7 BENTHIC MACROINVERTEBRATE PROTOCOLS

Rapid bioassessment using the benthic macroinvertebrate assemblage has been the most popular set of protocols among the state water resource agencies since 1989 (Southerland and Stribling 1995). Most of the development of benthic Rapid Bioassessment Protocols (RBPs) has been oriented toward RBP III (described in Plafkin et al. 1989). As states have focused attention on regional specificity, which has included a wide variety of physical characteristics of streams, the methodology of conducting stream surveys of the benthic assemblage has advanced. Some states have preferred to retain more traditional methods such as the Surber or Hess samplers (e.g., Wyoming Department of Environmental Quality [DEQ]) over the kick net in cobble substrate. Other agencies have developed techniques for streams lacking cobble substrate, such as those streams in coastal plains. State water resource agencies composing the Mid-Atlantic Coastal Streams (MACS) Workgroup, i.e., New Jersey Department of Environmental Protection (DEP), Delaware Department of Natural Resources and Environmental Control (DNREC), Maryland Department of Natural Resources (DNR) and Maryland Department of the Environment (MDE), Virginia DEQ, North Carolina Department of Environmental Management (DEM), and South Carolina Department of Health and Environmental Control (DHEC), and a workgroup within the Florida Department of Environmental Protection (DEP) were pioneers in this effort. These 2 groups (MACS and FLDEP) developed a multihabitat sampling procedure using a D-frame dip net. Testing of this procedure by these 2 groups indicates that this technique is scientifically valid for low-gradient streams. Research conducted by the U.S. Environmental Protection

STANDARD BENTHIC MACROINVERTEBRATE SAMPLING GEAR TYPES FOR STREAMS
(assumes standard mesh size of 500 µ nytex screen)

- **Kick net:** Dimensions of net are 1 meter (m) x 1 m attached to 2 poles and functions similarly to a fish kick seine. Is most efficient for sampling cobble substrate (i.e., riffles and runs) where velocity of water will transport dislodged organisms into net. Designed to sample 1 m^2 of substrate at a time and can be used in any depth from a few centimeters to just below 1m (Note -- Depths of 1m or greater will be difficult to sample with any gear).

- **D-frame dip net:** Dimensions of frame are 0.3 m width and 0.3 m height and shaped as a "D" where frame attaches to long pole. Net is cone or bag-shaped for capture of organisms. Can be used in a variety of habitat types and used as a kick net, or for "jabbing", "dipping", or "sweeping".

- **Rectangular dip net:** Dimensions of frame are 0.5 m width and 0.3 m height and attached to a long pole. Net is cone or bag-shaped. Sampling is conducted similarly to the D-frame.

- **Surber:** Dimensions of frame are 0.3 m x 0.3 m, which is horizontally placed on cobble substrate to delineate a 0.09 m^2 area. A vertical section of the frame has the net attached and captures the dislodged organisms from the sampling area. Is restricted to depths of less than 0.3 m.

- **Hess:** Dimensions of frame are a metal cylinder approximately 0.5 m in diameter and samples an area 0.8 m^2. Is an advanced design of the Surber and is intended to prevent escape of organisms and contamination from drift. Is restricted to depths of less than 0.5 m.

Agency (USEPA) for their Environmental Monitoring and Assessment Program (EMAP) program and the United States Geological Survey (USGS) for their National Water Quality Assessment Program (NAWQA) program have indicated that the rectangular dip net is a reasonable compromise between the traditional Surber or Hess samplers and the RBP kick net described the original RBPs.

From the testing and implementation efforts that have been conducted around the country since 1989, refinements have been made to the procedures while maintaining the original concept of the RBPs. Two separate procedures that are oriented toward a "single, most productive" habitat and a multihabitat approach represent the most rigorous benthic RBP and are essentially a replacement of the original RBP III. The primary differences between the original RBP II and III are the decision on field versus lab sorting and level of taxonomy. These differences are not considered sufficient reasons to warrant separate protocols. In addition, a third protocol has been developed as a more standardized biological reconnaissance or screening and replaces RBP I of the original document.

Kicknet

D-frame Dipnet

Rectangular Dipnet

Hess sampler

(*Mary Kay Corazalla, Univ. of Minnesota*)

7.1 SINGLE HABITAT APPROACH: 1 METER KICK NET

The original RBPs (Plafkin et al. 1989) emphasized the sampling of a single habitat, in particular riffles or runs, as a means to standardize assessments among streams having those habitats. This approach is still valid, because macroinvertebrate diversity and abundance are usually highest in cobble substrate (riffle/run) habitats. Where cobble substrate is the predominant habitat, this sampling approach provides a representative sample of the stream reach. However, some streams naturally lack the cobble substrate. In cases where the cobble substrate represents less than 30% of the sampling reach in reference streams (i.e., those streams that are representative of the region), alternate habitat(s) will need to be sampled (See Section 7.2). The appropriate sampling method should be selected based on the habitat availability of the reference condition and not of potentially impaired streams. For example, methods would not be altered for situations where the extent of cobble substrate in streams influenced by heavy sediment deposition may be substantially reduced from the amount of cobble substrate expected for the region.

7.1.1 Field Sampling Procedures for Single Habitat

FIELD EQUIPMENT/SUPPLIES NEEDED FOR BENTHIC MACROINVERTEBRATE SAMPLING —SINGLE HABITAT APPROACH

- standard kick-net, 500 μ opening mesh, 1.0 meter width
- sieve bucket, with 500 μ opening mesh
- 95% ethanol
- sample containers, sample container labels
- forceps
- pencils, clipboard
- Benthic Macroinvertebrate Field Data Sheet*
- first aid kit
- waders (chest-high or hip boots)
- rubber gloves (arm-length)
- camera
- Global Positioning System (GPS) Unit

* It is helpful to copy fieldsheets onto water-resistant paper for use in wet weather conditions

1. A 100 m reach representative of the characteristics of the stream should be selected. Whenever possible, the area should be at least 100 meters upstream from any road or bridge crossing to minimize its effect on stream velocity, depth, and overall habitat quality. There should be no major tributaries discharging to the stream in the study area.

2. Before sampling, complete the physical/chemical field sheet (see Chapter 5; Appendix A-1, Form 1) to document site description, weather conditions, and land use. After sampling, review this information for accuracy and completeness.

3. Draw a map of the sampling reach. This map should include in-stream attributes (e.g., riffles, falls, fallen trees, pools, bends, etc.) and important structures, plants, and attributes of the bank and near stream areas. Use an arrow to indicate the direction of flow. Indicate the areas that were sampled for macroinvertebrates on the map. Estimate "river mile" for sampling reach for probable use in data management of the water resource agency. If available, use hand-held Global Positioning System (GPS) for latitude and longitude determination taken at the furthest downstream point of the sampling reach.

