PROTOCOLS FOR MEASURING BIODIVERSITY:

Benthic Macroinvertebrates in Fresh Waters

by:

D.M. Rosenberg, I.J. Davies, D.G. Cobb, and A.P. Wiens

Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, R3T 2N6

TABLE OF CONTENTS

List of Figures

Introduction

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 mm (Rosenberg and Resh 1993).

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 (Table 1). More than 4000 species of aquatic insects and water mites have been reported from Canada (Table 2). Thus, as a highly diverse group, benthic macroinvertebrates are excellent candidates for studies of changes in biodiversity.

The use of benthic macroinvertebrates in biodiversity studies of lakes and streams is also supported by the extensive background knowledge available for these organisms. This covers everything from study design to data analysis (Table 3). Other general sources of information include Rosenberg (1978), Elliott and Tullett (1978, 1983, 1993), and Murkin et al. (1994).

A number of technical developments enable the effective use of benthic macroinvertebrates in biodiversity studies (Rosenberg and Resh 1993):

1. qualitative sampling and sample analyses is possible using simple, inexpensive equipment;
2. the taxonomy of many groups is well known and identification keys are available; and
3. many well-developed methods of data analysis are available.

However, benthic macroinvertebrates can be difficult to work with unless the proper study design is used (Rosenberg and Resh 1993). For example:

1. quantitative sampling is difficult because the contagious (i.e. clumped or patchy) distribution of benthic macroinvertebrates requires large numbers of samples to achieve reasonable precision in estimating population abundance. The resulting processing and identification requirements for samples can be costly and time consuming. An alternative would be to use rapid assessment procedures;
2. the distribution and abundance of benthic macroinvertebrates are affected by a large number of natural factors, which have to be accounted for to determine changes in biodiversity; and
3. some groups of benthic macroinvertebrates are taxonomically difficult, although the development of new and improved keys is a high priority in research.

The collection of benthic macroinvertebrates from lakes and streams is usually a straightforward procedure using standard equipment. However, the removal of

organisms from background material can be tedious and time-consuming unless available labor-saving strategies are used (see below) and the identification of organisms to the species level, when possible, requires substantial training and skill. The processing of samples can be successfully accomplished by non-specialists, but the involvement of systematists is recommended for species-level identifications. Data-analysis procedures are standard, and can be done by anyone trained in elementary statistics. The following account describes sampling methods for benthic macroinvertebrates in lotic (stream) and lentic (lake) habitats, valuable ancillary information, different analytical paths to follow, and techniques for efficient operation in the field and laboratory.

Abiotic Factors

An optimal sampling program should characterize the sampled habitat at several different spatial scales: (1) map variables such as latitude, longitude, and altitude; (2) area variables such as land use, extent of forest cover along the shoreline of a lake, and extent of canopy coverage over a stream channel; (3) sampling site variables such as water depth, substrate composition, and primary productivity; and (4) water variables such as pH, dissolved oxygen concentration, and total suspended solids. The extent of use of these scales will depend on the available time and money. For example, submitting water samples to a laboratory for analysis of major ions is expensive and should only be done if there is a specific use for the data. The following discussion describes a few important variables that can be comfortably and inexpensively measured in both streams and lakes and should be part of a general biodiversity sampling program for benthic macroinvertebrates. More specific measurements are discussed under the protocols for sampling lotic and lentic habitats.

Map variables — Maps (e.g. National Topographic Series) can be a source of easily obtained general information (Appendix II). In addition to latitude, longitude, and altitude, as mentioned above, it is possible to calculate catchment area surrounding a lake or upstream of a site on a stream, or stream order (e.g. Newbury 1984; Newbury and Gaboury 1993; McCullough and Campbell 1993). Specialized maps can also provide information on ecoprovinces or ecoregions, if that information is important to the study.

Area variables — It is often very useful to sketch, photograph, or videotape a sampling site to provide a permanent record of the surrounding area and its use. A video record places images in an easy-to-interpret sequence, saves extensive writing, and can include audio comment. All three visual records can record significant changes to habitat (e.g. storm damage) between sampling periods. Percentage scales can be devised to measure canopy coverage over a stream, macrophyte coverage in the water, or the extent of logging in the riparian zone; categories of riparian vegetation also can be established (e.g. B.C. Ministry of Environment, Lands, and Parks and Department of Fisheries and Oceans [BCMELPDFO] 1994). For streams, it is important to describe the riffle-pool sequence.

Sampling site variables — It is important to document the exact location of sites on lakes and streams if sampling there is to be repeated. Coordinates from maps of adequate scale are the starting points; electronic global positioning systems are also useful. Sketches, photos, or videos of the sampling site (see above) can be used to pinpoint previous sampling locations. For lakes, it is important to know which region is being sampled: littoral (shoreward), profundal (deep, below the light-controlled limit of plant growth), or pelagic (open water) (see Ruttner 1963 and Cole 1988 for definitions). For streams, sampling in either riffles or pools should be noted.

Physical and chemical factors of the environment are among the strongest determinants of the biological structure of benthic macroinvertebrates at any location. Substrate is perhaps the most important environmental factor for benthic macroinvertebrates and so it needs to be adequately described. A number of tedious methods using weights of various substrate fractions after sieving are available, but relative descriptions would probably suffice for most biodiversity surveys. Weber (1973; section on macroinvertebrates, table 1) describes a field evaluation method based on percentages of sizes of inorganic components or types of organic components for characterizing the substrate of lakes or streams. In lakes, substrate composition can be noted as proportional cover in up to three categories by using an alternating number/letter scheme as follows: 3B/2S/5G would represent an area that is 30% bedrock, 20% sand, and 50% gravel. 7M/3D/- would be a bottom type that is 70% mud and 30% debris (sticks, leaves, needles, etc.). B/-/- would be an area that is all bedrock.

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 thematerial surrounding the predominant substrates, and the degree of embeddedness. Photographic analysis of substrate is also possible (Nielsen et al. 1983) but is tedious. A recent straightforward method developed by an Australian worker (Thoms 1997) enables determination of whether the substrate at a stream site is framework- or matrix-dominated (i.e. do the cobbles provide interstitial spaces for benthic invertebrates, or are these spaces mainly filled by fine sediments?).

Water depth, shoreline slope, and exposure to winds are important measures for shoreline-dwelling lake fauna (Newbury 1984). Discharge and its associated variables are important measurements for stream fauna (Newbury and Gaboury 1993).

Primary productivity measurements (usually the concentration of chlorophyll-a) are generally useful in both lentic and lotic habitats (e.g. see Lamberti and Resh 1985; Turner et al. 1991; BCMELPDFO 1994). However, the collection, processing, and determination of chlorophyll-a concentrations is labour-intensive, time-consuming, and requires specialized equipment.

Water characteristics — In addition to pH, dissolved oxygen, and total suspended solids, used as examples above, measurements of temperature, conductivity, alkalinity, total phosphorus, nitrate, and major ions should be considered. Some of these variables can be measured by portable field instruments (e.g. pH, dissolved oxygen,

conductivity), whereas others may require laboratory analysis (e.g. total phosphorus, nitrate, major ions). Details of the chemical analysis of fresh water are discussed by Stainton et al. (1977).

Frequency and timing of sampling — In addition to using standard methods, it is important to establish the frequency and timing of sampling. Freshwater communities are non-equilibrium systems that are maintained by a flow-through of energy and materials. Interannual variability of benthic communities is high because of the many physical, chemical, and biotic factors that impinge on these communities. For example, weather, nutrient supply, and interspecific interactions all serve to regulate the benthos. For this reason, a single survey is usually insufficient to fully characterize an aquatic system; several years of data may be required to establish adequately the range of variation in community structure and productivity.

Seasonal variability of community structure and productivity is high because many species of benthic macroinvertebrates have annual (or shorter) life cycles, which culminate in an adult phase during the open-water period. Thus, the presence of mature larvae, pupae, or adults (the life stages most useful for taxonomic work: see below) may be short-lived and easily missed if seasonal development rates differ from year to year and mid-summer survey dates are chosen. It is best to sample either just after ice-out in the spring when late-stage larval forms are present but have not yet begun their final maturation, or in late fall after most species have mated and the immatures have had a chance to develop throughout the summer in preparation for over-wintering.

Sampling Procedures: Lentic

General considerations — This protocol emphasizes practical strategies for sampling biodiversity in lakes. The methods that are recommended are simple, inexpensive, easily standardized, and broadly applicable but they do not sample all of the biota. Qualitative, repeatable sampling strategies allow the broadest range of habitat possible to be surveyed for reasonable cost and effort. The objective of this approach is to provide a broad, repeatable characterization of the benthic macroinvertebrate fauna of lakes, which can document temporal change. The recommended sampling methods are additive to ensure that the program remains sufficiently flexible to suit monitoring needs at individual sites and the time and resources available.

Site selection — The reasons for selecting a particular lake are many and varied and should be the purview of the investigator. The reasons could include: (1) it is of a rare type (e.g. meromictic); (2) it is protected and so offers a long-term data set unperturbed by direct anthropogenic activity; (3) it has a special chemical status; (4) it is co-located with other monitoring activities; or (5) it has some important historical, commercial, or recreational importance.

Choice of sampling sites within lakes should maximize the diversity of habitat types sampled. For example, shoreline sites should include areas of bedrock exposure,

cobbles, sand, mud, organic debris, rooted macrophytes, regions of groundwater upwelling, different degrees of wave exposure, different types of shoreline vegetation, and lake inflows and outflows. Each of these habitats are likely to harbour different fauna. Offshore stations should span a range of depths above and below the summer thermocline in dimictic lakes. Sampling in the deepest part of a lake can often be given low priority because summer deoxygenation frequently renders the lake bottom uninhabitable to most benthic macroinvertebrates.

Care should be taken to provide an adequate description of the sampling site (see above). Special features of the surrounding area that may influence the biota found there should be noted. For example, stoneflies, mayflies, and terrestrial beetles may appear in a beach area at the mouth of an inflowing stream because the stream transported them there. Depth and substrate type are important variables to note in offshore sample locations.

Site locations must be adequately described to permit future re-sampling if a time series is planned (see above). It is often helpful to mark or note special reference points for sites that are to be revisited at regular intervals. For example, floats can be anchored at sites (e.g. Davies 1984; Campbell and Salki 1992). Headlands or prominent shoreline features such as trees can be lined up at a site from two different directions, or a compass can be used to establish bearings to two different landmarks. Once on site, an electronic fish finder can be used to pinpoint a depth or a known underwater structure.

Sample collection — Next follows a series of techniques that are recommended for use in the shoreline and offshore zones of lakes with a surface area of 20-40 ha. Each technique carries a recommendation for the minimum number of stations per lake needed to sample the fauna adequately. The initial sampling should be used to calibrate both the number of stations and the number of replicate samples needed to characterize a lake and a site, and the amount of effort devoted to collecting the samples. The samples collected can also serve to calibrate sample processing and specimen identification effort (see below). Examples of calibration efforts for lake studies can be found in Downing (1984), Stephenson et al. (1994), and Reid et al. (1995).

