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**PACIFIC NORTHWEST AQUATIC MONITORING PARTNERSHIP (PNAMP)**

# METHODS FOR THE COLLECTION AND ANALYSIS OF BENTHIC MACROINVERTEBRATE ASSEMBLAGES IN WADEABLE STREAMS OF THE PACIFIC NORTHWEST

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# I. BACKGROUND AND OBJECTIVES

## A. BACKGROUND AND PURPOSE OF THIS DOCUMENT

Benthic macroinvertebrates, or benthos, (benthic = bottom, macro = large, invertebrate = animal without a backbone) are organisms that live on the bottom of streams and rivers. The sampling protocols described in this document were designed to generate data sufficient to characterize the benthic macroinvertebrate assemblage and evaluate impacts from human caused disturbances. The purpose of this protocol is to characterize the macroinvertebrate assemblage for a stream reach as an indicator of biological integrity and stream health. It is not intended to characterize the condition of individual stream habitats or to provide data to analyze the macroinvertebrates as a food source for fish.

These protocols describe field, laboratory and data analysis protocols for benthic macroinvertebrates and do not cover safety issues which are covered in other documents (Peck et al., 2006). It is highly recommended that other physical habitat, biological and/or water quality data also be collected at any given site, however the protocols for this type of data will not be described in this document.

These benthic macroinvertebrate protocols apply only to streams that are perennial and wadeable. These protocols were synthesized from the following protocols:

- Oregon Department of Environmental Quality
- Washington Department of Ecology (Ecology, 2001)
- Multi-federal agency sponsored monitoring program (U.S.F.S., 2004)
- U.S. Environmental Protection Agency (EPA)'s Environmental Monitoring and Assessment Program (Peck et al., 2006)
- Bureau of Land Management (BLM)'s National Aquatic Monitoring Center (Hawkins et al., 2001)

These collection procedures, along with other environmental data, will allow analysis of biotic data with either multi-metric [e.g., an Index of Biotic Integrity (IBI)] or predictive models [e.g., River InVertebrate Prediction and Classification System (RIVPACS)] methods. Use of these procedures will allow users to share data, express their data in terms of standardized bioassessment measures, and thus directly compare their results with all other parties using these methods. Their general use does not preclude use of other sampling procedures that may be needed to address the specific objectives of individual projects.

In this document, we are attempting to strike a balance between providing flexibility while still ensuring that the resultant data are sufficiently compatible to be combined for analysis. There are subjects where there is agreement between all of the protocols listed above, for example mesh size. In these cases, we make a single recommendation. However, there are subjects where there is not such agreement, for example placement of the sampling device in the stream. For these subjects, we examined the different methods and as long as the different methods **did not** appreciably affect the ability of the data to be combined, we have included multiple recommendations.

## B. RATIONALE FOR SELECTING THIS RESOURCE TO MONITOR

Macroinvertebrates are good indicators of watershed health because they live in the water for all or most of their life, are easy to collect, differ in their tolerance to amount and types of pollution/habitat alteration, can be identified in a laboratory, often live for more than one year; have limited mobility, and are integrators of environmental condition.

## C. MEASURABLE OBJECTIVES

The presence and numbers of the different types of benthic macroinvertebrates provide accurate information about the health of a stream and watershed. It is the objective of the Clean Water Act - to "restore and maintain the chemical, physical and biological integrity of the Nation's waters". Biological integrity is commonly defined as "the ability to support and maintain a balanced, integrated, and adaptive community of organisms having a species composition, diversity and functional organization comparable to those of natural habitats within a region" (Karr, J. R. and D. R. Dudley. 1981).

# II. SAMPLING DESIGN

## A. RATIONAL FOR SELECTING THIS SAMPLING DESIGN OVER OTHERS

Overall sample site selection or design, including the distribution and location of sample sites, the number of sites to sample, etc., is not part of these protocols. These protocols are limited to actual sampling/collecting methods once the site has been selected. These protocols apply to most types of sampling designs that incorporate benthic macroinvertebrate sampling for wadeable streams.

## B. SITE SELECTION

Stream reaches or sites for benthic macroinvertebrate monitoring are typically selected using either a targeted or probabilistic design depending on the study design. Sites selected using a targeted design generate data that is relevant for measuring impacts from a known source or answering other site specific questions. Sites selected using a probabilistic design provide information of the overall status or condition of the watershed, basin, or region. The type of sampling design chosen will depend upon the objectives of your monitoring program. These protocols apply to both targeted and probabilistic sampling designs.

## C. SAMPLING FREQUENCY AND REPLICATION

Stream reaches or sites are sampled at a minimum of once during the index period. To address annual variation 10% of the sites or reaches in your study should be re-sampled annually. Targeting individual sites for sampling at the beginning and end of the index will generate data that explains temporal variation.

## D. RECOMMENDED NUMBER AND LOCATION OF SAMPLING SITES

The number and location of your sampling reach or site will depend upon your monitoring objectives and sampling design.

## E. RECOMMENDED FREQUENCY AND TIMING OF SAMPLING

Sampling and comparisons of data from the same seasons (or index periods) as the previous year's sampling provides some correction and minimization of annual variability. The index period recommended in this protocol is July 1st -October 15$^{th}$. This is discussed in detail in section 3.

# III. FIELD METHODS AND QUALITY ASSURANCE

## A. FIELD METHODS

### 1. Type of Sampler

The most commonly used gear types in the Pacific Northwest are the D-frame kick net and Surber sampler. Either type of gear will work for the methods described below. An important factor in the choice of gear is the desire to be consistent with others in your state or watershed and/or to use an existing data set in your analysis. For many monitoring activities, this is likely to be the overriding factor in gear type selection. Cazier (1993) found very little difference between the sampler types for their use in the collection of organisms for bioassessment metrics in Northern Idaho Palouse streams. Barton and Metcalfe-Smith (1992) also found no differences in several benthic sampling devices for summarized index data. Cao et al (2005) compared surber and D-frame kicknet samples and found that subsamples with the same number of individuals were highly and consistently comparable between sampling devices.