4. All riffle and run areas within the 100-m reach are candidates for sampling macroinvertebrates. A composite sample is taken from individual sampling spots in the riffles and runs representing different velocities. Generally, a minimum of 2 m^2 composited area is sampled for RBP efforts.

5. Sampling begins at the downstream end of the reach and proceeds upstream. Using a 1 m kick net, 2 or 3 kicks are sampled at various velocities in the riffle or series of riffles. A *kick* is a stationary sampling accomplished by positioning the net and disturbing one square meter upstream of the net. Using the toe or heel of the boot, dislodge the upper layer of cobble or gravel and scrape the underlying bed. Larger substrate particles should be picked up and rubbed by hand to remove attached organisms. If different gear is used (e.g., a D-frame or rectangular net), a composite is obtained from numerous kicks (See Section 7.2).

ALTERNATIVES FOR STREAM REACH DESIGNATION

- **Fixed-distance designation**—A standard length of stream, such as a reach, is commonly used to obtain an estimate of natural variability. Conceptually, this approach should provide a mixture of habitats in the reach and provide, at a minimum, duplicate physical and structural elements such as a riffle/pool sequence.

- **Proportional-distance designation**—Alternatively, a standard number of stream "widths" is used to measure the stream distance, e.g., 40 times the stream width is defined by EMAP for sampling (Klemm and Lazorchak 1995). This approach allows variation in the length of the reach based on the size of the stream.

6. The jabs or kicks collected from different locations in the cobble substrate will be composited to obtain a single homogeneous sample. After every kick, wash the collected material by running clean stream water through the net 2 to 3 times. If clogging does occur, discard the material in the net and redo that portion of the sample in a different location. Remove large debris after rinsing and inspecting it for organisms; place any organisms found into the sample container. Do not spend time inspecting small debris in the field. [Note — an alternative is to keep the samples from different habitats separated as done in EMAP (Klemm and Lazorchak 1995).]

7. Transfer the sample from the net to sample container(s) and preserve in enough 95 percent ethanol to cover the sample. Forceps may be needed to remove organisms from the dip net. Place a label indicating the sample identification code or lot number, date, stream name, sampling location, and collector name into the sample container. The outside of the container should include the same information and the words "preservative: 95% ethanol". If more than one container is needed for a sample, each container label should contain all the information for the sample and should be numbered (e.g., 1 of 2, 2 of 2, etc.). This information will be recorded in the "Sample Log" at the biological laboratory (Appendix A-3, Form 2).

8. Complete the top portion of the "Benthic Macroinvertebrate Field Data Sheet" (Appendix A-3, Form 1), which duplicates the "header" information on the physical/chemical field sheet.

9. Record the percentage of each habitat type in the reach. Note the sampling gear used, and comment on conditions of the sampling, e.g., high flows, treacherous rocks, difficult access to stream, or anything that would indicate adverse sampling conditions.

10. Document observations of aquatic flora and fauna. Make qualitative estimates of macroinvertebrate composition and relative abundance as a cursory estimate of ecosystem health and to check adequacy of sampling.

11. Perform habitat assessment (Appendix A-1, Form 2) after sampling has been completed; walking the reach helps ensure a more accurate assessment. Conduct the habitat assessment with another team member, if possible.

12. Return samples to laboratory and complete log-in form (Appendix A-3, Form 2).

QUALITY CONTROL (QC) IN THE FIELD

1. Sample labels must be properly completed, including the sample identification code, date, stream name, sampling location, and collector's name, and placed into the sample container. The outside of the container should be labeled with the same information. Chain-of-custody forms, if needed, must include the same information as the sample container labels.

2. After sampling has been completed at a given site, all nets, pans, etc. that have come in contact with the sample should be rinsed thoroughly, examined carefully, and picked free of organisms or debris. Any additional organisms found should be placed into the sample containers. The equipment should be examined again prior to use at the next sampling site.

3. Replicate (1 duplicate sample) 10% of the sites to evaluate precision or repeatability of the sampling technique or the collection team.

7.2 MULTIHABITAT APPROACH: D–FRAME DIP NET

Streams in many states vary from high gradient, cobble dominated to low gradient streams with sandy or silty sediments. Therefore, a method suitable to sampling a variety of habitat types is desired in these cases. The method that follows is based on Mid-Atlantic Coastal Streams Workgroup recommendations designed for use in streams with variable habitat structure (MACS 1996) and was used for statewide stream bioassessment programs by Florida DEP (1996) and Massachusetts DEP (1995). This method focuses on a multihabitat scheme designed to sample major habitats in proportional representation within a sampling reach. Benthic

FIELD EQUIPMENT/SUPPLIES NEEDED FOR BENTHIC MACROINVERTEBRATE SAMPLING —MULTI-HABITAT APPROACH

- standard D-frame dip net, 500 μ opening mesh, 0.3 m width (~ 1.0 ft frame width)
- sieve bucket, with 500 μ opening mesh
- 95% ethanol
- sample containers, sample container labels
- forceps
- pencils, clipboard
- Benthic Macroinvertebrate Field Data Sheet*
- first aid kit
- waders (chest-high or hip boots)
- rubber gloves (arm-length)
- camera
- Global Positioning System (GPS) Unit

* It is helpful to copy fieldsheets onto water-resistant paper for use in wet weather conditions

macroinvertebrates are collected systematically from all available instream habitats by kicking the substrate or jabbing with a D-frame dip net. A total of 20 jabs (or kicks) are taken from all major habitat types in the reach resulting in sampling of approximately 3.1 m^2 of habitat. For example, if the habitat in the sampling reach is 50% snags, then 50% or 10 jabs should be taken in that habitat. An organism-based subsample (usually 100, 200, 300, or 500 organisms) is sorted in the laboratory and identified to the lowest practical taxon, generally genus or species.

7.2.1 Habitat Types

The major stream habitat types listed here are in reference to those that are colonized by macroinvertebrates and generally support the diversity of the macroinvertebrate assemblage in stream ecosystems. Some combination of these habitats would be sampled in the multihabitat approach to benthic sampling.

Cobble (hard substrate) - Cobble will be prevalent in the riffles (and runs), which are a common feature throughout most mountain and piedmont streams. In many high-gradient streams, this habitat type will be dominant. However, riffles are not a common feature of most coastal or other low-gradient streams. Sample shallow areas with coarse (mixed gravel, cobble or larger) substrates by holding the bottom of the dip net against the substrate and dislodging organisms by kicking the substrate for 0.5 m upstream of the net.

