada);
- First Aid Level One (St. John's Ambulance);
- Life jackets, throw ropes, waders and a first aid kit present at all field sites;
- Completing a Task Hazard Analysis(THA) for "Streamflow Measurement: Wading";
- Safe Work Procedures (SWP) for "Streamflow Measurement: Wading";
- Site Inspection Sheet filled out for each site upon arrival;
- Providing your itinerary for field sampling to your supervisor prior to departure;
- Participating in a 'Call-in service' for end of the day so your supervisor knows you finished the field day safely.

2.4.2 Handling chemicals and sample preservatives

Once you have obtained your benthic macroinvertebrate sample in the field, it needs to be preserved in 95% ethyl alcohol to prevent your sample from changing (e.g. organisms feeding on others) or degrading before it is analyzed. If the sample contains a large amount of water, it needs to first be drained so that the liquid in the sample jar remains very close to 95% ethanol. As a fixative, ethanol penetrates throughout the organism's tissue to stop microbial activity that leads to decomposition. Ethanol can be harmful and should be handled with caution. Use of ethanol requires knowledge and understanding of Transport of Dangerous Goods (TDG), Workplace Hazardous Materials Information System (WHMIS) and the

Material Safety Data Sheet (MSDS) for this chemical. Links to all of these are provided in the References and Further Reading section. This training, largely available online, can be acquired through http://www.yowcanada.com/ for example, and should be taken prior to field sampling.

In the field, we recommend that you:

1) Keep the ethanol in a leak proof plastic bottle within a sealed container so that the fumes do not escape into your vehicle.
2) Store the ethanol separately from any sample bottles as ethanol will dissolve permanent markers.
3) Ensure the container and lid are properly labeled with name, MSDS reference, and date
4) Wear gloves and protective eyewear when handling ethanol and preserve samples outside of the vehicle where it is well ventilated.
5) Ensure it is secured in your vehicle while traveling, if possible, store outside passenger compartment (e.g. back of the pickup truck or in the trunk).
6) Do not use ethanol on the bank of the stream in case of spillage.

2.5 Choosing an analytical laboratory for your water samples

This choice will depend on what parameters are required as determined by your study objective and your budget. There are a few required parameters for CABIN that may be important for the predictive model and a suite of recommended parameters for CABIN that may aid in interpretation of your test site assessment. In order for water chemistry parameters for model building or the interpretation to be useful, the parameters must be collected at both your reference and your test sites. These factors must be considered in designing the study. You'll need to arrange analyses with your chosen lab prior to sampling and in many cases the lab will also provide you with the bottle sets needed.

2.6 Check the equipment list before departure....!

Finally, before heading out to the field you must ensure you've packed all the necessary equipment for sampling. An equipment checklist should be used to ensure everything is prepared according to CABIN protocols for field sampling (Table 1).

Item	
Kick net	Tote box or duffle bag
Stopwatch	Camera (& film if not digital)
Tweezers	Clipboard

- Squeeze bottle
- Sample jars ~ 3 per site
- Sample labels (waterproof paper)
- Permanent markers (for labeling jars)
- 95% Ethyl alcohol ~ 1-2 L per site
- Water chemistry bottles (as per lab requirements)
- 30 m tape measure/range finder
- Metre stick (stream depth)
- Metal ruler (substrate measurements)
- Velocity metre or tennis balls
- Handheld GPS
- Densiometer
- White tray
- In situ water quality probes (pH, DO, conductivity, turbidity, temperature)
- HB pencils
- Field sheets per site (waterproof paper)
- Topographic maps
- Field Manual
- Duct tape & Utility knife
- Extra batteries (as required for all equipment)
- Waders, life jackets, throw ropes
- first aid kit
- Cooler and ice packs
- Plastic bags
- Buckets
- Clothing for inclement weather
- Material Safety Data sheets for any chemicals being used
- 1 8”x 2” 250 micron mesh sieve (in case bucket swirling method is needed)

Table 1 - Field equipment checklist for CABIN sampling

3.0 Field Sampling Procedures

3.1 Complete your site inspection sheet upon arrival

On arrival in the field, a Site Inspection Sheet (see Appendix), must be filled out. The completion of this sheet allows for any potential dangers to be defined and ensures that all field members are aware of the precautions that need to be taken (e.g. avoidance of high flow areas upstream or a waterfall downstream). This sheet is important in the prevention of accidents in the field and must be taken seriously.

3.2 What types of samples and measurements do I take?

In the field, there are two types of measurements and samples to be taken:

1. A sample of the benthic macroinvertebrates using the kick net, and
2. Measurements and samples describing the environment, including habitat measurements and water samples.

It is critical that **all** measurements be completed, otherwise, the site might have to be discarded from the development of the model if there is missing data, or, a test site might not be assessed due to lack of data to compare with the reference model. Before leaving the site, the field sheets should be re-checked to ensure that all measurements and samples have been taken. It is useful to pass field sheets to other members of the sampling team to look for missing information and to also double check that the recorded values are legible. One of the analytical methods in CABIN employs multivariate statistics. This method is very sensitive to missing data and the only options available when data are missing are to either discard the site from analysis or discard the missing variable from all sites. In either case, significant time and money will have been lost. Hence, care should be taken to measure all the variables at the sites.

3.3 What is the order for doing these measurements and sampling?

Habitat assessment has been separated from the invertebrate sample collection in the description of methods below. It is suggested that one follows the order below when in the field:

General information can largely be collected before arriving at the site. Photographs and site inspection should be taken on arrival.

Reach characteristics can be assessed before entering the water.

Water quality measurement should be done **first** as disturbance of the sediment can affect some variables, particularly nutrients. This should be done slightly downstream of where the benthic sample will be taken so as not to disturb the sampling location. Samples could also be taken after the invertebrate samples but in this instance, the samples should be taken upstream of the invertebrates sampling to avoid sediment disturbance.

Benthic invertebrate samples should be collected before other stream measurements are taken (e.g. channel profile) which will disturb the stream bottom in a riffle representative of the “site” (6x bankfull width).

Substrate composition can be assessed directly in the stream if the water is shallow and has good visibility, or on an exposed gravel bar or the bank that is similar to the stream bottom where the invertebrate sampling took place.

Channel measurements should be taken in the area (or similar to the area) where the benthic sample was taken.

If you have a field crew of three or four people, some of these steps can be occurring at the same time. For instance, while one person is filling out the field sheet for the general information and the reach characteristics, a second person can be labeling a benthic jar and preparing to do the kick sample while a third person is taking the water samples downstream. In the time required for one person to do the kick sample and preserve the sample, the other two people will have completed most of the field sheet, taken the water quality samples and are continuing with channel characteristics. At this point the person who did the kicking can proceed with recording the substrate characteristics. If it is a fast flowing stream, you may need a fourth person to belay the sampler in the river. Each of these steps is described in more detail in the next sections.

3.4 General information

3.4.1 Determine the sample reach

The actual sampling reach should be determined and identified to all crew members. The sampling site is defined as a river reach six times the bankfull width (Figure 5a); this typically represents a complete pool/riffle/pool sequence contained within the meander wavelength (Figure 5b). If desired, marking tape, sticks or some other indicators can be used to define the reach (site). The sampling site should be typical of the general area.

When you arrive at the site, you do not want to disturb the stream before the water chemistry or the benthic sample is taken, so bankfull width will be estimated for the purposes of determining the sample reach but will not be measured until after you have completed your water and benthic sampling. You want to know this in order to determine how far upstream and downstream you will look to evaluate things like riparian vegetation, canopy cover, habitats present, etc. (see section 3.5).

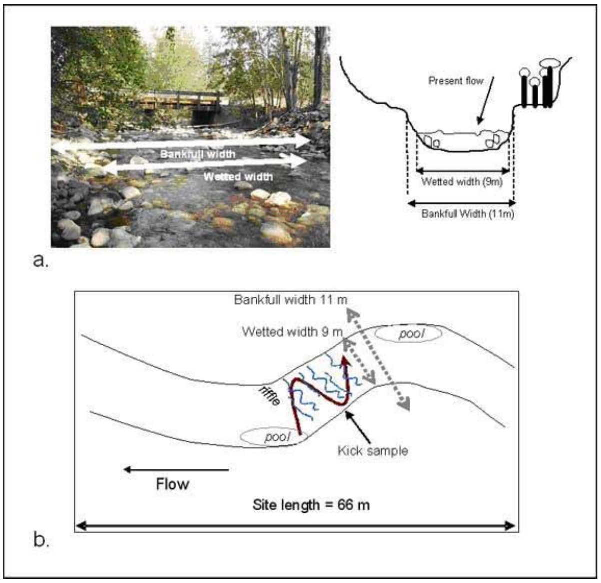


Figure 6 - Measurement of bankfull and wetted width (a), and diagram of pool-riffle-pool sequence and determination of site length and sampling location (b)

3.4.2 What should be recorded upon arrival at the site?

Variables including: latitude, longitude, and altitude are obtained (or verified) using a GPS or other similar equipment (if available).

