Freshwater Biological Sampling Manual

BC MINISTRY OF ENVIRONMENT, LANDS AND PARKS.
Water Management Branch

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BIOLOGICAL SAMPLING MANUAL

1. Introduction

This manual covers the minimum requirements to ensure quality and consistency of the field aspects of biological data collection. The essential tasks in biological sampling are to collect representative samples that meet the requirements of the program, and to prevent deterioration and contamination of the samples before analysis. The procedures outlined in this manual are oriented primarily towards BC Environment employees, consultants, or those under a legal requirement to undertake a sampling program for the Ministry. Following the protocols outlined in this manual will aid field staff in collecting reliable, representative samples.

The protocols presented here are the most acceptable ones used at present. It should be emphasized that in unusual circumstances or with development of new methods, experienced professional judgment is a necessary component of method choice and application. It is intended that this document will be updated as the need arises to incorporate new knowledge. For specialized sampling needs, considerable literature exists and should be consulted. This is particularly the case with benthic stream invertebrates.

The importance of entering standardized field data into a database (Environmental Monitoring System, EMS, for BC Environment) that is accessible to others, needs to be stressed. Field data become useful information when they have been collected following standard protocols and exist in a form that is easily retrieved for a variety of purposes.

This manual does not address the collection of samples for the purpose of providing legal evidence. For information regarding legal sampling refer to *Guidelines for the Collection and Analyses of Water and Wastewater Samples for Legal Evidence* (Lynch and van Aggelen, 1994).

This document also does not address project design (site locations, frequency of sampling, duration, quality assurance program, etc.) or data interpretation. These topics are the subject of a separate document.

The sample containers, preservatives and sampling procedures described in this manual reflect those generally used by BC Environment staff. Shipping procedures and safety measures are also outlined. Different agencies or laboratories may have specifications which differ from those described here.

It should be acknowledged that funding for the initial manuscript upon which this manual is based was provided by the Aquatic Inventory Task Force of the Resource Inventory Committee.

2. General Considerations

2.1 Preparing to Go to the Field

Preparation for each sampling trip is critical since oversights are not usually noticed until staff reach the first station. The most effective way to prepare for a sampling trip is with a **checklist** that is designed to meet the requirements of each project. Other than considering site-specific instructions, the checklist should identify the following needs:

- Type and number of (labeled) bottles, including extras
- Field equipment such as sediment grabs, invertebrate samplers, fish nets, tow nets, etc.
- Preservatives
- Appropriate quantity of ice packs and coolers
- Log books
- Personal gear (for all possible weather conditions such as survival suits, raincoats, protective footwear, etc.)
- First aid kit and other safety equipment (life jackets, survival suits)
- Camera or video equipment as required
- Laboratory requisition forms (partially filled out)

A general operating procedure is to have the essential equipment in a box or plastic "tote" which is dedicated to this activity. See Appendix 1 of this chapter for an example of a generic checklist.

2.2 Locating the Site in the Field

It is the responsibility of the field staff to locate all sampling stations accurately. Only if the same location is sampled consistently can temporal changes in the water quality be interpreted with confidence. Therefore, accurate station location descriptions (that identify key landmarks) must be prepared on the first visit to every sampling site. Good photographic documentation is the best way of ensuring that each site is easily recognized. It is essential that each site be referenced by watershed code. The *User's Guide to the British Columbia Watershed/Waterbody Identifier System, Version 2.1* (RIC 1997) describes the process of identifying the appropriate codes. As well, a map that labels the sample sites should accompany the **site identification log book**. This log book can be in the form of a 3-ring binder with a 1:50 000 map. The basic site location data (latitudes, longitudes, map sheet number, watershed code, site identification number, etc.) should be incorporated into the database (EMS in the case of BC Environment). GPS is rapidly becoming the standard for location in the field and this technology should be used whenever it is available.

2.3 Field Notes/Observations

Good sampling practice always involves the use of detailed field notes. Specific information about seemingly unimportant facts such as the time of day or weather conditions are often important when interpreting data. A **field log book** (3-ring binder with waterproof paper) for each project is mandatory. All field measurements (DO, temperature, conductivity, etc.) should be entered (by date) directly into this field log book. The following list emphasizes those observations that should be recorded for biological variables:

- Site name and EMS code
- Date and time
- Weather
- Names of all the personnel on the sampling crew
- Station depth (phytoplankton, zooplankton, fish)
- Tow volumes (zooplankton) (net mouth size, mesh size, tow depth)
- Surface area sampled (periphyton)
- Fish species collected including sex, weight, length, and comments regarding general appearance (health)
- Preservatives used (for each sample)

All information recorded in the log book should be initialed by the data recorder and entered into the database as soon as possible upon return from the field. The field note book is a very important document and it should be stored as an archival document for future reference.

3. Quality Assurance/Quality Control

3.1 Field Quality Assurance

The field quality assurance program is a systematic process which, together with the laboratory and data storage quality assurance programs, ensures a specified degree of confidence in the data collected for an environmental survey. The field quality assurance program involves a series of steps, procedures, and practices which are described below.

3.1.1 Technique/Diligence

The quality of data generated in a laboratory depends, to a large degree, on the integrity of the samples that arrive at the laboratory. Consequently, the field investigator must take the necessary precautions and have the appropriate knowledge to collect representative samples and protect samples from contamination and deterioration. Included in this category

are consistency of sampling, correct use of equipment, and detailed field notes.

There are many sources of contamination; the following are some basic precautions to heed for biological samples particularly where **tissue samples** are to be obtained and processed:

- Sample containers, new or used, must be cleaned according to the recommended methods and certified by the issuing laboratory as 'contamination free' (if pre-cleaned by the laboratory). Pre-cleaned bottles must be supplied with caps attached. Always label and store pre-cleaned bottles in plastic bags to avoid confusion and contamination during transport.

- Only the recommended type of sample container for each analysis should be used. Use only certified contamination free preservatives.

- The inner portion of sample bottles and caps must not be touched with **anything** (e.g., bare hands, gloves, etc.) other than the sample itself .

- Sample containers must be kept in a clean environment, away from dust, dirt, fumes and grime. Bottles must be capped at all times and stored in clean shipping coolers both before and after the collection of the sample. Vehicle cleanliness is an important factor in eliminating contamination problems.

- Petroleum products (gasoline, oil, exhaust fumes) are prime sources of contamination. Spills or drippings (which are apt to occur in boats) must be removed immediately. Exhaust fumes and cigarette smoke can contaminate samples with lead and other heavy metals. Air conditioning units are also a source of trace metal contamination.

- Samples must never be permitted to get warm; they should be stored in a cool place. Coolers packed with ice packs are recommended (most samples must be cooled to 4°C during transit to the laboratory; some are required to be frozen through the use of dry ice). Conversely, samples must not be permitted to freeze unless freezing is part of the preservation protocol.

- Samples must be shipped to the laboratory without delay so that they arrive within 24 hours of sampling. Samples must be analyzed within the time limits set out in Appendix 2 of this chapter.

- The sample collectors should keep their hands clean and refrain from smoking or eating while working with biological samples.

For **taxonomic samples**:

The following is the basic information which must accompany samples:

- Collection site (gazetted name, preferably with latitude and longitude and the sample site number),
- Collection date (using the Y/M/D convention written as "1999 June 12" not 99/06/12!!),
- Sampler's name,
- Collection method and details (e.g., net mouth size).

All of these data should be recorded in such a way that they will remain legible. Labels inside bottles filled with wet samples should be written with pencil on paper labels designed for this use.