Snags - Snags and other woody debris that have been submerged for a relatively long period (not recent deadfall) provide excellent colonization habitat. Sample submerged woody debris by jabbing in medium-sized snag material (sticks and branches). The snag habitat may be kicked first to help dislodge organisms, but only after placing the net downstream of the snag. Accumulated woody material in pool areas are considered snag habitat. Large logs should be avoided because they are generally difficult to sample adequately.

Vegetated banks - When lower banks are submerged and have roots and emergent plants associated with them, they are sampled in a fashion similar to snags. Submerged areas of undercut banks are good habitats to sample. Sample banks with protruding roots and plants by jabbing into the habitat. Bank habitat can be kicked first to help dislodge organisms, but only after placing the net downstream.

Submerged macrophytes - Submerged macrophytes are seasonal in their occurrence and may not be a common feature of many streams, particularly those that are high-gradient. Sample aquatic plants that are rooted on the bottom of the stream in deep water by drawing the net through the vegetation from the bottom to the surface of the water (maximum of 0.5 m each jab). In shallow water, sample by bumping or jabbing the net along the bottom in the rooted area, avoiding sediments where possible.

Sand (and other fine sediment) - Usually the least productive macroinvertebrate habitat in streams, this habitat may be the most prevalent in some streams. Sample banks of unvegetated or soft soil by bumping the net along the surface of the substrate rather than dragging the net through soft substrates; this reduces the amount of debris in the sample.

7.2.2 Field Sampling Procedures for Multihabitat

1. A 100 m reach that is representative of the characteristics of the stream should be selected. Whenever possible, the area should be at least 100 m upstream from any road or bridge crossing to minimize its effect on stream velocity, depth and overall habitat quality. There should be no major tributaries discharging to the stream in the study area.

2. Before sampling, complete the physical/chemical field sheet (see Chapter 5; Appendix A-1, Form 1) to document site description, weather conditions, and land use. After sampling, review this information for accuracy and completeness.

ALTERNATIVES FOR STREAM REACH DESIGNATION

- **Fixed-distance designation**—A standard length of stream, such as a reach, is commonly used to obtain an estimate of natural variability. Conceptually, this approach should provide a mixture of habitats in the reach and provide, at a minimum, duplicate physical and structural elements such as a riffle/pool sequence.

- **Proportional-distance designation**—Alternatively, a standard number of stream "widths" is used to measure the stream distance, e.g., 40 times the stream width is defined by EMAP for sampling (Klemm and Lazorchak 1995). This approach allows variation in the length of the reach based on the size of the stream.

3. Draw a map of the sampling reach. This map should include in-stream attributes (e.g., riffles, falls, fallen trees, pools, bends, etc.) and important structures, plants, and attributes of the bank and near stream areas. Use an arrow to indicate the direction of flow. Indicate the areas that were sampled for macroinvertebrates on the map. Approximate "river mile" to sampling reach for probable use in data management of the water resource agency. If available, use hand-held GPS for latitude and longitude determination taken at the furthest downstream point of the sampling reach.

4. Different types of habitat are to be sampled in approximate proportion to their representation of surface area of the total macroinvertebrate habitat in the reach. For example, if snags comprise 50% of the habitat in a reach and riffles comprise 20%, then 10 jabs should be taken in snag material and 4 jabs should be take in riffle areas. The remainder of the jabs (6) would be taken in any remaining habitat type. Habitat types contributing less than 5% of the stable habitat in the stream reach should not be sampled. In this case, allocate the remaining jabs proportionately among the predominant substrates. The number of jabs taken in each habitat type should be recorded on the field data sheet.

5. Sampling begins at the downstream end of the reach and proceeds upstream. A total of 20 jabs or kicks will be taken over the length of the reach; a single *jab* consists of forcefully thrusting the net into a productive habitat for a linear distance of 0.5 m. A *kick* is a stationary sampling accomplished by positioning the net and disturbing the substrate for a distance of 0.5 m upstream of the net.

6. The jabs or kicks collected from the multiple habitats will be composited to obtain a single homogeneous sample. Every 3 jabs, more often if necessary, wash the collected material by running clean stream water through the net two to three times. If clogging does occur that may hinder obtaining an appropriate sample, discard the material in the net and redo that portion of

the sample in the same habitat type but in a different location. Remove large debris after rinsing and inspecting it for organisms; place any organisms found into the sample container. Do not spend time inspecting small debris in the field.

7. Transfer the sample from the net to sample container(s) and preserve in enough 95% ethanol to cover the sample. Forceps may be needed to remove organisms from the dip net. Place a label indicating the sample identification code or lot number, date, stream name, sampling location, and collector name into the sample container. The outside of the container should include the same information and the words “preservative: 95% ethanol”. If more that one container is needed for a sample, each container label should contain all the information for the sample and should be numbered (e.g., 1 of 2, 2 of 2, etc.). This information will be recorded in the "Sample Log" at the biological laboratory (Appendix A-3, Form 2).

8. Complete the top portion of the “Benthic Macroinvertebrate Field Data Sheet” (Appendix A-3, Form 1), which duplicates the “header” information on the physical/chemical field sheet.

9. Record the percentage of each habitat type in the reach. Note the sampling gear used, and comment on conditions of the sampling, e.g., high flows, treacherous rocks, difficult access to stream, or anything that would indicate adverse sampling conditions.

10. Document observations of aquatic flora and fauna. Make qualitative estimates of macroinvertebrate composition and relative abundance as a cursory estimate of ecosystem health and to check adequacy of sampling.

11. Perform habitat assessment (Appendix A-1, Form 3) after sampling has been completed. Having sampled the various microhabitats and walked the reach helps ensure a more accurate assessment. Conduct the habitat assessment with another team member, if possible.

12. Return samples to laboratory and complete log-in forms (Appendix A-3, Form 2).

QUALITY CONTROL (QC) IN THE FIELD

1. Sample labels must be properly completed, including the sample identification code, date, stream name, sampling location, and collector’s name and placed into the sample container. The outside of the container should be labeled with the same information. Chain-of-custody forms, if needed, must include the same information as the sample container labels.

2. After sampling has been completed at a given site, all nets, pans, etc. that have come in contact with the sample should be rinsed thoroughly, examined carefully, and picked free of organisms or debris. Any additional organisms found should be placed into the sample containers. The equipment should be examined again prior to use at the next sampling site.

3. Replicate (1 duplicate sample) 10% of the sites to evaluate precision or repeatability of sampling technique or collection team.