Note: This may have already been completed from a topographic map. On-site measurement may also be necessary if a site has to be moved in the field.

The station number, stream name, and date should also be recorded on every field sheet as well as the field crew names. This is very important if the field sheets get separated.

Note: If the location coordinates and geographic descriptions have not been recorded on the field sheets ahead of time, this should be the first data recorded at the site.

3.4.3 Photograph your site

Because of the expense of field collection, it is often impractical or impossible to go back to a site. Photographs of the location can often help to resolve any questions that may arise during data entry or analysis (Figure 6). Also, the photographs may illustrate how a site has changed if it is revisited. First, a photograph of the field sheet with the site number is taken to identify the ensuing series of photographs. These include an upstream, downstream and an across the stream photograph. In addition, a photograph should be taken of the substrate in an area most similar to where the invertebrate sample was collected (e.g. in a dry area along the channel edge similar to the wetted sampling area). A wetted substrate photo can be taken but often the substrate is difficult to photograph below the surface of the water without distortion, unless you have an underwater camera in which case the water should be deep enough to capture more than one rock in the photo. These photographs provide a valuable record of conditions at the site. If a digital camera is available it should be used, as images can be stored in the CABIN database.

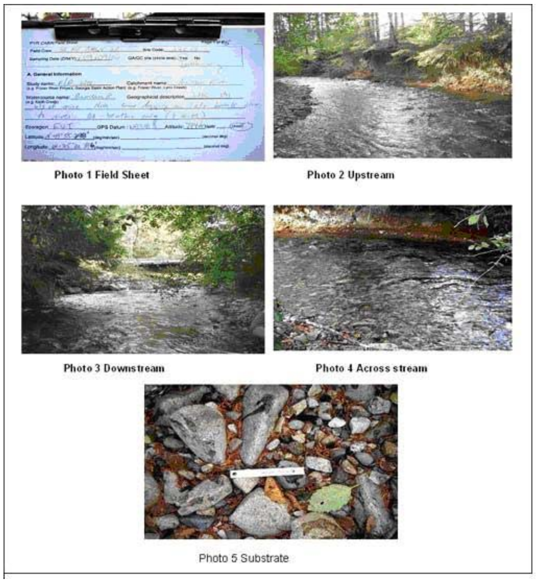


Photo 1 Field Sheet

Photo 2 Upstream

Photo 3 Downstream

Photo 4 Across stream

Photo 5 Substrate

Figure 7 - Sample site photographs

3.4.3.1 Summary

We recommend you take photos of 1) the field sheet 2) upstream of your site 3) downstream of your site 4) across the stream, and 5) the substrate.

Tip: A metal ruler or something of a known size should be positioned in the photograph for scale.

3.4.4 Hand-draw a map of your site and other geographical notes

A hand-drawn map is useful to illustrate major landmarks or features of the channel morphology or orientation, vegetative zones, points of entry and exit, compass orientation, flow direction, buildings, roads etc. that might be used to aid in data interpretation as well as to describe to others for potential revisits how to get to your site and where you sampled (Figure 7). There is space on the field sheet where this map can be drawn.

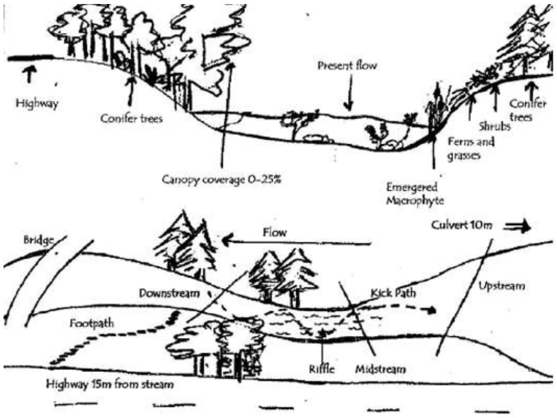

Present flow
Highway
Conifer trees
Conifer trees
Shrubs
Ferns and grasses
Canopy coverage 0–25%
Emergered Macrophyte
Bridge
Flow
Culvert 10m
Downstream
Kick Path
Upstream
Footpath
Riffle
Midstream
Highway 15m from stream

Figure 8 - Diagram of site map and site characteristics

3.5 Reach Characteristics

3.5.1 Habitat

There are two descriptions of habitat that can be recorded on the field sheets: habitat present at the site (6x the bankfull width) and habitat sampled during the kick net sample collection. The habitat to be sampled is usually a riffle or straight run. These are usually the most common habitat types. In some cases, other habitat types may be more common in a particular region and may be the focus of the regional reference database. This should be discussed with your regional EC CABIN team member or Ministry of Environment regional biologist. The habitats types present at a site (Figure 8) provide a description of the stream and provide an indication of what type of organisms may be expected. Aim to sample the dominant habitat type in the watershed/region, i.e. typically riffles or straight runs.

Method: This is a simple categorical description of whether the area sampled is predominantly riffle/rapids, a straight run, or a pool/back eddy (Figure 8). Simply checkmark the appropriate description for the sampling site as well as the actual kick net sampling location.

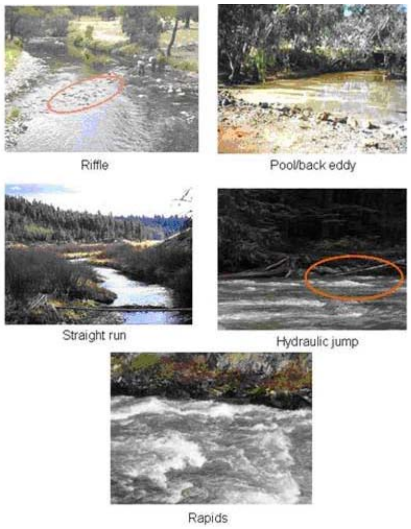

Riffle
Pool/back eddy
Straight run
Hydraulic jump
Rapids

Figure 9 - Habitat types found in streams

3.5.2 Canopy coverage

The degree of canopy coverage is important for two reasons. First, canopy cover provides shade and thus prevents the stream from overheating in the summer, effecting the degree of both temperature and oxygen stress. Second, extensive canopy can determine the relative amount of external versus internal plant material in the stream and the types of organisms present.

Method: This is a simple approximation of the percentage of the stream (i.e. from bankfull width) covered by the tree canopy. Stand in the middle of the stream and estimate the percent shading of the stream channel provided by overhanging vegetation (Figure 9). It is advisable to get all the individuals present at the site to estimate the coverage and then take the average value. This is usually not too difficult because the CABIN protocols requires only a broad categorical range such as 0%, 1-25%, 26-50%, 51-75% and 76-100%.

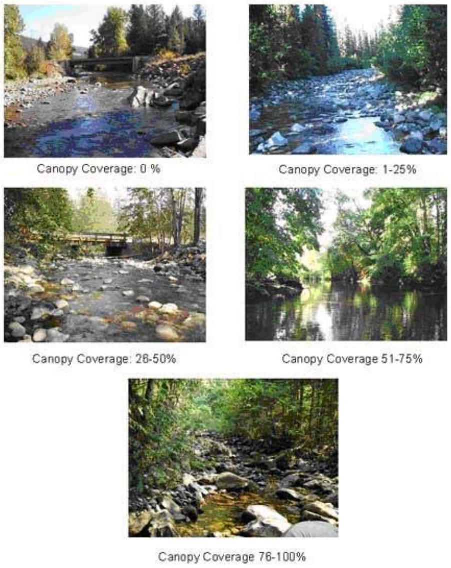

Canopy Coverage: 0 %
Canopy Coverage: 1-25%
Canopy Coverage: 26-50%
Canopy Coverage 51-75%
Canopy Coverage 76-100%

Figure 10 - Canopy cover estimates

3.5.3 Macrophyte coverage

Macrophyte coverage refers to the quantity of aquatic plants or vegetation that is present at the sample site. There are three types of macrophytes; submergent (e.g. oxygen weed), emergent (e.g. water cress, bulrushes, reeds), and floating (e.g. water lilies, duck weed) (Figure 10). Many organisms are adapted to specifically living among emergent plants, which tend to be associated with slower flow conditions and higher nutrient levels. Macrophytes also determine the form of material available to herbivores in the invertebrate community. Algal cover is not included in your estimates of macrophyte coverage.