As part of the lab taxonomic identification process, verification of taxa by recognized experts should be routine.

Voucher specimens should be retained for future reference and reverification. Voucher material should be stored using museum standards for preservation. At present, this is 125 mL Flint glass jars with wide mouth, plastic caps with a plastic gasket and liner. Preservatives should be noted under different sample types.

3.2 Field Quality Control

Quality control is an essential element of a field quality assurance program. In addition to standardized field procedures for biological sampling, field quality control requires, at the very minimum, the submission of replicate samples for all variables and reference samples for macro-invertebrate and fish tissue samples. Replicate samples detect heterogeneity within the environment, allow the precision of the measurement process to be estimated, and provide a check on the reproducibility of the sampling. There are many aspects to laboratory QA/QC noted below, and the field and lab aspects typically have to be considered together. One essential component is reference samples. They are used to document the bias of the analytical (laboratory) process. The timing and the frequency of replicate samples are established in the project design and will vary with each project.

3.2.1 Replicate Samples

To determine the degree of heterogeneity within the biological community being sampled, it is necessary to take replicate samples. These replicates can consist of multiple samples (grabs, tows, or whole fish) from the same general area (to measure how well a single sample represents the community or how many samples are necessary to achieve some level of sampling confidence), or portions of a single sample (i.e., sectioned grabs - to measure more localized invertebrate heterogeneity).

3.2.2 Split Samples

Split samples are aliquots taken from the same container and assumed to be identical. These samples can be sent to two or more laboratories for separate analysis and the results can be used to determine interlab variability of the different laboratories or the consistency of results within one lab.

3.2.3 Reference Samples

For tissue samples, laboratory tested and preserved reference materials are available. For example, the National Research Council of Canada has dogfish liver and muscle tissue and lobster hepatopancreas tissue for the determination of trace elements and organomercury. These reference tissues have been subjected to a large number of analyses performed by independent laboratories using several different analytical techniques. Consequently, the NRC provides mean values and confidence intervals for these substances. Other reference tissues are available from other sources.

For taxonomy samples, some basic taxonomic reference materials are available. The US EPA is one source of these at least for algal taxonomy, chlorophyll *a* and some bacterial species.

These reference samples should be submitted to the analyzing laboratory along with the samples collected during a field trip. They should be transferred to a regular sample container and labeled with plausible site names and numbers (the codes used for identification must be documented in the field log book).

4. Collecting and Processing Biological Samples

4.1 Lake Biological Samples

The collection of deep water samples requires that at least one member of the sampling group be very familiar with boat operation and safety. **If the sampling trip involves the use of a boat, then the weather forecast or marine conditions should be obtained prior to departure from home. If conditions are poor, then the sampling trip should be postponed.**

4.1.1 Bacteria

Samples collected for bacterial analysis are simply grab water samples collected at designated sites. They can be collected at near shore or deep water stations. Deep samples require the use of a Van Dorn bottle for the sample collection (Figure 1). **All bacteriological samples must be cooled (to 4°C) as soon as they are collected and must be shipped to the lab as soon as possible**. The following are the protocols for collecting shore samples, deep water surface samples, and depth profile samples.

4.1.1.1 Shore Samples

To avoid contamination from suspended sediments, the sample collector must wade out past the point where wave action affects the lake bottom. In most cases, this distance is not far from shore. **The sampler should not exceed a depth where there exists a reasonable possibility that water might unexpectedly enter the gum-boot or hip-wader**. This is particularly important during colder weather when getting wet poses a health risk (such as hypothermia).

PROTOCOL
(shore samples)

(a) Obtain labeled, sterilized, 250 mL bottles and wade into the lake at the most accessible point.

(b) Once you reach a sufficient depth (where bottom material will not interfere with the sample), stop and orient yourself towards the center of the lake. Often samplers wait standing in place for 2 - 3 minutes to ensure sediment disturbed by wading can settle.

(c) Remove the lid and hold it aside without allowing the inner surface to touch anything.

(d) With your other hand, grasp the bottle well below the neck. Lean out towards the center of the lake and in one continuous motion

plunge the bottle beneath the surface to a standardized depth (two frequently used depth for surface samples are 0.1 m and 0.5 m) and slowly force it through the water until it is entirely full. This motion creates a current over the mouth of the bottle such that water entering the bottle has not come in contact with your hand.

(e) Pour out enough water to provide 2.5 - 5 cm air space above the sample. Replace the cap immediately.

(f) Return to shore and pack the sample in a cooler containing a sufficient quantity of ice packs (twice the volume of ice to samples in summer months or equal volume when sampling in winter months).

4.1.1.2 Surface Bacteriological Samples at Deep Stations

PROTOCOL
(for collecting surface samples at deep stations)

(a) The person at the bow (front) should always collect the samples. This is because the bow is the anchor point and even in a slow current the boat will drift so that the bow is upstream. This precaution reduces the potential of contamination from the boat or motor. The person in the stern (rear) can be responsible for holding the boat's position (when not anchored), taking the field measurements, and making field notes, and helping to stabilize boat when bow person is working near gunwales.

(b) Obtain a labeled, sterilized, 250 mL sample bottle and remove the lid without touching the inner surface of either the bottle or lid.

(c) Reach out an arm length from the boat to take the sample. Ensure that the person in the stern is providing counterbalance (working over the opposite side of the boat).

(d) Plunge the bottle under the surface and move it slowly towards the current (the direction the boat is facing) until the bottle is entirely full.

(e) Pour out enough water to provide a 2.5-5 cm air space above the sample.

(f) Recap the bottle and place in a cooler immediately.

4.1.1.3 Deep Samples

Deep samples are usually collected by Van Dorn sampler. Note that Van Dorn samplers are available in both horizontal and vertical configurations. The advantage of the vertical configuration is that the water within the open bottle is flushed out as the bottle is lowered so that one can be guaranteed that the water was collected from the indicated depth. The advantage of the horizontal configuration is that a very narrow depth range is sampled. Vertical configurations are usually used in large lakes. Horizontal configurations are used for samples to be collected at or just above/below a very sharp thermocline, or to be collected near the lake bottom. Horizontal configurations are mandatory for very shallow lakes.

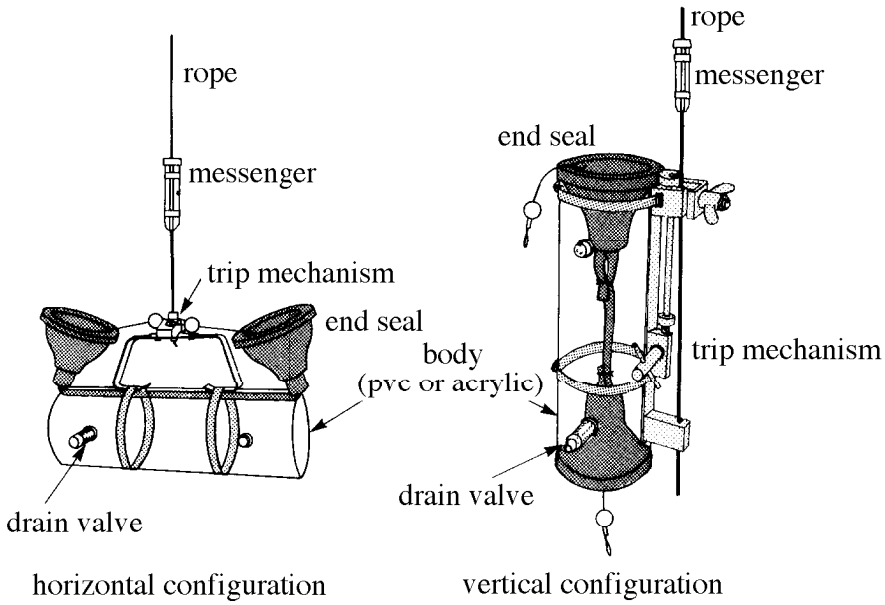


Figure 1. Van Dorn Sampler

PROTOCOL
(for taking deep samples)

(a) Open the Van Dorn sampler by raising the end seals. Do not touch any of the inner surfaces of the apparatus.