*D-frame kick net:* The D-shaped frame for the net commonly used by laying the spine of the net firmly onto the stream bottom. The dimensions of the D-shaped frame are 1 ft. wide (along the spine) and 1 ft. tall where the widest part of the "D" attaches to a long pole. The net is either cone or bag-shaped for the capture of organisms. This type of net is easy to transport and can be used in a variety of habitat types. However, the D-net must have a defined or delimited area that is sampled/kicked. This area will either be $1ft^2$ or 1ft x 2ft.

*Surber:* The dimensions of the Surber frame are approximately 1 ft. x 1 ft.. It is horizontally placed on cobble substrate to delineate an approximately 1 $ft^2$ area. A vertical section of the frame has the net attached and captures the dislodged organisms from the sampling area. The use of the Surber is generally restricted to depths of less than 1 ft..

### 2. Mesh Size

The mesh size refers to the size of the openings in the net of the sampling device. A 500 µm mesh size is recommended for use in stream bioassessments in the Pacific Northwest regardless of the type of sampler (D-frame kick net or surber). A mesh size of approximately 500 µm is consistently used across all states and federal biological assessment programs in the Pacific Northwest.

### 3. Sample Reach Length

Sample reaches need to be long enough to incorporate local habitat-scale variation. In the Pacific Northwest, the use of sample reaches that increase in proportion to stream size (e.g. multiples of wetted- or bankfull stream width), is by far the most commonly used reach length method. Forty times the width of the sample reach length, while based on research related to fish assemblages, is also considered adequate for characterizing the benthic assemblage and the associated habitat. This length is adequate to insure that the repeating patterns of variation that are associated with riffle-pool sequences and meander bend morphology are accounted for most wadeable streams in the Pacific Northwest. At each site, the stream reach location is determined by identifying the lower end of the study unit and estimating an upstream distance of 40 times the average wetted stream width. Note that other parameters that you may

be sampling such as physical habitat or fish community may influence the length of the sample reach. Use of this reach length identification strategy assumes the channel segment type does not change within the estimated distance.

### 4. Habitats sampled

Riffles (or fast moving water habitats, this document will use the term riffle for simplicity) are the primary habitat type recommended for sampling macroinvertebrates in the Pacific Northwest. Riffle areas have relatively fast currents, moderate to shallow depth, and cobble/gravel substrates. These areas generally have the most diverse macroinvertebrate assemblage. Also, standardization of field methods is simplified by using a single, readily identifiable habitat type. Throughout most of Washington, Oregon and Idaho, riffles are common features of wadeable streams. Past research has demonstrated that biological signals from riffles are consistent and easily detected from surveys in this habitat type.

We also provide a transect based approach in this protocol. It is useful in places where riffles do not occur or for programs that apply to very broad geographic areas. Gerth and Herlihy (2006) compared transect based and riffle sampling results and found that these sample type differences did not influence the detection of important environmental gradients.

### 5. Compositing

Compositing is taking multiple macroinvertebrates samples from the study reach and combining them into a single sample. From this combined sample, a portion of the sample is identified and enumerated in the laboratory. Carter and Resh (2001) found that across the nation 74.4% of state bioassessment programs composited their samples. The primary advantages of composting sample are that it is less expensive (one sample for laboratory analysis versus many samples) and that it represents more individual microhabitat patches. Compositing samples generates data sets with a larger amount of taxonomic information.

### 6. Area of stream bottom sampled

Due to the patchy nature of macroinvertebrate distribution, a very important factor is how much of the stream bottom is actually sampled. The area of stream bottom sampled will have significant consequences on how representative your sample is of a reach. Sampling larger areas will yield more species and therefore be more representative of the stream. However, there is a point of diminishing returns, combined with the feasibility (and habitat destruction) of collecting samples from an extremely large portion of the stream bottom. In the Palouse region of Washington and Idaho, Cazier (1993) found that 75% of the taxa were collected by sampling 5.3$ft^2$ of stream bottom and that 100% of the 45 taxa were collected within 19.4$ft^2$ of stream bottom. Clearly, collection of a single Surber sample is inadequate. Sampling from 8$ft^2$ of stream bottom is recommended. Be aware that the outcome of sampling a greater area be results with larger values (for both multi-metric indices and predictive models) than that produced from a standard 8$ft^2$ sample.

### 7. Number of samples in the composite

There is more that one method to attain a single composited sample that represents 8$ft^2$ of stream bottom. A total of 4 or 8 samples can be distributed within the reach length using the gear types described above. Taking more, smaller samples would increase the number of individual microhabitat patches

encountered. However, there is little evidence, given a standard amount of stream bottom sampled ($8ft^2$), that there is a difference between using 4 or 8 samples to make up a composite sample. Therefore, the following are all recommendations for methods to distribute the sample collection sites within a reach. Macroinvertebrate samples should be taken from either:

- *4 different riffle habitats.* Two separate 1 $ft^2$ fixed-area samples are taken from each habitat unit for a total of 8 samples (a total of $8ft^2$ of stream bottom sampled), or ;
- *8 different riffle habitats.* One 1 $ft^2$ fixed-area sample is taken from each habitat unit for a total of 8 samples, (a total of $8ft^2$ of stream bottom sampled), or;
- *4 different fast-water habitats.* One 2 ft. by 1 ft. fixed area sample is taken from each habitat unit for a total of 4 samples (a total of $8ft^2$ of stream bottom sampled).
- *8 evenly spaced transects along the reach.* One 1 $ft^2$ fixed-area sample is taken at 8 evenly spaced transects along the entire reach (a total of $8ft^2$ of stream bottom sampled).

The 4 or 8 individual samples will be composited into a single sample for taxonomic identification and enumeration that will be used to represent the sample reach.

### 8. Placement of sampling device

Once the stream reach of interest has been identified, the selection of which riffle/fast-moving habitat(s) to sample within this reach needs to be decided (see above, Section III.A.7). Then once the riffle/fast-moving habitat is identified, the location within that riffle/fast-moving habitat of where the sampler is placed must be determined.

The transect-based method does not rely on the identification of riffle/fast-moving habitat(s). Once evenly spaced transects are laid out along the reach, place the ¼ of the way across the stream width on the left side. Then continue to sample at each transect, alternating between left, center (1/2 way across the stream) and right (3/4 of the way across) for each of the 8 transects.