It is always a good idea to sweep shoreline vegetation with a net to collect adult insects when visiting a site to do aquatic collections (e.g. Lindeberg 1967; Rosenberg et al. 1988). If necessary, collections can be standardized by sweeping either over a fixed-time period or over a fixed area. The specimens collected not only provide a qualitative characterization of biodiversity, but also can serve as valuable reference material for the identification of immature aquatic forms of the same species. Adult specimens can also be collected by light trapping (e.g. Kovats and Ciborowski 1989) or pan trapping (e.g. Giberson and Rosenberg 1994). A rearing program to obtain taxonomically identifiable life stages is also recommended (see below).

1. *Shoreline sampling*

a. Rock pick (5 locations) - Investigators should spend 15-20 min on rocky shorelines turning over rocks and examining them for invertebrates. It is easier to collect 2-3 rocks at a time, place them in a white plastic pan, and return to the shoreline to examine them than to juggle equipment while still standing in the water. Organisms can be removed from the rocks using forceps and then preserved in a properly labelled vial or jar. (Preservatives and labelling hints are given below and in Appendix I). Leeches and aquatic worms often contract when they are preserved, which makes them hard to identify. They should be placed in soda water first and then preserved. Cleaned rocks should be replaced to minimize site damage.

b. Kick and sweep (5 locations) - This technique is highly versatile; it can be used on rock, sand, gravel, and mud bottoms, although it is difficult to do on highly organic substrates. The kick net is a triangular (or D-shaped) metal frame holding a mesh bag of 400-mm size (Fig. 1a). 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.

The investigator walks back and forth over the chosen area kicking up the substrate and then sweeping above the disturbed area to capture dislodged or escaping invertebrates, but leaving behind much of the debris. The net should be kept moving forward or lifted out of the water between sweeps to prevent specimens from escaping. Frequent emptying into a bucket will reduce sample loss resulting from net clogging. A standard time interval of 5 min should be used initially, but different times should be examined in the calibration study. The investigator should pass over the sampling area (~5-10 m2) twice in the allotted time because

second passes often provide more specimens than are caught during the first pass.

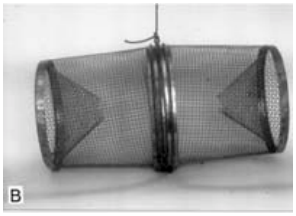
Sampling is done in depths up to 1 m, so chest waders or wet-suit pants and hard-soled, wet-suit boots are required. Chest waders are hazardous if the investigator falls; wet-suit equipment is safer and warmer.

When sampling is completed, the kick net is removed from the water and material on the sides of the net is washed down into the cup by splashing water onto the outside of the net. The cup is removed and its contents are emptied into a Whirl-Pakâ bag or a plastic jar. Material remaining in the cup can be washed into the bag by spraying the outside of the mesh (bottom of the cup) with water from a squeeze bottle. The net and cup should be checked for remaining invertebrates. If a bucket was used (see above), its contents should be washed over a fine-mesh screen (400 mm) and added to a sample jar or bag.

Sufficient preservative is added to the sample container to produce a final concentration of 4-10% formalin, 5-10% Kahle's fluid (recipe in Appendix I), or 70% ethanol. Samples preserved in Kahle's fluid or formalin should be transferred into 70% ethanol back in the laboratory that same day, refrigerated overnight, drained, and represerved in 70% ethanol the next day. This process kills specimens quickly in the field with a minimum of preservative, provides tissue fixation without dissolving calcareous deposits in the exoskeletons of some taxa, preserves colour, replaces most of the water in organisms with alcohol, and makes sorting more comfortable by reducing the amount of formalin in the sample.

A label (non-recycled photocopier paper or waterproof paper marked by soft pencil or alcohol-proof pen) accurately describing the sampling location (stream name, site number), date, replicate, and collector, is added to the inside of the bag or jar (Appendix I, II). The outside of the container should be similarly labelled using a waterproof felt pen. Careful records of sampling sites, times, and other germane observations should be kept in a waterproof, field note book (Appendix II).

c. Activity trap (10 locations) - This is a good method to sample larger invertebrates such as crayfish, leeches, and dragonfly larvae. Wire minnow traps, which have been modified to enlarge the entrances to ~30-40 mm diameter (Fig. 1b) are baited with pieces of hot dog or cheesecloth bags containing cat food, set in the evening, and retrieved the following morning (I.J. Davies, unpublished data). Samples obtained do not require sorting, and can be preserved in 70% ethanol directly. Another kind of activity trap, consisting of a bottle with a funnel suspended in the water column, is described by Murkin et al. (1994; fig. 7E). Depth of placement may significantly affect total catch and taxa collected by activity traps (T.D. Galloway, University of Manitoba, personal communication).



2. *Offshore sampling*

a. Grab (5 locations at mid-depths and/or 5 in the profundal zone) - Grab sampling is especially effective in fine-grained, soft substrate. Grab samples are taken from a boat at mid-depth (less than 2m but above the maximum depth of the mid-summer thermocline; usually ~5 m) or in the oxygenated profundal zone (from the base of the maximum summer thermocline down to the upper boundary of the zone of oxygen depletion; usually 7-10 m). Marker buoys can be set out prior to sampling to mark the stations (see above).

The tall Ekman (15.2 x 15.2 x 30 cm) is the most popular grab used in lakes (Burton and Flannagan 1973; Fig. 1c). This grab can be fitted with lead weights on the sides to ensure penetration into the substrate. However, care should be taken not to drive the grab too deeply into the sediments because most surface-dwelling organisms will be missed, and the grab may be hard to retrieve. The grab should be carefully lowered to the substrate, raised slightly and lowered again (while maintaining tension on the rope) to ensure that the grab stays upright. The messenger is then dropped down the rope to trigger the jaws to close, and the grab is retrieved. Sample depth should be noted as a check on position.

The quality of the sample should be examined before the sample is sieved. A bucket should be brought under the grab just below the surface of the water; the bucket and grab are then brought into the boat. If the jaws are closed and the grab is 20-80% full (open the top flaps to see),

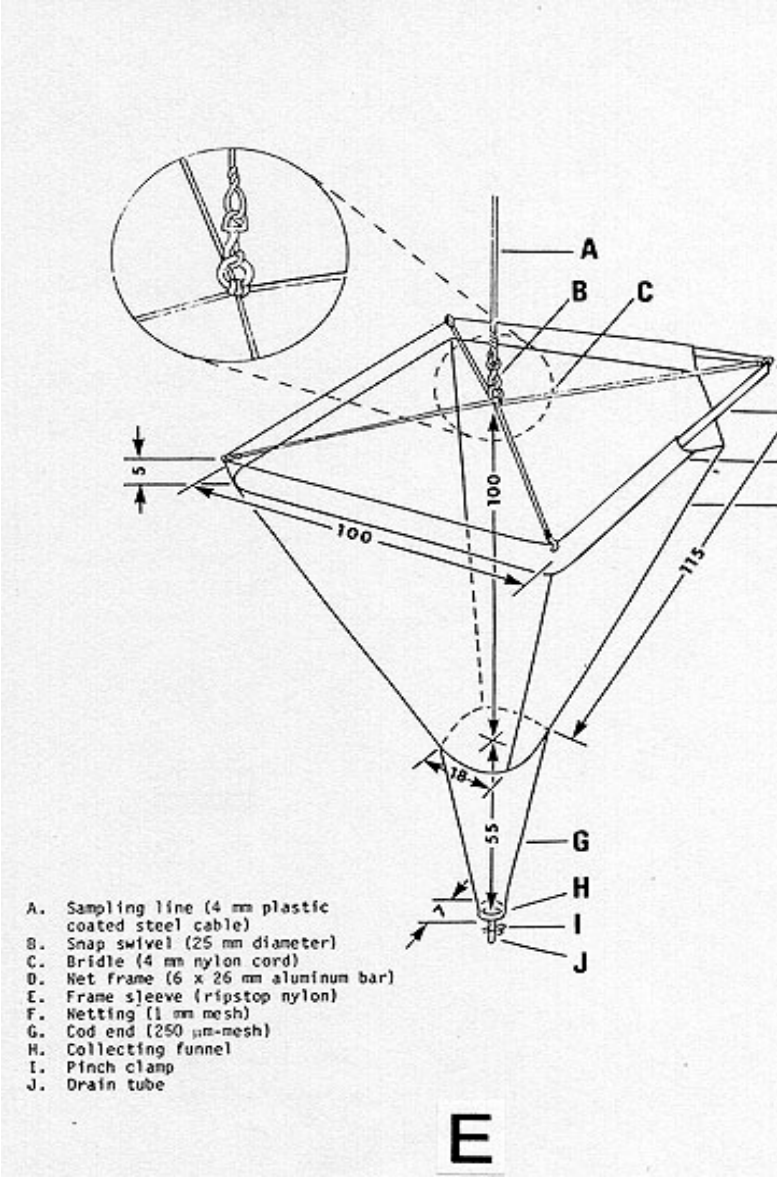
then the sample is probably acceptable. The jaws are then opened so the mud sample falls into a mesh bag (400 mm) for sieving and sample-size reduction. The mesh bag is attached to a circular metal frame by a canvas sleeve and may be fitted with a removable cup to facilitate sample removal (Fig. 1d). The sieve net is agitated vigorously until most of the substrate passes through. The net is washed down, the contents of the cup are added to a container, and the sample is preserved and labelled as outlined above.

A 400-mm mesh sieve is recommended for general purposes in lakes. However, the mesh size should be adjusted depending on the objective of the study. For example, a requirement to collect early instars may mean a smaller mesh size should be used. Small mesh sizes will require longer sieving times and will result in larger samples than when using larger mesh sizes.

The Ekman grab samples a uniform surface area of substrate, which produces a quantitative estimate of the number of organisms per m2, or standing stock. The conversion factor to number per m2 for a 15.2 x 15.2 cm grab is 43. The grab is one of the few lentic sampling devices that is quantitative. Some investigators prefer to measure the volume of mud collected and express the number of organisms on a volumetric basis.

b. Nighttime vertical net tow (3 locations) - A nighttime, vertical, net tow will capture invertebrates that are benthic by day but planktonic at night (e.g. Mysis relicta Lovén, Chaoborus spp.). Such organisms usually can escape sampling by grabs or are present on the bottom in such low numbers that they are missed by grab sampling. The net to be used is described in Nero (1982) and Nero and Davies (1982) (Fig. 1e). The net has an aperture of 0.5 m2 and is fitted with a 500-800-mm mesh net. It is lowered to the bottom, raised 1 m to prevent filling with sediment, held stationary for 1 min to allow redistribution of organisms in the overlaying water column, and then pulled to the surface at a rate of 0.25-0.5 m·s-1. Net contents are emptied into a labelled bottle (see above) through a 1 cm

or larger ball valve or petcock in the codend and the net is rinsed three times by backwashing to ensure complete sample removal. Seventy-percent ethanol is the best preservative for these samples because it avoids distortion of the more delicate organisms collected; the samples are usually free of organic debris.



Sampling stations should be located at mid-depths or in the oxygenated profundal zone. Sampling should begin 1 h after dark, preferably on a cloudy night because organisms such as M. relicta are quite sensitive to light and will not migrate upward under a strong moon.

Sampling for biomass — The methods described above can also be used to collect samples for biomass determination. Either a separate set of samples is taken, or the samples taken for number counts can be split

into halves. Samples meant for biomass determination should not be preserved because most preservatives leach organic substances and this will affect biomass (see Rosenberg 1978 for references). However, freezing these samples is acceptable.

