

STREAM MACROINVERTEBRATE PROTOCOL

Oregon Plan for Salmon and Watersheds

By the Water Quality
Interagency Workgroup
For the Oregon Plan

MENTOR CONTACTS

As with any monitoring project, questions come up that are not answered or covered sufficiently in this protocol. Therefore, the CSRI Monitoring Team is gathering together a group of mentors that are agency experts concerning monitoring each specific parameter. These mentors may be contacted with specific questions about your particular monitoring goals and effort. Questions about macroinvertebrate monitoring should be directed to:

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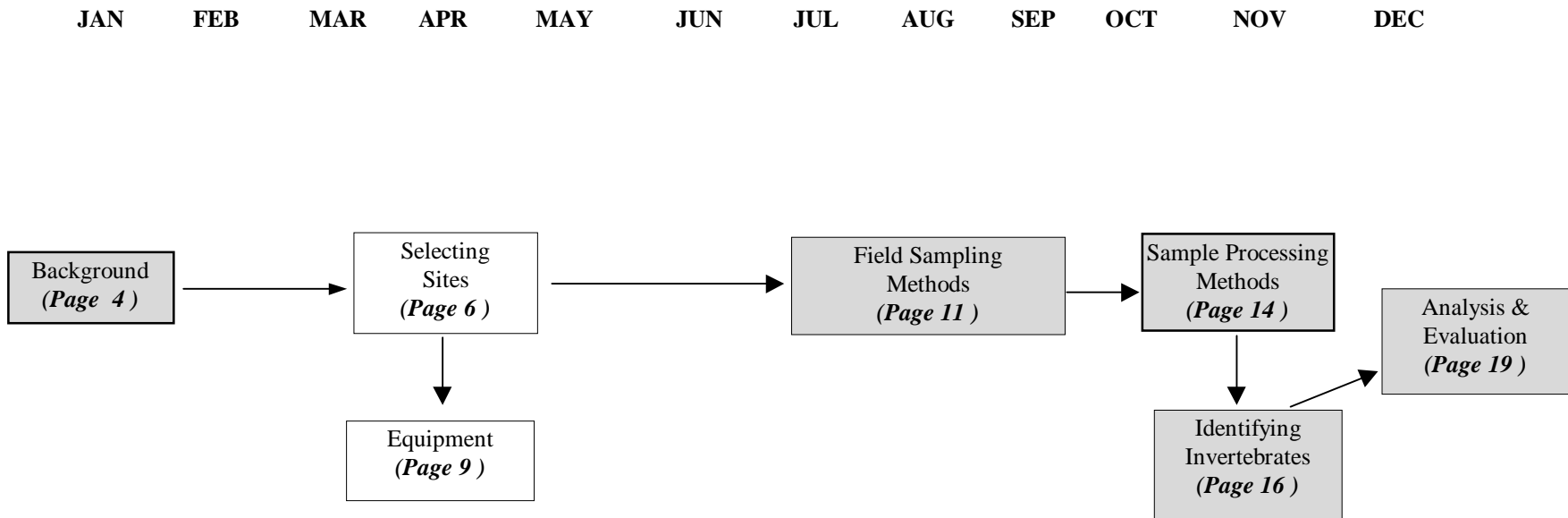
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STREAM MACROINVERTEBRATE MONITORING FLOW CHART

The chart below depicts each step one needs to complete during a typical stream invertebrate monitoring season. Each task and the timelines are relevant for persons interested in initiating a formal invertebrate monitoring project. Each section box has a corresponding page number you can refer to for the specific protocols to perform that task. Shaded boxes refer to steps which will normally be performed the first year and every succeeding year of a long-term study. Unshaded boxes are steps which only need to be completed the first year of a long-term study.



BACKGROUND: WHY MONITOR MACROINVERTEBRATES?

Evaluating the biological community of a stream provides a sensitive and cost effective means of assessing stream condition, particularly when stream impacts are from non-point sources, sporadic events, or cumulative low level pollution. The premise of the method described in the EPA Rapid Bioassessment Protocols (Plafkin, et.al., 1989) is that the habitat quality and biological community condition of impacted sites are compared with minimally impacted reference sites to evaluate the water quality of a stream.