For those methods, using riffle/fast-moving habitats, there are four primary methods for determining where to place your sampling device in the riffle/fast-moving habitat unit. They are referred to here as the random, systematic, grid, and best professional judgment methods. Any of these methods are acceptable, the random, systematic, or grid methods are recommended for field crews with less experience.

*Random method:* Determine net placement within riffle/fast-moving habitat by generating 2 pairs of random numbers between 0 and 9 for each sample. The first number in each pair (multiplied by 10) represents the percent upstream along the habitat unit's length. The second number in each pair represents the percent of the stream's width from bank left. Take samples where the length and width distances intersect (estimate by eye). If it is not possible to take a sample at the locations (log in the way, too deep, etc.), draw additional random numbers until you can.

*Systematic method:* The beginning sampling point either left side, center, or right side, within the riffle/fast-moving habitat is assigned at random using a die or other suitable means (e.g., digital watch). Once this first sampling point has determined, points at successive riffles are assigned in order (Left, Center, Right as you face downstream) as 25%, 50%, and 75% of the wetted width, respectively.

*Grid method:* Beginning at the downstream end of the reach, select the first riffle/fast-moving habitat and collect one sample from each riffle/fast-moving habitat. Visualize a 3 x 3 grid over each riffle/fast-moving habitat. As shown below, for the first habitat area, select the lower left square, for the second select the lower center; third, the lower right; etc.

| | | |
|---|---|---|
| 7 | 8 | |
| 4 | 5 | 6 |
| 1 | 2 | 3 |

*Best Professional Judgment method:* A variety of riffle/fast-moving habitat habitats are chosen within the reach to ensure representativeness of the biological community. The locations within a reach are determined by finding representative combinations of the following variables: depth of riffle/fast-moving habitat, substrate size, and location within a riffle/fast-moving habitat area of the stream (forward, middle, back). This method assumes the largest variety of benthic macroinvertebrate taxa will be collected and that any differences identified through numerical analyses will represent change over time (if the same site) or divergence from a reference condition.

## 9. Field Processing

Field processing includes activities such as sorting, removing debris and sieving macroinvertebrate samples in the field. In the Pacific Northwest, generally very little field processing is done other than removing the largest pieces of organic debris (i.e. sticks) and rocks from the sample, after ensuring that any attached organisms are removed. Removal of these large objects from the sample reduces damage to the organisms and allows for a smaller sample so that less preservative is needed.

## 10. Preservatives

All states and other agency programs in the Pacific Northwest preserve organisms with ethanol ranging in concentration from 70 - 95%. The use of 95% ethanol is recommended. Care is taken that adequate quantity of preservative is used (i.e. 3 parts preservative to 1 part sample by volume) and that the preservative is added in a timely manner (within an hour or two of collection). This often requires that preservative be packed into the field. Preservatives should be added to the lip of the container and as much air removed as possible to minimize damage to the organisms.

## 11. Sampling season (index period)

Macroinvertebrate assemblages integrate stressor effects over the course of the year, and their seasonal cycles of abundance and taxa composition are fairly predictable within the limits of interannual variability (Gibson et al., 1996). Sampling and comparing data from the same season (or index period) as the previous year's sampling provides some correction and minimization of annual variability. The index period is a time frame during the year in which samples are collected and are assumed to contain uniformly representative life stages of species in the community.

The index period recommended in this protocol is July 1st -October 15th and it was chosen for the following reasons: adequate time is available for the instream environment to stabilize following natural disturbances (e.g. spring floods); many macroinvertebrates reach body sizes that can be readily identified,

and; representation of benthic macroinvertebrate species reaches a maximum, particularly during periods of pre-emergence (typically mid-spring to late-summer).

**Table 1 Summary of Field Method Recommendations**

| ISSUE | RECOMMENDATION |
|---|---|
| Type of Sampler | Either D-frame or Surber |
| Mesh Size | 500 micron |
| Sample Reach Length | Depends on monitoring objectives, but generally 40 times the channel width. |
| Habitats sampled | Riffle (fast water habitat) or transect based |
| Area of stream bottom sampled | 8 $ft^2$ of stream bottom (requires 4-8 subsamples). |
| Compositing | Yes, multiple samples are combined in the field into one sample for laboratory identification and enumeration. |
| Number of samples in composite | Multiple, either:<br>**4 different riffle/fast-moving habitats** – two separate 1 $ft^2$ fixed-area samples from each riffle/fast-moving habitat for a total of 8 samples to composite, or ;<br>**8 different riffle/fast-moving habitats** - one 1 $ft^2$ fixed-area sample from each riffle/fast-moving habitat for a total of 8 samples to composite, or;<br>**4 different riffle/fast-moving habitats** - One 2 ft. by 1 ft. fixed area sample from each riffle/fast-moving habitat for a total of 4 samples to composite.<br>**8 evenly spaced transects along the reach -** One 1 $ft^2$ fixed-area sample is taken at evenly spaced transects along the entire reach.<br><br>**All methods total 8$ft^2$ stream bottom being sampled.** |
| Placement of sampling device | Any of the following methods are acceptable:<br><br><u>Riffle/fast-moving habitats:</u><br>**Random**: Determine net placement within each riffle/fast-moving habitat unit by generating 2 pairs of random numbers between 0 and 9. The first number in each pair (multiplied by 10) represents the percent upstream along the habitat unit's length. The second number in each pair represents the percent of the stream's width from bank left. Take samples where the length and width distances intersect (estimate by eye).<br>**Systematic:** The beginning sampling point within the riffle/fast-moving habitat is selected at random, points at successive transects are selected in order as 25%, 50%, and 75% of the wetted width (left, center, right as you face downstream).<br>**Grid method:** Beginning at the downstream end of the reach, select the first riffle/fast-moving habitat and collect one sample from each riffle/fast-moving habitat. Visualize a 3 x 3 grid over each riffle/fast-moving habitat. For the first habitat area, select the lower left square, for the second select the lower center; third, the lower right; etc.<br>**Best Professional Judgment:** A variety of riffle/fast-moving habitat habitats are chosen within the reach by finding representative combinations of the following variables: depth of riffle/fast-moving habitat, substrate size, and location within a riffle/fast-moving habitat area of the stream (forward, middle, back).<br><br><u>Transect-based approach:</u><br>Once evenly spaced transects are laid out along the reach, place the ¼ of the way across the stream width on the left side. Then continue to sample at each transect, alternating between left, center (1/2 way across the stream) and right (3/4 of the way across) for each of the 8 transects. |
| Field Processing | Minimal removal of large material. |
| Preservatives (field) | 95% ethanol |
| Sampling season (index period) | July 1 – October 15 |

## B. FIELD QUALITY ASSURANCE

The overarching quality assurance objective for field data is to ensure that data of known quality are generated. To achieve this goal, data must be reviewed for 1) precision, 2) representativeness, 3) comparability, and 4) completeness.