Another, more time-consuming method is to develop length:weight regressions (e.g. Rosenberg et al. 1988; Nolte 1990). This allows biomass to be predicted from measurement of body length or length of a single body part on an organism.

Sampling Procedures: Lotic

General considerations — The general protocol described below is meant to apply to most situations of sampling for benthic macroinvertebrates in wadeable streams or the wadeable shoreline of large, deep rivers. The latter habitats require safety precautions: (1) sample with a partner onshore, (2) stay in water less than 1 m deep, (3) wear a lifevest, and (4) have a bundled safety line stationed downstream that can be tossed out by the partner in the event the person sampling falls and is carried downstream by the current.

The method recommended below is designed to integrate different habitats (e.g. see Cuffney et al. 1993) within a stream reach. As such, it usually produces samples with relatively low coefficients of variation (see below). Nonetheless, calibration steps are advisable to determine the optimal number of samples, as described for lake sampling. The method works best in cobble substrates and least well in slow-moving, sand- or mud-bottom streams. Kellogg (1994) describes sampling in mud-bottom streams. This protocol uses a D-frame kick net and distinguishes four habitats: (1) steep banks/vegetated margins, (2) silty bottom with organic matter, (3) woody debris with organic matter, and (4) sand/rock/gravel substrate. Alternatively, lake protocols may be adapted to sampling depositional habitats in streams or rivers.

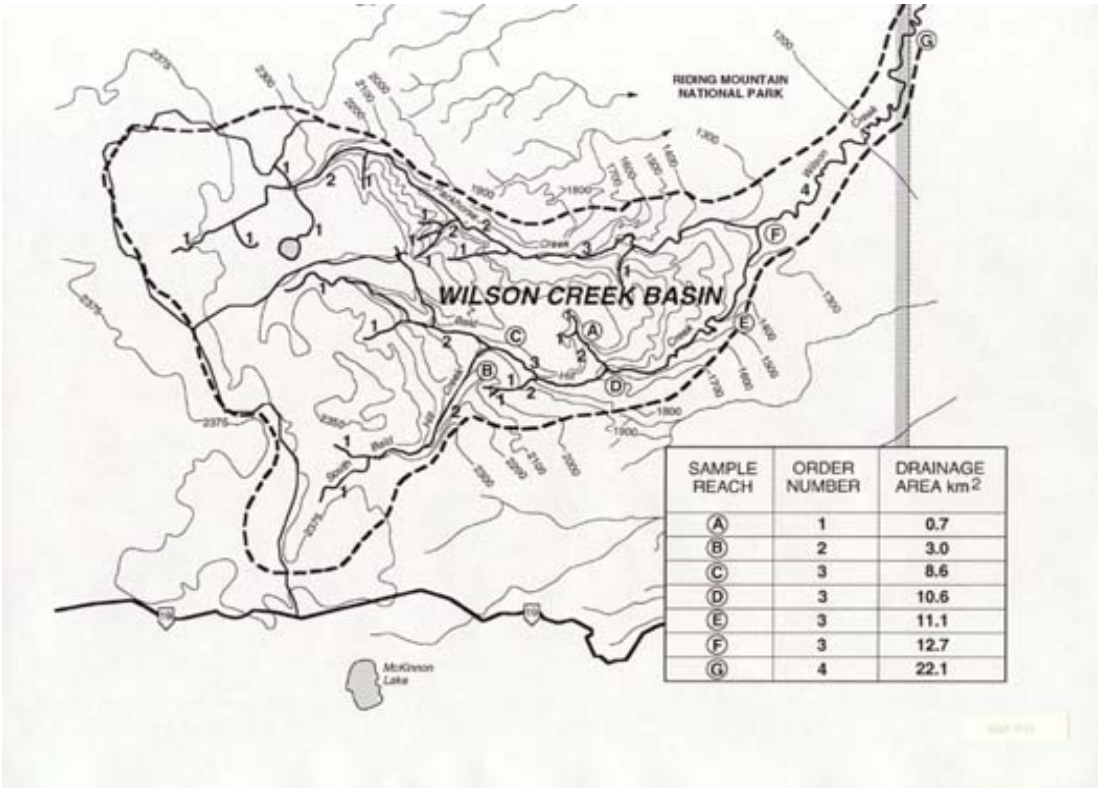
Alternative methods may be used, depending on the purpose of the survey, and a few examples are given below. If a habitat-related question is being examined, then it may be necessary to sample specific habitats such as detritus, woody debris, etc. The selected procedure should be used at all sites.

Timing of sampling — The general consideration presented for lentic sampling above also applies to streams (see also Cuffney et al. 1993). Most stream species are best sampled in the early spring and late fall, as for lakes, but several lotic species have unique life histories that require special timing. For example, winter stoneflies are most easily sampled as nymphs during the winter or as adults in the spring, just prior to ice break-up (Flannagan and Cobb 1983). Some mayflies have short life histories and may be missed by a single sampling. More frequent sampling will be necessary if the study intends to collect these species.

When to sample a stream is a trade-off between time of year and accessibility. Late spring sampling will provide many large, near-mature specimens, but high discharge or spate may limit access to the sampling site. On the other hand, flows are usually minimal in late fall but many species may be in early stages of their development and so identification, which is usually based on mature specimens, will be difficult.

Site selection — A general biodiversity survey requires coverage of different reaches in a stream catchment. Sampling sites can be defined in the context of a single catchment and in relation to regional hydrology. Maps and aerial photographs can be used to select typical stream reaches that are not affected by either anthropogenic influences (e.g. dams, bridges, clear-cut areas) or natural influences (e.g. tributary confluences, unique modifying factors such as waterfalls).

For stream catchments greater than 50 km2 in area, 1:250,000-scale National Topographic Series maps are suitable for selecting sample sites, but for small catchments 1:50,000-scale maps are recommended. The catchment can be delineated by following the drainage divides, or the highest points from which water flows in one direction or the other (Fig. 2). The line eventually meets the stream at the lower end of the catchment. Once the catchment has been outlined, stream-channel segments can be determined using the Strahler (1964) method (Fig. 2). Thus, stations that represent different stream orders can be established within a catchment.



SAMPLE REACH	ORDER NUMBER	DRAINAGE AREA km^2
A	1	0.7
B	2	3.0
C	3	8.6
D	3	10.6
E	3	11.1
F	3	12.7
G	4	22.1

Figure 2

Sampling stations can then be chosen at the scale of the stream reach, which can be variously defined as 6x bankfull width (including one riffle and one pool) or 12x bankfull width (including two riffles and two pools: a meander; Newbury and Gaboury 1993). However, Newbury (1984) recommends that hydrological exploration should include a reach that is 20-30x channel width to determine average channel characteristics. It is important to take photographs (or videos) and make sketches at the sampling scale chosen. Photographs should include upstream and downstream views of the reach in which the sampling site is located, habitat types such as riffles and pools, and the substrate (see above). The sketch to scale should include the stream channel; floodplain and riparian vegetation; major flow habitats such as riffles, pools, and chutes; natural obstructions such as logs and boulders; and locations of hydrological transects and biological sampling sites (Fig. 3).

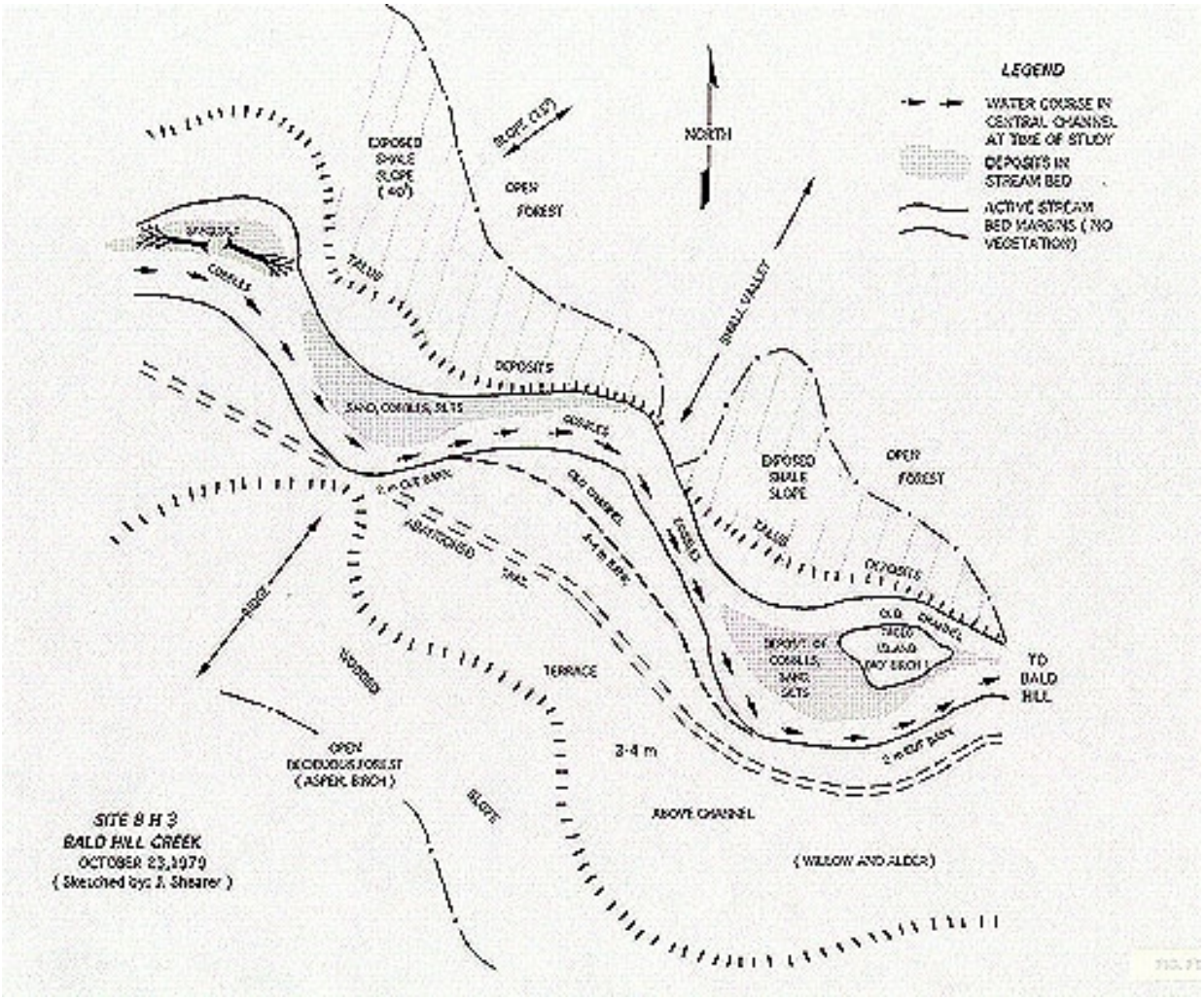


Figure 3

To relocate sites on subsequent visits, trees can be flagged on opposite banks, and a measured line pinpointing the site can be stretched between them. The same result can be achieved by driving an iron stake into the shoreline and measuring distance from it to the site or from a prominent boulder to the site. Details of the site can be marked directly on the ground-level photographic prints already taken (see above).

Characterizing the stream channel — 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 spates 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).