Method: This is an approximation of the amount of the stream bed that is covered by macrophyte vegetation (Figure 11). Simply circle the approximate degree of the wetted channel covered by aquatic plants.

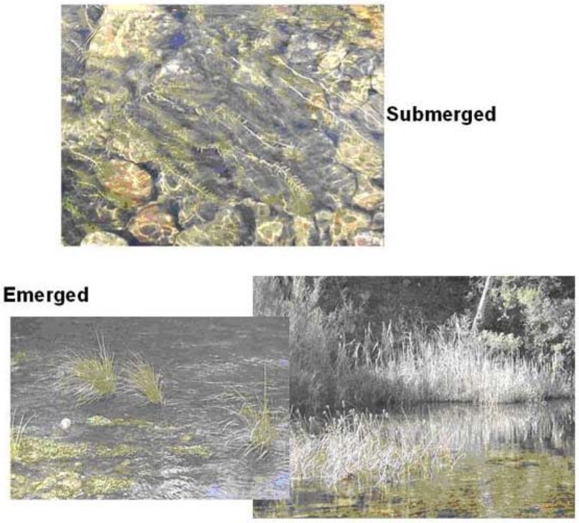


Figure 11 - Emergent and submerged macrophytes found in streams

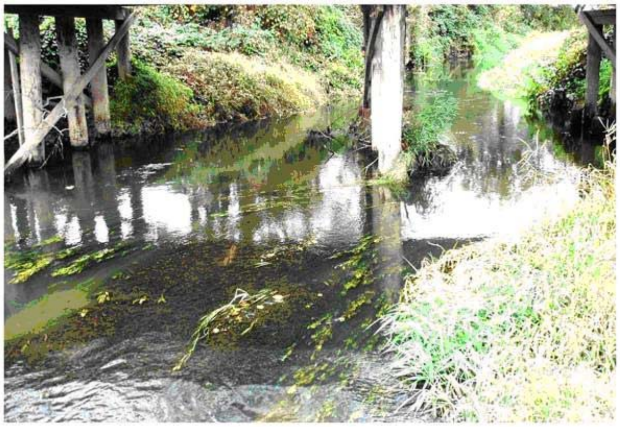


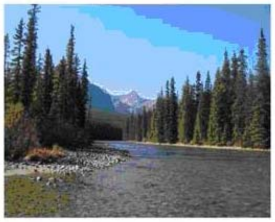
Figure 12 - Example of macrophyte coverage 26-50%

3.5.4 Riparian vegetation

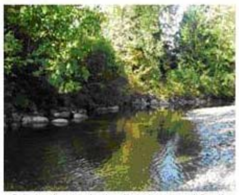
The terrestrial zone, which may or may not be forested, along rivers, streams, and lakes is known as the "riparian zone". Riparian zones are areas of transition between aquatic and terrestrial ecosystems. Riparian vegetation bordering a stream protects the water from disturbance, acts as a buffer between the stream and general activities in the watershed, and protects the banks from erosion. In describing the riparian zone you are interested in the vegetation that can influence the stream either by providing nutrients in the form of leaves or needles or by drawing water from the channel for growth. The CABIN protocols for describing the riparian vegetation are very general and focused on the quality of nutrients from the allochthonous material (e.g. coniferous needles are less nutritious than leaves, and grasses can consume a lot of oxygen from the stream).

Method: This describes the vegetation found in the riparian zone along the stream sampling site. Check if any of the four vegetation types is present: ferns/grasses, shrubs, deciduous trees, coniferous trees (Figure 12). There are four distinct categories: coniferous tress, deciduous trees, shrubs, grasses/ferns. A separate row of boxes is provided on the field sheet to note the Dominant Riparian Vegetation as opposed to all those that are present.

Grasses/ferns, shrubs, Deciduous trees



Conifers, shrubs and grasses



Grasses/ferns, shrubs, Deciduous and Conifer trees.

Figure 13 - Examples of riparian vegetation types

3.5.5 Periphyton Coverage

Periphyton is a complex mixture of algae, cyanobacteria, heterotrophic microbes, and detritus that is attached to submerged surfaces in most aquatic ecosystems. It is composed of primary producers and acts as an important food source for other aquatic organisms, including benthic macroinvertebrates and fish. These organisms also stabilize substrata and serve as habitat for many other organisms.

Method: Five descriptive categories are used on the CABIN field sheet to describe periphyton coverage found at each site and approximate thickness, modified from Stevenson, J. and L.L. Bahls (2007).

3.6 Water Quality Samples

Dissolved materials in the water are determined by erosional processes in the catchment, underlying bedrock, and surficial geology. Measurement of several key variables that either directly or indirectly affect the invertebrates can provide a great amount of information about the types of pollutants present, and their impact on a stream. Specific activities produce specific pollutants; by identifying specific pollutants we can identify activities that may be having an impact on the stream. For example, nutrients are likely to come from agricultural activities such as animal feedlots, grazing land, and runoff from fertilized cropland but could also come from land use changes, such as forestry or construction. Wastewater treatment plants are also key sources of nutrients and pollution.

Other parameters must be obtained by taking water samples and having them analyzed at a laboratory. To collect water samples, pre-washed and ready-to-sample bottles are usually supplied by the analytical laboratory you have chosen to analyze them. The lab will analyze certain parameters from each specific bottle so it is important to acquire your bottle sets from the lab rather than using your own. This is also necessary to help ensure your water samples are not contaminated.

3.6.1 What samples need to be measured by a lab?

- Nutrients including phosphorus (measured as total unfiltered phosphorus) and nitrogen;
- Alkalinity;
- Major ions (e.g. Ca, Mg, Na, K);
- If you do not have access to field probes to measure basic parameters such as conductivity and pH, the lab can do this for you also although the field readings are often better;
- Metals may also be measured in a lab (optional).

3.6.2 What are the specific handling restrictions of samples?

Some parameters have specific restrictions for handling time and temperature of samples. Most samples have a 72 hr maximum handling time and should be turned over to the lab for analysis within this time period. All should be kept in the dark at 4 °C to prevent any change due to bacterial growth.

Note: In general, water quality conditions are not the lone predictors for the benthic community, but they are used to aid in the interpretation of results. It is useful to have as much water quality data available should anomalies within the data set occur.

3.6.2.1 Collect, preserve and label samples

Before taking water samples you should consult your lab for proper handling procedures. The lab will also indicate to you whether you will need to add preservative, such as sulphuric acid, to your nutrient bottle while in the field. Depending on the lab, some bottles may come with preservative already in the bottle in which case sampling should be done carefully and with gloves.

Label each bottle with proper, legible labels, with the appropriate 'Site Code', and date using a water- and solvent-proof marker. Samples should be collected from flowing water in the middle of the stream or in the vicinity of the benthic invertebrate sample. At each site a sample will be taken for alkalinity, major ions and nutrients. Care should be taken not to touch the mouth of the bottles or the inside of the caps/lids (Figure 13). Bottles should be filled 10 cm below the surface of the water leaving a slight air space at the top. Keep your samples cool (ideally at 4 °C), in the dark, and submit them to the lab as soon as possible.

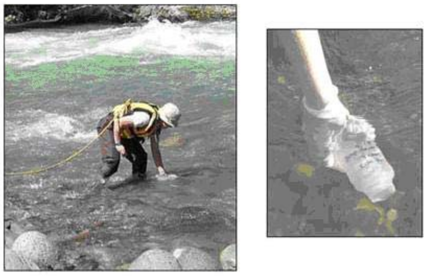


Figure 14 - Safe and proper water sampling technique

3.6.2.2 Record water quality measurements on site

The following water quality parameters can be measured on site using individual field sensors or a multi-parameter field probe *in-situ* (measured in the stream rather than back at the lab from a sample bottle) (Figure 14). Below (Table 2) are descriptions of each parameter explaining why they provide important data for your CABIN site.