(b) Set the trigger mechanism as in Figure 1.

(c) Lower the sampler to the desired depth. Ensure that the loose end of the rope is tied to the boat.

(d) Send the messenger down to "trip" the mechanism that closes the end seals.

(e) Retrieve the sampler to the surface.

(f) Flush the drain valve with some of the sample water so as to reduce the possibility that bacteria from previous sample sites will contaminate the current sample. Then, transfer the water sample from the Van Dorn bottle to sterilized, labeled, 250 mL sample bottles via the drain valve. Leave a 2.5-5 cm air space at the top of the bottle. **Take care to avoid contact with the drain spout as contamination often occurs at this stage.**

(g) Recap the bottle and place in the cooler immediately.

4.1.2 Zooplankton

Zooplankton, or planktonic animals, are free floating and suspended in open or pelagic waters. They are generally collected with a conical net (Figure 2) that has a specific mesh size (ranging from as small as 64 µm to as large as 256 µm). Small mesh openings will clog more readily than larger ones, but small organisms will pass readily through larger openings. The mesh size required for a particular lake will depend on the productivity of the lake and the purpose of the study. The size of the mesh and the diameter of the net opening used for each BC Environment project will be clearly outlined in the project design. The preferred net mesh, when appropriate, is 64 µm with a net mouth diameter of 20 cm.

The net is lowered to a particular depth and pulled directly up through the water column (known as a vertical tow). Alternatives to the vertical tow are the horizontal and oblique tows in which various strata of the lake are sampled individually (a horizontal tow) or as a composite (oblique tow). These are elaborate techniques that require specialized equipment rigged to the boat and a tow net that has remote open and close capabilities. Unless there is specific need for data from horizontal and oblique tows

they are not used. Therefore, the vertical tow is the only protocol that will be mentioned here.

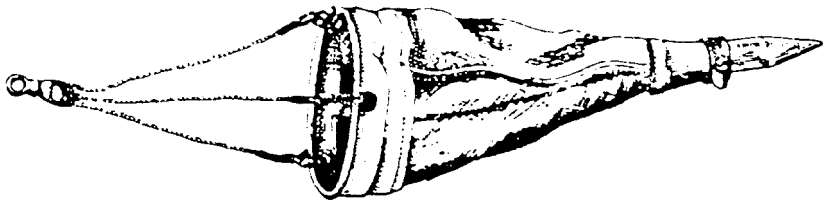


Figure 2. Plankton tow net

PROTOCOL
(vertical tow)

(a) Ensure rope is securely fastened at the plankton net opening and that the dead end is tied to the boat.

(b) At the designated site, lower the net to depth outlined in the project design.

Note: The actual distance that the net travels through the water must be recorded and the total volume of water that passes through the net must be calculated (see Figure 3).

(c) In smaller lakes, haul the net hand over hand with a steady, unhurried motion at a rate of 0.5 m/s. In large lakes, when long net hauls are conducted, use a davit, meter wheel, and winch. The maximum tow speed used should be 1 m/s.

(d) Once the net is at the surface, wash the net by raising and lowering the net body below the net mouth in the water. Then squirt de-ionized water against the outside of the netting and from the top downward. This washes any adhered plankton down into the cod-end (removable 'cup' at the end of the net).

(e) Disconnect the cod-end and carefully decant the water and plankton into a pre-labeled bottle. Rinse the cod-end several times, pouring each rinsate into the bottle (this ensures that all plankton are collected).

(f) Wash the net by rinsing (pulling it through the water without the cod-end). This is an absolute necessity before proceeding to the next sample site (particularly between lakes).

(g) Preserve the sample with 70% ethanol (70mL of 100% ethanol for each 30 mL of sample volume) and place in the cooler for shipping.

Volume of water through a zooplankton tow

$$V = \Pi r^2 d$$

where:

v = volume of water filtered through sampler

Π = 3.1416

r = radius of net mouth

d = depth of net sampler at start of vertical haul (total length of course through water)

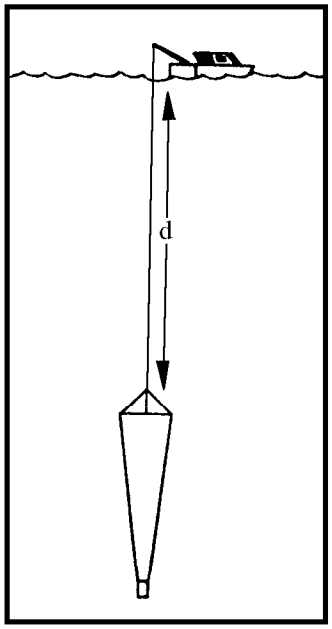


Figure 3. Tow volume calculation

4.1.3 Phytoplankton

Phytoplankton collection in the open water of a lake consists of surface and/or deep grab sampling. A Van Dorn bottle is the most common tool for collecting the deep samples.

4.1.3.1 Surface Samples

PROTOCOL
(for collecting surface phytoplankton samples)

(a) Anchor the boat at the designated sample site (alternatively, if the water is too deep or a buoy is not present, the person in the stern will have to maintain position while the person in the bow takes the samples).

(b) Obtain a labeled, 1 litre sample bottle and remove the lid without touching the inner surface of either the bottle or lid.

(c) Reach out an arm length from the boat to take the sample. Ensure that the person in the stern is providing counterbalance (working over the opposite side of the boat).

(d) Plunge the bottle under the surface and move it slowly towards the current (the direction the boat is facing) until the bottle is entirely full. Standard sampling depths for "surface" water samples are 0.1 m and 0.5 m.

(e) Preserve the sample with 1-3 mL of Lugol's solution (1-3 mL per 1 litre of sample). Different water chemistry and density of algal material require different concentration of preservative. A general guideline is that there be sufficient Lugol's to turn the sample the colour of weak tea.

Note: Plankton nets for phytoplankton samples are <u>not</u> recommended for quantitative samples. They are size selective and very non-quantitative. They can be used however for identification of the species present as the higher density assists in the taxonomic work, particularly of rarer species.

4.1.3.2 Deep Samples

PROTOCOL
(for collecting deep water samples)

(a) Open the Van Dorn sampler by raising the end seals.

(b) Set the trigger mechanism as in Figure 1.

(c) Lower the sampler to the desired depth (epilimnion, hypolimnion or thermocline - the location of which should have been determined by prior DO/temperature profile data collection - see Ambient Freshwater Sampling chapter). Ensure that the loose end of the rope is tied to the boat.

(d) Send the messenger down to "trip" the mechanism that closes the end seals.

(e) Retrieve the sampler to the surface.

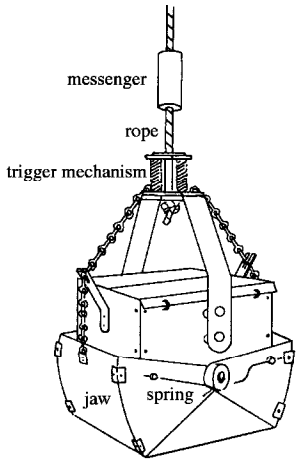
(f) Transfer the water sample from the Van Dorn bottle to labeled, 1 liter sample containers via the drain valve.