Average channel width and depth for a reach can be determined by measuring several pools and riffles. Transects are established at right angles to the flow; a tape measure is stretched across the stream and secured to each bank; and width and depth of the present and bankfull flows are measured at 1/6 intervals across the total width (Fig. 4).

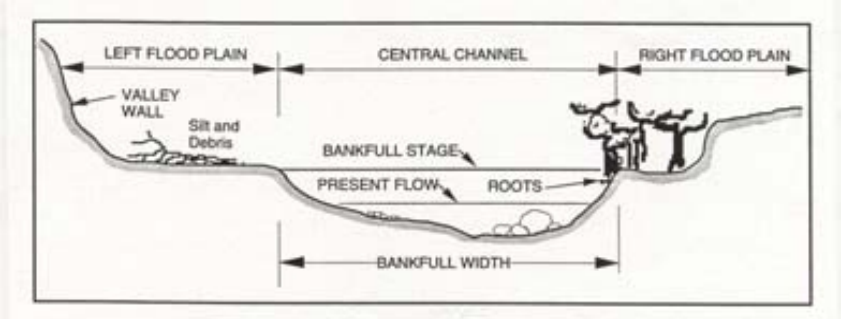


Figure 4

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

Substrate characterization — The many different ways to characterize stream substrates have been discussed under "Abiotic factors", above. However, the "random walk" technique of Newbury and Gaboury (1993) is a simple, quantifiable method meant for streams. The investigator walks through the stream reach and every several steps randomly selects a substrate particle. He or she measures the length, width, and

height of each of 48 or 96 particles. The mean diameter is determined by averaging the three dimensions. The averages are then ranked and a cumulative frequency distribution curve is plotted (Fig. 5). The characteristic or median particle size for the reach, can be obtained from the plot.

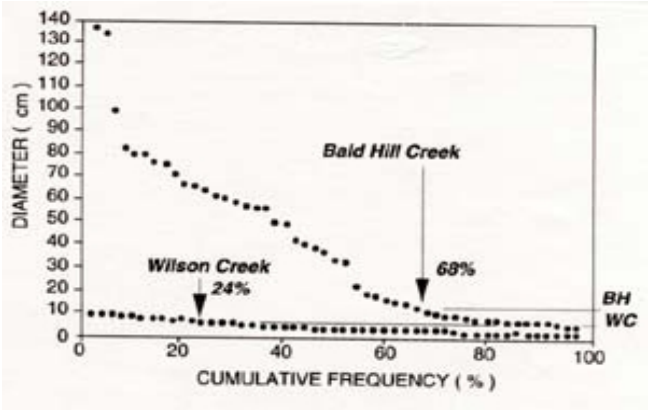


Figure 5

These procedures should be adequate for site characterization in biodiversity studies. More detailed methods (e.g. water velocity and discharge, surface slopes) are described in Newbury and Gaboury (1993) and Harrelson et al. (1994).

One final note concerns the strategy of stream sampling. *In situ* measurements such as for temperature, dissolved oxygen, and conductivity, collection of water samples for laboratory analysis, and biological sampling should be done before hydrological analyses to avoid disruption of the substrate.

Sample collection — Next follows a description of the kick-net technique recommended for general biodiversity sampling in wadeable streams. An alternative, stratification of habitats for separate sampling, is also discussed.

Most biodiversity studies require species-level identifications, so it is advisable to collect adult specimens to aid in identification of immatures taken from a stream. Therefore, as for lakes, it is useful to take sweep-net samples along shoreline vegetation. Light trapping and pan trapping (see references above) will also help provide adult specimens. A rearing program is also recommended (see below).

1. *Kick-net sampling* — The kick net (Fig. 1a) is described under the lentic protocols, above. A 400-mm mesh net is recommended for general sampling; use of finer or coarser mesh will depend on the objectives of the study. For example, a life-history study of certain species will probably require no larger than 200-mm mesh, whereas a 1000-mm mesh is all that is required for a broad survey of large forms.

   The kick net is placed downstream of the collector, flat side of the triangle resting

on the substrate of the stream. The collector walks backward, away from the net, kicking the substrate to disturb it to a depth of ~5 cm. 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 collector zig-zags over the stream bottom from bank to bank in an upstream direction for a timed period (e.g. 2-5 min). Standard time collections (e.g. 3 min) allow comparisons among sites. The zig-zag coverage allows collection of invertebrates from a variety of stream habitats (pools, riffles, runs, etc.). 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.

When sampling is completed, the net and cup are washed, the sample is preserved, and the container is labelled as described above under the lentic protocols (see also Appendix I, Appendix II). The comments made about biomass samples under the lentic procedures described above are also applicable to lotic sampling.

A modified procedure is used by Manitoba Environment (D. Williamson, personal communication). The kick net is equipped with a light anchor and a rope of sufficient length that the distance travelled by the kick net yields an area of ~1 m2. The light anchor is dropped into the stream and kicking proceeds upstream until the rope becomes taut. The net is then emptied. Five replicates are taken in this way at each site.

2. *Additional sampling methods* — It may be necessary to distinguish among habitats that are being sampled if you are interested in taxa that only occur in certain habitats or in separate estimates of the benthic macroinvertebrates in different habitats. For example, the deep parts of large rivers can be sampled using a variety of grabs or suction devices (Rosenberg 1978; Elliott and Tullett 1978, 1983, 1993; Downing 1984). If it is not possible to sample this habitat directly, then indirect sampling methods such as artificial substrates (e.g. Rosenberg and Resh 1982), light traps, or pan traps can be used.

   Areas of groundwater infiltration provide unique habitats for benthic macroinvertebrates. Such areas can provide refugia by remaining open in the winter when the rest of the stream freezes or during summer droughts when much of the channel desiccates. The water chemistry of these areas is more typical of ground water than stream water (i.e. cooler, higher conductivity), so they can be easily identified. The kick-net method described above is satisfactory in these special habitats, but care needs to be taken not to obliterate the habitat if it is rare.

   You may be interested in the macroinvertebrate fauna of woody debris. Manual collection of wood particles and removal of specimens as described for the lake rock pick (see above) may be required (see also Cuffney et al. 1993). Some invertebrates that adhere tightly to stony substrates and trailing vegetation may

be missed by kick-net sampling. These two habitats require close visual inspection. Other special habitats may require specialized approaches and sampling equipment. Examples of such habitats include, but are not restricted to, the hyporheic (stream-interstitial) zone (e.g. Williams 1984) and phytotelmata (plant-container habitats; e.g. Barton and Smith 1984).

Sample Processing

General considerations — Samples from lakes or streams can be sorted either before or after preservation. Live sorting can be done either on shore, adjacent to the sampling site, or in a laboratory within a few hours of collection. Unpreserved samples should be sorted quickly to avoid the possibility that invertebrate predators will alter species composition. Small amounts of the sample should be placed in a shallow, light-coloured pan with sufficient water to allow organism movement. Small portions of the sample are moved toward the empty part of the pan, and freed animals are collected by forceps, spoon, or eye dropper. Alternatively, the sample can be added to a bucket of water and swirled to suspend invertebrates and organic matter. Small amounts of the supernatant are then successively poured through a fine sieve, and the invertebrates are removed and preserved. The procedure is followed until only inorganic particles remain in the bucket. The organic matter remaining in the sieve can either be discarded or preserved for closer examination in the laboratory.

If the goal of the study is a qualitative characterization of biodiversity, then it may be sufficient to sort for a 15-min period or to collect the first 300 animals, providing that as many different kinds of invertebrates as possible are removed. Removing only the largest or the most abundant kinds is not recommended. Rare species may be missed, especially if several similar-looking species are present; only microscopic examination of preserved specimens can avoid this problem.

Samples that are preserved and returned to the laboratory can be sorted in more leisurely and more quantitative fashion. Formalin-fixed samples should be rinsed thoroughly before sorting, and preserved in 70% ethanol if they need to be further stored before sorting. It is probably easiest to do so in two stages: (1) a coarse sort, in which large invertebrates or pieces of debris are removed from bits of the sample placed in a shallow, white pan, followed by (2) a fine sort in which bits of the rest of the sample are examined by eye, under a magnifying glass, or under a binocular microscope at 12-16x power. Each bit of sample is examined closely, debris is teased apart, and all macroinvertebrates are removed and placed into water- or alcohol-tight 10 ml glass vials containing 70% ethanol, according to the most-easily-identified taxon (see below). Accurate counts of each of the taxa are kept on laboratory sheets specially designed for this purpose. The sheets have a caption for essential information such as the date of sampling, name of the lake or stream, site location, type of sample, sample collector, and sample sorter. Column headings include taxon, life stage (larvae, pupa, or adult), counts, and conversion factors (if applicable) to number per m2.

All residues of sorted samples should be retained for as long as possible after the study is over, and 10% of the samples in a set should be resorted by different people. Sorting efficiency should be at least 95% in the resorted samples or further training of laboratory staff may be indicated.

Sorting aids — A number of methods facilitate the removal of organisms from background debris and their enumeration, as follows: (1) vital stains, (2) flotation or elutriation, (3) subsampling, and (4) automated counters (Rosenberg 1978; Murkin et al. 1994; Table 3).

1. *Vital stains* — The following stains will turn organic material bright pink and make it easier to spot amidst the debris in a white pan:
   a) Rose Bengal - 1-1.5 g of crystals dissolved in 100 ml of 70% ethanol.
   b) Eosin B and Biebrich Scarlet - 1 g of each compound dissolved in 10 ml of 95% ethanol and added to 100 ml of distilled water.

   A few ml of either stain are added to ~500 ml of sample to be sorted and the sample is left for 24 h. The sample can be washed with water to remove excess stain. Organisms will be preferentially stained; for example, Rose Bengal works best on soft tissues but does not stain mollusc shells or heavily chitinized insects. Rose bengal may not be useful in samples containing large amounts of organic debris because it stains the growing tips of roots. Eosin/Biebrich does not stain plant debris to the same extent, so it may be better suited to highly organic samples.

2. *Flotation and elutriation* — These techniques work best for highly inorganic samples. Flotation involves submersing the sample in a solution with a specific gravity higher than that of the organisms (Murkin et al. 1994). The invertebrates then float to the surface where they can be removed. However, light organic matter will also float, and invertebrates with heavy shells, soil tubes, or cases may not. Common flotation solutions include: sugar, sodium chloride, calcium chloride, D-mannitol, magnesium sulfate, and kerosene. Use of kerosene tends to be messy. References to the use of flotation to clean samples can be found in Rosenberg (1978) and Murkin et al. (1994).

   Elutriation depends on invertebrates being carried upward in a column by bubbles of air (e.g. Stewart 1975; Kingsbury and Beveridge 1977; see also Rosenberg 1978). An overflow trough collects the organisms and light debris. The process may not work for heavy invertebrates such as clams.

   Both methods require that sorting efficiency be determined. Thus, the original residues of at least 10% of the samples should be hand-sorted to determine the proportion of benthic macroinvertebrates left behind. Efficiency of separation should be high (~95%), but if it is lower it should be consistently so, and there should be no obvious taxonomic bias.