Figure 15 – Example of field crew collecting water quality information with a multi-probe (wearing appropriate personal protective equipment)

Variable	**Description**
Temperature (ºC)	Temperature is a key physical variable that directly affects many of the physical, biological and chemical factors influencing aquatic organisms. If temperatures are outside the range of tolerance for organisms for extended periods of time they can become stressed and die, resulting in a change in the types of organisms inhabiting the stream. Temperature can be modified by various factors such as: weather, removal of riparian vegetation, turbidity, dams, etc.
pH	The relative acidity of water is ranked on a pH scale (percent hydrogen) of 0 – 14. A pH of 0 is strongly acidic while 14 is strongly basic (alkaline). Pure water has a pH of 7 (neutral). The pH scale is logarithmic, thus for every change in 1 unit there is a 10 fold change in acidity. A stream with a pH of 6 is 100 times more acidic than one with a pH of 8. Water with pH 6.5 to 8.5 is suitable for the greatest diversity of aquatic organisms. Young fish and aquatic insects are especially sensitive to extreme pH values outside the optimum range. Stream pH is usually determined by the surrounding geological makeup, but acid rain, wastewater discharges, and drainage from coniferous forests (acidic) can contribute to low stream pH.
Conductivity (µS/cm)	Conductivity is the ability of a solution to carry an electrical current, which is dependent on the total concentration of ionized substances dissolved in the water – measured in microsiemens/centimeter. For example pure water has a conductivity of 0. Conductivity is affected by temperature so many probes provide the option of recording Specific Conductance. This parameter is adjusted to a temperature of 25 °C so that no matter what the temperature was at any site, specific conductance can be compared. Conductivity is a useful tracer of point source discharges and sudden increases along a stream can indicate a pollution source.
Dissolved Oxygen (DO) mg/L or % saturation	The amount of oxygen dissolved (DO) in water effects which kind of organisms are found there. Water with higher concentrations of DO are generally considered to be higher quality and support many types of animals. Natural occurrences of low DO (hypoxia) can occur and many of the animals considered pollution-tolerant have naturally evolved to live in these types of environments (e.g., bottom waters of productive lakes). Low DO concentrations can create unfavourable conditions for many organisms and can change population structure. Under conditions of extremely low DO, organisms that require high oxygen levels (e.g. mayflies, stoneflies) will emigrate or die leaving other organisms that can tolerate low oxygen (e.g. midges, worms). Low DO in streams can be caused by several factors. Temperature is a major influence as cold water can contain more oxygen. Streams with low flow in the summer when air temperature is high are subject to reduced DO levels. Slow moving water has less natural aeration. Organic wastes such as agricultural runoff and sewage discharges

	reduce oxygen because of bacterial decomposition of organic matter. Aquatic plants and algae replenish oxygen during daylight but consume oxygen during the night.
Turbidity (NTU)	Turbidity refers to the cloudiness of water due to the presence of suspended solids such as silt, clay, living organisms, and organic matter. It is the measure of the extent to which light penetration in water is reduced from suspended materials. The more turbid the water, the murkier it is. Turbid waters become warmer as suspended particles absorb heat from sunlight, causing oxygen levels to fall (Warm water holds less oxygen than cooler water). Photosynthesis decreases with less light, resulting in even lower oxygen levels. Suspended solids in turbid water can clog fish gills, reduce growth rates, decrease resistance to disease, and prevent egg and larval development. Settled particles smother eggs of fish and aquatic insects. Higher turbidity levels are also often associated with higher levels of disease-causing micro-organisms such as viruses, parasites and some bacteria.

Table 2 – Description of *in-situ* variables collected using a multi-probe

Method: Methods for measuring these parameters in the field will vary depending on the instrument being used. Most field probes need to be calibrated before use. When using any instrument be sure to follow the manufacturer's instructions for care, calibration, and use. Here are some further tips for when you are recording the data:

- Always allow time for the reading to stabilize, especially for DO, turbidity, and temperature.
- For conductivity and pH only, a reading can be taken in a water sample collected in a clean container from the main flow.
- DO may vary significantly along the length of a river. In riffle areas where re-aeration occurs, DO will be higher than in slow moving areas and pools. Therefore, it is important that DO readings be taken in a similar habitat at each site.
- Where possible, express DO results both as concentration (mg/L) and percent saturation as a function of temperature (Figure 15).
- Turbidity must be measured from a water sample sent to the laboratory if you do not have a meter to use in the field.

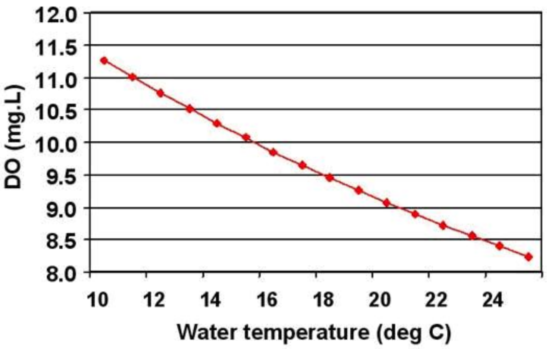


Figure 16 - Maximum dissolved oxygen (DO) concentration at specific water temperatures

3.7 Benthic Invertebrate Sampling

What is a kick net?

CABIN uses a kick net sampling method standardized by sampling effort (i.e. time – 3 minutes). The kick net is a triangular metal frame holding a mesh bag of 400 µm size (Figure 16) with a collection cup at the end. One end of the metal frame is attached to a rake handle. The part of the bag that attaches to the frame is made of canvas or ripstop-plastic tarpaulin to withstand abrasion. A detachable cup can be added to the end of the bag to facilitate removal of the sample. A 400 µm mesh net is recommended for general sampling.

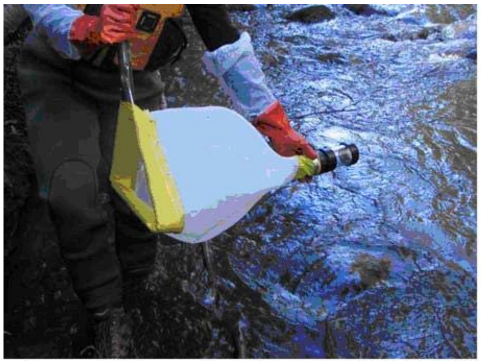


Figure 17 - Triangular kick net with 400 µm mesh net and detachable collection cup

3.7.1 Sample using a kick net

The kick net is placed downstream of the collector, flat side of the triangle resting on the substrate of the stream. The sampler walks backward in the upstream direction, dragging the net along the bottom of the stream as the sampler walks, kicking the substrate to disturb it to a depth of ~5-10 cm if possible. For large cobble, often only the surface can be disturbed and the cobble are turned over to dislodge any invertebrates clinging to the interstitial spaces. For large boulders, the net is held downstream while the boulder is brushed by hand. The net is held near to the area being disturbed so the current will carry dislodged animals into it. The net should be held close to the sampler’s feet at all times to ensure that most of the disturbed sediment, substrate and organisms are swept into the net rather than around the net.

The sampler continuously zigzags over the stream bottom from bank to bank in an upstream direction for a timed period of **3 minutes**. The standard collection time allows for comparisons among sites based on standardized effort rather than standardized area as with other sampling devices. The zigzag coverage allows for an integrated collection of invertebrates from a variety of stream microhabitats (areas around large boulders, riffle, runs, and bank overhang) (Figure 17). The timed kick allows for the collection of invertebrates from microhabitats in proportion to their occurrence at a site which is important when streams of various sizes are sampled for one study. It is important that sampling be extended directly adjacent to the stream bank because this region may have aquatic macrophytes that support a unique fauna but it is also important to ensure that the water level hasn’t recently risen so that you are sampling an area that was dry the day before and may not likely have many invertebrate living there. If the sampler needs to stop to get around a difficult obstruction, the stopwatch is paused until the sampler is ready to continue.