(g) Preserve the sample with Lugol's solution (1 to 3 mL per 1 liter of sample).

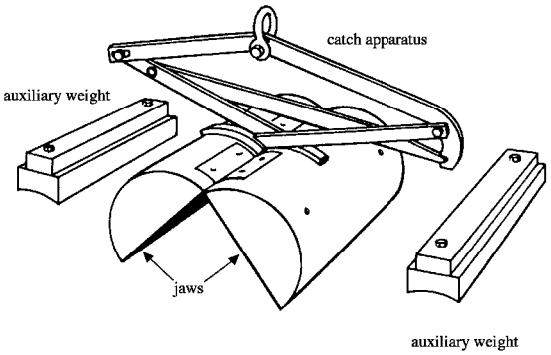
(h) Recap the bottle and place in the cooler.

4.1.4 Benthic Fauna

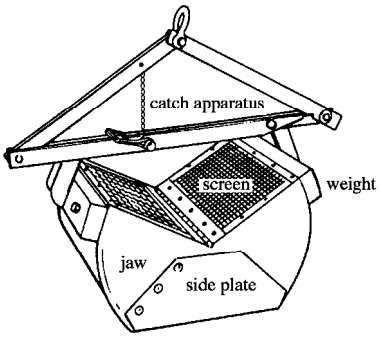
Benthic invertebrates in lakes or large slow moving rivers are generally collected in the same fashion as sediment samples (see Lake and Stream Bottom Sediment Sampling document). The processing of the sample once it has been collected is where the techniques differ. The type of grab sampler to be used at a particular site will depend on the site conditions and the purpose of the study. The equipment to be used will be dictated by the project design and must be outlined in the field log book and pre-sampling checklist. Figure 4 presents some of the grab samplers that are available, while Figure 5 presents the core sampler most commonly used.



Ekman grab



Petersen Grab



Ponar grab

Figure 4. Common Sediment Grab Samplers

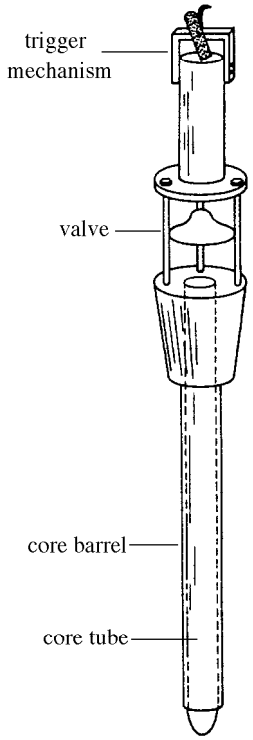


Figure 5. Kajak-Brinkhurst Sediment Core Sampler

4.1.4.1 Grab Sampling

PROTOCOL
(boat sampling with a grab sampler)

(a) Ensure that the rope is securely fastened to the sampler and that the loose end of the rope is tied to the boat.

(b) Set the grab sampling device with the jaws cocked open (see Figure 4). **Great care should be taken while dealing with the device while it is set as accidental closure can cause serious injuries.**

(c) Lower the sampler until it is resting on the sediment (its own weight is adequate to penetrate soft sediments). At this point, the slackening of the line activates the mechanism to close the jaws of the Ponar and Petersen grabs.

(d) For the Ekman grab, send the messenger down to 'trip' the release mechanism.

(e) Retrieve the sampler slowly to minimize the effect of turbulence (that might result in loss/disturbance of surface sediments).

(f) Place a container (i.e., a shallow pan) beneath the sampler just as it breaks the surface of the water.

Note: If the jaws were not closed completely, the sample must be discarded. Discard the sample into a bucket if the second collection attempt is made from the same general area. Dump the unwanted sample only after the "real" sample has been successfully collected.

(g) Place a sieve between the sampler and the pan and gently open the jaws and allow the sediments to empty into the sieve. The size of the sieve mesh will depend on the purpose of the study, but a common mesh size is 0.20 mm (200 µm). This size represents the practical lower limit for general study of benthic organisms. It is not as crucial to have small mesh size when the only analysis to be conducted is biomass.

(h) Immediately record (in the field log book) observations regarding the appearance of the sediment (i.e., texture, colour, odour, presence of biota, presence of detritus).

(i) Rinse the sieve with de-ionized or on-site water to remove as much sediment as possible.

(j) Transfer the organisms to a pre-labeled sample bottle and preserve with 70% ethanol. Formalin may be used as a fixative for initial preservation but should be subsequently washed and transferred to 70% ethanol.

4.1.4.2 Core sampling

PROTOCOL

(boat sampling with a core sampler)

(a) Open the valve and set the trigger mechanism (as per Figure 5). Ensure the rope is securely fastened to the corer and attach the loose end of the rope to the boat.

(b) Most corers are designed to be simply lowered into the sediments and fill the core tube by their own weight and need not be dropped from any hight. Consideration of the type of corer used and the nature of the sediment being sampled will need to be taken into account.

(c) Send the messenger down to release the trigger mechanism.

(d) Carefully retrieve the sampler and place a stopper into the bottom opening **before** removing from the water to prevent loss of the sample.

(e) Remove the core tube or liner from the corer and stopper the upper end.

Note: Once on shore, the sample can be treated as a bulk sample or it can be sectioned and the organisms separated from the sediment in strata.

(f) For bulk samples all the sediment may be sieved as per the grab samples above. Otherwise, the sediment should be sectioned in regular intervals as it is extruded (record the thickness of each stratum the length of entire core). Each stratum may be sieved and its contents placed in pre-labeled sample bottles or, the unsieved sediments can be placed directly into pre-labeled sample bottles.

(g) Preserve the samples with 70% ethanol.

Note: It is preferable to section the core as soon as possible after it is retrieved. As the sediment warms, it tends to expand in the core tube. With warming, decomposition gases are liberated at a much faster rate and if they bubble through the core, they will disturb the stratigraphy.

4.1.5 Macrophytes

Aquatic plants are collected for one of three purposes: first, biomass studies for the determination of how much plant material is present on an area or lake basis; second, tissue analyses for the detection of metals, pesticides and plant products; or third, taxonomic analyses as part of ecological or impact studies (permanent records of community structure over time) and to serve as reference specimens for the above two analyses. These reference specimens are a valuable scientific record and their collection and subsequent handling should be done with care so that much of the time and expense that has gone into their collection is not wasted. Reference specimens are stored in herbaria which provide permanent records of what was found and allow identifications to be re-verified if necessary. Even when the major thrust of the work is for biomass studies or tissue analyses, representative specimens must still be collected and saved as vouchers of the species that were analyzed. With respect to sampling for taxonomic purposes, Warrington (1994) documents techniques regarding the processing of individual taxa within each of the three groups of aquatic plants (floating, submergent, and emergent). This manual presents a general overview of the protocols for collecting aquatic plants for taxonomic purposes.

4.1.5.1 Taxonomy

Generally, the whole plant should be collected. Some groups cannot be identified to species without mature fruits or flowers. Large plants are pressed and mounted on a 30 by 40 cm white card that is dimensionally stable when wetted. Small plants like the duckweeds do not make satisfactory pressed and dried specimens. In this case, small screw cap vials make good collecting and preservation containers.

PROTOCOL
(small floating species collection)

(a) Scoop a few individual plants into a pre-labeled vial.