3. *Subsampling* — Samples are usually subsampled to save processing time because either the samples are excessively large or there are large numbers of them. Thus, the sample is quantitatively reduced, the invertebrates from a known portion of the sample are counted, and these counts are extrapolated back to the entire sample. Samples need to be homogeneous, so large organisms or pieces of debris should be removed prior to subsampling. Several methods are available (e.g. see Rosenberg 1978; Murkin et al. 1994); for example, the volumetric method of Wrona et al. (1982), the weight-based method of Sebastien et al. (1988), and the spatial (sample-splitting) method of Marchant (1989) have proven reliable. In the Marchant (1989) method, a predetermined number of invertebrates (100, 200, or 300) is removed from a box subdivided into 100 cells (Fig. 6) and then counting stops. No matter what method is used, precision and accuracy need to be assessed initially by comparing selected subsamples to the total sample (Gibbons et al. 1993; Murkin et al. 1994). Subsampling error should be estimated in at least 10% of the samples being processed by sorting another subsample of equal size (Environment Canada and Department of Fisheries and Oceans 1993); allowable deviations between counts should be set at a reasonable but consistent level.

Figure 6

Rare species may be missed by subsampling, which is an important concern in biodiversity studies. Thus, rare species deserve special consideration when selecting a subsampling method (Murkin et al. 1994); alternatively, avoid subsampling completely.

4. *Automated counters* — The process of counting organisms of various taxa can be facilitated by using mechanical, desk-top devices that are subdivided into

8-10 keys. These counters can also be worked by foot pedals. It is also possible to use a voice-activated electronic counting system that enters the data directly into a database (Williams and Briton 1986; A. Salki, Freshwater Institute, personal communication). The products of mechanical counters should be added to laboratory sheets before entry into electronic databases. A hard copy should also be kept of the voice-activated counts.

Data Analysis

Four different types of biodiversity studies may be undertaken: (1) pilot or reconnaissance studies at the beginning of a full-fledged program; (2) descriptions of population or community characteristics; (3) detection of differences in populations or communities between or among sites; and (4) initiation of a long-term, rapid-assessment program. Each of these studies has its own data-analytical requirements, so it is important to decide the objective of a biodiversity study at the outset.

Pilot/reconnaissance studies — Initial, small-scale studies are needed to calibrate sampling methods for a definitive study. Pilot or reconnaissance studies can be used to reveal what organisms occur, their approximate densities, and their spatial aggregations, all essential elements in the design of a full-scale program. For example, the number of replicate sample units is usually determined at this stage of a study (Resh and McElravy 1993). The size of the mean, the degree of aggregation, and the desired precision will influence how many samples should be taken to estimate the mean of a benthic measure (Resh and McElravy 1993). The reviews cited in Table 3 and most statistics texts provide appropriate formulae.

In some small-scale biodiversity studies, it may be necessary to take a pre-determined number of sample units because of budget considerations. In this case, six sample units will generally provide estimates ±40% of the mean total number of individuals (95% confidence intervals) in a community (Resh and McElravy 1993).

Population/community characteristics — Conservation studies may require an estimate of the density of a particular species. Therefore, precise estimates of the mean would be an appropriate goal (Table 3). Large numbers of samples may be required to obtain such estimates (e.g. Merritt et al. 1996).

Detection of differences — Studies of the effects of disturbance often require the determination of the difference in means over time or at different sites. This involves a different approach than estimating specific population or community measures with a given level of precision; the reader is directed to Resh and McElravy (1993), Norris and Georges (1993), and Merritt et al. (1996) for a discussion of the subject and an introduction to the literature.

The detection of differences among sites or times has traditionally relied on quantitative approaches using inferential statistics (e.g. Green 1979; Stewart-Oaten et al. 1986; Underwood 1991). However, a new approach called the "reference condition"

(Reynoldson et al. 1995, 1997; Wright 1995), which uses qualitative sampling and multivariate statistics, circumvents many of the problems inherent in quantitative, inferential approaches (Reynoldson et al. 1997).

Rapid assessment approaches (RAAs) — The high cost of quantitative approaches has led to the development of qualitative methods called RAAs (e.g. Plafkin et al. 1989). The original purpose of using RAAs was to identify water quality problems and to document long-term regional changes in water quality (Resh and Jackson 1993), but these methods can also be applied to measuring changes in biodiversity.

Qualitative techniques have been used in Europe for decades (e.g. biotic indices and scoring systems; see Metcalfe 1989). Their chief advantage is the reduction of the intensity of study required at individual sites (relative to what is required by quantitative approaches: see above), which permits a greater number of sites to be examined (Resh and Jackson 1993). Key to the use of RAAs are the following considerations: (1) What population and/or community measures are relevant? (2) What are the baselines against which these measures are being compared? (3) How much deviation from a baseline indicates change? Resh and Jackson (1993) provide a comprehensive review of RAAs.

Quality Assessment/Quality Control (QA/QC)

A QA/QC plan is essential to the success of any monitoring program. It provides a basis of believability for the results and helps to structure the work. This structure takes the form of field notes, checklists, chain-of-custody forms for samples, sample-processing forms, check samples for taxonomic identifications, third-party verification of taxonomic identifications (or even statistical analyses), data screening, and database maintenance (e.g. see Norris and Georges 1993 for a review). For example, the development of standard field/laboratory forms (Appendix I) should be considered for large-scale biodiversity programs. QA/QC requirements should be woven through the entire fabric of the biodiversity program.

A few suggestions for QA/QC have been included in the discussion of sampling and sample processing above. Investigators are referred to the QA/QC chapter of this binder for a more detailed treatment of the subject, and to Gibbons et al. (1993), and Environment Canada and Department of Fisheries and Oceans (1992, 1993, 1995) for actual examples of QA/QC programs pertinent to benthic macroinvertebrates.

Roughly 20% of program resources should be devoted to QA/QC, although more will be required if the results are intended for legal or policy use. Investigators should be able to design the QA/QC procedures that are best suited to their needs and available resources from the literature provided here.

Volunteer Involvement

Freshwater benthic macroinvertebrates are a prime subject for biodiversity studies by the non-specialist volunteer. They are used extensively by lay groups for monitoring water quality in the USA (e.g. Kerr et al. 1994) and Canada. The Save Our Streams (SOS) program sponsored by the Izaak Walton League of America (707 Conservation Lane, Gaithersburg, MD, 20878-2983) is an excellent example.

We encourage volunteers interested in biodiversity studies to get involved in already existing, national programs rather than striking out on their own. Biodiversity programs, like water-quality monitoring programs, require a great deal of coordination in the training of individuals, collection of samples, identification of specimens, and maintenance of records. These activities are best done by a central organization. The Ecological Monitoring Coordinating Office (P.O. Box 5050, Burlington, Ontario, Canada, L7R 4A6) may be able to inform you about biodiversity studies in your area. Contact with local provincial or federal departments of environment or natural resources is also encouraged.

For people interested in restricted surveys (e.g. a lake or stream on which they have a cottage), it is possible to follow scaled-down versions of the protocols presented above. A lake survey would be restricted to the shoreline where rock picks, kick and sweep samples, or activity traps could be used. The kick-net technique could be used in a stream survey. Sites should be adequately described in both systems. Qualitative sorting should be sufficient. Needham and Needham (1962), Clegg and Anthon (1968), Lehmkuhl (1979), Kellogg (1994), or any of the commonly available field guides to aquatic insects should suffice for identification. A local reference collection should be established if the survey is to be repeated. It would also be advisable for the volunteer to establish liaison with a nearby expert who can advise on sampling design and taxonomy.

Volunteers represent an extremely valuable potential source of interest and manpower in doing benthic macroinvertebrate surveys (e.g. Reynoldson et al. 1986). However, care must be taken to coordinate their activities so their efforts are not wasted.

Acknowledgements

We thank B. Finnamore, T. Galloway, D. Williamson, and two anonymous reviewers for their helpful comments. R. Janusz provided originals of the Wilson Creek figures.

Experts to Contact For More Information

A number of Canadian specialists can be consulted for help in the design and analysis of in-depth benthic macroinvertebrate sampling programs in stream and rivers. These specialists include:

1. Dr. J.J.H. Ciborowski
Department of Biology
University of Windsor
Windsor, Ontario, Canada
N9B 3P4

2. Dr. M.H. Colbo
Department of Biology
Memorial University
St. John's, Newfoundland, Canada
A1B 5S7

3. Dr. L.D. Corkum
Department of Biology
University of Windsor
Windsor, Ontario, Canada
N9B 3P4

4. Dr. D.A. Craig
Department of Biological Sciences
University of Alberta
Edmonton, Alberta, Canada
T6G 2E9

5. Dr. J.M. Culp
Environment Canada
National Hydrology Research Institute
11 Innovation Boulevard
Saskatoon, Saskatchewan, Canada
S7N 2X8

6. Dr. T.D. Galloway
Department of Entomology
University of Manitoba
Winnipeg, Manitoba, Canada
R3T 2N2

7. Dr. D.J. Giberson
Department of Biology
University of Prince Edward Island
Charlottetown, Prince Edward Island,
Canada
C1A 4P3

8. Dr. L. Hare
INRS-EAU
Université du Québec
C.P. 7500
Sainte-Foy, Québec, Canada
G1V 4C7

9. Dr. P.-P. Harper
Departement de Sciences Biologiques
Université de Montréal
C.P. 6128, Succ. A.
Montréal, Québec, Canada
H3C 3J7

10. Dr. A. Morin
Ottawa-Carlton Institute of Biology
University of Ottawa
P.O. Box 450, Station A
Ottawa, Ontario, Canada
K1N 6N5

11. Mr. R.A. Reid
Ontario Ministry of Environment
and Energy
Dorset Research Centre
Bellwood Acres Road, P.O. Box 39
Dorset, Ontario, Canada
POA 1EO

12. Dr. J.B. Rasmussen
Department of Biology
McGill University
1205 Dr Penfield Avenue
Montréal, Québec, Canada
H3A 1B1

13. Dr. J.S. Richardson
Westwater Research Centre
University of British Columbia
Vancouver, British Columbia
Canada
V6T 1Z2

14. Dr. I.R. Walker
Department of Biology
Okanagan University College
1000 K.L.O. Road
Kelowna, British Columbia, Canada
V1Y 4X8

15. Dr. H.E. Welch
Freshwater Institute
501 University Crescent
Winnipeg, Manitoba, Canada
R3T 2N6
(Arctic Canada Specialist)

16. Dr. D.D. Williams
Division of Life Sciences
Scarborough Campus
University of Toronto
West Hill, Ontario, Canada
M1C 1A4

Appendix I: Special Considerations in Field Sampling and Laboratory Processing

Fixatives and Preservatives

The terms "fixative" and "preservative" have been used interchangeably throughout this protocol. However, formalin and Kahle's fluid (see 1 and 2 below) are properly referred to as fixatives, and are used to kill and fix organisms in the field, whereas ethanol (see 3 below) is properly referred to as a preservative, and is intended for long-term use.

1. **10% formalin** (100% formalin = 37% aqueous formaldehyde solution; 100 mL of 100% formalin + 900 mL of water = 10% formalin) - A good general-use fixative, although specimens will lose colour and become brittle and the shells of molluscs will dissolve after long-term storage. Buffered formalin will avoid the latter. It is best to use formalin as a field fixative and transfer specimens into 70% ethanol for long-term storage. Many investigators prefer not to use formalin at all because it is a skin and eye irritant and may be carcinogenic under prolonged exposure (National Cancer Institute 1996).