7.3 LABORATORY PROCESSING FOR MACROINVERTEBRATE SAMPLES

Macroinvertebrate samples collected by either intensive method, i.e., single habitat or multihabitat, are best processed in the laboratory under controlled conditions. Aspects of laboratory processing include subsampling, sorting, and identification of organisms.

All samples should be dated and recorded in the "Sample Log" notebook or on sample log form (Appendix A-3, Form 2) upon receipt by laboratory personnel. All information from the sample container label should be included on the sample log sheet. If more than one container was used, the number of containers should be indicated as well. All samples should be sorted in a single laboratory to enhance quality control.

LABORATORY EQUIPMENT/SUPPLIES NEEDED FOR BENTHIC MACROINVERTEBRATE SAMPLE PROCESSING

- log-in sheet for samples
- standardized gridded pan (30 cm x 36 cm) with approximately 30 grids (6 cm x 6 cm)
- 500 micron sieve
- forceps
- white plastic or enamel pan (15 cm x 23 cm) for sorting
- specimen vials with caps or stoppers
- sample labels
- standard laboratory bench sheets for sorting and identification
- dissecting microscope for organism identification
- fiber optics light source
- compound microscope with phase contrast for identification of mounted organisms (e.g., midges)
- 70% ethanol for storage of specimens
- appropriate taxonomic keys

7.3.1 Subsampling and Sorting

Subsampling benthic samples is not a requirement, and in fact, is frowned upon by certain scientists. Courtemanch (1996) provides an argument against subsampling, or to use a volume-based procedure if samples are to be subsampled. Vinson and Hawkins (1996) and Barbour and Gerritsen (1996) provide arguments for a fixed-count method, which is the preferred subsampling technique for RBPs.

Subsampling reduces the effort required for the sorting and identification aspects of macroinvertebrate surveys and provides a more accurate estimate of time expenditure (Barbour and Gerritsen 1996). The RBPs use a fixed-count approach to subsampling and sorting the organisms from the sample matrix of detritus, sand, and mud. *The following protocol is based on a 200-organism subsample, but it could be used for any subsample size (100, 300, 500, etc.)*. The subsample is sorted and preserved separately from the remaining sample for quality control checks.

1. Prior to processing any samples in a lot (i.e., samples within a collection date, specific watershed, or project), complete the sample log-in sheet to verify that all samples have arrived at the laboratory, and are in proper condition for processing.

2. Thoroughly rinse sample in a 500 µm-mesh sieve to remove preservative and fine sediment. Large organic material (whole leaves, twigs, algal or macrophyte mats, etc.) not removed in the field should be rinsed, visually inspected, and discarded. If the samples have been preserved in alcohol, it will be necessary to soak the sample contents in water for about 15 minutes to hydrate the benthic organisms, which will prevent them from floating on the water surface during sorting. If the sample was stored in more than one container, the contents of all

containers for a given sample should be combined at this time. Gently mix the sample by hand while rinsing to make homogeneous.

SUBSAMPLE PROCEDURE MODIFICATIONS

Subsampling procedures developed by Hilsenhoff (1987) and modified by Plafkin et al. (1989) were used in the original RBP II and RBP III protocols. As an improvement to the mechanics of the technique, Caton (1991) designed a sorting tray consisting of two parts, a rectangular plastic or plexiglass pan (36 cm x 30 cm) with a rectangular sieve insert. The sample is placed on the sieve, in the pan and dispersed evenly.

When a random grid(s) is selected, the sieve is lifted to temporarily drain the water. A "cookie-cutter" like metal frame 6 cm x 6 cm is used to clearly define the selected grid; debris overhanging the grid may be cut with scissors. A 6 cm flat scoop is used to remove all debris and organisms from the grid. The contents are then transferred to a separate sorting pan with water for removal of macroinvertebrates.

These modifications have allowed for rapid isolation of organisms within the selected grids and easy removal of all organisms and debris within a grid while eliminating investigator bias.

3. After washing, spread the sample evenly across a pan marked with grids approximately 6 cm x 6 cm. On the laboratory bench sheet, note the presence of large or obviously abundant organisms; *do not remove them from the pan*. However, Vinson and Hawkins (1996) present an argument for including these large organisms in the count, because of the high probability that these organisms will be excluded from the targeted grids.

4. Use a random numbers table to select 4 numbers corresponding to squares (grids) within the gridded pan. Remove all material (organisms and debris) from the four grid squares, and place the material into a shallow white pan and add a small amount of water to facilitate sorting. If there appear (through a cursory count or observation) to be 200 organisms ± 20% (cumulative of 4 grids), then subsampling is complete.

Any organism that is lying over a line separating two grids is considered to be on the grid containing its head. In those instances where it may not be possible to determine the location of the head (worms for instance), the organism is considered to be in the grid containing most of its body.

If the density of organisms is high enough that many more than 200 organisms are contained in the 4 grids, transfer the contents of the 4 grids to a second gridded pan. Randomly select grids for this second level of sorting as was done for the first, sorting grids one at a time until 200 organisms ± 20% are found. If picking through the entire next grid is likely to result in a subsample of greater than 240 organisms, then that grid may be subsampled in the same manner as before to decrease the likelihood of exceeding 240 organisms. That is, spread the contents of the last grid into another gridded pan. Pick grids one at a time until the desired number is reached. The total number of grids for each subsorting level should be noted on the laboratory bench sheet.

TESTING OF SUBSAMPLING

Ferraro et al. (1989) describe a procedure for calculating the "power-cost efficiency" (PCE), which incorporates both the number of samples and the cost (i.e. time or money) for each alternative sampling scheme. With this analysis, the optimal subsampling size is that by which the costs of increased effort are offset by the lowest theoretical number of samples predicted from the power analysis to provide reliable resolution (Barbour and Gerritsen 1996).

There are 4 primary steps in assessing the PCE of a suite of alternative subsampling strategies:

Step 1: For each subsampling strategy (i.e., 100-, 200-, 300- organism level, or other) collect samples at several reference and impaired stations. The observed differences in each of the core metrics is defined to be the magnitude of the difference desired to be detected. The difference is the "effect size" and is equivalent to the inverse coefficient of variation (CV).

Step 2: Assess the "cost" (c_i), in time or money, of each subsampling scheme *i* at each site. The cost can include labor hours for subsampling, sorting, identification, and documentation. Total cost of each subsampling alternative is the product of cost per site and required sample size.

Step 3: Conduct statistical power analyses to determine the minimum number of replicate samples (n_i) needed to detect the effect size with an acceptable probability of Type I ($\propto$; the probability that the null hypothesis [e.g., "sites are good"] is true and it is rejected. Commonly termed the significance level.) and Type II (β; the probability that the null hypothesis is false and it is accepted) error. Typically, $\propto$ and β are set at 0.05. This step may be deleted for those programs that already have an established number of replicate samples.