(b) Preserve the specimens in a solution of 70% ethanol, 25% water and 5% formalin.

(c) These specimens must be submitted to a herbarium where they will be identified to species and stored for future reference.

PROTOCOL

(collection of large emergent or rigid plants for taxonomic purposes)

(a) Field notes should be written directly on the card stock **before** you place the plants onto the card (you will not be able to write on the card once it is wet). The notes should be written in pencil in the lower right hand corner of the card where it will later be covered by the permanent label.

(b) Collect an entire specimen and store it until conditions are appropriate to mount.

Note: Do not leave them out in the sun even briefly as they will wither very quickly and become useless as specimens. Emergent plants should not be submerged, but kept in a bag with a little water in the bottom to maintain high humidity. It is best to keep each species in its own bag and all the bags from one lake or site together in one large (garbage) bag.

(c) To mount each specimen, lay the plant on the card stock with the roots in the bottom left corner (fold over the top if it is too tall to fit).

Note: Do not cover the label area in the bottom right corner.

(d) Spread out leaves and flowers, turn some over so the bottoms can be seen, and try to make a neat and tidy specimen that covers the whole sheet.

Note: For small plants fill the sheet with more specimens from the same clump or clone, to show as much variability as possible.

(e) For fruiting plants where the seeds may be shed on drying, collect the seeds into small paper or cellophane pouches and attach these pouches to the finished herbarium sheet.

(f) After mounting the aquatic plant on the card stock, place a piece of heavy blotting paper on top of the specimen to help dry the plant quickly.

(g) Wrap the card stock, plant, and blotter in a newsprint folder. The newsprint should be 30 by 90 cm in size and folded in half to form a folder in which the mounted plant is placed.

(h) Once you have accumulated several of these wrapped packages (each containing one card stock/specimen), place them into a plant press with a piece of corrugated cardboard separating each package.

Note: The corrugations should run in the same direction so that air flow through the press is facilitated.

(i) Drying should take place within several days to prevent fungal growth and rotting and to preserve colours and shapes as much as possible. If you will be in a laboratory or herbarium the same day, the plant presses may be dried in a proper plant drier or a forced draft oven at 40°C. In the field, use motel hot air registers, baseboard heaters, or hair dryers to move warm air through the corrugated cardboard. If the weather is dry, secure the plant press on the roof of the truck and allow air to blow through the corrugated cardboard as you drive from site to site. As the plants in the presses dry it will be necessary to retighten the presses periodically, at least daily, to maintain the pressure and hold the plants flat.

(j) These specimens must be submitted to a herbarium where they will be identified to species and stored for future reference.

PROTOCOL

(collection of large submergent or
non-rigid plants for taxonomic purposes)

(a) Field notes should be written directly on the card stock **before** you place the plants onto the card (you will not be able to write on the card once it is wet). The notes should be written in pencil in the lower right hand corner of the card, where it will later be covered by the permanent label.

(b) Collect an entire specimen and store it until conditions are appropriate to mount.

Note: Do not leave them out in the sun even briefly as they will wither very quickly and become useless as specimens. Keep them in a bag or bucket of water at all times until you are ready to press them. It is best to keep each species in its own bag and all the bags from one lake or site together in one large bag.

(c) Since these plants are flaccid and clump together when removed from water, they need to be floated onto the card stock and arranged neatly to keep them from becoming a useless mat. Start by slowly lifting the bottom of the card stock out of the water at the root end and arranging and spreading the plant as you continue. Once a portion is out of the water, it will stay in place. Some, but not all, plants will allow a limited amount of rearranging once they are out of the water. Hold the card stock with one corner down and let most of the excess water drain off. Floating is best done in 36 by 50 cm photographic trays or directly in the lake from which the specimen was collected (it will be difficult in lakes if there is any wind or waves). A tray on a picnic table at a site with running water is an ideal mounting and pressing site.

Note: Do not take plants to a different lake to float them onto the card stock; you risk spreading weeds from lake to lake.

(d) Continue as in (e) through (j) on the previous page.

4.1.5.2 Tissue Analysis

Plant tissues may be collected for analyses of metals, pesticides, nutrients, plant products, for dry weight/wet weight ratios, or for other laboratory analyses. In all cases an entire, intact, voucher specimen of each species should be collected and filed in a herbarium as a record of what was analyzed. The procedures outlined above under the section headed **Taxonomy** (4.1.5.1) should be followed.

For analyses of chemicals, it may be necessary to analyze distinct portions of the plant separately to determine where the material is localized in the plant (the portion to be collected will be outlined in the project design).

PROTOCOL
(collecting samples for tissue analysis)

(a) Collect entire specimens and keep them submerged and covered until they can be processed (plants should never be allowed to desiccate).

(b) Place each specimen in individual air tight bags (e.g., Zip Lock) or tissue cups or glass bottles (for analysis of trace organics). Ensure each is fully labeled. **Ensure the quantity of tissue and the type of container are appropriate for the analysis that will be conducted (see Appendix 2 of this chapter).**

(c) Place the sample container in the required cooler for shipping (some analyses need to be frozen, therefore, these samples will be placed in a cooler containing a sufficient quantity of dry ice). **Always wear gloves when handling dry ice.**

4.1.5.3 Biomass Studies

Biomass studies, mass of plants per unit area, are not routine and are usually conducted as part of a research project. They are not performed as part of standard sampling programs. They often require the use of SCUBA to map out and harvest the designated area. Refer to Warrington (1994) for further information regarding biomass protocols.

4.1.6 Fish

For fish collection and processing protocols, refer to *Fish Collection Standards and Methods* (RIC 1997), which has replaced the *Fish Collection, Preservation, Measurement and Enumeration Manual* (Triton Environmental Consultants, 1994) as the standard manual. The *Field Key to Freshwater Fishes of British Columbia* (RIC 1994), is used to identify fishes in the field.

4.2 River/Stream Biological Samples

In some instances, the protocols for collecting biological specimens in rivers are similar to those used for sampling in lakes. But in the case of benthic fauna and fish, the collection techniques are considerably different. The following are the protocols for the collection of biological samples from flowing waters.

4.2.1 Bacteria

As is the case for lakes, bacteria are collected as grab samples and immediately cooled to 4°C to minimize metabolic activity (growth/reproduction) until they can be analyzed by the laboratory.

Wherever practical, samples should be collected at mid-stream rather than near-shore. Samples collected from mid-stream reduce the possibilities of contamination (i.e., shore effects - back eddies, seepage from near shore soils, etc.). The most important issue to consider when deciding where the sample should be collected from is **SAFETY**. If the flow is sufficiently slow that the collector can wade into the stream without risk, then the sample can be collected at a depth that does not pose a threat (discretion is the key - **never wade into water that appears deep or fast flowing**). When conditions dictate that the sample be taken from the stream bank, deviations from the standard protocol should be accurately documented in the field log book and transferred to the database as soon as possible.

PROTOCOL
(for wading into flow)

(a) Obtain a labeled, sterile 250 mL bottle and wade into the river downstream from the point at which you will collect the sample. Wade upstream to the sample site. This ensures that you will not disturb sediments upstream from the sample point.

(b) Stand perpendicular to the flow and face upstream.

(c) Remove the lid and hold it aside without allowing anything to touch the inner surface.

(d) With your other hand, grasp the bottle well below the neck. Plunge it beneath the surface with the opening facing directly down, then immediately orient the bottle into the current.

(e) Once the bottle is full, remove it from the water by forcing it forward (into the current) and upwards. Pour out some water to ensure there is an air space.