2. **10% solution of Kahle's fluid in water** (to make 1L: 15 parts by volume (pbv) of 95% ethanol [290 mL]; 6 pbv of 100% formalin [115 mL]; 1 pbv of glacial acetic acid [20 mL]; and 30 pbv of distilled water [575 mL]; see Wiggins 1977) - This is a good fixative for most insects but should be replaced within hours with 70% ethanol for molluscs and crayfish. It is acidic and will remove calcium from shells and carapaces.

3. **70% ethanol in water** (750 mL of 95% ethanol topped up to 1 L with water » 70% ethanol) - A good preservative, especially for crustaceans and insects, but unlike formalin it does not fix tissues. Its main advantage is low toxicity to humans. However, large volumes are required, it is expensive, and concentrations >40% can only be obtained with a permit. Denatured ethanol may be available in higher concentrations. Isopropyl (rubbing) alcohol or the more toxic methanol (methyl hydrate) can often be used as substitutes.

The volatility of ethanol means that it evaporates readily. Thus, collections stored in ethanol must be periodically monitored. Addition of a few drops of glycerine to vials containing alcohol-preserved material will protect them from desiccation, and will keep the specimens from becoming brittle.

Labelling

Field labels must be added to the inside and outside of vials, jars, or plastic bags. Labelling can be done directly on the vessel or, if it is to be reused, on a piece of duct tape stuck to the outside. Inside labels should be written with a soft-lead pencil on non-recycled, high-quality paper (Appendix II). Labels should include the station number/location, the lake or stream, and the date. It is important that persons receiving the samples for processing understand any date abbreviations used. The samples

taken should be entered in the field data book.

Laboratory labels must be added to the inside of vials containing organisms sorted to higher taxa as well as those that have been identified to lower taxa and verified by an expert. Such labels should be written in indelible ink on high-quality or waterproof paper (Appendix II), and should include the following information: (1) collection site, (2) date of sampling, (3) identity of taxon, (4) number of specimens, and (5) identifier.

Blank labels for field and laboratory use can be prepared en masse on a sheet using a word processor and a laser printer or laser photocopier. (Ink-jet printing runs in water or ethanol). They are then cut apart for use. Packs can be prepared for field use by applying rubber cement to one end of a bundle of labels. Sheets of blank labels can be photoreduced for use in small vials.

Standard Field/Laboratory Forms

Large-scale biodiversity surveys are well-served by the development of standard field and laboratory forms, which document characteristics of the habitat sampled, include site drawings or photos, and track samples from the point at which they were taken through to processing and identification of organisms. Examples of standard forms can be found in Cuffney et al. (1993) and Kellogg (1994). (A site form developed for a large biomonitoring study on the Fraser River, British Columbia, is available from either D.M. Rosenberg or A.P. Wiens).

Identification of Specimens

The value of sweep-net collections of adult insects along shorelines to the identification of immature aquatic forms has been discussed above. Another, more time-consuming endeavor is to establish rearing programs to provide associated stages. The taxonomy of aquatic insects is based mainly on the adult form, although the immatures are the forms most frequently collected in aquatic sampling. If the adult, cast pupal skin, and cast last larval skin are available for holometabolous insects (i.e. those with complete metamorphosis such as midge flies), or the adult and a series of cast nymphal skins are available for hemimetabolous insects (i.e. those with incomplete metamorphosis such as mayflies), then the immature forms can often be identified by working backward from the adult. Merritt et al. (1996) review field-based and laboratory rearing methods for major insect groups.

The specimens sorted into major taxa and stored in 10-ml glass vials (see above) can be identified to lower levels by using two excellent, North American texts: Thorp and Covich (1991) for non-insect benthic macroinvertebrates and Merritt and Cummins (1996) for the insects. Both texts provide keys to genera and references to the more specialized literature for species-level determinations.

Once identified, specimens belonging to the same taxon should be stored in their own vial or in a group of shell (tiny) vials plugged with cotton and placed together in a larger

vial. Accurate labelling is essential. Counts should be carefully entered onto the sorting sheets described above, or onto sheets specially designed for lower taxa. These data can eventually be entered into an electronic database.

Non-specialists may find it difficult to identify most benthic invertebrates to the species level. Hence, it is wise to send representative, identified material to qualified systematists for verification or get the systematists directly involved in the study. The experts listed above will be able to recommend systematists who specialize in taxa of great relevance to the study. A voucher collection of identified/verified material should be prepared (and curated) for future reference. Curation is important because vials containing alcohol will dry out over time. Voucher collections often prove invaluable in rechecking data, and in taxonomic revisions.

Appendix II: Sources of Equipment and Supplies for Studies of Macroinvertebrate Biodiversity

Disclaimer: Listing of suppliers or sampling devices does not constitute endorsement by the authors of this report. It is intended only as an initial source of information.

Maps and Planning Material

1. The most comprehensive source for maps in Canada is The National Atlas Information Service, Room 650, 615 Booth St., Ottawa, Ontario, Canada, K1A OE9; phone 1-800-465-6277. The National Topographic Series in different scales may provide the most information for planning a study, but land-use maps are also often available. Website: http://ellesmere.ccm.emr.ca/.

2. Provincial Natural Resources offices may have small-scale maps or aerial photographs of local study areas. They may also have hydrographic maps of local waterways.

3. Hunting and fishing stores may have commercially produced terrain maps or contour maps of lakes.

Basic Supplies

Waterproof notebooks and papers suitable for data sheets and labels are available from J.L. Darling Corporation, Tacoma, Washington, USA, 98421-3696, as the "Rite in the Rain" series. One of the distributors in Canada is Western Technical Supplies, 845 West 15th St., West Vancouver, British Columbia, V7P 1M5; phone 604-986-2391. All-Weather Copier Pak paper, No. 8511 is available for photocopying waterproof data sheets and labels. It comes in an 8.5" x 11", 200-sheet package. An HB or 2B pencil will produce water- and alcohol-proof labels on this paper.

Sorting and bug-picking forceps are available from a variety of places: locally from medical or dental supply houses, first-aid or safety supply stores, or from large, national, scientific supply outlets such as Canadawide Scientific, Fisher Scientific (USA Website: http://www.fisher1.com/) or VWR Canlab (USA Website: http://www.vwrsp.com/). Straight #5s or #4s are useful for smaller invertebrates such as midge larvae but larger forceps are needed for the mayflies and stoneflies.

Vials, jars, bottles, caps, stoppers, and plastic bags such as the Whirl-Pakâ are available from scientific supply companies but also may be available from local distributors of plastic or glass containers. Tongue-and-groove-type bags of various sizes are common in grocery stores.

Preservatives such as ethanol are available from scientific supply companies, usually in a denatured (toxic-if-ingested) form. They may also be acquired from specialty companies such as Commercial Alcohols Inc., 2 Chelsea Lane, Brampton, Ontario,

Canada, L6T 3Y4, also of Toronto and Montreal. Ethanol is a substance controlled by law, so a permitting process will have to be followed. Formaldehyde may also be purchased from scientific supply companies and occasionally from agricultural suppliers where it is sold as a soil fungicide. Purchases of preservatives are usually made through a sponsoring scientific or educational institution because many companies are reluctant to sell to individuals. However, liquors such as vodka (40% ethanol) will preserve small samples if not diluted by water. Samples preserved in vodka should have the liquor changed within 24 h to eliminate any possible dilution. A temporary preservative could also be made of a solution of 70% automobile antifreeze (ethylene glycol) in water. It is not recommended for long-term storage of specimens, and it is toxic to animals.

Field Equipment

Grabs, corers, dip nets, and other sampling equipment are available from a variety of sources but the most often-cited one is the Wildlife Supply Company (WILDCO), 301 Cass Street, Saginaw, Michigan, USA, 48602; phone 517-799-8100 (Website: http://www.wildco.com/). WILDCO has a number of distributors in Canada, including Egetec Enterprises of Barrie, Ontario and Hoskin Scientific of Vancouver, British Columbia, Burlington, Ontario, and Montreal, Québec. The Website for Hoskin Scientific is http://www.teamkd.com/HOSKIN.HTM; e-mail may be sent to Hoskin@direct.ca. WILDCO catalogues are available from each of these distributors or from the parent company.

Equipment for physical and chemical sampling such as thermometers, and O_2, pH, conductivity, and alkalinity meters can be found in scientific supply company catalogues. An excellent source for surveying, mapping, and general environmental equipment is Forestry Supplies Inc., 205 West Rankin St., P.O. Box 8397, Jackson, Mississippi, USA, 39284-8397; phone 1-800-647-5368.

Self-made sampling equipment such as dip nets, Ekman sieve nets, and kick-sampling nets are often equal in quality to the commercial variety. Local machine shops can bend 1/4" (6.4 mm) stainless steel rod into any shape, such as a hoop for a 36-cm diameter Ekman sieve net or a 35-cm base triangle for a kick net, welded to a 10-cm long, 1 1/8" (28.6 mm) ID stainless steel pipe as a handle socket. Nitexâ nylon netting of the desired mesh size for stream or lake sampling is often available from local silk-screen, print-making shops, or from WILDCO. Heavy "duck" canvas will suffice for nets not used roughly, but ripstop trucker’s tarpaulin material from tent and awning suppliers is superior for nets to be used for kick-sampling streams. This material is harder to sew but may be fastened to the net frame by snaps or velcro on the outside of the hoop, which facilitates replacement. Tent-door and window screening (woven ripstop nylon), although usually a dark colour, makes inexpensive mesh sweep nets for capturing aerial adults of many aquatic insects.

Entomological supplies such as adult sweep nets, light traps, activity traps, and taxonomic keys are available from numerous suppliers, including Canadian scientific

supply companies, but specialized companies abound. These include BioQuip® Products, 17803 LaSalle Ave., Gardena, California, USA, 90248-3602; Carolina Biological Supply Company, 2700 York Rd., Burlington, North Carolina, USA, 27215 (Website: http://www.carolina.com/), and many others. Excellent source lists of suppliers and equipment are found at the following websites: http://insects.ummz.lsa.umich.edu/ entostuff.html (mostly USA suppliers), and http://www.ex.ac.uk/~gjlramel/six.html (a very good source of information).

Laboratory Supplies

Invertebrate-viewing and handling equipment such as microscopes, slides, mounting, and staining supplies are available through large scientific supply companies but some of the specialized subsampling equipment such as sample-splitters are available from entomological suppliers and WILDCO. White-enamel steel trays for live-sorting specimens in debris are often sold as "lasagna pans" at restaurant-supply stores. Office Depot (phone 1-800-685-8800) sells a fluorescent lamp with a central magnifying lens suitable for live-sorting.

The above sources should be regarded only as starting points in the search for equipment and information for biodiversity studies of macroinvertebrates; numerous other suppliers exist. Many other services are available, such as companies offering customized statistical programs for data analysis, and contract services for sorting and identification.

References

APHA (American Public Health Association). 1989. Benthic macroinvertebrates, p.10-95 to 10-113. *In*: Standard methods for the examination of water and wastewater. 17th ed. American Public Health Association, Washington, D.C.

Barton, D.R. and S.M. Smith. 1984. Insects of extremely small and extremely large aquatic habitats, p. 456-483. *In*: V.H. Resh and D.M. Rosenberg (eds.) The ecology of aquatic insects. Praeger, New York.