Step 4: Calculate the PCE for each sampling scheme by:

$$PCE_i = \frac{(n \mathrm{X} c)_{\min}}{(n_i \mathrm{X} c_i)}$$

where $(n \mathrm{X} c)_{min}$ = minimum value of (n X c) among the *i* sampling schemes. The PCE formula is equivalent to the "power efficiency" ratio of the sample sizes attained by alternative tests under similar conditions (Ferraro et al. 1989) with the n's multiplied by the "cost" per replicate sample. Multiplying n by c puts efficiency on a total "cost" rather than on a sample size basis. The reciprocal of PCE_i is the factor by which the optimal subsampling scheme is more efficient than alternative scheme *i*. When PCE is determined for multiple metrics, the overall optimal subsampling scheme may be defined as that which ranks highest in PCE for most metrics of interest.

5. Save the sorted debris residue in a separate container. Add a label that includes the words "sorted residue" in addition to all prior sample label information and preserve in 95% ethanol. Save the remaining unsorted sample debris residue in a separate container labeled "sample residue"; this container should include the original sample label. Length of storage and archival is determined by the laboratory or benthic section supervisor.

6. Place the sorted 200-organism (± 20%) subsample into glass vials, and preserve in 70% ethanol. Label the vials inside with the sample identifier or lot number, date, stream name, sampling location and taxonomic group. If more than one vial is needed, each should be labeled separately and numbered (e.g., 1 of 2, 2 of 2). For convenience in reading the labels inside the

vials, insert the labels left-edge first. If identification is to occur immediately after sorting, a petri dish or watch glass can be used instead of vials.

7. Midge (Chironomidae) larvae and pupae should be mounted on slides in an appropriate medium (e.g., Euperal, CMC-9); slides should be labeled with the site identifier, date collected, and the first initial and last name of the collector. As with midges, worms (Oligochaeta) must also be mounted on slides and should be appropriately labeled.

8. Fill out header information on Laboratory Bench Sheet as in field sheets (see Chapter 5). Also check subsample target number. Complete back of sheet for subsampling/sorting information. Note number of grids picked, time expenditure, and number of organisms. If QC check was performed on a particular sample, person conducting QC should note findings on the back of the Laboratory Bench Sheet. Calculate sorting efficiency to determine whether sorting effort passes or fails.

9. Record date of sorting and slide monitoring, if applicable, on Log-In Sheet as documentation of progress and status of completion of sample lot.

QUALITY CONTROL (QC) FOR SORTING

1. Ten percent of the sorted samples in each lot should be examined by laboratory QC personnel or a qualified co-worker. (A lot is defined as a special study, basin study, entire index period, or individual sorter.) The QC worker will examine the grids chosen and tray used for sorting and will look for organisms missed by the sorter. Organisms found will be added to the sample vials. If the QC worker finds less than 10 organisms (or 10% in larger subsamples) remaining in the grids or sorting tray, the sample passes; if more than 10 (or 10%) are found, the sample fails. If the first 10% of the sample lot fails, a second 10% of the sample lot will be checked by the QC worker. Sorters in-training will have their samples 100% checked until the trainer decides that training is complete.

2. After laboratory processing is complete for a given sample, all sieves, pans, trays, etc., that have come in contact with the sample will be rinsed thoroughly, examined carefully, and picked free of organisms or debris; organisms found will be added to the sample residue.

7.3.2 Identification of Macroinvertebrates

Taxonomy can be at any level, but should be done consistently among samples. In the original RBPs, two levels of identification were suggested — family (RBP II) and genus/species (RBP III) (Plafkin et al. 1989). Genus/species provides more accurate information on ecological/ environmental relationships and sensitivity to impairment. Family level provides a higher degree of precision among samples and taxonomists, requires less expertise to perform, and accelerates assessment results. In either case, only those taxonomic keys that have been peer-reviewed and are available to other taxonomists should be used. Unnamed species (i.e., species A, B, 1, or 2) may be ecologically informative, but may be inconsistently handled among taxonomists and will, thus, contribute to variability when a statewide database is being developed.

1. Most organisms are identified to the lowest practical level (generally genus or species) by a qualified taxonomist using a dissecting microscope. Midges (Diptera: Chironomidae) are

mounted on slides in an appropriate medium and identified using a compound microscope. Each taxon found in a sample is recorded and enumerated in a laboratory bench notebook and then transcribed to the laboratory bench sheet for subsequent reports. Any difficulties encountered during identification (e.g., missing gills) are noted on these sheets.

2. Labels with specific taxa names (and the taxonomist's initials) are added to the vials of specimens by the taxonomist. (Note that individual specimens may be extracted from the sample to be included in a reference collection or to be verified by a second taxonomist.) Slides are initialed by the identifying taxonomist. A separate label may be added to slides to include the taxon (taxa) name(s) for use in a voucher or reference collection.

3. Record the identity and number of organisms on the Laboratory Bench Sheet (Appendix A-3, Form 3). Either a tally counter or "slash" marks on the bench sheet can be used to keep track of the cumulative count. Also, record the life stage of the organisms, the taxonomist's initials and the Taxonomic Certainty Rating (TCR) as a measure of confidence.

4. Use the back of the bench sheet to explain certain TCR ratings or condition of organisms. Other comments can be included to provide additional insights for data interpretation. If QC was performed, record on the back of the bench sheet.

5. For archiving samples, specimen vials, (grouped by station and date), are placed in jars with a small amount of denatured 70% ethanol and tightly capped. The ethanol level in these jars must be examined periodically and replenished as needed, before ethanol loss from the specimen vials takes place. A stick-on label is placed on the outside of the jar indicating sample identifier, date, and preservative (denatured 70% ethanol).

QUALITY CONTROL (QC) FOR TAXONOMY

1. A voucher collection of all samples and subsamples should be maintained. These specimens should be properly labeled, preserved, and stored in the laboratory for future reference. A taxonomist (the reviewer) not responsible for the original identifications should spot check samples corresponding to the identifications on the bench sheet.

2. The reference collection of each identified taxon should also be maintained and verified by a second taxonomist. The word "val." and the 1st initial and last name of the person validating the identification should be added to the vial label. Specimens sent out for taxonomic validations should be recorded in a "Taxonomy Validation Notebook" showing the label information and the date sent out. Upon return of the specimens, the date received and the finding should also be recorded in the notebook along with the name of the person who performed the validation.

3. Information on samples completed (through the identification process) will be recorded in the "sample log" notebook to track the progress of each sample within the sample lot. Tracking of each sample will be updated as each step is completed (i.e., subsampling and sorting, mounting of midges and worms, taxonomy).