### 1. Precision

Precision is the degree of agreement among repeated measurements of the same characteristic, or parameter, and gives information about the consistency of methods. Precision is estimated by re-sampling 10% of the reaches sampled annually using the same protocols and the same field crew.

### 2. Representativeness

Representativeness is the extent to which measurements actually represent the true environmental condition. It is the degree to which data from the project accurately represent a particular characteristic of the watershed that is being tested. Representativeness of samples is ensured by adherence to standard field sampling and measurement and laboratory protocols. The sampling protocol is designed to produce consistent and repeatable results in each stream reach. Physical variability within riffle/fast-moving habitats is accounted for through sampling based on depth, substrate distribution, and location within the riffle habitat.

### 3. Completeness

Completeness is defined as the proportion of useable data gathered (Kirchmer and Lombard 2001). Sample loss is minimized with sturdy sample storage vessels, adequate labeling of each vessel, adequate and timely addition of preservatives. Sample contamination occurs when containers are improperly sealed or stored. Loss of benthic material or desiccation diminishes the integrity of the sample. The goal for completeness of benthic macroinvertebrate data sets is 95% of the total samples collected. Completeness is defined as the total number of samples that we are confident in using for further data analysis following field collection (Ecology, 2001).

### 4. Comparability

Comparability describes the confidence in comparing one data set to another. Comparability of data sets is primarily achieved through adherence to commonly accepted protocols (e.g. field sampling, analytical methods and objectives). The primary purpose for this document is to improve comparability among monitoring programs in the Pacific Northwest Region.

## C. SEQUENCE OF EVENTS

- Check field equipment against list before leaving.
- Review protocols.
- Evaluate and delineate the reach (how this is done will depend upon your objectives and is not covered in this protocol).
- Collect or measure water quality parameters of interest (not covered in this protocol).

- Collect macroinvertebrates as described in section D.
- Conduct physical habitat site measurements (not covered in this protocol).
- Send macroinverebrate samples to the laboratory for identification and enumeration.

## D. DETAILS OF TAKING MEASUREMENTS

Field procedures follow a sequence of measurements that ensure quality information is collected and a reasonable amount of time is spent at each site. Sampling will begin at the first fast-water habitat encountered at the site and will continue upstream with the next 3 (or 7) fast-water habitat units.

Once the location for sampling within the riffle/fast-moving habitat is determined, with the net opening facing into the flow of water, position the net quickly and securely on the stream bottom to eliminate gaps under the frame.

Collect benthic macroinvertebrates from within the 1ft² (or 1ft x 2ft) sampling frame in front of the net. If no sampling frame is used, visually imagine the square sampling plot in front of the net and restrict your sampling to within that area.

Work from the upstream edge of the sampling plot backward and carefully pick up and rub stones directly in front of the net to remove attached animals. Quickly inspect each stone to make sure you have dislodged everything and then set it aside. If a rock is lodged in the stream bottom, rub it a few times concentrating on any cracks or indentations.

After removing all large stones, keeping the sampler securely in position, starting at the upstream end of the quadrat, vigorously kick the remaining finer substrate within the sampling area for 30 seconds.

Pull the net up out of the water. Immerse the net in the stream several times or splash the sides of the net with stream water to remove fine sediments and to concentrate organisms at the end of the net. After completing the sample, hold the net vertically and rinse material to the bottom of the net.

After taking a sample, empty the net's contents into the bucket. If the net has a cup at the end, remove the cup over the top of the bucket and wash it out.

Carefully transfer the material from the bucket (benthic macroinvertebrates and organic matter) into the sample jar. Inspect the bucket for any organisms that might remain. Remove any remaining organisms by hand and place in the sample jar.

Repeat the above procedure at the remaining riffle/fast-water habitats.

The composite sample will be preserved in one or more sample jars (generally one jar) depending on the amount of material collected.

The macroinvertebrate field samples are preserved in 95% ethanol.

Storage containers can be either heavy-duty freezer bags or plastic wide-mouth jars. Plastic wide-mouth jars are preferred. A double bag system is used when storing samples in freezer bags. Extra care must be taken when using freezer bags due to the increased risk of breakage and leakage that would result in sample loss and a hazardous situation. Sample labels are placed in the dry space between the inner- and outer freezer bags. Jars are labeled both inside with a paper label and outside on both the lid and bottle.

Label information should contain at the following information, at a minimum, the following: name of stream, date of collection, County and State, project name (if applicable), and collector's name.

# IV. LABORATORY METHODS AND QUALITY ASSURANCE

After samples are collected in the field, they will be shipped to a taxonomic laboratory. While it is possible to conduct your own taxonomic identifications and enumerations, it requires a great deal of specialized knowledge. It is recommended that samples be sent to a laboratory that specializes in the identification and enumeration of macroinvertebrates and is familiar with the taxa from the Pacific Northwest.

## A. LABORATORY METHODS

### 1. Subsampling/number of organisms identified

Taking a benthic sample and only using a fraction of the sample obtained from the field for identification and enumeration is called "subsampling". Subsampling has been crucial to the reduction in the cost and time associated with processing benthic samples (Barbour, et al. 1999). The goal of subsampling is to provide an unbiased representation of a larger sample (Barbour and Gerritsen, 1996). Subsampling procedures developed by Hilsenhoff (1987) and modified by Plafkin et al. (1989) were used in the original Rapid Bioassessment Protocols. Over half of the state bioassessment programs in the Nation use only a 100 organism count (Carter and Resh, 2001). In Rocky Mountain streams of Wyoming, it was found that a 200 organism subsample was adequate in terms of information return for the investment (Gerritsen et al., 1996). However, others (Kerans and Karr, 1994) have advocated identifying all organisms in the sample. Most agencies in the Pacific Northwest use a 300 to 500 organism subsample as a balance between maximizing the information obtained from a sample while keeping costs feasible. A 300 organism count is the minimal level recommended. However, if data will be analyzed using a predictive model (e.g., RIVPACS), then a 350 count or greater is recommended (Ostermiller and Hawkins, 2004).