Bioassessments can involve a variety of components of the stream biological community including algae, benthic macroinvertebrates, and fish. Monitoring each of these components has it's own advantages and disadvantages, and they all have published protocols (Plafkin, et.al., 1989; EPA, 1996). The protocols described here are for macroinvertebrates.

Macroinvertebrate communities tend to have greater diversity than fish communities in the same stream, which makes evaluation with some community diversity metrics more meaningful. Also, the natural integrity of fish communities is often compromised by sport fishing, stocking of sport fish, and the introduction of exotic species.

Macroinvertebrates are fairly stationary, easy to collect, and are responsive to human disturbance. In addition, the relative sensitivity or tolerance of many macroinvertebrates is well known. In general they provide a simple hands-on approach to understanding and measuring stream health.

In order to adequately evaluate the overall ecological integrity of aquatic systems it is necessary to develop a monitoring program that encompasses chemical, physical, and biological integrity (EPA, 1990). The macroinvertebrate bioassessment protocol described here is part of a comprehensive approach that involves analyzing the stream habitat condition, physical and chemical parameters, and the biological community.

The physical and chemical water quality parameters routinely measured are listed in Appendix A. The biological community evaluation methods described in this manual are adapted from the EPA Bioassessment Protocols (EPA, 1996) and other referenced sources.

Types of Methods –

Three different levels of macroinvertebrate sampling procedures are described in this protocol. They have unique objectives and require different levels of expertise.

LEVEL I:

Level 1 methods are the simplest to use and require the least experience. They also provide the least amount of information about the health of the macroinvertebrate community. Education is the main goal for Level 1 studies. If your objective is to inform citizens or students about what lives in streams, and only need a very basic assessment of stream conditions, Level 1 methods would be appropriate.

LEVEL II:

The Level 2 protocol is designed to provide a screening level assessment of stream conditions. This means sites can be classed as heavily disturbed, slightly disturbed, or non-disturbed. Finer levels of impairment will be difficult to detect. If your objective is to screen the condition of a variety of sites for prioritizing more in-depth studies, or if you don't have the budget or expertise to complete Level 3 studies, then the Level 2 protocol would be appropriate.

LEVEL III:

The Level 3 protocol provides a sensitive measure of stream condition using macroinvertebrate communities as the primary indicator. Four classes of stream condition can be determined: no disturbance, slight disturbance, significant disturbance and severe disturbance. Applied correctly, studies following this protocol can be used for a variety of objectives such as, identifying levels of stream disturbance within a watershed or region, effectiveness monitoring of restoration projects, trend assessments, and biocriteria evaluations.

SELECTING SITES

Overview:

This section describes the considerations involved in selecting bioassessment sampling sites and designing a monitoring program. The concepts presented here apply to any of the monitoring Levels (I, II, or III). Level 1 studies, designed primarily for education, don't require the same consideration as studies designed to assess stream condition within or between different streams. A site with easy access and a good diversity of invertebrates will be adequate for most educational projects.

For Level 2 or Level 3 studies it is important to consider that stream habitats are complex and change over distance and time. Different communities can inhabit different portions of the same stream, due to natural and anthropogenic factors. Also, the composition and abundance of the macroinvertebrate assemblage can change dramatically between seasons due to life-cycle patterns of the different species.

Careful site selection and monitoring timing is critical to insure that data collected are not biased, and that the differences noted between sites are not due to some artifact of the monitoring program design.

Choosing sites

Several types of sites may be selected for bioassessment surveys:

- *Study sites* are sites selected to answer specific questions. These could include questions about the effects of certain land uses, improvement following resto-

ration work, effectiveness of Best Management Practices (BMPs), etc.

- *Reference sites* are sites that reflect the best available conditions present within a specific stream, watershed, basin or ecoregion. An ideal reference site would be in a pristine, natural condition. A realistic reference site usually represents the best attainable conditions and has experienced some level of human affect. Ideally more than one reference site is used. Five to 10 reference sites should be sampled for studies that include several streams over a range of habitats.
- *Randomly selected sites* are sites chosen completely at random, without regard to the level of human disturbance. In most cases random site selection is stratified according to certain factors such as, stream order, land use, or ecoregion. Random site selection provides an unbiased assessment of the range of conditions present within a study area. (*Note: In Oregon, EPA Research Lab in Corvallis, can provide a list of randomly selected sites for specific projects. Contact Phil Larson at (541) 754-4362.*)

Sample sites need to be representative of the larger study area. Physiographic characteristics like vegetation, soils, geology, land use, gradient, riparian characteristics, and substrate frequency need to be considered to assure that your sample sites are representative of the larger population. For example, sample sites should not be directly downstream from anomalies such as culverts, bridges, roads, landslides, or waterfalls unless these are the conditions that the moni-

toring program is evaluating. Likewise, a sample site should not be located in a riffle with a large bedrock outcropping if the remainder of the stream is characterized by boulder and cobble substrate.