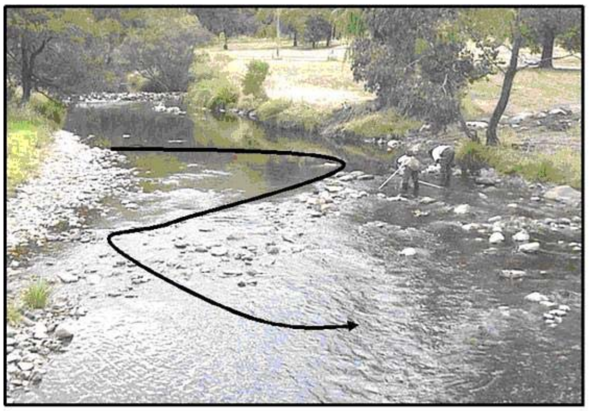


Figure 18 - Kick net transect through a riffle

3.7.1.1 When sampling is completed

When sampling is completed, the cup is removed and its contents are emptied into a 250 µm mesh sieve. Material remaining in the cup can be washed into the sieve by spraying the outside of the mesh with water from a squeeze bottle. The net and cup should be checked for remaining invertebrates. Sample material can then easily be transferred from the sieve to a wide-mouth plastic sample jar using a squeeze bottle. An extra sample jar lid containing a hole which has been covered with a fine mesh will allow extra water that has entered the sample jar to be drained.

At some sites, large amounts of debris and sand are collected and would fill many jars if not "cleaned" on site with elutriation. As a rule of thumb, if your sample is going to fill more than three 500 mL plastic jars then you will want to elutriate your sample of the excess debris (Figure 18). In most cobble streams, this procedure is not required. See the instructions for "Bucket Swirling and Sieving" (section 3.7.1.2).

A label (waterproof paper marked by soft pencil) is added to the inside of the jar accurately describing the:

- Sampling location (site code);
- Date;
- Sample jar number if more than one was needed;
- Sampler's name or initials.

The outside and lid of the container should be similarly labeled using a waterproof felt pen. Be sure to leave enough room in the sample jar to add ethanol to the sample. Be careful not to spill the ethanol as much as possible as ethanol will dissolve waterproof felt pen markings (i.e. sample jar labeling).

Remember that often the sample collected will require more than one jar. Write the number on the labels (i.e. 1 of 3, 2 of 3 and 3 of 3).

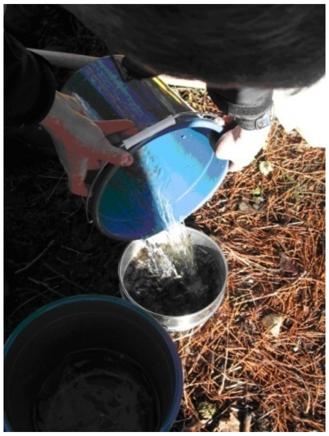


Figure 19 – Elutriation using several buckets

3.7.1.2 Bucket Swirling and Sieving to remove excess debris (only used in special situations)

If, after removing the sample from the net, there is a large amount of inorganic matter (sand/gravel) in the sample, this material should be removed in the field as described by Needham and Needham (1962). This procedure has been shown to reduce sorting time by as much as 50% (Rosillon 1987, Ciboroski 1991). Furthermore, this elutriation process minimizes the adverse effects that large amounts of inorganic matter placed in the sample jar can have on invertebrates (damaged specimens are more difficult to identify), and increases laboratory subsampling efficiency (easier distribution on sieves, trays, cones etc.).

Method:

1. A bucket that is 30-40 cm in height, 20-30 cm width, circular, has a handle and or a spout to pour off the sample is required. It should be light weight, compact and light coloured as it needs to be transported to the field; small waste baskets work well.
2. Place the entire sample in the circular bucket with 10-20 cm of stream water to a depth of less than half the depth of the container.
3. Wash the macroinvertebrates from large stones and pebbles into the water in the bucket, discard.
4. Swirl the water in bucket to achieve a vortex. The organisms and fine particulate matter being lighter than the inorganic sand and gravel will float up and can be poured off with the water into a sieve. This sieve should be no greater than the mesh size of the net but can be smaller (i.e., 250 µm) especially as the “effective” mesh size is usually less than the net mesh size
5. Repeat this process of adding water (a second bucket can be useful to add stream water to the first) and swirling until there is no organic matter rinsing out of the sand/gravel. Gently swirling the sand/gravel with your hand may also help to suspend organic matter and organisms into the water.
6. If there is an excessive amount of inorganic material this process can be performed in batches as there is an optimal amount of inorganic material desired in the bucket.
7. Although a small amount of sand may end up in the sieve, try to swirl so that the sand and gravel remain in the bucket and minimize any amount poured into the sieve.

8. Before discarding any of the inorganic sample portions, a thorough visual inspection should be conducted to detect any invertebrates missed (note: elmid beetles tend to remain in the inorganic portion of the sample so watch very carefully for them), especially cased or shelled invertebrates (e.g., rock encased caddisflies) that may be retained in the inorganic portion. This can easily be done if the bucket has a white bottom or the material can also be poured in to a white flat bottom tray for inspection.
9. Finally, it is recommended that if this is a field protocol utilized for a study, a subset (1 in 10 samples) of the gravel be retained in a separate jar, preserved as per a regular sample. This QA/QC component will be examined under a microscope in the laboratory.
10. Indicate on your field sheet if this method was used at the site and if the remaining debris was kept for QA/QC purposes.

3.7.2 Preserve the sample

The sample is preserved with 95% ethanol. This kills the organism quickly, fixing the tissue of soft bodied organisms so the body remains firm for identification and does not decompose. Additionally, unlike other fixatives, ethanol will not degrade taxa with calcified shells or exoskeletons, and preserves some colour. These benefits make sorting and identification more accurate, and allow sample collections to be kept for longer periods.

3.7.3 Summary of the Benthic Macroinvertebrate Sampling Procedure

- The kicking area should be defined with the field crew so everyone knows what area of the stream not to disturb until the sample has been taken.
- Kicking must start at the downstream end of the defined area.
- The net should be placed firmly on the substrate as close to the stream border as is possible and applicable (i.e. not in a previously dry area) as you step into the stream.
- Sampling begins by kicking and twisting your feet through the substrate, rolling over rocks and stones keeping the net close to your feet at all times. As you disturb the substrate, the loosened organisms and debris should be carried into the net with the current. Be careful not to let the disturbed material flow past the net. In slow moving streams you will need to move slower to ensure that all your disturbed material is caught within the net. The kicking style of different samplers will vary. Some people like to shuffle and others like to toe kick. Either method will work. The idea is to disturb the substrate.
- Large rocks or those deeply embedded should be rubbed using your hand to loosen the organisms. Note: the net should always be in contact with the substrate and should always be directly downstream of the person kicking.
- The area to be sampled should be traversed in a zigzag pattern going from bank to bank and always working in an upstream direction. In large rivers or very fast flowing streams, bank to bank sampling may not be possible. In these cases the same procedure is followed, zigzagging through the wadeable area that has been defined for sampling.
- At the end of **3 minutes**, the net should be quickly lifted from the current. The sample is examined for large rocks and sticks than can be washed and removed. They

should be rinsed carefully in the net, checking for organisms, and removed so they do not fill your sample container.

- If you have a large sample with a lot of debris, you may need to elutriate your sample before putting it into the sample container. Refer to 3.7.1.2.
- The sample is then washed into a sieve with a squeeze bottle and the net checked carefully for organisms clinging to it. The net may need to be rinsed several times with the squeeze bottle or in the stream to remove all of the organisms. The sample is then transferred into a sample bottle and the excess water removed from the bottle.
- Note: Seams and folds should be checked carefully for hidden organisms.
- Water is then drained from the sample and 95% ethanol is then added to preserve the sample.
- Containers are labeled outside and a label is also placed inside each sample. If there are multiple containers, be sure to indicate how many containers for each sample (i.e. 1 of 3, 2 of 3, etc).
- Records of the person doing the kicking and the typical depth of the kicked area are noted on the field sheets, as well as the number of sample jars required to contain the sample and the sampling time.

3.8 Substrate Characteristics

The composition of the stream bed material is important in identifying hydrological characteristics of the river and the type of habitat available to aquatic organisms. Insects need to attach themselves to the stream bottom or live within the bed materials. The more attachment or living spaces available the greater will be the variety and number of organisms found. Optimally, the stream bed of riffle habitats will be dominated by cobbles, gravel and boulders. As the percentage of sand and silt increases, the suitability and availability of living space for invertebrates decreases.

To sample the surface particle size distribution, a random pebble count is conducted measuring the median diameter of the substrate. This information can be very important for describing the power of the river or stream. This variable describes the particles that are moved down the river by the force of the water at flood periods.