### 2. Subsample approach: Fixed Count/known area of tray

A "fixed count" refers to approach where a predetermined number of organisms are sorted and numerated from a sample (not to be confused with actual sample size). It is useful for rapid return of data and appropriate for generation of proportional metrics. The most commonly use type of tray for this method is the Caton tray, which is a sampling tray with 30 6x6 cm grids.

### 3. Amount of tray area evaluated

The sample is spread out in the tray and grids are randomly selected. Macroinvertebrates are then picked out of the grid. A minimum of 3 randomly selected grids of the tray are evaluated until the desired number of organisms has be subsampled.

### 4. Large and Rare Organisms

After subsampling, the remainder of the sample is visually examined for large and rare organisms for a fixed amount of time (i.e. 10 minutes). These organisms are identified and added to the total taxa list either directly into the database or in the comments. In the scientific community there is some disagreement about whether to conduct these searches, and if they are conducted, what to do with the resulting data.

Vinson and Hawkins (1996) found that conducting a large-rare search before subsampling the remaining sample increased the number of taxa that will be encountered. They also found that adding these taxa to the raw taxa richness values (from subsamples with >300 organisms) produces data that are defensible regardless of the metrics that these data were used to calculate. VanSickle (2005) found that excluding rare taxa from predictive models either increased average predictive model or had no effect.
In the Pacific Northwest, the use of this method varies and is therefore an optional method depending upon the type of project.

### 5. Taxonomic levels and specific taxa

In the Pacific Northwest, most organisms are identified to the lowest practical taxonomic level (generally genus or species) by a qualified taxonomist using a dissecting microscope. Genus/species provides more accurate information on ecological relationships and sensitivity to environmental impairment. All States and most Federal agencies in the Pacific Northwest use lowest practical level (genus/species) of identification with the exception of oligochaetes, molluscs, microcrustaceans, and mites. Each taxa found in a sample is recorded and enumerated. Each taxa should also be assigned the appropriate Integrated Taxonomic Information System (ITIS) code.

**Table 2 Summary of Laboratory Method Recommendations**

| ISSUE | RECOMMENDATION |
|---|---|
| Sub-sampling | Yes |
| Minimum number of organisms identified | 500 optimal (300 minimum, 350 if using predictive models) |
| Subsample approach: Fixed Count/known area of tray | Yes |
| Amount of tray area evaluated | Minimum of 3 randomly selected grids of the Caton tray. |
| Large and rare search | Optional |
| Taxonomic level and specific taxa | Lowest practical level (genus/species) of identification with the exception of oligochaetes, molluscs, microcrustaceans, and mites. |

## B. LABORATORY QUALITY ASSURANCE

### 1. Macroinvertebrate Sorting

Precision of the sub-sampling process is evaluated by re-sorting a new sub-sample of the original samples. Ten percent of the benthic macroinvertebrate samples (e.g. 1 of 10 samples) are re-sorted by a second laboratory technician. Sorting results that are less than 95% similar would indicate the need for more thorough distribution of sample materials in the sub-sampling tray or more special attention given to easily missed taxa when sorting (i.e. increased magnification).

### 2. Taxonomic Accuracy and Precision

Correct identification of benthic organisms is important for accurate description of community structure and function. Taxonomic misidentification results in inadequate stream biology characterization. Errors in identification of benthic macroinvertebrate taxa should be $\leq 5\%$ of the total taxa in the sample. Re-identification of samples is done for 10% of the total number of samples collected in each year. Secondary identification is conducted by experienced taxonomists in order to maintain confidence in the data set. Difficult taxa are sent to museum curators whose specialty includes members of a particular taxa.

# V. DATA HANDLING, ANALYSIS, AND REPORTING

In this section, we will describe how to handle, analyze and report macroinvertebrate data. This section will assume that you have collected your data according to the protocols described in this document and have sent your samples to a professional taxonomist for identification and enumeration.

## A. METADATA

"Metadata" is data about the data. In the context of macroinvertebrate field data collection or laboratory identification and enumeration, the metadata would include who collected the data, who identified and enumerated the organisms, the project that the effort is a part of, when and where these tasks were completed, and what methods were used.

In designing a data collection and reporting effort it is important to identify how much data and metadata is needed and what is the most efficient way to collect it. It is possible, with thoughtful design to minimize duplication of data collection effort. For example a field form (or an electronic version of the same) might be designed to capture common metadata – for example project name, date of sample, data collectors name, sample site and method in a header - while variable information – sample numbers and other details are captured in a details section. This will depend on the individual circumstances, for example it may be that the location of sampling changes with each sample so that detail would need to be captured with each sample. When a system can be devised to capture common metadata it is necessary to develop a way to link the metadata to the detailed data. This can be completed with a code – often some combination of the data collectors name and date.

When you send your samples to the laboratory for taxonomic identification and enumeration, be sure to place both an exterior label and an interior label into each sample container. Use a soft lead pencil or waterproof marker on the labels for the following information (at a minimum):

| | |
|---|---|
| Sample Identification - | sample number or alphanumeric code |
| Stream Name - | name of the stream where your sample was collected. |
| State - | name of the State |
| Latitude - | latitude* decimal degree |
| Longitude - | longitude* decimal degree |
| Sampling Date and Time - | date and time* when the sample was taken (YYYY-MM-DD, HH:MM:SS 24hr clock) |
| Sampling Method - | description or citation of what field methods were used |
| Sampling area ($ft^2$) - | amount of stream bottom sampled, in this protocol ($8ft^2$) |
| Collector(s) | name(s) of people who collected the sample |
| Collector phone number | phone number of data collector (___) ___ ____ |
| Collector e-mail | |

If a sample must be divided between two or more containers, please indicate that clearly. For example, a single sample comprising 3 containers requires three separate labels marked (sample #) 1-3, (sample#) 2-3, (sample#) 3-3, or some equivalent. Sample numbers must be unique.