Reference and study streams should be in the same ecoregion or ecologically similar area (watershed or basin) and be within an acceptable range of elevation, gradient, and stream order (Omernik, 1987).

Ecoregions are areas of relative ecosystem homogeneity containing essentially similar physiographic characteristics such as vegetation, geology, hydrology, soils, and climate. Similar streams in the same ecoregion would be expected to have similar water chemistry and habitat conditions, and support similar biological communities. Differences between well chosen reference and study sites should be due to human or natural disturbance and not due to natural differences between the streams.

Locating minimally impacted reference streams in the same ecoregion as study streams can sometimes prove difficult, especially at the lower elevation sections of streams. In cases where unimpaired reference sites are not available, one should select the least impaired areas possible.

Generally, impacted and reference site selection is done in three stages:

- *Office Reconnaissance*: Using maps, aerial photos, published reports, and other materials the monitoring area is studied and likely streams are identified.
- *Consult the Experts*: Federal and State resource management agency personnel are very knowledgeable of the natural characteristics and human impacts in the

areas they administer. They can also provide information on work planned for the future in the basins being considered for study, such as proposed timber sales or stream improvement work. Local fisheries biologists are a particularly good resource.

- *Field Reconnaissance*: The streams identified in the previous two steps are visited and visually surveyed to verify the representativeness and similarity of the streams and to select specific stream reaches for sampling.

How many sites per stream?

The location and number of sites per stream depends on the objectives of the study, the type of impacts, and the resources available. Generally, program designs are of three types:

- *Paired stream approach*, with a single, or few, site(s) per stream. A study stream is paired with a nearby unimpacted reference stream where several sites are also selected.
- *Upstream/downstream approach*, with several sites along a single stream. One or more sites upstream of some disturbance, with the best attainable conditions, are used as the reference sites. One or more sites are then selected within and/or downstream from the area of concern.
- *Ecoregion approach*, where a number of least impacted reference sites within a single physiographic type or ecoregion are selected to determine the natural reference condition.

Whichever approach is used it is important to sample enough sites to determine the inherent variability within and between different sites. Gathering additional data collected by other agencies or groups, can improve the effectiveness of your own study to detect differences between sites. The collection and analysis methods used by other studies needs to be comparable however.

Selecting specific sample locations

Flowing water can generally be divided into several habitat types; pools, runs, glides, riffles, bends, undercuts, etc. Within the major habitat types there can be other smaller habitat categories. Examples would be inorganic substrate like rocks and gravel, or organic substrate like submerged logs and leaf packs. Since each habitat type can have a different macroinvertebrate assemblage, it is necessary to decide what habitat(s) you will sample.

Two approaches to habitat selection are commonly followed: multiple and single habitat assessment.

Multiple habitat assessment involves a stratified sample design that evaluates two or more habitat types. Each habitat type is sampled, processed, and evaluated separately. Pools and riffles are the most common habitat types sampled in a multiple habitat design, but other habitats might be included. The habitats most typical of the study stream should be chosen.

In a single habitat assessment usually only riffles are sampled. Riffles tend to contain the most diverse and sensitive invertebrate assemblage compared to other habitats (Plafkin, et.al., 1989). However, sampling only riffles may not always be adequate. Defining your questions in the sampling plan

will help you determine whether single or multiple habitats should be collected.

When are sites sampled ?

Stream habitats will have different macroinvertebrate communities, habitat conditions, and chemical water quality at different times of the year. Bioassessment surveys are typically done over the course of several years, so it is important to repeat sampling at the same time of year to make year-to-year comparisons possible. Sampling several times per year may be desirable to describe the seasonal variability of the stream and to determine the best time of the year to evaluate a specific type of impact. Once the seasonality of a stream has been adequately characterized it may be possible to reduce the sampling to a single critical season that best indicates impacts.