In streams, Nielsen et al. (1983) describe the use of a substrate score that is determined by the sizes of the two predominant substrates, the size of the material surrounding the predominant substrates, and the degree of embeddedness. In undisturbed streams, fine sediments (< 2mm) do not accumulate in large quantities on gravel and cobble in riffles. In areas modified by agriculture or other stream side activity, increased erosion results in accumulation of fine material in the interstitial spaces and fills the spaces utilized by clinging organisms. Embedded riffle substrates provide less desirable habitat for invertebrates and reduces productivity.

3.8.1 Determine the substrate composition using a 100-pebble count

In the random 100 pebble count, the intermediate axis of the substrate is measured, which is not the largest axis but is the axis on which the rock will roll down the river. This information is one of many variables collected by stream surveyors when they need to estimated the discharge of a stream.

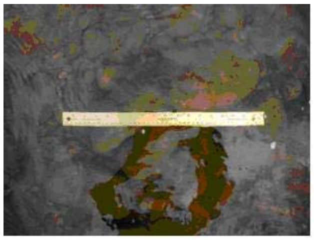


Figure 20 – Photographing representative substrate size with the stream reach

Method: With a metal ruler in hand, zig-zag through the sampling area and stops every two steps or so. Lean your index finger on the edge of your foot pointing down and touch the stream substrate without looking. Pull out the material that the tip of your finger is touching and measure its intermediate axis (Figure 20 axis b) in millimeters. This is the diameter perpendicular to the longest axis (Figure 20 axis a). If the item cannot be pulled out then measure it in the water. Be careful not to bias the substrate to the largest pebble that you touch.

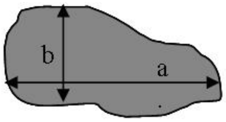


Figure 21 - The intermediate axes of a substrate particle

The Wolman D50 (i.e. median diameter), Wolman Dg (i.e. geometric mean diameter) and the % composition of the Wentworth substrate classes will be calculated automatically in the CABIN database using the 100 pebble data that is entered.

3.8.2 Determine the substrate embeddedness category

The embeddedness category refers to how deeply the dominant substrate is buried in the surrounding material (i.e. sand and gravel). The more embedded the substrate is, the fewer interstitial spaces there are for invertebrates to live in. Embeddedness is often confused with compactedness. Compactedness is how difficult it is to remove the substrate from the surrounding material. It is possible that the substrate will be very compacted but only ¼ embedded. Be sure not to confuse the two. Compactedness is not recorded as a standard variable for the CABIN protocols.

Method: This can be done at the same time as the 100 pebble count. Wade into the middle of the area you sampled for invertebrates and pick up at least five pieces of large pebble or cobble, avoiding rocks disturbed by sampling. Estimate the percentage depth of rock buried in the fine material (Figure 21). A stain line on the rock may indicate the level of burial and aid in the estimation. Record the average degree of embeddedness.

NOTE: This is NOT compactedness (how loose the substrate is).

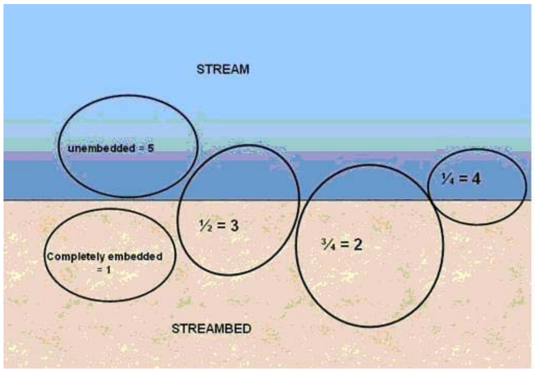


Figure 22 - Scoring categories of substrate embeddedness

3.9 Channel Measurements

Characteristics of the channel in a stream reach often determine the abundance and distribution of benthic macroinvertebrates, so it is important to describe these attributes. The dimensions and shape of the channel and the substrate paving the bottom of the channel, a factor of critical importance to the benthos, are a result of the geology of the area and peak flows. Peak flows occur, on average, in two out of three years, are called "bankfull discharge" and are related to flash floods caused by snow melt or summer rain storms. Erodible materials are carried through the stream reach, shape the dimensions of the channel (width, depth), and leave behind substrate material that the stream does not have enough energy to transport. The substrate material is crucial to the development of benthic macroinvertebrate communities. It is possible to relate faunal distributions with a particular suite of hydrological variables by measuring channel characteristics (e.g. Cobb et al. 1992).

3.9.1 Bankfull Width, Wetted Stream Width, and Bankfull-Wetted Depth

As flow decreases, water will cover less of the stream bottom, which will limit the available habitat for aquatic organisms. As flow increases, it erodes the banks of the stream and carries erodible materials down the stream and deposits them when the flow decreases again providing habitat for aquatic organisms. Two measures of stream width are made, **bankfull width** to represent high flow conditions and **wetted stream width** to represent low flow conditions at the time of sampling. A third measurement, for bankfull-wetted depth, is also made to determine the height from the water surface to bankfull. This measurement, added to the wetted depths at each point in the transect, provides a user with the tools necessary to calculate bankfull discharge.

1. A transect is established at right angles to the flow.
2. A tape measure is stretched across the stream and secured to each bank.
3. Width of the present (wetted) and bankfull flows are measured (Figure 22).
4. Measure bankfull-wetted depth by measuring the height of the tape at bankfull width from the water surface. Be sure the bankfull measurement (e.g. the tape) is level to the water surface. The bankfull-wetted depth measurements should be the same across the stream.
5. Bankfull flows are usually short in duration and seldom observed; however, they can be determined by locating points of vegetation change on the stream banks, where

algae or marl have been scoured from boulders, or where sediment texture abruptly changes.

6. Three cross sections may be taken to estimate the average channel width at the site but is not necessary. For the purposes of CABIN and the predictive model, one cross section is sufficient. The increased precision will not generally change the model predictions.

Note: Detailed determination of bankfull dimensions is described in Newbury and Gaboury (1993) and Harrelson et al. (1994).

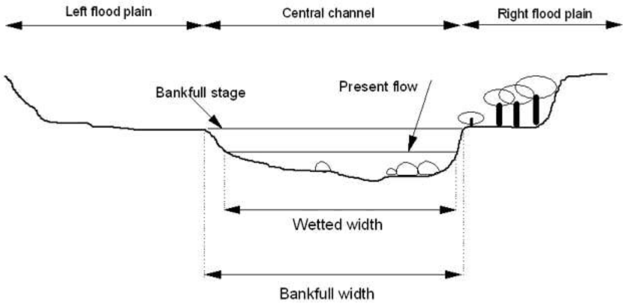


Figure 23 - Various channel attributes (adapted from Newbury and Gaboury 1993).

3.9.2 Velocity and Depth of Stream

It is important to obtain a measurement of the velocity and depth from the area from which the benthic sample was taken, as this represents the conditions to which the invertebrates are exposed. Often the length of the site is quite large. Therefore, determining where the sampling will take place is an important first step. It needs to be representative for both the riffle or run habitat and representative for the cross sectional measurements such as width, depth and velocity. As best as possible, try to do the cross sectional measurements at the location where the benthic sample was taken as these measurements are to represent both the site as well as the conditions where the invertebrates were collected.

3.9.2.1 Method A: Velocity Meter

The area travelled during the kick sample should have been noted. If a velocity meter (e.g., Sontek, Price meter) is available, the stream flow should be measured at 3-5 points across the channel following the procedures outlined in the operation of these meters. In smaller streams (5 m or less width) this should be done at ¼, ½ and ¾ of the way across the stream. In larger streams (> 5m) we would recommend five equidistant measurement points across the stream (Figure 23). It may not be possible to get measurements at the exact equidistant point due to the presence of a large boulder which creates an eddy. Move your point slightly to the left or right of this obstruction. The goal here is not to accurately measure discharge but to get an average of the current conditions to which the invertebrates are exposed. One cross-section may be adequate to get an average velocity and depth at the site if the reach is relatively simple and uniform, however, three cross-sections are advisable if the channel has variable form (e.g. meander bends, point bars, cutbanks). The area where the kick sample was taken is the most important cross section.