Note: The detail of minimum information above identifies minimum metadata information needed for collection of one sample (or possibly one sample in multiple containers). If many samples are being collected by the same person using the same methods, as is often the case, it may be more efficient to link the multiple sampling events to a common set of metadata using an appropriate code.

***Best Practices for Reporting Location and Time Related Data have been developed by the Northwest Environmental Data Network and are available at http://www.nwcouncil.org/ned/time.pdf**

There is also metadata that you will want to ensure that you receive back from the taxonomic laboratory for each sample. This could include:

Name of the taxonomist completing the identification and enumeration
Name of the Laboratory
Address of Laboratory
Date of Analysis
Method of analysis used
% of Sample Identified - how much of the actual sample was used to obtain the desired number of organisms.
Number of Organisms - the number of organisms identified in the laboratory

## B. DATA ENTRY, VERIFICATION AND EDITING

In the taxonomic laboratory data will either be initially entered on bench sheets and then entered into an electronic database, or directly entered into an electronic data entry program. In either case, you should ask for a description of the analytical method used to help you determine the level of data quality for your data management system. The most common data entry mistakes include: typographic errors, duplicates, etc.

## C. DATA FORMAT

From the taxonomic lab you will generally receive for each sample a taxa list with associated counts and often a list of community metric calculations (depending upon what you request). You may also request a biological reference table from the taxonomic lab which includes tolerance values, habits, feeding guilds and taxonomic codes for each taxon. You will also receive QA/QC reports for the project. You can receive your data both in hard copy and in an electronic format that you will need to specify such as: Access databases, Excel, etc. When you receive your data you should follow sound data management practices to maintain the original data and any derived data. You should clearly identify and mark the original data. In particular make sure that the data, and associated metadata is backed up using an approved method. Typically this involves creating a hard copy and keeping the copy at a secure site separate from the primary location of your work. The data management planning for the collection effort

(see VIa) below should specify the steps you need to take. It is also important to make sure that a copy of the data is registered, where possible, with regional metadata portals or other repositories so that it can be located and used by other researchers for other purposes.

## D. RECOMMENDATIONS FOR DATA ANALYSIS AND INTEPRETATION

This section is provided as an introduction to analyzing and interpreting aquatic macroinvertebrate sample results. The data collected by these protocols can be used for any of the analyses described below. Many different approaches have been used to prepare and analyze macroinvertebrate assemblage data. But they all start out with a list of macroinvertebrates that were collected, identified and counted. Depending upon your objectives, there are many other ways to examine macroinvertebrate assemblage data (such as ordination and other statistical tools) that are not presented in this document. The data analysis tools presented in this section those that are commonly used by State and Federal agencies to analyze and interpret macroinvertebrate data.

All of the data analysis procedures described in this document have as a foundation the concept of using a "reference condition" in order to determine divergence from expected conditions. Whether you develop this information yourself, or rely on what others have developed, the following is a brief description of the reference condition.

### 1. Reference Conditions

Reference conditions are the expectations on the state of aquatic biological communities (in this case macroinvertebrates) in the absence of human disturbance and pollution. For the purposes of assessing stream condition, biological integrity is defined as the biological attributes (species composition, diversity, functional organization) of anthropogenically undisturbed or minimally disturbed aquatic systems (reference condition). Impairment (diminished or lack of integrity) is evident when the biological attributes of a particular site differ from those of undisturbed or minimally disturbed systems. Biological changes in a waterbody that are associated with anthropogenic disturbances are evidence of impairment.

The reference condition establishes the basis for making comparisons and for detecting stream impairment. Depending upon the objectives of your monitoring activity, the reference condition can be defined on one of two scales. One scale is site specific, where the reference condition is specifically applicable to an individual waterbody. Another scale is ecoregional, where the reference condition represents biological expectations for streams across larger landscape areas (e.g., ecoregion scale).

Reference sites must be selected with care because the resulting database will be used as a benchmark against which test sites are compared. Additional information is available concerning reference site selection from many sources including Hughes, R.M. (1994), Plafkin et al. (1989), and U.S.EPA, (1996).

The State of Washington (Department of Ecology) monitors 10 reference sites annually throughout the State and calculates metrics, RIVPACs scores and provides IBI information. This information is available through the following website:

http://www.ecy.wa.gov/programs/eap/fw_benth/ambient.html#tenrefsites

## 2. Data Analysis

There are three major categories of data analyses that can be conducted with your macroinvertebrate data they are individual metrics, a biological index (often called an IBI, or Index of Biological Integrity) and predictive models (i.e. RIVPACs). The type of analysis you do will depend upon your capabilities, the type of questions that you are trying to answer (your monitoring objectives), the amount of data that you have and, if you are using existing reference condition, then type of tool that was used to develop this reference condition.

## 3. Individual Metrics

A quick and easily interpreted method for analyzing biological data is to evaluate individual metrics. A metric is a measurable characteristic of the macroinvertebrate assemblage that changes in some predictable way with increased human influence. Using a benthic macroinvertebrate taxa list several numerical values can be calculated. Many taxonomic labs will calculate these metrics for you. Calculated values are then compared to values from the reference condition. This is a useful and simple way to get a general idea of stream condition; however it is not appropriate for more regulatory decision making.

There are generally four categories of metrics (additional information can be found in Barbour, et al., 1999):

**Taxa richness metrics**, or the number of distinct taxa, represents the diversity within a sample. There are a variety of taxa richness metrics; the most common being total Taxa Richness which is simply the number of identified taxa within a sample. Taxa richness 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. For example, EPT richness, a commonly used metric, is the number of taxa in the sample that belong to the insect Orders Ephemeroptera, Plecoptera, and Trichoptera (EPT). These orders are commonly considered sensitive to pollution.

**Composition metrics** reflect key taxa and their relative abundance in the sample. Key taxa are those that are of special interest or ecologically important. For example, as noted above organisms in the insect order Plecoptera are a key taxa that are considered sensitive to pollution. Percentage of the assemblage that is made up of organisms in the order Plecoptera (% Plecoptera) generally decreases with increasing human influence.