Effective periods for macroinvertebrate sampling in Oregon include:

Winter: December, January, February

Spring: March, April, May, June

Summer: July, August, September

Fall: October, early November.

Depending on a stream's elevation or region in the State, the months of May/June and October/November tend to be transition months between seasons, and for that reason invertebrate communities may be changing faster than at other times. Whatever sampling period has been selected, sampling should be avoided during or immediately after high water. High flows can significantly effect the ability to collect representative samples.

EQUIPMENT

LEVEL I ASSESSMENTS:

Equipment:	Costs:
<ul style="list-style-type: none"> • Collection net - Kick screen, or D-frame kick net are the most versatile. If these are not available a large fish aquarium net with fine mesh netting could also be used. Simply picking up stones from the stream bottom is also an option. 	<ul style="list-style-type: none"> • \$10 - \$50
<ul style="list-style-type: none"> • Small buckets 	<ul style="list-style-type: none"> • \$20
<ul style="list-style-type: none"> • Waterproof boots or waders 	
<ul style="list-style-type: none"> • Waterproof, insulated, elbow-length gloves (if working in polluted or very cold water). 	<ul style="list-style-type: none"> • \$35
<ul style="list-style-type: none"> • Shallow white plastic tray (ex. 12" x 16" or larger, 1 to 3 inches deep). 	<ul style="list-style-type: none"> • \$5
<ul style="list-style-type: none"> • 2 – 4 white ice cube trays 	<ul style="list-style-type: none"> • \$5
<ul style="list-style-type: none"> • Tweezers 	<ul style="list-style-type: none"> • \$5 - \$10
<ul style="list-style-type: none"> • Sample vials 	<ul style="list-style-type: none"> • \$10
<ul style="list-style-type: none"> • Hand lens 	<ul style="list-style-type: none"> • \$5 - \$30
<ul style="list-style-type: none"> • Macroinvertebrate field guides 	<ul style="list-style-type: none"> • \$10 – 50
<ul style="list-style-type: none"> • Pencils and paper 	<ul style="list-style-type: none"> • \$5
<ul style="list-style-type: none"> • Denatured ethanol (80-90%) 	<ul style="list-style-type: none"> • \$20
	Total Costs: \$100 - \$200

LEVEL II & III ASSESSMENTS:

Equipment:	Costs:
• Sub-sampling sorting tray (Caton Tray)	• \$150
• Tripod for field sorting (optional)	• (\$50) - optional
• Random number table, or other random number generator	
• D-frame Kick net, 30 cm. wide D-shaped hoop net with 500 micrometer mesh opening and a long handle	• \$50
• Plastic sieve bucket with a 500 micrometer mesh bottom	• \$50
• Plastic jars with tight fitting lids or zip-lock bags, 0.5 to 1.0 liter	• \$10
• Denatured ethanol (80-90%)	• \$20
• Shallow white plastic tray (ex. 12" x 16" or larger, 1 to 3 inches deep).	• \$5
• Waterproof, insulated, elbow-length gloves (if working in polluted or very cold water).	• \$35
• Labeling tape and alcohol-resistant marking pens (ethanol dissolves most inks)	• \$10
• Small vegetable scrub brush	• \$5
• Tweezers	• \$10
• Sample vials	• \$20
• Hand lens	• \$30
• Macroinvertebrate field guides	• \$50
• Paper and pencils	• \$5
	Total Costs: \$450

FIELD SAMPLING METHODS

LEVEL I ASSESSMENTS:

Field procedures for Level 1 assessments can follow a variety of techniques using simple, inexpensive equipment. The main objective is to collect a representative variety of species from your selected area.

Procedure:

- If possible select a shallow area with a gravel/cobble bottom and fairly fast current (make sure the current is not too fast to safely wade in). Other habitats may also be sampled; ex. Wood and leaf debris, pools, and stream margins.
- If using a kick screen or D-frame net, place the bottom of the net firmly against the stream bottom and disturb the area upstream of the net by picking up pieces of large gravel and cobble and rubbing their surfaces with your hands or with a small vegetable brush. After most of the cobble sized pieces have been moved continue disturbing the stream bottom with your hands or feet to a depth of several inches.