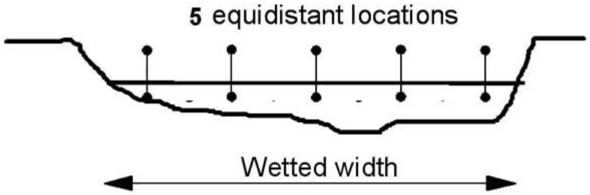


Figure 24 - Velocity and depth cross-sectional profile

3.9.2.2 Method B: Velocity Head Rod (if a velocity meter is unavailable)

If a velocity meter is unavailable, a simpler alternative is to indirectly estimate surface-water velocity using a metal ruler and following the Velocity Head Rod method. Place a metal ruler or meter stick vertically in the stream with the narrow edge in line with the oncoming flow of water and measure the water level (e.g. the depth of flowing water). With the narrow edge facing the flow there is little effect on the flow. Then, turn the ruler so the flat face is against the flow. You'll see that the water is superelevated (e.g. piles up) on the upstream side causing the depth to increase at the point of zero velocity (stagnation zone). This superelevated water mass is called the "velocity head". Record the level of the flowing water with the flat face facing the flow (e.g. the depth of stagnation zone). The difference between the flowing water depth and the depth of the 'stagnation zone' is the head (h or ΔD). These measurements should be made at 5 locations across the stream. The distance from shore of each measurement is also recorded on the field sheet.

Velocity is then calculated using the following equation:

Velocity (m/s) = SQRT (2(ΔD)*9.81)
or
Velocity (m/s) = (2gh)0.5

Note: ΔD and h are expressed in meters and g (gravity) = 9.81 m/s2

3.9.3 Slope

Slope represents the stream gradient, which in turn influences sediment transport and discharge characteristics. Channel slope is the difference in elevation at the upstream and downstream ends of a stream segment, divided by the length of that segment. We suggest two methods for acquiring slope for your CABIN sites.

3.9.3.1 Method A

Use the following steps to determine slope from a topographic map modified from: http://geology.isu.edu/geostac/Field_Exercise/topomaps/slope_calc.htm

1. Find the area of your site on the map, draw a straight line perpendicular to the contours on the slope. For the best accuracy, start and end your line on, rather than between, contours on the map.

2. Measure the length of the line you drew and, using the scale of the map, convert that distance to the appropriate units of the map you are using (e.g. meters or feet).

3. Determine the total elevation change along the line you drew (subtract the elevation of the lowest contour used from the elevation of the highest contour used). You do not need to do any conversions on this measurement, as it is a real-world elevation change.

4. To calculate a percent slope, simply divide the elevation change by the distance of the line you drew. Multiply the resulting number by 100 to get a percentage value equal to the percent slope of the hill. If the value you calculate is, for example, 20, then what this means is that for every 100 feet or meters you cover in a horizontal direction, you will gain (or lose) 20 feet or meters in elevation.

3.9.3.2 Method B:

For high gradient streams (steep slopes), slope can be measured with a hand level (expressed as a percentage). It is only accurate to 1% or 0.01m/m so it is not recommended for typical low gradient streams. Usually surveyor's equipment such as a transit, tripod and level, or total station is used to measure slope in the field. Slope is entered into CABIN as a ratio of the height in metres over the distance in metres and is usually quite low (i.e. <0.01). Channel slope can also be measured using 1:50 000 scale topographic maps for large stream segments which will be much larger than you defined sampling reach but will be representative of the slope of the upstream drainage basin.

3.10 Quality Assurance and Control in Sampling

Quality Assurance and Control (QA/QC) is an ongoing process which has the goal of prevention, early detection, and correction of field and analytical data collection errors.

The first step to ensuring quality data is training the participants (Culp et al. 1999). This is the reason for this online CABIN program in combination with the in-person certification workshop. Regional Environment Canada staff and the Canadian Rivers Institute will provide the in-person training to participants who have completed online modules. Participants will then receive CABIN certification after demonstrating their knowledge and understanding of the field procedures.

A coordinator/project manager should be identified who will ensure that field crews are trained and who will also coordinate the management of the data. Only trained participants will receive a username and password to enter data into the CABIN database.

All members of the field crew must ensure all data sheets are filled in correctly and completely before leaving the site.

All members of the field crew must determine if the data are reasonable before they leave the field, and if not, the measurements should be repeated before leaving. This may require taking a calculator to determine if some of your measurements seem reasonable.

When planning a CABIN study it is required that QA/QC is completed on 10% of all samples taken using the CABIN protocol. This means that at every tenth site sampled in the entire process should be repeated in triplicate within the site to provide some assurance of the spatial variability associated with the benthic community and the habitat measurements. There are two assessments of variation that can be examined at a site.

1. The variation in the measurement of habitat variables due to operator variability where the habitat measurements would be measured at the same spot three times (this cannot be done for invertebrate sampling since the riffle community has already been disturbed and a sample would have to be taken upstream of the disturbance). This provides a measure of within-site variability.
2. The variation in sampling location, wherein three riffles are evaluated in a study reach, starting at the downstream end. The spatial variation of both the benthic community and the corresponding habitat can be measured to provide an estimate of within-reach variability. The three kick net samples are bagged separately and labeled as replicate samples for the given site. If there is little spatial variation at a test site, the CABIN assessments will not differ among the three samples. If there is little spatial variation at a reference site, the samples will group together biologically and the habitat data will also provide similar results from the predictive model.

4.0 Post-sampling Procedures and Instructions for Taxonomic Analyses

4.1 Post-sampling procedures for sample inspection, storage, and preparation for laboratory processing

Just as the sampling process for any study is critical to the success of the research, so is the management and analysis of those samples once they arrive at the laboratory from the field. A carefully structured framework for managing both the samples themselves, as well as data generated from their analysis is required. Without such a framework, samples can be lost or unanalyzed and results can become misplaced or misconstrued. Guidelines have been established for sample and data management. These guidelines are broken down into sections below, each identifying the proper procedure(s) at each stage. Module 4 of the online training course goes into detail regarding taxonomic sample processing procedures, however, the standard CABIN procedures are also described in this section as they are important for data comparability and should be communicated to your taxonomist.

4.1.1 Create a project folder

Upon return from the field, a hard-copy Project Folder should be started by the project manager. The Project Folder acts as a data warehouse for original field sheets, analytical laboratory results, sampling permits and any other hard-copy information generated during a project, until their entry into the electronic data base (see the online Module 5 and/or Data Entry Module). Individuals responsible for specific results must ensure that their data sheets are placed in the Project Folder once analysis has been completed.

4.1.2 Benthic Macroinvertebrate sample preparation for shipping and storage

Invertebrate samples should be checked against the Project Folder log to ensure that no samples have been lost during delivery to the laboratory. Once all of the samples have been accounted for, they should be prepared for storage until processing can be done.

4.1.3 Distribute your samples

After project samples have been collected in the field, they should be inspected and distributed to the appropriate laboratories for analysis. The inspection of samples includes:

1. Entering each sample into the Project Folder log.
2. Checking against the project field sheets to ensure the sample ID and correct number of sample containers from all sampling locations are accounted for.
3. Identifying any samples which have been damaged/lost during transport from the field. Damaged/lost samples are noted, and a comment must be placed in the 'Comments' section of the CABIN database.
4. Benthic macroinvertebrate samples sent to a professional taxonomist for sample processing, sorting and identification are sent with a sample submission sheet listing the site code, sampling date and number of jars for each sample. Ensure all samples are clearly labeled with a waterproof label inside and permanent label on the outside of the container.

5. Water chemistry samples should be stored in the dark at 4°C until delivery to the laboratory for analysis. Samples should be listed on a sample submission sheet supplied by the analytical lab. Generally, major ions and nutrients have limited holding time, usually up to 72 hrs. Consult the analytical lab for required bottles, preservatives and holding times. Usually the analytical lab will supply the bottles necessary for the analysis and if samples will be analyzed within the holding time, preservatives are not necessary.

4.1.4 Benthic invertebrate sample processing and taxonomy

Typically, macroinvertebrate samples are sent to a professional taxonomist or taxonomy laboratory for analysis. It is important to choose a qualified taxonomy lab to ensure that the following sources of QA/QC errors are avoided:

- Sorting efficiency (S.E.)
- Identification error
- Labeling error
- Sub-sampling error
- Washing error
- Data entry error
- Calculation error

Therefore while choosing a taxonomy lab it is important to look for:

- Training and experience (NABS certification or regular attendance at taxonomy workshops)
- Adequate technical taxonomic literature/equipment
- Standard operating procedures
- Reference collection verified by experts
- Internal and external QA checks
- Interaction with recognized taxonomic experts

When delivering the sample to the taxonomist, CABIN protocols for laboratory procedures should be discussed with the taxonomist in order to ensure CABIN standard processing and sub-sampling procedures are used.