**Feeding group metrics** provide information on the type and balance of feeding strategies (food acquisition and morphology) in the benthic assemblage. The taxonomic lab will generally provide you information on the functional feeding group for each taxa. Feeding groups include scrapers, shredders, gatherers, filterers, and predators. In stressed conditions there can be unstable food dynamics and an imbalance in functional feeding groups can result. For example, the metric of % shredders, which are generally considered more sensitive organisms and are thought to be well represented in healthy streams, will decrease with increased human disturbance.

**Tolerance/Intolerance metrics** 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). The taxonomic lab will generally provide you information on the tolerance/intolerance level for each taxa. An example metric in this category is percent of the organisms that are considered to be tolerant to pollution (% tolerant taxa) which generally increases with increasing stress.

**Habit (or behavioral) metrics** refer to the 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. Percent clingers is a metric commonly used in the northwest.

## 4. Multimetric index

A multimetric index combines indicators, or metrics, into a single index value, often called an Index of Biological Integrity or IBI. The individual metrics that provide information on diverse biological attributes are integrated into an index to provide an overall indication of biological condition. This document will not cover how to develop an IBI, however, detailed methods for the multi-metric IBI development are outlined in Kerans and Karr (1994) and Barbour et al. (1999). Multi-metric Indexes (IBIs) that have been developed in the northwest and their applicability to data collected using the field protocol are cited in this document.

Use of a multi-metric index requires use of comparable field protocols and taxonomic laboratory protocols for generating biological data. Multi-metric indexes are developed for specific regions on the landscape and use of the analytical tool is restricted to streams within this region (i.e. small streams in the Puget Lowland ecoregion).

The Washington State Department of Ecology (Wiseman, 2003) has developed and calibrated multi-metric indices for the Puget Lowland and Cascades ecoregions. If you are sampling streams in either of these ecoregions using the protocol described in this document, then this index is applicable. Below are summary tables (Tables 3 and 4) showing the applicable metrics and scoring criteria for the indices developed for the Puget Lowlands (Table 3) and Cascades (Table 4) ecoregions. To use this index, following sample collection, the laboratory processing should include generation of the metrics described in one of the two tables depending on ecoregion of the stream.

**Table 3 Scoring criteria for Puget Lowland metrics for Ecology's multi-metric index.**

| Category | Metric | Scoring Criteria | | |
|---|---|---|---|---|
| | | 1 | 3 | 5 |
| Richness | total richness | <24 | 24-33 | >33 |
| Richness | Ephemeroptera Richness | <4 | 4-6 | >6 |
| Richness | Plecoptera Richness | <3 | 3-5 | >5 |
| Richness | Trichoptera Richness | <4 | 4-6 | >6 |
| Tolerance | intolerant richness (bi) | <2 | 2 | >2 |
| Tolerance | % Tolerant (TV7) | >19 | 11-19 | <11 |
| Tolerance | % top 3 abundant | >70 | 54-70 | <54 |
| Trophic/Habit | % Predators | <11 | 11-19 | >19 |
| Trophic/Habit | % Clingers | <26 | 26-47 | >47 |
| Voltinism | Long-Lived Richness | <3 | 3-5 | >5 |

**Table 4 Scoring criteria for Cascade metrics for Ecology's multi-metric index.**

| Category | Metric | Scoring Criteria | | |
|---|---|---|---|---|
| | | 1 | 3 | 5 |
| Composition | % Ephemeroptera | <35 | 35-57 | >57 |
| Richness | total richness | <37 | 37-52 | >52 |
| Richness | Plecoptera Richness | <5 | 5-9 | >9 |
| Richness | Trichoptera Richness | <9 | 9-12 | >12 |
| Richness | Clinger Richness | <12 | 12-16 | >16 |
| Tolerance | intolerant richness | <6 | 6-9 | >9 |
| Tolerance | % Tolerant (bi) | >23 | 12-23 | <12 |
| Tolerance | HBI | >3.8 | 2.8-3.8 | <2.8 |
| Trophic/Habit | % Clingers | <36 | 36-54 | >54 |
| Trophic/Habit | % Filterers | >28 | 15-28 | <15 |

The scores for each metric (1, 3 or 5) are then added for an overall index score. The minimum multi-metric index score possible is 9 and the maximum score is 50. This overall score is then assessed as good, fair and poor using the benchmarks in Table 5.

**Table 5 Narrative assessments by Ecoregion using Ecology's index values**

| Narrative Assessment | Puget Lowlands | Cascades |
|---|---|---|
| Good | > 30 | > 28 |
| Fair | 20-30 | 23-28 |
| Poor | < 30 | <23 |

If you are planning on using Ecology's index, you will need the additional detailed information on this index and is available from (Wiseman, 2003) at the following website:

http://www.ecy.wa.gov/programs/eap/fw_benth/index.html

Currently, multi-metric indexes are unavailable for other ecoregions in Washington State. Construction of this analytical tool is dependent on characterization of adequate numbers of reference sites within a landscape area.

In addition, EPA's Office of Research and Development developed a set of multi-metric indices for large ecological regions of the western United States (Table 6). Additional details on the application and development of these indices is available in Stoddard et al (2005a and 2005b) which is available at the following website:

http://www.epa.gov/wed/pages/publications/authored.htm

**Table 6 Metrics from Stoddard et al (2005a and 2005b).**

| Mountains Ecoregion Metrics | Xeric Ecoregion Metrics |
|---|---|
| % non-insect individuals | % non-insect taxa |
| % individuals in top 5 taxa | Shannon Diversity |
| % omnivore taxa | Shredder taxa richness |
| % burrower individuals | % clinger taxa |
| EPT taxa richness | EPT taxa richness |
| % tolerant taxa | % non-tolerant taxa |

## 5. RIVPACS (River InVertebrate Prediction and Classification System)

RIVPACs is an empirical (statistical) model that predicts the macroinvertebrate taxa that would be expected to occur at a site in the absence of environmental stress. The number of observed taxa (O) at a test site is divided by the expected taxa (E), yielding a ratio. A ratio of 1 indicates that all expected taxa are present. As a site becomes more degraded, fewer expected taxa are observed, yielding a smaller ratio.