(f) Replace the cap immediately.

(g) Return to shore and place the sample in a cooler with sufficient ice packs (twice the volume of ice as the sample in summer, same volume in winter).

PROTOCOL

(for sampling from the stream bank)
(when the current is too strong, water is too deep, or ice is too thin)

(a) Secure yourself to a solid object on shore (with a safety harness and line if necessary).

(b) Remove lid from a labeled, sterile 250 mL bottle and place into a clean resealable bag (e.g., Zip Lock).

(c) Hold the bottle well below the neck.

(d) Reach out (arm length only) and plunge the bottle under the water and immediately orient it into the current.

(e) When the bottle is full, pull it up through the water while forcing into the current. Pour some water out so as to leave an air space.

(f) Immediately recap the bottle.

(g) Place the sample in a cooler with sufficient ice packs (twice the volume of ice as the sample in summer, same volume in winter).

Note: If conditions are such that sampling from a bridge is an option, refer to the chapter, Ambient Freshwater and Effluent Sampling, 4.2.2, for the protocol.

4.2.2 Periphyton

Periphyton are defined as attached species of microflora (algae) and are primarily found on hard, immobile surfaces such as large stones (cobbles, boulders) and on aquatic plants. Analysis of periphyton communities, particularly for the purpose of biomass calculations, involves collecting the algae from a known surface area. To do so, the following pieces of equipment are required:

- A piece of equipment to outline a known surface area and contain the removed biomass - for example, a toilet bowl plunger that has had the handle insertion hole cut larger. The diameter of the hole is known, therefore the surface area of the exposed rock below the hole is known. A rim of neoprene is glued around the hole to prevent water and algae from escaping when collecting the sample. It is also possible to use a template if the algal mass is gelatinous or coherent enough to be picked off the growing surface. A template is a piece of flexible rubber or plastic with a square or round hole cut in it to define the area to be scraped.
- A water bottle (squirt bottle) containing de-ionized water

- A tooth brush (to scrape the algae free from the rock)
- A turkey baster (to transfer the sample from the scraping cup to the sample bottle
- Pre-labeled sample bottles
- Preservative (Lugols)

PROTOCOL
(natural substrate)

(a) Choose a location of fairly uniform substrate over wetted width. Try to work in upstream but random direction. Select rocks that are relatively flat and large enough to accommodate at least three scraping cups. Unless the project design states that rocks with the most dense growth are to be sampled, the sample collector should select the rocks randomly. To do so, the collector should not specifically look for an area from which to sample, but instead should wade into the water and stop without regard for apparent algal concentration and then grab the first rock that is suitable for scraping. Return to shore with the selected rock and repeat the process until 3 or 4 rocks are collected. **Don't attempt to lift rocks that are too heavy and don't enter water that may pose a threat to your safety. Watch your footing while returning to shore with the rock.**

(b) Hold the scraping cup firmly over a selected patch on a rock. Squirt some de-ionized water into the cup. With the tooth brush, scrape the algae (within the cup) off the rock. Each new algal patch that is scraped can be referred to as a disc.

(c) Transfer the water-algae mixture to a pre-labeled bottle (a turkey baster with the tip cut off works very well as the transfer tool). Do not remove the cup yet.

(d) Squirt some fresh de-ionized water into the cup, re-scrape and collect the rinsate. At this point you have collected one disc. Collect and composite more discs until you have transferred a sufficient quantity of algae to the sample bottle [see (e)].

(e) If algal density is low or patchy, collect at least five discs from each rock. If algal density is high or homogeneous, then take fewer discs per rock, but ensure that you use at least five rocks to characterize the periphyton at the site. **Always record the total number of discs collected**.

(f) If the sample is to be analyzed for biomass, place the bottle in a black garbage bag and then immediately into a cooler that contains a sufficient quantity of ice packs (twice the volume of ice as the total

sample when sampling in summer, equal volumes when sampling in winter).

(g) For measurement of biomass, the samples are prepared for chlorophyll analysis by filtering the sample onto a membrane filter (0.45 or 1.0 μ pore size), then folding this filter and placing it inside a larger (8 to 10 cm.) Whatman type paper filter labeled (date, site, area scraped), then clipped with a plastic paperclip and placed into a jar with desiccant and placed in the freezer or in a cold cooler for shipment to the lab.

Note: In washing and transferring the sample, keep the volume of water used to a minimum. Filtering periphyton samples can be very difficult due to suspended sediments that generally accompany the periphyton.

It may be necessary to use a larger pore size filter than for phytoplankton (typically 0.45 μ). Periphyton cells are typically much larger than phytoplankton. An analysis of the filtrate from the periphyton sample will indicate if significant biomass is passing through the larger filter size chosen.

(h) If the samples are to be analyzed for taxonomy (only two or three discs are required), then the sample must be preserved with Lugol's solution. Use at least 1 mL of Lugol's per 250 mL of sample (with heavy biomass, more preservative is needed). No filtration is required. Tip once or twice to mix preservative and sample water.

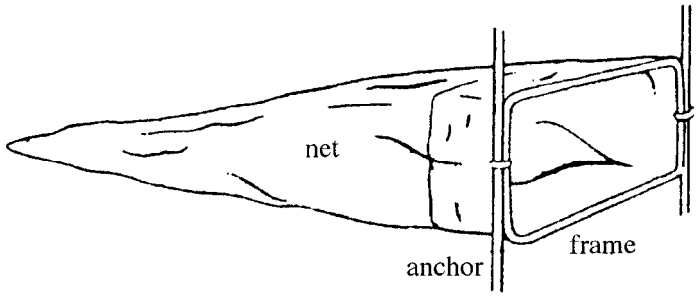
(i) Different conditions or circumstances may require different methods than described here. Other methods are acceptable as long as they are described and documented and approved by Ministry authority. For example, where heavy periphyton growth occurs, a method for sampling heavy growth could use a template disc to define a sampling area and scrape the area around it, then remove the disc of periphyton with a scalpel. This method reduces the volume of wash water that must be used and potential clogging of filters.

4.2.3 Benthic Fauna (macro-invertebrates)

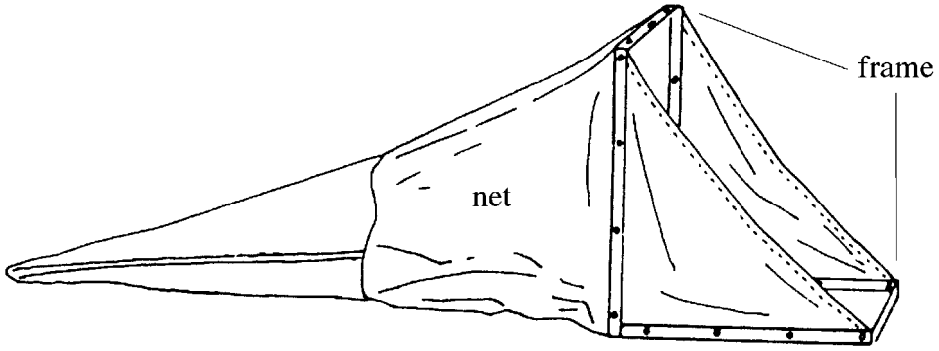
Samplers used for the collection of invertebrates in the shallow portions (<one metre deep) of rivers or streams fall into two categories: those sampling natural substrates, and those using artificial substrates. Two of the more common samplers used for natural substrates are the Surber and Hess samplers (Figure 8). The Drift net sampler can also be used to collect the emerging or drifting invertebrate stages. Kick nets are also used for inventory type surveys,

Natural Substrate. The advantage of these types of sampler is that the sample reflects the natural community of the stream. The disadvantages are that such samples have a very high variability and many samples need to be taken to characterize the benthic community. The large number of samples necessary generally means relatively high cost.