BCMELPDFO (B.C. Ministry of Environment, Lands, and Parks and Department of Fisheries and Oceans). 1994. Stream inventory manual. Fisheries Branch, B.C. Ministry of Environment, Lands, and Parks, Victoria, British Columbia.

Burton, W. and J.F. Flannagan. 1973. An improved Ekman-type grab. Journal of the Fisheries Research Board of Canada 30: 287-290.

Campbell, P. and A.G. Salki. 1992. A durable all-season marker float and mooring buoy for limnological field studies. CanadianTechnical Report of Fisheries and Aquatic Sciences 1852. iv + 5 p.

Clegg, J. and H. Anthon. 1968. Pond and stream life. Blandford Press, London, England. 108 p.

Cobb, D.G., T.D. Galloway, and J.F. Flannagan. 1992. Effects of discharge and substrate stability on density and species composition of stream insects. Canadian Journal of Fisheries and Aquatic Sciences 49: 1788-1795.

Coffman, W.P. and L.C. Ferrington, Jr. 1996. Chironomidae, p. 635-754. *In*: R.W. Merritt and K.W. Cummins (eds.) An introduction to the aquatic insects of North America. 3rd ed. Kendall/Hunt, Dubuque, Iowa.

Cole, G.A. 1988. Textbook of limnology. 3rd ed. Waveland Press, Prospect Heights, Illinois. 401 p.

Collins, J.N. and V.H. Resh. 1989. Guidelines for the ecological control of mosquitoes in non-tidal wetlands of the San Francisco Bay area. California Mosquito and Vector Control Association Inc. and the University of California Mosquito Research Program, Sacramento, California.

Comiskey, C. and C. Brandt. 1982. Marine ecosystem monitoring. Quantitative impact assessment final report, Appendix A. Science Advisory Board, U.S. Environmental Protection Agency, Washington, D.C.

Cuff, W. and N. Coleman. 1979. Optimal survey design: lessons from a stratified random sample of macrobenthos. Journal of the Fisheries Research Board of Canada 36: 351-361.

Cuffney, T.F., M.E. Gurtz, and M.R. Meador. 1993. Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program. U.S. Geological Survey Open-File Report 93-406. U.S.G.S., Raleigh, North Carolina. 65 p. (http:// wwwrvares.er.usqs.gov/nawqa/protocols/OFR-93-406/inv1.html).

Cummins, K.W. 1975. Macroinvertebrates, p. 170-198. *In*: B.A. Whitton (ed.) River ecology. University of California Press, Berkeley, California.

Danks, H.V. and D.M. Rosenberg. 1987. Aquatic insects of peatlands and marshes in Canada: synthesis of information and identification of needs for research, p. 163-174. *In*: D.M. Rosenberg and H.V. Danks (eds.) Aquatic insects of peatlands and marshes in Canada. Memoirs of the Entomological Society of Canada 140.

Davies, I.J. 1984. Sampling aquatic insect emergence, p. 161-227. *In:* J.A. Downing and F.H. Rigler (eds.) A manual on methods for the assessment of secondary productivity in fresh waters. 2nd ed. IBP Handbook 17. Blackwell Scientific Publications, Oxford, England.

Dawson, C.L. and R.A. Hellenthal. 1986. A computerized system for the evaluation of aquatic habitats based on environmental requirements and pollution tolerance associations of resident organisms. EPA/600/S3-86/019. Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, Oregon.

Downing, J.A. 1984. Sampling the benthos of standing waters, p. 87-130. *In:* J.A. Downing and F.H. Rigler (eds.) A manual on methods for the assessment of secondary productivity in fresh waters. 2nd ed. IBP Handbook 17. Blackwell Scientific Publications, Oxford, England.

Elliott, J.M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. 2nd ed. Freshwater Biological Association Scientific Publication No. 25. 156 p.

Elliott, J.M. and P.A. Tullett. 1978. A bibliography of samplers for benthic invertebrates. Freshwater Biological Association Occasional Publication No. 4. 61 p.

Elliott, J.M. and P.A. Tullett. 1983. A supplement to a bibliography of samplers for benthic invertebrates. Freshwater Biological Association Occasional Publication No. 20. 27 p.

Elliott, J.M. and P.A. Tullett. 1993. A new bibliography of samplers for freshwater benthic invertebrates. Freshwater Biological Association Occasional Publication No. 30. 92 p.

Environment Canada and Department of Fisheries and Oceans. 1992. Aquatic environmental effects monitoring requirements. Annex 1. Aquatic environmental effects monitoring requirements at pulp and paper mills and off-site treatment facilities regulated under the Pulp and Paper Effluent Regulations of the *Fisheries Act*, Environment Canada and Department of Fisheries and Oceans, Ottawa. 19 p. and attachments.

Environment Canada and Department of Fisheries and Oceans. 1993. Technical guidance document for aquatic environmental effects monitoring related to Federal *Fisheries Act* requirements. Version 1.0. Environment Canada and Department of Fisheries and Oceans, Ottawa. 128 p.

Environment Canada and Department of Fisheries and Oceans. 1995. Further guidance for the invertebrate community survey for aquatic environmental effects monitoring related to Federal *Fisheries Act* requirements. EEM 2. Environment Canada and Department of Fisheries and Oceans, Ottawa. 206 p.

Flannagan, J.F. and D.G. Cobb. 1983. New records of winter stoneflies (Plecoptera) from Manitoba with notes on their zoogeographical origins. Canadian Entomologist 115: 673-677.

Gibbons, W.N., M.D. Munn, and M.D. Paine. 1993. Guidelines for monitoring benthos in freshwater environments. Report prepared for Environment Canada, North Vancouver, BC. EVS Consultants, North Vancouver, British Coumbia. 81 p.

Giberson, D.J. and D.M. Rosenberg. 1994. Life histories of burrowing mayflies (*Hexagenia limbata* and *H. rigida*, Ephemeroptera: Ephemeridae) in a northern Canadian reservoir. Freshwater Biology 32: 501-518.

Green, R.H. 1979. Sampling design and statistical methods for environmental biologists. John Wiley, New York. 257 p.

Harrelson, C.C., C.L. Rawlins, and J.P.Potyondy. 1994. Stream channel reference sites: an illustrated guide to field technique. General Technical Report RM-245. U.S. Department of Agriculture, Forestry Service, Ft. Collins, Colorado. 61 p.

Hellawell, J.M. 1978. Biological surveillance of rivers. National Environment Research Council and Water Research Centre, Stevenage, England. 332 p.

Hellawell, J.M. 1986. Biological indicators of freshwater pollution and environmental management. Elsevier, London, England. 546 p.

Kellogg, L.L. 1994. Save Our Streams. Monitor’s guide to aquatic macroinvertebrates. Izaak Walton League of America, Gaithersburg, Maryland. 60 p.

Kerr, M., E. Ely, V. Lee, and A. Desbonnet. 1994. National directory of volunteer environmental monitoring programs. 4th ed. EPA 841-B-94-001. U.S. Environmental Protection Agency, Narragansett, Rhode Island. 531 p.

Kingsbury, P.D. and W.J.G. Beveridge. 1977. A simple bubbler for sorting bottom fauna samples by elutriation. Canadian Entomologist 109: 1265-1268.

Kovats, Z.E. and J.J.H. Ciborowski. 1989. Aquatic insect adults as indicators of organochlorine contamination. Journal of Great Lakes Research 15: 623-634.

Lamberti, G.A. and V.H. Resh. 1985. Comparability of introduced tiles and natural substrates for sampling lotic bacteria, algae and macroinvertebrates. Freshwater Biology 15: 21-30.

Lehmkuhl, D.M. 1979. How to know the aquatic insects. The Pictured Key Nature Series, W.C. Brown, Dubuque, Iowa. 168 p.

Lindeberg, B. 1967. Sibling species delimitation in the *Tanytarsus lestagei* aggregate (Diptera, Chironomidae). Annales Zoologici Fennici 4: 45-86.

Marchant, R. 1989. A subsampler for samples of benthic invertebrates. Bulletin of the Australian Society of Limnology 12: 49-52.

McCullough, G.K. and P. Campbell. 1993. Lake variation and climate change study: ELA lakes, 1986-1990. II. Watershed geography and lake morphology. Canadian Technical Report of Fisheries and Aquatic Sciences 1898: iv + 29 p.

McIntyre, A.D., J.M. Elliott, and D.V. Ellis. 1984. Introduction: design of sampling programmes, p. 1-26. *In:* N.A. Holme and A.D. McIntyre (eds.) Methods for the study of marine benthos. 2nd ed. Blackwell Scientific, Oxford, England.

Merritt, R.W. and K.W. Cummins (eds.) 1996. An introduction to the aquatic insects of North America. 3rd ed. Kendall-Hunt, Dubuque, Iowa. 862 p.

Merritt, R.W., K.W. Cummins, and V.H. Resh. 1996. Design of aquatic insect studies: collecting, sampling and rearing procedures, p. 12-28. *In:* R.W. Merritt and K.W. Cummins (eds.) An introduction to the aquatic insects of North America. 3rd ed. Kendall-Hunt, Dubuque, Iowa.

Metcalfe, J.L. 1989. Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe. Environmental Pollution 60: 101-139.

Murkin, H.R., D.A. Wrubleski, and F.A. Reid. 1994. Sampling invertebrates in aquatic and terrestrial habitats, p. 349-369. *In:* T.A. Bookhout (ed.) Research and management techniques for wildlife and habitats. The Wildlife Society, Bethesda, Maryland.

National Cancer Institute. 1996. CancerWEB. Formaldehyde. CancerNet from the National Cancer Institute. 2 p. (http://www.graylab.ac.uk/cancernet/600038.html).

Needham, J.G. and P.R. Needham. 1962. Guide to the study of freshwater biology. 5th ed. McGraw-Hill, New York. 108 p.

Nero, R.W. 1982. A description of three nets suitable for estimating the abundance of Mysis relicta. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1046. 8 p.

Nero, R.W. and I.J. Davies. 1982. Comparison of two sampling methods for estimating the abundance and distribution of *Mysis relicta*. Canadian Journal of Fisheries and Aquatic Sciences 39: 349-355.

Newbury, R.W. 1984. Hydrologic determinants of aquatic insect habitats, p. 323-357. *In:* V.H. Resh and D.M. Rosenberg (eds.) The ecology of aquatic insects. Praeger, New York.

Newbury, R.W. and M.N. Gaboury. 1993. Stream analysis and fish habitat design. A field manual. Newbury Hydraulics, Gibsons, British Columbia. 256 p.

Nielsen, L.A., D.L. Johnson, and S.S. Lampton. 1983. Fisheries techniques. American Fisheries Society, Bethesda, Maryland. 468 p.

Nolte, U. 1990. Chironomid biomass determination from larval shape. Freshwater Biology 24: 443-451.

Norris, R.H. and A. Georges. 1986. Design and analysis for assessment of water quality, p. 555-572. *In:* P. De Deckker and W.D. Williams (eds.) Limnology in Australia. Junk Publishers, Dordrecht, The Netherlands.

Norris, R.H. and A. Georges. 1993. Analysis and interpretation of benthic macroinvertebrate surveys, p. 234-286. *In:* D.M. Rosenberg and V.H. Resh (eds.) Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.