Repeat at two or three locations in the same habitat type and combine contents from each net into a single sample.

- Remove the net from the stream and wash it's contents into a small bucket. Clean and discard large pieces of gravel, leaves, twigs, etc. from the sample.

If no net is used, pick up pieces of large gravel or cobble and hold over the bucket while you rub the rock's surface clean.

Pieces of wood and leaf packs can also be gently washed in the bucket

- Pour the material in the bucket into the white plastic tray, and remove all the invertebrates you can find.
- Turn to *Sample Processing Methods* section for final processing steps.

LEVEL II & III ASSESSMENTS:

Both Level 2 & 3 assessments follow the same field sampling methods.

Method Overview:

The goal of the field sampling technique is to collect an unbiased, representative sample of macroinvertebrates. First a representative stream reach approximately 40 times the mean wetted channel width in length is selected. From within this reach two riffles are chosen (if pools will be sampled select two pools also). Two 0.18 square meter (2 square feet) kick samples are randomly selected in each riffle or pool. The four kick samples from each habitat type (riffle & pool) are composited resulting in one riffle and one pool sample to either process in the field or the lab.

Procedure:

1. Randomly select two kick-net sites within the downstream riffle or pool. Random numbers in the table used by DEQ

have four digits. The first two identify the percent up from the bottom of the riffle or pool, and the second two are the percent of stream width across the channel. For example, a random number of 3225 would place the sample at 32 percent up from the bottom and one quarter across the stream width. These percentages are determined by visual estimates

3. After locating the random sample site place the net into the stream with the flat part of the hoop resting on the bottom and perpendicular to the stream flow. Collect the macroinvertebrate sample by disturbing a 30 by 60 centimeter area (1ft x 2ft) of stream bottom directly in front of the net so that the current carries the macroinvertebrates into the net.

4. Carefully rub by hand, or with a small scrub brush, all substrate larger than five centimeters (golf ball size and larger) in front of the net to dislodge any clinging macroinvertebrates. After rubbing place the substrate outside of the sample plot.

5. Thoroughly disturb the remaining substrate to a depth of five to ten centimeters with the hands or feet.

6. Collecting a sample in slow moving water is a little more difficult. It may involve pulling the net through the water as the substrate is disturbed to capture suspended organisms.

7. After the sample is collected and the net removed, the large substrate is returned to the sample plot.

8. The contents of the net are placed in a sieve bucket and the sampling procedure is repeated at three more plots for that habitat

type. The preferred order for sampling is from downstream to upstream.

9. All four samples for the same habitat type are composited in the sieve bucket. Large organic material and rocks are rinsed, carefully inspected for clinging macroinvertebrates, and removed. As much fine sediment as possible is washed away. Leaf packs from pool samples may require considerable rinsing and removal of debris before preserving the composite sample.

10. For lab sorting and analysis the composite sample is placed in a labeled jar or double zip-lock bag and preserved with 90% ethanol for sorting and subsampling in the lab. It is recommended that the alcohol in the sample be changed with fresh alcohol within one week to ensure adequate preservation. A paper and pencil label inside the jar is recommended, as well as an exterior label.

11. For field sorting do not preserve the specimens. Keep them alive and follow the sub-sorting procedures described in the next section. Field sorting is faster since live, moving specimens are easier to see. Field sorted macroinvertebrates also tend to be in better condition than lab sorted specimens, making identification easier.

The disadvantage to field sorting is that it adds one to three hours to the field time per site, especially in low productivity streams that may require sorting most, if not all, of the sample to get the minimum number of specimens required for analysis.

Field Sample Label Information

Sample Site: _____
Location: _____ Date: _____
Habitat Sampled: riffle___ pool___ other___
Collected by: _____
Sampler Type: D-net_____ Other_____
of Kicks composited _____ # of squares sorted _____

SAMPLE PROCESSING METHODS

LEVEL I ASSESSMENTS:

Level 1 assessments follow a simplified sample processing procedure compared to Level 2 or 3 assessments. For example, it does not utilize a specific sub-sorting method or require a minimum number of invertebrates for identification. The main objective is to group the invertebrates by order and determine the number of sensitive or tolerant taxa present. As a result Level 1 studies help volunteers recognize the importance of the invertebrate community and provides a general indication of disturbance.