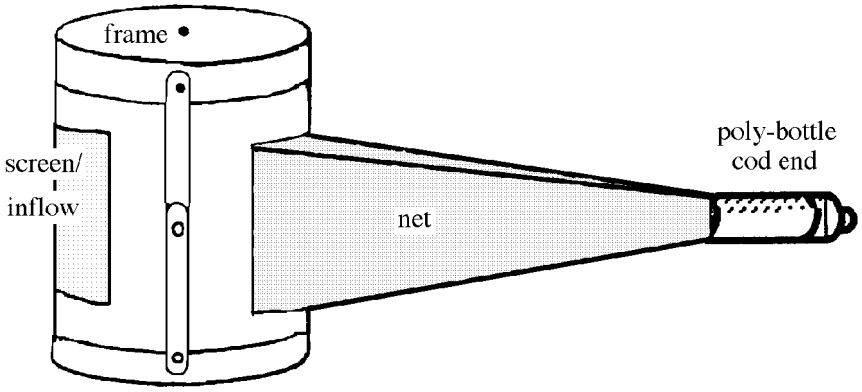
The recommended mesh size is 210 µm (microns) for most samples. In some specialized studies a different mesh size may be appropriate. It may be appropriate to collect the samples using a 210 µm mesh size but to do a preliminary sieving at 500µ. The sample material retained on the 210 µm mesh is stored to be processed later if the program design calls for it.



Drift net sampler



Surber sampler



Hess sampler

Figure 8. Common benthic macro-invertebrate samplers

4.2.3.1 Surber Sampler

The Surber sampler consists of two interlocking frames that support a capturing net. One frame outlines the area of stream bed to be sampled while the other supports the net. The sampler is intended for use in shallow (30 cm or less) flowing waters. Use A1 tent pegs (hooked) to anchor surber in fast moving water. Repetitive sampling should be timed (i.e., 5 minutes each for more uniform sampling).

PROTOCOL
(Surber sampler)

(a) Choose a wetted width location where substrate is fairly uniform. Provide a description of general habitat (e.g., is this a run, pool or riffle section, etc.). Position the sampler securely at a random location on the stream bottom parallel to water flow with the net portion down-stream. The mesh size of the net should be compatible with the goal of the program.

Note: Take care not to disturb the substrate upstream from the sampler.

(b) Carefully turn over and lightly rub all rocks and large stones that lie within the frame. This process dislodges organisms that are clinging to the stones. Examine each large stone for organisms, including larval or pupal cases, that may be clinging to the stone before discarding (downstream or to the side of the sampler). In order to maintain comparability between stations, a limit on time spent on handling and rubbing the substrate should be set (5 minutes recommended).

(c) Stir remaining gravel with your hands to a depth of 5 to 10 cm.

(d) Move the sampler **upstream** to a new randomly selected patch of stream bottom and repeat steps (a) through (c). Continue this process until five patches of stream bottom have been sampled, each upstream from the last. This creates a composite of the five samples. The total area sampled will depend on the size of the sampler (x 5) and should be calculated and recorded in the **field log book**.

(e) Return to shore and carefully invert the net into a shallow pan containing stream water. Ensure all invertebrates are rinsed from the net into the pan.

(f) Transfer the organisms into a pre-labeled plastic sample bottle and preserve with 70% ethanol (may be initially fixed with 10% formalin). **Rinse sample net after each use.**

4.2.3.2 Hess Sampler

The Hess sampler is a metal cylinder with a screened opening on one side and an opposite opening with a net attached (Figure 8). The sample collector places the Hess sampler in the stream with the screen oriented into the current and the net trailing behind. The water is able to flow freely through the sampler and out through the net. With a known radius, the stream bed area that is sampled is easily calculated. This value must be recorded in the **field log book**.

PROTOCOL
(Hess sampler)

(a) Position the frame securely on the stream bottom. Ensure the screened opening is facing into the current and the net portion is trailing down-stream. Hold the sampler in position by applying pressure with your knees.

Note: Take care not to disturb the substrate upstream from the sampler.

(b) Reach into the cylinder and carefully turn over and lightly rub all rocks and large stones. This process dislodges organisms that are clinging to the stones and washes them into the net. Examine each stone for organisms, including larval or pupal cases, that may be clinging to it before discarding it outside of the cylinder. In order to maintain consistency between samples, a standard time should be assigned to sampling each site (5 minutes recommended).

(c) Stir remaining gravel with your hands to a depth of 5 to 10 cm.

(d) Move the sampler **upstream** to a new patch of stream bottom and repeat steps (a) through (c). Continue this process until five patches of stream bottom have been sampled, each upstream from the last. This creates a composite sample of the five areas.

(e) Return to shore and carefully wash the contents of the net into the cod-end then transfer to a shallow pan. Ensure all invertebrates are rinsed into the pan.

(f) Transfer the organisms into a pre-labeled sample bottle and preserve with 70% ethanol. **Rinse sample net after each use.**

4.2.3.3 Drift Net Sampler

Drift net samplers are designed to be anchored in flowing water to capture macro-invertebrates that have migrated or have been dislodged from the bottom surfaces into the current. They are limited to use in small, shallow streams. Ideally, drift nets should span the entire width of the stream that is being sampled. Several nets can be placed across the stream channel to capture all drift organisms and to measure spatial variation in the drift.

PROTOCOL
(Drift Net sampler)

(a) Anchor drift nets in water sufficiently shallow that they will extend above the water surface. Set drift net samples for the time specified by the project design (the length of time is designed to collect a representative sample, but not so long that flow through the nets becomes impaired by clogging from captured particulate matter).

(b) Transfer the organisms into pre-labeled sample bottles.

(c) Preserve with 70% ethanol and place in cooler. Initial fixation may be done with 10% formalin.

(d) Record time, area of net opening, stream discharge per unit time (see the Ambient Fresh Water and Effluent Sampling chapter, 6.7), and volume of water filtered in the **field log book**.

Artificial substrates. The above techniques sample the benthos directly from the natural substrate. Another technique is to use artificial substrate and to place it in the stream so it is colonized by the organisms in the stream then removed later and the community analyzed. These types of samplers are best suited for upstream/downstream studies or studies designed to test for changes over time. They do not necessarily provide a representative sample of the actual community which is living in the stream.

There are advantages and disadvantages to artificial substrates.

Advantages:

- Allow collection of samples from locations that can't be sampled because of substrate or depth
- Reduced variability
- Non-destructive sampling of a location
- Flexibility in sampling design

Disadvantages:

- Colonization rates differ from site to site
- Species in sampler may be different than stream bottom
- Long incubation/exposure times (6-10 weeks)
- Vulnerability of samplers to vandalism

The most frequently used artificial substrate sampler is the "barbecue basket" sampler. The basket sampler is fabricated by filling the basket (available in a number of variations from hardware stores) with gravel (2.5 to 7.5 cm diameter) that is then placed in the stream bottom. The substrate becomes colonized and is removed after some predetermined length of time.

PROTOCOL

(basket sampler)

(a) Place the basket sampler in the stream and anchor if necessary. Leave in place for the necessary colonization time.

(b) When the samplers are removed, take particular care not to dislodge organisms from the sampler. A general technique is to carefully place the basket sampler in a plastic bag underneath the water before it is lifted out.