A check list of questions to ask the taxonomist and a list of the specific requirements needed for the sample should be brought to the taxonomists' attention. The following sections describe some key points to discuss with your taxonomist.

4.2 CABIN standard protocol for sample processing

The taxonomist must follow the CABIN protocols outlined in Rosenberg et al. (1999) and in the online CABIN modules to process samples. They are outlined here. This is to ensure that differences observed among sites are not attributed to differences in the way the samples were processed. This is an important component to a nationally consistent and standardized program.

- CABIN samples are subsampled using a Marchant Box subsampler (Marchant 1989).
- The taxonomist shall not use a sieve greater than 400 µm when transferring, washing or processing the samples.
- Samples must be processed and subsampled such that a count of 300 organisms is obtained. Cells/fractions that have been started must be finished. When subsampling, the cell or sample fraction where the taxonomist achieves the count of 300 must be completed before the subsampling is complete. For example, 15 out of 100 cells may be counted to achieve a final count of 323 organisms. If specimens are broken or damaged, only heads are counted. The entire sample is subsampled including the large (>2mm) fraction. CABIN does not separate out the large fraction (>2mm) as is required by some protocols to ensure that large rare organisms are not missed.
- The lab will report sorting efficiency by having someone other than the sorter examine the picked sample residue to determine the number of organisms missed. A minimum **sorting efficiency of 95%** must be achieved.
- During identification, each organism is identified to family level using the taxonomic key and tallies are recorded on the bench sheets. Organisms from the same families are kept in single vials in ethanol and labeled appropriately. Bench sheets are stored in the Project Folder until data entry takes place.
- Some taxa are not included in the CABIN subsample counts but are noted as 'present' (e.g. Porifera, Nematoda, Platyhelminthes, Ostracoda, Copepoda, and Cladocera). Refer to section 4.3 for a detailed list of what is not included and why.
- Tallies are transferred from bench sheets to the electronic database, and the bench sheets are placed in the Project Folder.
- Whenever possible, particularly for reference sites, the taxonomist will identify all 300+ organisms to the lowest possible level as specified in 'taxonomic effort' below. Due to early instars and the condition of the organism, higher level identification (i.e. family) may only be possible and this is acceptable.
- Voucher reference collections must be properly archived on a species per study basis. This means that of all the samples submitted to the taxonomist for a particular study, one vial would contain 1-3 specimens of a particular taxon from a study. An appropriate label must be placed inside the container or on the mounted slide. This is a standard requirement for any project. However, if you are unable to maintain a reference collection, speak with you regional Environment Canada CABIN team member or Ministry of Environment regional biologist to discuss other options.
- The taxonomist can enter the lowest level identification into the CABIN database using a CABIN username and password provided by Environment Canada. The trained CABIN participant will enter site codes in the CABIN database. Taxa not found in the CABIN database may be searched for and retrieved from the Integrated Taxonomic Information System (ITIS). Taxa from ITIS may be loaded directly into the CABIN database for use during data entry. To maintain quality control, taxa retrieved from ITIS (http://www.itis.gov/) require that a voucher specimen be sent to the national taxonomy lab for verification and inclusion in the national reference collection.
- All new taxa, not yet entered into the CABIN database must be verified by the Environment Canada taxonomist and submitted for the national reference collection for CABIN.

4.3 Taxonomic effort required for CABIN samples

CABIN analysis is currently performed on family-level data. Family-level is the minimum required level of identification for CABIN. However, when reference data is collected, we strongly recommend lower level identification for the future use of other potential projects such as those related to biodiversity assessments, development of diagnostic tools and possibly genus level predictive models.

Insects that need to be identified down to genus/species level where possible (listed by order):

- Coleoptera
- Diptera (Note: Chironomidae need to be slide mounted)
- Ephemeroptera
- Heteroptera
- Lepidoptera
- Megaloptera
- Odonata
- Plecoptera
- Trichoptera

Non insect taxa that should be identified to genus/species where possible:

- Chelicerata
- Crustacea (except classes not included in CABIN counts)
- Hydroida
- Branchiura
- Mollusca (identification should be done to a minimum of family and genus where possible)
- Annelida (leeches, polychaetes, and oligochaeta should be identified to a minimum of family level. Oligochaeta require slide mounts)
- Nemertea (to genus level)

Taxa **NOT** to be included in CABIN counts:

- Ostracoda (not adequately sampled with a 400 µm kick net)
- Cladocera (generally not benthic and can bias samples collected close to reservoirs)
- Copepoda (generally not benthic and can bias samples collected close to reservoirs)
- Porifera (not adequately sampled with a 400 µm kick net)
- Nemata (not adequately sampled with a 400 µm kick net)

- Platyhelminthes (not adequately sampled with a 400 µm kick net)
- Non-benthic taxa (terrestrial drop-ins)

4.4 Quality Assurance/ Quality Control (QA/QC) in the taxonomy laboratory

The taxonomist must be aware of the QA/QC protocols for the CABIN program and must be carried out on a regular basis, in order to establish a standard sorting efficiency for the sampling process.

Determination of sorting efficiency is established:

- The residue from every picked sample (enumerated cells only) is replaced in a sample container and stored. These containers should be labeled inside and out with sample # and a label identifying it as sorted.
- 20% of residue samples (minimum 3) are randomly selected from the total and re-picked; and the number of new organisms found is counted.
- The percentage of organisms missed (% OM) is calculated using the equation:

$$\frac{\#OrganismsMissed}{TotalOrganismsFound} * 100 = \%OM$$

- The average %OM is calculated based on the 3 samples re-picked, and represents the standard sorting efficiency for that project.
- Greater than or equal to 95% of the total organisms must be recovered for an acceptable sorting efficiency. If the sorting efficiency is < 95%, all the samples must be redone. The kinds of organisms missed should be brought to the attention of the person sorting and picking to rectify the problem.

Taxonomic error, which is the misidentification that results in the loss or addition of taxa, will be determined by:

- All of the first five samples all verified
- 2 of the next 10 samples are verified (randomly selected by project manager)
- 2 of every 30 samples are verified (randomly selected by project manager)

Two measurements of taxonomic error are calculated. The errors must not exceed 5% error rate at the family level of identification, or 10% at the genus level. Errors must be brought to the attention of the taxonomist to rectify the problem

$$\frac{\#TaxaErrors}{TotalOriginalTaxa} * 100 = \%TaxaError$$

$$\frac{\#IncorrectIdentification}{TotalOrganismsFound} * 100 = \%Misidentifications$$

4.5 NABS Taxonomic Certification

Although CABIN does not currently require use of a certified taxonomist it is strongly recommended to ensure consistency among taxonomists and the data provided to the national database for long term monitoring and assessment. The North American Benthological Society (NABS) is currently running a Taxonomic Certification Program at family and genus level. It has been operational since 2005 with most certification exams held in the USA and more exams being held in Canada recently. Local certified taxonomists and information on how to become certified in Canada can be found on the NABS website (http://www.benthos.org ; www.nabstcp.com).

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Further Reading

CABIN website: http://cabin.cciw.ca/

CABIN Staff Contacts: http://cabin.cciw.ca/Main/cabin_contact.asp

Transport of Dangerous Goods: http://www.tc.gc.ca/tdg/training/menu.htm

WHMIS: http://www.hc-sc.gc.ca/ewh-semt/occup-travail/whmis-simdut/index_e.html

WHMIS online training: http://www.yowcanada.com/

Know your watershed: http://map.ns.ec.gc.ca/kyw/

Background on Ecozones: http://www.ec.gc.ca/soer-ree/English/Framework/Nardesc/default.cfm

Water Quality: http://www.ec.gc.ca/water/en/manage/qual/e_qual.htm
http://www.env.gov.bc.ca/wat/wq/

Background on Water Quality parameters:
http://waterontheweb.org/under/waterquality/index.html

NABS: http://www.benthos.org

Integrated Taxonomic Information System (ITIS): http://www.itis.gov/