Key Elements:

- Remove all invertebrates from samples collected within the same habitat at the same reach.
- Sort specimens into individual containers (ice cube trays are often used) by order: Mayflies, Stoneflies, Caddisflies, etc.
- Visually estimate the number of different types of taxa within each order. Ex. How many different looking mayflies are there?
- Record the number of different taxa within each order and count how many are present.
- Based on the numbers recorded above a general water quality rating can be calculated as described in the *Analysis and Evaluation* Section.

LEVEL II & III ASSESSMENTS:

The goal of the sample processing procedures for Level 2 & 3 studies is to remove an unbiased, random representative sub-sample of macroinvertebrates from the composited stream bottom sample of debris.

The size of the sub-sample is a minimum of 300 individuals. The same size sub-sample should be used for all sites for effective comparisons.

Equipment:

- Sub-sampling tray (see Caton 1991, Appendix ?)
- Tripod with sorting tray platform for field sorting (optional)
- Random number table, or other random number generator
- 'Cookie cutter' 6 cm X 6 cm
- Denatured ethanol
- Vials, approximately 20 mls.
- Labeling tape and alcohol-resistant marking pens
- Forceps
- Tally counter (optional)

Procedure:

1. To sort the sample, place the composited sample into the mesh bottomed sorting tray. DEQ uses the equipment described by Caton (1991)

2. Place the mesh bottomed tray into the plastic outer tray and add approximately 3 cm of water to facilitate the even distribution of debris. In the field, place the tray on a level tripod platform.
3. Evenly distribute the material in the tray and lift the mesh bottom tray out of the water.
4. The sorting tray is divided into thirty, 6 X 6 cm squares. Use the random number table to select a minimum of four of these squares. Use the 'cookie cutter' to segregate and remove the selected squares.
5. Distribute the contents of the four squares into a separate white plastic tray with a small quantity of clean water. All the macroinvertebrates are removed with forceps and placed in a labeled vial of alcohol. An inside paper and pencil label is recommended as well as an exterior label.
6. A minimum of 300 specimens and four squares are sorted. If necessary, additional one or more squares must be sorted to attain the 300 organism minimum sample size. All organisms are completely removed from all sub-sampled squares to avoid biasing the macroinvertebrate sample toward the larger, more visible species. Using a tally counter is recommended. Keep track of the number of squares subsampled in order to estimate the original macroinvertebrate density in the stream.
7. The Caton sorting tray has thirty squares, each six centimeters square. When four D-frame kick samples are composited, each square represents approximately sixty square centimeters of stream bottom.

IDENTIFYING INVERTEBRATES

Method overview:

Three different levels of taxonomic identification can be used after sorting is completed: order, family or genus/species level. The level of taxonomic identification is important in determining the cost and expertise needed to do the analysis, as well as the resolution and sensitivity of the data to detect environmental impacts.

Level 1 assessments do not identify organisms beyond the Order level (Ephemeropter, Plecoptera, Diptera, etc.). Within each order organisms are simply lumped into similar looking groups. This approach is useful for demonstrating the variety of organisms living in a stream reach, but has limited value in assessing differences between sites. In general a rough approximation of the invertebrate community can be determined and sample sites categorized as having either an adequate or limited invertebrate community. Further sampling and more detailed analysis should be performed using level 2 or level 3 assessment methods if there are concerns about a stream's condition.

Level 2 assessments rely on family level identification for assessing the invertebrate community. Family level identification is faster and requires less expertise than genus/species level, but is less sensitive. Three levels of biological conditions may be determined from family level identification: non-impaired, moderately impaired, and severely impaired.

Level 3 assessments rely on genus/species identification for most orders. This is the most effective level for evaluating stream condition and evaluating differences between sites. It also requires the most time and expertise. Because of the identification skills required at this level, it is often most effective to contract out identification to a qualified taxonomist for level 3 assessments.

Four impairment categories may be discerned at this level: non-, slightly, moderately, and severely impaired. Table 1 shows the recommended level of taxonomy for each order.

Level II & III Identification Methods:

Equipment:

- Dissecting microscope (10X - 60X zoom)
- Light source
- Forceps
- Macroinvertebrate taxonomic keys, see references for recommended keys
- Data recording form

Procedure:

1. If the sample was not sorted in the field then lab sort according to the procedure described in the "Sample Processing" section.
2. Identify the macroinvertebrates to the taxonomic level desired. Table 1 lists

the level of taxonomic identification for different macroinvertebrate groups that is recommended for Level 3 assessments.