(c) Record time, any site related data such as flow, temperature and pH (see the Ambient Freshwater and Effluent Sampling document), and data about the appearance and condition of the basket sampler in the **field log book**.

(d) In the lab, remove the animals from the sampler by carefully washing each rock into a sieve. Transfer the organisms into pre-labeled sample bottles. Preserve with 70% ethanol and place in cooler. Initial fixation may be done with 10% formalin before transfer to ethanol for longer term storage.

4.2.4 Macrophytes

Due to the similarity in the techniques, refer to 4.1.5 (in lake sampling) for the protocols for collecting and processing macrophytes in rivers.

4.2.5 Fish

Refer to *Fish Collection Standards and Methods* (RIC 1997) for protocols involving fish collection and processing.

5. Shipping

The day's sampling schedule must be designed to ensure that the samples arrive at the shipping agency's terminal well before the end of business hours. Since some variables have very limited hold times (Appendices 2 and 3), every effort must be made to avoid delays in shipping. The following is the procedure to be followed to maintain the integrity of the samples during transit.

Note: Generally, all samples, except those for bacteriological and taxonomic identification, should be securely packed in large coolers. Bacteriological samples are typically packed in a smaller cooler. Taxonomy samples should be preserved and do not require cooling during shipment.

PROTOCOL
(shipping)

Note: Ice packs should be used as opposed to loose ice or bagged ice. When loose ice melts, the contents of the cooler are free to shift, potentially allowing contamination of samples with melted ice water and/or breakage of glass bottles.

(a) Pack the samples upright in the cooler with at least 1 (winter) to 2 (spring, summer, fall) times as much ice as the total volume of the samples. Ensure that the glass containers are separated from each other by ice packs, plastic bottles or clean packing material to prevent them from shifting, falling over and/or breaking. For some analyses, tissues need to be hard frozen - dry ice is needed.

(b) Complete the laboratory requisition forms, enclose them in a sealed plastic bag, and place them in the cooler on top of the samples. The recommended minimum information that should accompany samples to the laboratory (on each requisition form) includes:

- Name of the source,
- Site name

- EMS site numbers
- Date and time of collection
- Name of collector
- Field measurements
- Comments on sample appearance, weather conditions, and any other observations that may assist in interpreting data.

(c) Seal the cooler with heavy duty packing tape to reduce the possibility of it accidentally opening and to prevent tampering with the samples. Coolers arriving at the laboratory with torn or absent tape alert the lab staff that tampering might have occurred during transit.

(d) Attach a label prominently displaying the destination.

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Appendix 1 Generic Field Checklist

(including water, sediments, biota and effluents)

General:

Log Books ____
Cooler (with ice packs) ____
Rope ____
Camera (film) ____
Way bills ____
De-ionized water (4L) ____
Resealable bags ____
Pencils ____
Felt Markers (waterproof) ____
Tape ____
Requisition forms ____
Shipping labels ____
Squirt bottle ____
Ice packs ____ Dry Ice ____

Labeled Sample Bottles:

General chemistry (1 L) # ____
Dissolved Metals # ____
Total Organic Carbon # ____
Coliforms # ____
Zooplankton # ____
Periphyton # ____
Tissue cups # ____
Extras - two of each
General chemistry (2 L) # ____
Total Metals # ____
Low-level nutrients # ____
Sediments # ____
Phytoplankton # ____
Invertebrates # ____
Macrophytes ____

Sampling Equipment (clean, in working order, batteries charged):

DO Sampler (BOD bottle, Winkler reagents) ____
Thermometer ____
pH meter ____
Hydrolab ____
Van Dorn, rope ____
Auger (bit sharpened, skimmer) ___
Sediment grab ____
Sieves ____
DO meter ____
Conductivity meter ____
Secchi disc ____
Through Ice Sampler ____
Spare probe membranes (repair kit) ____
Sediment corer ____
Zooplankton tow nets ____
Benthic invertebrate sampler (Hess, drift net, Surber) ____
Periphyton kit (cup, denture brush, baster) ____
Macrophyte sample kit (buckets, garbage bags, float tray, plant press, blot paper, herbarium sheets, newsprint, corrugated cardboard) ____
Electrofishing equipment ____
Fish nets ____

Filtration and Preservation Equipment:

Filter Pots ____
Tweezers ____
Preservative Vials ____
70% ethanol ____
Lugol's solution ____
Syringe(s), Hose ____
0.45 µ Sartorius filter papers ____
Disposal Container (for used vials) ____
Formalin _____
Magnesium carbonate ____

Boat Equipment:

Canoe (or boat) ____	Paddles ____
Motor ____	Fuel ____
Life jackets ____	Rope ____
Anchor ____	Tool kit ____

Personal Gear:

Lunch ____	Survival suit ____
Rain gear ____	Gum boots ____
Waders (hip, chest) ____	Sun screen ____
Flash light ____	

Safety:

WHMIS guidelines ____	First Aid Kit ____
Goggles (or safety glasses) ____	Rubber gloves ____

Appendix 2 Lab Sample Container, Preservation, and Hold Times for Sediments and Tissues

TYPE OF ANALYSIS	MINIMUM SIZE	CONTAINER TYPE	PRESERVATION	MAXIMUM HOLD TIME
SEDIMENTS, TISSUES AND ORGANICS				
General chemistry	200 g	P, W	keep cool, 4°C	72 h
EOX, Extractable Organic Halides	50 g	G, Solv, Fc	freeze (dry ice)	6 mo
Metals	100 g	P, W (T)	keep cool, 4°C	72 h
Organic Carbon	100 g	P or G	keep cool, 4°C	72 h
Organics - Semivolatile	200 g	G, A, W, Solv	freeze (dry ice)	6 mo
Organics - Volatile	50 g	G, W, Solv, B	freeze (dry ice)	14 d
Particle Size Analysis	100 g dry wt	P or G	keep cool, 4°C	72 h
PQ-8 (copper 8, copper quinolate)	100 g	G (A), W, Solv	freeze (dry ice)	6 mo
ANALYSES WITH LIMITED SHELF LIFE				
pH, Turbidity, Acidity, Alkalinity				72 h
Ammonia, TKN, Nitrate, Nitrite				72 h
Portho, total, total dissolved				72 h
bacteria				48 h

LEGEND

P = plastic	G = glass
B = Baked	T = Tissue Cup
Solv = solvent cleaned	A = amber
Fc = foil lined cap	W = wide mouth

Appendix 3 Laboratory Sample Container, Preservation, and Hold Times for Fresh Water Biological Sampling

TYPE OF ANALYSIS	MINIMUM SIZE	CONTAINER TYPE	PRESERVATION	MAXIMUM HOLD TIME
BACTERIOLOGICAL				
Total Coliform	250 mL	P, Ster	keep cool, 4°C	48 h
Fecal Coliforms	250 mL	P, Ster	keep cool, 4°C	48 h
Fecal Streptococci	250 mL	P, Ster	keep cool, 4°C	48 h
E. coli	250 mL	P, Ster	keep cool, 4°C	48 h
Enterococci	250 mL	P, Ster	keep cool, 4°C	48 h
TAXONOMIC				
Phytoplankton	1 L	P	1-3 mL Lugol's	6 mo
Periphyton	250 mL	P	1 mL Lugol's	6 mo
Zooplankton	250 mL	P	70% ethanol	6 mo
Benthic invertebrates	500 mL	P,W	70% ethanol	6 mo

LEGEND
P = plastic
Ster = sterile
W = wide mouth