4. A library of basic taxonomic literature is essential in aiding identification of specimens and should be maintained (and updated as needed) in the taxonomic laboratory (see attached list). Taxonomists should participate in periodic training on specific taxonomic groups to ensure accurate identifications.

7.4 BENTHIC METRICS

Benthic metrics have undergone evolutionary developments and are documented in the Invertebrate Community Index (ICI) (DeShon 1995), RBPs (Shackleford 1988, Plafkin et al. 1989, Barbour et al. 1992, 1995, 1996b, Hayslip 1993, Smith and Voshell 1997), and the benthic IBI (Kerans and Karr 1994, Fore et al. 1996). Metrics used in these indices evaluate aspects of both elements and processes within the macroinvertebrate assemblage. Although these indices have been regionally developed, they are typically appropriate over wide geographic areas with minor modification (Barbour et al. 1995).

The process for testing the efficacy and calibrating the metrics is described in Chapter 9. While the candidate metrics described here are ecologically sound, they may require testing on a regional basis. Those metrics that are most effective are those that have a response across a range of human influence (Fore et al. 1996, Karr and Chu 1999). Resh and Jackson (1993) tested the ability of 20 benthic metrics used in 30 different assessment protocols to discriminate between impaired and minimally impaired sites in California. The most effective measures, from their study, were the richness measures, 2 community indices (Margalef's and Hilsenhoff's family biotic index), and a functional feeding group metric (percent scrapers). Resh and Jackson emphasized that both the measures (metrics) and protocols need to be calibrated for different regions of the country, and, perhaps, for different impact types (stressors). In a study of 28 invertebrate metrics, Kerans and Karr (1994) demonstrated significant patterns for 18 metrics and used 13 in their final B-IBI (Benthic Index of Biotic Integrity). Richness measures were useful as were selected trophic and dominance metrics. One of the unique features of the fish IBI presently lacking in benthic indices is the ability to incorporate metrics on individual condition, although measures evaluating chironomid larvae deformities have recently been advocated (Lenat 1993).

Four studies that were published from 1995 through 1997 serve as a basis for the most appropriate candidates for metrics, because the metrics were tested in detail in these studies (DeShon 1995, Barbour et al. 1996b, Fore et al. 1996, Smith and Voshell 1997). These metrics have been evaluated for the ability to distinguish impairment and are recommended as the most likely to be useful in other regions of the country (Table 7-1). Other metrics that are currently in use in various states are listed in Table 7-2 and may be applicable for testing as alternatives or additions to the list in Table 7-1.

Taxa richness, or the number of distinct taxa, represents the diversity within a sample. Use of taxa richness as a key metric in a multimetric index include the ICI (DeShon 1995), the fish IBI (Karr et al. 1986), the benthic IBI (Kerans et al. 1992, Kerans and Karr, 1994), and RBP's (Plafkin et al. 1989, Barbour et al. 1996b). Taxa richness usually consists of species level identifications but can also be evaluated as designated groupings of taxa, often as higher taxonomic groups (i.e., genera, families, orders, etc.) in assessment of invertebrate assemblages. Richness measures reflect the diversity of the aquatic assemblage (Resh et al. 1995). The expected response to increasing perturbation is summarized, as an example, in Table 7-2. Increasing diversity correlates with increasing health of the assemblage and suggests that niche space, habitat, and food source are adequate to support survival and propagation of many species. Number of taxa measures the overall variety of the macroinvertebrate assemblage. No identities of major taxonomic groups are derived from the total taxa metric, but the elimination of taxa from a naturally diverse system can be readily detected. Subsets of "total" taxa richness are also used to accentuate key indicator groupings of organisms. Diversity or variety of taxa within these groups are good indications of the ability of the ecosystem to support varied taxa. Certain indices that focus on a pair-wise site comparison are also included in this richness category.

Table 7-1. Definitions of best candidate benthic metrics and predicted direction of metric response to increasing perturbation (compiled from DeShon 1995, Barbour et al. 1996b, Fore et al. 1996, Smith and Voshell 1997).

Category	Metric	Definition	Predicted response to increasing perturbation
Richness measures	Total No. taxa	Measures the overall variety of the macroinvertebrate assemblage	Decrease
	No. EPT taxa	Number of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)	Decrease
	No. Ephemeroptera Taxa	Number of mayfly taxa (usually genus or species level)	Decrease
	No. Plecoptera Taxa	Number of stonefly taxa (usually genus of species level)	Decrease
	No. Trichoptera Taxa	Number of caddisfly taxa (usually genus or species level)	Decrease
Composition measures	% EPT	Percent of the composite of mayfly, stonefly, and caddisfly larvae	Decrease
	% Ephemeroptera	Percent of mayfly nymphs	Decrease
Tolerance/Intolerance measures	No. of Intolerant Taxa	Taxa richness of those organisms considered to be sensitive to perturbation	Decrease
	% Tolerant Organisms	Percent of macrobenthos considered to be tolerant of various types of perturbation	Increase
	% Dominant Taxon	Measures the dominance of the single most abundant taxon. Can be calculated as dominant 2, 3, 4, or 5 taxa.	Increase
Feeding measures	% Filterers	Percent of the macrobenthos that filter FPOM from either the water column or sediment	Variable
	% Grazers and Scrapers	Percent of the macrobenthos that scrape or graze upon periphyton	Decrease
Habit measures	Number of Clinger Taxa	Number of taxa of insects	Decrease
	% Clingers	Percent of insects having fixed retreats or adaptations for attachment to surfaces in flowing water.	Decrease

Composition measures can be characterized by several classes of information, i.e., the identity, key taxa, and relative abundance. Identity is the knowledge of individual taxa and associated ecological patterns and environmental requirements (Barbour et al. 1995). Key taxa (i.e., those that are of special interest or ecologically important) provide information that is important to the condition of the targeted assemblage. The presence of exotic or nuisance species may be an important aspect of biotic interactions that relate to both identity and sensitivity. Measures of composition (or relative abundance) provide information on the make-up of the assemblage and the relative contribution of the

populations to the total fauna (Table 7-2). Relative, rather than absolute, abundance is used because the relative contribution of individuals to the total fauna (a reflection of interactive principles) is more informative than abundance data on populations without a knowledge of the interaction among taxa (Plafkin et al. 1989, Barbour et al. 1995). The premise is that a healthy and stable assemblage will be relatively consistent in its proportional representation, though individual abundances may vary in magnitude. Percentage of the dominant taxon is a simple measure of redundancy (Plafkin et al. 1989). A high level of redundancy is equated with the dominance of a pollution tolerant organism and a lowered diversity. Several diversity indices, which are measures of information content and incorporate both richness and evenness in their formulas, may function as viable metrics in some cases, but are usually redundant with taxa richness and % dominance (Barbour et al. 1996b).