If you have used the field and lab methods described in this protocol, you can use the RIVPACs model if a model is available for streams in your area. Companion information to the biological samples collected for each site serves as the "predictor" variables and are used for determining expected species composition. These predictor variables are often a mix of features that can be derived from map sources and some are from field measurements. You may need to collect additional water column and habitat variables in addition to macroinvertebrates to use RIVPACs. Additional detailed information is available at the Western Center for Monitoring and Assessment of Freshwater Ecosystems (Western Center) website:

www.cnr.usu.edu/wmc

In addition to information on constructing predictive models like RIVPACS, users can access the Western Center website to run data on existing models. Use of the software on the website requires manipulation and creation of input files that must be in text (ASCII) format. Because some data files may have more than 256 columns, you may not be able to use a spreadsheet program to manipulate and create these files. A full set of instructions for use of this on-line analytical tool is available from the web site.

The Western Center has constructed models for streams and rivers in Oregon, Washington, and Idaho, among other States and regions. These models all differ in their specific data requirements, largely associated with the predictor variables and level of taxonomy used to describe the biological community. When running these models, you will need to follow the specific data guidelines as well as the general guidelines outlined on the website.

For the State of Oregon, the Predictive Assessment Tool for Oregon (PREDATOR) consists of three regional RIVPACs type models that assess the biological integrity of wadeable streams. The Oregon Department of Environmental Quality (DEQ) developed the models with the intent of supplying a scientifically rigorous bioassessment tool that is easy to use and understand by a large audience. The overall goal is to promote better understanding of the conditions of Oregon's streams.

The Xerces Society for Invertebrate Conservation has developed a web guide for using Oregon DEQ's PREDATOR models. The information presented on the website below provides users with backgound to the model and guidance to convert existing data, run the models, and interpret your results.

http://www.xerces.org/aquatic/predator/predator.htm

# VI. PERSONEL REQUIREMENTS AND TRAINING

## A. ROLES AND RESPONSIBILITIES

Field operations can be completed with a minimum of two people to gather benthic macroinvertebrate samples and measure other environmental variables at each site. The project leader will design and direct the biological monitoring project. The project leader should develop a brief plan of how the data will be collected, reported and subsequently managed as a part of the biological monitoring project design effort. A two page "Check List for Organizing Field Collection and Management of Data" can be used to guide this task and is available at http://www.nwcouncil.org/ned/Checklist.pdf. The plan will identify data management roles and responsibilities.

Laboratory identification and enumeration of benthic macroinvertebrates should be conducted by a laboratory with documented standard operating procedures.

## B. QUALIFICATIONS

For the field collections, the personnel must be able to safely operate stream sampling equipment for measuring biological communities and physical variables.

The personnel who conduct the laboratory identification and enumeration must have sufficient knowledge and training to be able to identify organisms to the genus/species level.

## C. TRAINING PROCEDURES

One advantage of field sampling of benthic macroinvertebrates is that it is relatively simple. A one day training session in the field with a experienced aquatic biologist should be sufficient for training in the field collection of benthic macroinvertebrates.

Laboratory sorting of benthic macroinvertebrates can be conducted by trained technicians. The amount of training that is required for sorting will depend upon the other duties, such as data management, that may or may not be assigned to the sorting technician.

The identification of benthic macroinvertebrates requires someone educated in aquatic biology and experience in taxonomic identification of benthic macroinvertebrates. This need for specialized experience is why this protocol recommends the use of a professional taxonomic lab.

# VII. OPERATIONAL REQUIREMENTS

## A. FACILITY AND EQUIPMENT NEEDS

The following are suggested lists of facility and equipment needs:

Field sampling equipment:
0.09 m² Surber sampler with 500 µm mesh net or D-frame kick net and 4 ft. handle
Buckets, plastic, 8-10 qt. Capacity
Sieve with 500µm mesh
Sieve bottom bucket, 500µm mesh openings
Wash bottle, 1-L capacity
White plastic wash tub
Plastic wide-mouth jars with screw caps
Small spatula, scoop or spoon to transfer sample
Forceps
Funnel with large bore spout
95% ethanol
Rubber gloves
Cooler
Labeling materials
Field data forms
If using a RIVPACs model, additional field equipment may be needed to collect predictor variable data

Laboratory equipment/supplies for macroinvertebrate identification:
Standardized gridded pan, such as a Caton tray, which is a sampling tray with 30 6X6 cm grids (30 cm x 36 cm)
500 micron sieve
Forceps
White plastic or enamel pan for sorting
Specimen vials with caps or stoppers
Sample labels
Dissecting microscope (generally 10x power)
Compound microscope
Light source
Ethanol
Laboratory data forms

Appropriate taxonomic keys, which should include, ***but are not limited to***:

- An Introduction to the Aquatic Insects of North America, 3rd ed. (Merritt and Cummins 1996), please note that a 4th edition is expected to be published this year.
- The Stoneflies (Plecoptera) of the Rocky Mountains (Baumann et al. 1977)
- Nymphs of North American Stonefly Genera (Plecoptera), 2nd ed. (Stewart and Stark 2002)

- Larvae of the North American Caddisfly Genera (Trichoptera), 2nd ed. (Wiggins, 1995)
- Manual of Nearctic Diptera, Volume 1 (McAlpine et al. 1981)
- Freshwater Snails (Mollusca: Gastropoda) of North America (Burch 1982)
- Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea, 4th Edition. (Smith 2001)
- Ecology and Classification of North American Freshwater Invertebrates, 2nd ed. (Thorp and Covich 2001)

## B. STARTUP COSTS AND BUDGET CONSIDERATIONS

Another advantage of macroinvertebrate field sampling is that it requires little expensive equipment. The most expensive piece of equipment is probably the sampling net which ranges in price from $180-$500, depending upon the type of net chosen. The following are some potential sources for sampling gear.

Wildlife Supply Company
http://www.wildco.com/
800-799-8301

Ward's Natural Science
http://www.wardsci.com/
800-962-2660

BioQuip
http://www.bioquip.com/
310-667-8800

Forestry Suppliers, Inc.
http://www.forestry-suppliers.com/
800-647-5368

Sending benthic macroinvertebrate samples to a laboratory for taxonomic identification and enumeration generally costs around $200 to $350 per sample. This will depend upon many factors and you will need to get a more accurate estimate from the taxonomic lab of your choice. The Xerces Society (a non-profit organization) aquatic invertebrate conservation program provides a list of taxonomic laboratories in the Pacific Northwest (http://www.xerces.org/aquatic/).

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