Ortal, R. and S. Ritman. 1985. Israel inland water ecological data base, p. 73-76. *In:* P.S. Glaeser (ed.) The role of data in scientific progress. Elsevier, New York.

Peckarsky, B.L. 1984. Sampling the stream benthos, p. 131-160. *In:* J.A. Downing and F.H. Rigler (eds.) A manual on methods for the assessment of secondary productivity in fresh waters. 2nd ed. IBP Handbook 17. Blackwell Scientific Publications, Oxford, England.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers. Benthic macroinvertebrates and fish. EPA/444/4-89/001. Office of Water Regulations and Standards, U.S. Environmental Protection Agency, Washington, D.C.

Prepas, E.E. 1984. Some statistical methods for the design of experiments and analysis of samples, p. 266-335. *In:* J.A. Downing and F.H. Rigler (eds.) A manual on methods for the assessment of secondary productivity in fresh waters. 2nd ed. IBP Handbook 17. Blackwell Scientific Publications, Oxford, England.

Reid, R.A., K.M. Somers, and S.M. David. 1995. Spatial and temporal variation in littoral-zone benthic invertebrates from three south-central Ontario Lakes. Canadian Journal of Fisheries and Aquatic Sciences 52: 1406-1420.

Resh, V.H. 1979. Sampling variability and life history features: basic considerations in the design of aquatic insect studies. Journal of the Fisheries Research Board of Canada 36: 290-311.

Resh, V.H., J.W. Feminella, and E.P. McElravy. 1990. Sampling aquatic insects. Videotape. Office of Media Services, University of California, Berkeley, California.

Resh, V.H. and J.K. Jackson. 1993. Rapid assessment approaches to biomonitoring using benthic macroinvertebrates, p. 195-233. *In:* D.M. Rosenberg and V.H. Resh (eds.) Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.

Resh, V.H., G.A. Lamberti, E.P. McElravy, J.R. Wood, and J.W. Feminella. 1984. Quantitative methods for evaluating the effects of geothermal energy development on stream benthic communities at The Geysers, California. California Water Resources Center Contribution No. 190. California Water Resources Center, University of California, Davis, California. 57 p.

Resh, V.H. and E.P. McElravy. 1993. Contemporary quantitative approaches to biomonitoring using benthic macroinvertebrates, p.159-194. *In:* D.M. Rosenberg and V.H. Resh (eds.) Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.

Resh, V.H., D.M. Rosenberg, and J.W. Feminella. 1985. The processing of benthic samples: responses to the 1983 NABS questionnaire. Bulletin of the North American Benthological Society 2: 5-11.

Reynoldson, T.B., R.C. Bailey, K.E. Day, and R.H. Norris. 1995. Biological guidelines for freshwater sediment based on BEnthic Assessment of SedimenT (the BEAST) using a multivariate approach for predicting biological state. Australian Journal of Ecology 20: 198-219.

Reynoldson, T., L. Hampel, and J. Martin. 1986. Biomonitoring networks operated by schoolchildren. Environmental Pollution (Series A) 41: 363-380.

Reynoldson, T.B., R.H. Norris, V.H. Resh, K.E. Day, and D.M. Rosenberg. 1997. The reference condition: a comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. Journal of the North American Benthological Society 16.

Rosenberg, D.M. 1978. Practical sampling of freshwater macrozoobenthos: a bibliography of useful texts, reviews, and recent papers. Canadian Fisheries and Marine Service Technical Report No. 790. 15 p.

Rosenberg, D.M. and V.H. Resh. 1982. The use of artificial substrates in the study of freshwater benthic macroinvertebrates, p.175-235. *In:* J. Cairns, Jr. (ed.) Artificial substrates. Ann Arbor Science, Ann Arbor, Michigan.

Rosenberg, D.M. and V.H. Resh. 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates, p. 1-9. *In:* D.M. Rosenberg and V.H. Resh (eds.) Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.

Rosenberg, D.M., A.P. Wiens, and B. Bilyj. 1988. Chironomidae (Diptera) of peatlands in northwestern Ontario, Canada. Holarctic Ecology 11: 19-31.

Ruttner, F. 1963. Fundamentals of limnology. University of Toronto Press, Toronto. 295 p.

Sebastien, R.J., D.M. Rosenberg, and A.P. Wiens. 1988. A method for subsampling unsorted benthic macroinvertebrates by weight. Hydrobiologia 157: 69-75.

Southwood, T.R.E. 1978. Ecological methods with particular reference to the study of insect populations. 2nd ed. Chapman and Hall, London, England. 391 p.

Stainton, M.P., M.J. Capel, and F.A.J. Armstrong. 1977. The chemical analysis of fresh water. 2nd ed. Canadian Fisheries and Marine Service Miscellaneous Special Publication 25. 180 p.

Stephenson, M., G. Mierle, R.A. Reid, and G.L. Mackie. 1994. Effects of experimental and cultural lake acidification on littoral benthic macroinvertebrate assemblages. Canadian Journal of Fisheries and Aquatic Sciences 51: 1147-1161.

Stewart, K.W. 1975. An improved elutriator for separating stream insects from stony substrates. Transactions of the American Fisheries Society 104: 821-823.

Stewart-Oaten, A., W.W. Murdoch, and K.R. Parker. 1986. Environmental impact assessment: "pseudoreplication" in time? Ecology 67: 929-940.

Strahler, A.N. 1964. Quantitative geomorphology of drainage basins and channel networks, p. 39-76. *In:* V.T. Chow (ed.) Handbook of applied hydrology. 4th ed. McGraw-Hill, New York.

Thoms, M.C. 1997. Sedimentation in the Pambula River, NSW, Australia. Australian Journal of Earth Science. (In press).

Thorp, J.H. and A.P. Covich (eds.). 1991. Ecology and classification of North American freshwater invertebrates. Academic Press, New York. 911 p.

Turner, M.A., E.T. Howell, M. Summerby, R.H. Hesslein, D.L. Findlay, and M.B. Jackson. 1991. Changes in epilithon and epiphyton associated with experimental acidification of a lake to pH 5. Limnology and Oceanography 36: 1390-1405.

Underwood, A.J. 1991. Beyond BACI: experimental designs for detecting human environmental impacts on temporal variations in natural populations. Australian Journal of Marine and Freshwater Research 42: 569-587.

Voshell, J.R., Jr., R.J. Layton, and S.W. Hiner. 1989. Field techniques for determining the effects of toxic substances on benthic macroinvertebrates in rocky-bottomed streams, p. 134-155. *In:* U.M. Cowgill and L.R. Williams (eds.) Aquatic toxicology and hazard assessment: 12th volume. American Society for Testing and Materials Special Technical Publication 1027. ASTM, Philadelphia, Pennsylvania.

Weber, C.I. (ed.) 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. EPA-670/4-73-001. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Wiggins, G.B. 1977. Larvae of the North American caddisfly genera (Trichoptera). University of Toronto Press, Toronto. 401 p.

Williams, D.D. 1984. The hyporheic zone as a habitat for aquatic insects and associated arthropods, p. 430-455. *In:* V.H. Resh and D.M. Rosenberg (eds.) The ecology of aquatic insects. Praeger, New York.

Williams, R. and D. Briton. 1986. Speech recognition as a means of enumeration in the analysis of biological samples. Marine Biology 92: 595-598.

Winterbourn, M.J. 1985. Sampling stream invertebrates, p. 241-258. *In:* R.D. Pridmore and A.B. Cooper (eds.) Biological Monitoring in Freshwaters: Proceedings of a

Seminar, Hamilton, November 21-23, 1984, Part 2. Water and Soil Miscellaneous Publication No. 83, National Water and Soil Conservation Authority, Wellington, New Zealand.

Wright, J.F. 1995. Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. Australian Journal of Ecology 20: 181-197.

Wrona, F.J., J.M. Culp, and R.W. Davies. 1982. Macroinvertebrate subsampling: a simplified apparatus and approach. Canadian Journal of Fisheries and Aquatic Sciences 39: 1051-1054.

Table 1. Approximate number of known species of major North American groups of freshwater benthic invertebrates (from Thorp and Covich 1991).

Taxon	Common Name	No. of Known Species
Turbellaria	Flatworms	>200
Gastropoda	Snails	~350
Bivalvia	Mussels and clams	>250
Oligochaeta	Worms	~150
Hirudinea	Leeches	~80
Acari	Water mites	>1500
Insecta	Mayflies	~575
Ephemeroptera	Dragonflies and damselflies	~415
Odonata	Stoneflies	~550
Plecoptera	True bugs	324
Heteroptera	Beetles	>1100
Coleoptera	Caddisflies	>1340
Trichoptera	Midge flies	>2000[a]
Diptera		
Chironomidae		
	Total	**~8834**

[a] Estimate is for the Nearctic region (Coffman and Ferrington 1996).

Table 2. Approximate number of known species of water mites and aquatic insects in Canada (from Danks and Rosenberg 1987).

Taxon	Common Name	No. of Known Species
Acari	Water mites	500
Ephemeroptera	Mayflies	301
Odonata	Dragons and damselflies	195
Plecoptera	Stoneflies	250
Hemiptera	True bugs	138
Celeoptera	Beetles	579
Trichoptera	Caddisflies	546
Dipteria	Flies	74
Culicidae	Mosquitoes	132
Tabanidae	Horse and deer flies	180
Ceratopogonidae	No-see-ums	480
Chironomidae	Midge flies	1170
Other families		
Others		90
	Total	**4635**

Table 3. Topics discussed by selected reports with the design and analysis of benthic research and biomonitoring. An asterisk indicates detailed treatment. (From Resh and McElravy 1993. Reprinted with permission of Chapman and Hall, New York).

Author	Lentic	Lotic	Survey Design-Stratification /Sampling Consideration	Study Design/ Frequency of Studies	Sampling Devices	Sorting Procedures	Data Storage and Retrieval / Quality Control
APHA (1989)	x	x			x	x	
Collins and Resh (1969)	x		x	x	x		x
Comiskey and Brandt (1982)	x	x	x*		x	x	x*
Cuff and Coleman (1979)	x		x*				
Cummins (1975)		x	x			x	
Dawson and Hellenthal (1986)	x	x					x*
Downing (1984)	x				x*	x	
Elliott (1977)	x	x	x				
Environment Canada and Department of Fisheries and Oceans (1993, 1995)	x	x	x	x	x	x	x
Gibbons *et al.* (1993)	x	x	x	x	x	x	x
Green (1979)	x	x	x	x			
Hellawell (1978)		x	x		x*	x	
Hellawell (1986)	x	x	x		x		
McIntyre *et al.* (1984)	x		x	x		x	
Merritt *et al.* (1996)	x	x	x		x		
Norris and Georges (1986)		x	x				x
Ortal and Ritman (1985)		x					x*
Peckarsky (1984)		x	x		x		

Prepas (1984)	x	x	x				
Resh (1979)		x	x		x	x	
Resh *et al.* (1984)		x			x*	x	x
Resh *et al.* (1985)		x			x	x	
Resh *et al.* (1990)	x	x			x		
Southwood (1978)	x	x	x		x		
Voshell *et al.* (1989)		x	x	x	x		
Weber (1973)	x	x	x		x	x	
Winterbourn (1985)		x	x		x	x	