Table 7-2. Definitions of additional potential benthic metrics and predicted direction of metric response to increasing perturbation.

Category	Metric	Definition	Predicted response to increasing perturbation	References
Richness measures	No. *Pteronarcys* species	The presence or absence of a long-lived stonefly genus (2-3 year life cycle)	Decrease	Fore et al. 1996
	No. Diptera taxa	Number of "true" fly taxa, which includes midges	Decrease	DeShon 1995
	No. Chironomidae taxa	Number of taxa of chironomid (midge) larvae	Decrease	Hayslip 1993, Barbour et al. 1996b
Composition measures	% Plecoptera	Percent of stonefly nymphs	Decrease	Barbour et al. 1994
	% Trichoptera	Percent of caddisfly larvae	Decrease	DeShon 1995
	% Diptera	Percent of all "true" fly larvae	Increase	Barbour et al. 1996b
	% Chironomidae	Percent of midge larvae	Increase	Barbour et al. 1994
	% Tribe Tanytarsini	Percent of Tanytarisinid midges to total fauna	Decrease	DeShon 1995
	% Other Diptera and noninsects	Composite of those organisms generally considered to be tolerant to a wide range of environmental conditions	Increase	DeShon 1995
	% *Corbicula*	Percent of asiatic clam in the benthic assemblage	Increase	Kerans and Karr 1994
	% Oligochaeta	Percent of aquatic worms	Variable	Kerans and Karr 1994
Tolerance/ Intolerance measures	No. Intol. Snail and Mussel species	Number of species of molluscs generally thought to be pollution intolerant	Decrease	Kerans and Karr 1994
	% Sediment Tolerant organisms	Percent of infaunal macrobenthos tolerant of perturbation	Increase	Fore et al. 1996

Table 7-2. Definitions of additional potential benthic metrics and predicted direction of metric response to increasing perturbation (continued).

Category	Metric	Definition	Predicted response to increasing perturbation	References
	Hilsenhoff Biotic Index	Uses tolerance values to weight abundance in an estimate of overall pollution. Originally designed to evaluate organic pollution	Increase	Barbour et al. 1992, Hayslip 1993, Kerans and Karr 1994
Tolerance/ Intolerance measures (continued)	Florida Index	Weighted sum of intolerant taxa, which are classed as 1 (least tolerant) or 2 (intolerant). Florida Index = 2 X Class 1 taxa + Class 2 taxa	Decrease	Barbour et al. 1996b
	% Hydropsychidae to Trichoptera	Relative abundance of pollution tolerant caddisflies (metric could also be regarded as a composition measure)	Increase	Barbour et al. 1992, Hayslip 1993
Feeding measures	% Omnivores and Scavengers	Percent of generalists in feeding strategies	Increase	Kerans and Karr 1994
	% Ind. Gatherers and Filterers	Percent of collector feeders of CPOM and FPOM	Variable	Kerans and Karr 1994
	% Gatherers	Percent of the macrobenthos that "gather"	Variable	Barbour et al. 1996b
	% Predators	Percent of the predator functional feeding group. Can be made restrictive to exclude omnivores	Variable	Kerans and Karr 1994
	% Shredders	Percent of the macrobenthos that "shreds" leaf litter	Decrease	Barbour et al. 1992, Hayslip 1993
Life cycle measures	% Multivoltine	Percent of organisms having short (several per year) life cycle	Increase	Barbour et al. 1994
	% Univoltine	Percent of organisms relatively long-lived (life cycles of 1 or more years)	Decrease	Barbour et al. 1994

Tolerance/Intolerance measures are intended to be representative of relative sensitivity to perturbation and may include numbers of pollution tolerant and intolerant taxa or percent composition (Barbour et al. 1995). Tolerance is generally non-specific to the type of stressor. However, some metrics such as the Hilsenhoff Biotic Index (HBI) (Hilsenhoff 1987, 1988) are oriented toward detection of organic pollution; the Biotic Condition Index (Winget and Mangum 1979) is useful for evaluating sedimentation. The Florida Index (Ross and Jones 1979) is a weighted sum of intolerant taxa (insects and crustaceans) found at a site (Beck 1965) and functions similarly to the HBI (Hilsenhoff 1987) used in other parts of the country. The tolerance/intolerance measures can be independent of taxonomy or can be specifically tailored to taxa that are associated with pollution tolerances. For example, both the percent of Hydropsychidae to total Trichoptera and percent Baetidae to total Ephemeroptera are estimates of evenness within these insect orders that generally are considered to be sensitive to pollution. As these families (i.e., Hydropsychidae and Baetidae) increase in relative abundance, effects of pollution (usually organic) also increase. Density (number of

individuals per some unit of area) is a universal measure used in all kinds of biological studies. Density can be classified with the trophic measures because it is an element of production; however, it is difficult to interpret because it requires careful quantification and is not monotonic in its response (i.e., density can either decrease or increase in response to pollution) and is usually linked to tolerance measures.

Feeding measures or trophic dynamics encompass functional feeding groups and provide information on the balance of feeding strategies (food acquisition and morphology) in the benthic assemblage. Examples involve the feeding orientation of scrapers, shredders, gatherers, filterers, and predators. Trophic dynamics (food types) are also included here and include the relative abundance of herbivores, carnivores, omnivores, and detritivores. Without relatively stable food dynamics, an imbalance in functional feeding groups will result, reflecting stressed conditions. Trophic metrics are surrogates of complex processes such as trophic interaction, production, and food source availability (Karr et al. 1986, Cummins et al. 1989, Plafkin et al. 1989). Specialized feeders, such as scrapers, piercers, and shredders, are the more sensitive organisms and are thought to be well represented in healthy streams. Generalists, such as collectors and filterers, have a broader range of acceptable food materials than specialists (Cummins and Klug 1979), and thus are more tolerant to pollution that might alter availability of certain food. However, filter feeders are also thought to be sensitive in low-gradient streams (Wallace et al. 1977). The usefulness of functional feeding measures for benthic macroinvertebrates has not been well demonstrated. Difficulties with the proper assignment to functional feeding groups has contributed to the inability to consider these reliable metrics (Karr and Chu 1997).

Habit measures are those that denote the mode of existence among the benthic macroinvertebrates. Morphological adaptation among the macroinvertebrate distinguishes the various mechanisms for maintaining position and moving about in the aquatic environment (Merritt et al. 1996). Habit categories include movement and positioning mechanisms such as skaters, planktonic, divers, swimmers, clingers, sprawlers, climbers, burrowers. Merritt et al. (1996) provide an overview of the habit of aquatic insects, which are the primary organisms used in these measures. Habit measures have been found to be more robust than functional feeding groups in some instances (Fore et al. 1996).

7.5 BIOLOGICAL RECONNAISSANCE (BioRecon) OR PROBLEM IDENTIFICATION SURVEY

The use of biological survey techniques can serve as a screening tool for problem identification and/or prioritizing sites for further assessment, monitoring, or protection. The application of biological surveys in site reconnaissance is intended to be expedient, and, as such, requires an experienced and well-trained biologist. Expediency in

FIELD EQUIPMENT/SUPPLIES NEEDED FOR BENTHIC MACROINVERTEBRATE SAMPLING —BIORECON

- standard D-frame dip net, 500 μ opening mesh, 0.3 meter width (~ 1.0 ft frame width)
- sieve bucket, with 500 μ opening mesh
- 95% ethanol
- sample containers
- sample container labels
- forceps
- field data sheets*, pencils, clipboard
- first aid kit
- waders (chest-high or hip boots), rubber gloves (arm-length)
- camera
- Global Positioning System (GPS) Unit

* It is helpful to copy fieldsheets onto water-resistant paper for use in wet weather conditions

this technique is to minimize time spent in the laboratory and with analysis. The "turn-around" time from the biosurvey to an interpretation of findings is intended to be relatively short. The BioRecon is useful in discriminating obviously impaired and non-impaired areas from potentially affected areas requiring further investigation. Use of the BioRecon allows rapid screening of a large number of sites. Areas identified for further study can then either be evaluated using more rigorous bioassessment methods for benthic macroinvertebrates and/or other assemblages, or ambient toxicity methods.

Because the BioRecon involves limited data generation, its effectiveness depends largely on the experience of the professional biologist performing the assessment. The professional biologist should have assessment experience, a knowledge of aquatic ecology, and basic expertise in benthic macroinvertebrate taxonomy.

The BioRecon presented here is refined and standardized from the original RBP I (Plafkin et al. 1989), and is based on the technique developed by Florida DEP (1996), from which the approach derives its name. This biosurvey approach is based on a multihabitat approach similar to the more rigorous technique discussed in Section 7.2. The most productive habitats, i.e., those that contain the greatest diversity and abundance of macroinvertebrates, are sampled in the BioRecon. As a general rule, impairment is judged by richness measures, thereby emphasizing the presence or absence of indicator taxa. Biological attributes such as the relative abundance of certain taxa may be less useful than richness measures in the BioRecon approach, because samples are processed more quickly and in a less standardized manner.

7.5.1 Sampling, Processing, and Analysis Procedures

1. A 100 m reach representative of the characteristics of the stream should be selected. For the BioRecon, it is unlikely that the alternative reach designation approach (i.e., x times the stream width), will improve the resolution beyond a standard 100 m reach. Whenever possible, the area should be at least 100 meters upstream from any road or bridge crossing to minimize its effect on stream velocity, depth and overall habitat quality. There should be no major tributaries discharging to the stream in the study area.

2. Before sampling, complete the "Physical Characterization/Water Quality Field Data Sheet" (Appendix A-1, Form 1) to document site description, weather conditions, and land use. After sampling, review this information for accuracy and completeness.

3. The major habitat types (see 7.2.1 for habitat descriptions) represented in the reach are to be sampled for macroinvertebrates. A total of 4 jabs or kicks will be taken over the length of the reach. A minimum of 1 jab (or kick) is to be taken in each habitat. More than 1 jab may be desired in those habitats that are predominant. Habitat types contributing less than five percent of the stable habitat in the stream reach should not be sampled. Thus, allocate the remaining jabs proportionately among the predominant substrates. The number of jabs taken in each habitat type should be recorded on the field data sheet.

4. Sampling begins at the downstream end of the reach and proceeds upstream. A total of four jabs or kicks will be taken over the length of the reach; a single *jab* consists of forcefully thrusting the net into a productive habitat for a linear distance of 0.5 m. A *kick* is a stationary sampling accomplished by positioning the net and disturbing the substrate for a distance of 0.5 m upstream of the net.

5. The jabs or kicks collected from the multiple habitats will be composited into a sieve bucket to obtain a single homogeneous sample. If clogging occurs, discard the material in the net and redo that portion of the sample in the same habitat type but in a different location. Remove large debris after rinsing and inspecting it for organisms; place any organisms found into the sieve bucket.

6. Return to the bank with the sampled material for sorting and organism identifications. Alternatively, the material can be preserved in alcohol and returned to the laboratory for processing (see Step 7 in Section 7.1.1 for instructions).

7. Transfer the sample from the sieve bucket (or sample jar, if in laboratory) to a white enamel or plastic pan. A second, smaller, white pan may be used for the actual sorting. Place small aliquots of the detritus plus organisms in the smaller pan diluted with a minimal amount of site water (or tap water). Scan the detritus and water for organisms. When an organism is found, examine it with a hard lens, determine its identity to the lowest possible level (usually family or genus), and record it on the Preliminary Assessment Score Sheet (PASS) (Appendix A-3, Form 4) in the column labeled "tally." Place representatives of each taxon in a vial, properly labeled and containing alcohol.

QUALITY CONTROL (QC)

1. Sample labels must be properly completed, including the sample identification code date, stream name, sampling location, and collector's name and placed into the sample container. The outside of the container should be labeled with the same information. Chain-of-custody forms, if needed, must include the same information as the sample container labels.

2. After sampling has been completed at a given site, all nets, pans, etc. that have come in contact with the sample will be rinsed thoroughly, examined carefully, and picked free of organisms or debris. Any additional organisms found should be placed into the sample containers. The equipment should be examined again prior to use at the next sampling site.

3. A second biologist familiar with the recognition and taxonomy of the organisms should check the sample to ensure all taxa are encountered and documented.

8. If field identifications are conducted, verify in the lab and make appropriate changes for misidentifications.

9. Analysis is done by determining the value of each metric and comparing to a predetermined value for the associated stream class. These value thresholds should be sufficiently conservative so that "good" conditions or non-impairment is verified. Sites with metric values below the threshold(s) are considered "suspect" of impairment and may warrant further investigation. These simple calculations can be done directly on the PASS sheet.

7.6 TAXONOMIC REFERENCES FOR MACROINVERTEBRATES

The following references are provided as a list of taxonomic references currently being used around the United States for identification of benthic macroinvertebrates. Any of these references cited in the text of this document will also be found in Chapter 11 (Literature Cited).

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