Identification to genus/species should be performed by experienced entomologists using current taxonomic keys (see “Taxonomic References”) under the supervision of a senior aquatic entomologist. Family level identification is possible by less experienced staff, but sufficient taxonomic training is still critical.

3. The number of each taxon is noted on a tally sheet along with other site identifier

information (see attached data recording forms).

4. Quality control procedures described in the Quality Assurance section should be completed to evaluate the quality of the sample identification.
5. The biometrics and biological condition assessments used to analyze the macroinvertebrate data are outlined in the Analysis and Evaluation section.

Table 1
Level of Macroinvertebrate Identification
for Level III Analysis

Taxon	Level of Identification				
	Order	Family	Sub-family	Genus	Species
Amphipoda				X	X
Arachnida	X				
Coleoptera (most)		X			
Elmidae				X	X
Diptera (most)				X	
Chironomidae			X		
Ephemeroptera				X	X
Gastropoda		some		X	
Hemiptera				X	
Lepidoptera				X	
Megaloptera				X	
Odonata		some		X	
Oligochaeta	X				
Ostracoda	X				
Pelecypoda	X				
Plecoptera		some		X	X
Trichoptera				X	
Turbellaria	X				

ANALYSIS & EVALUATION

OVERVIEW:

Data analysis and evaluation of stream condition relies on evaluating characteristics of the invertebrate community. This is often done through the use of “metrics.” Metrics are characteristics of the community that are known to change as a result of anthropogenic disturbances. Examples include total taxa richness, mayfly richness, % dominant taxa, etc. Each metric is scored (usually 1, 3, or 5) based on scoring criteria. All the individual metric scores are then summed together for an overall “Biotic Index” score for the site. The final biotic index falls within a known range indicating different level of impairment.

Individual metric scoring criteria and the impairment categories for the biotic index scores are based on data collected from reference sites in similar regions as the study sites being evaluated. The metric values presented here are based on reference site data collected by the Department of Environment Quality (DEQ) in the Oregon Coast Range. These criteria will work for assessing other Oregon coastal streams, but should not be used to assess streams from other areas of the State. The mentor contacts listed at the beginning of this document should be contacted for assessing streams outside the coast range for the most appropriate metric criteria.

LEVEL I ASSESSMENTS:

To develop a general evaluation of a site with Level 1 data the invertebrates are first separated by order, then the number of different “looking” organisms in each order are recorded and counted. The different orders of invertebrates can be generally classed as “sensitive,” or “tolerant.”

Sensitive organisms are those most sensitive to pollution and are first to disappear from the invertebrate community as a result of disturbance or pollution.

Orders considered sensitive are:

- Mayflies (Ephemeroptera)
- Stoneflies (Plecoptera)
- Caddisflies (Trichoptera)

Tolerant organisms are those that tolerate high levels of disturbance and pollution, and remain present after other groups have disappeared. This includes the orders:

- Aquatic worms (Oligochaeta)
- Leeches (Hirudinea)
- Blackflies (Diptera)
- Midges (Diptera)
- Snails (Gastropoda)

Since level 1 assessments are primarily an educational level, different levels of stream impairment can not be calculated. The generalized data only provides information to say the community appears to be adequate or limited.

Sites where each of the three sensitive orders (mayflies, stoneflies, and caddisflies) are all present and tolerant organisms make up less than 50% of the total organisms counted from the sample are considered adequate. If any one

of the three orders are absent and/or tolerant organisms equal more than 50% of the total in the sample the site has a limited invertebrate community. Level 2 or 3 assessments should be performed to evaluate the sites further.

LEVEL II ASSESSMENTS:

Level 2 site assessments are based on family level identifications. The number of organisms in each family are counted and recorded. These values are then used to determine metric values or scores. Metric scores are summed to determine the overall rating for the site. The following table outlines the family level metrics and scoring criteria.

Metric	Raw Value	Scoring Criteria			Score (circle)
		5	3	1	
Taxa Richness		>18	10-18	<10	5 3 1
Mayfly Richness		>4	2-4	<2	5 3 1
Stonefly Rich.		>3	1-3	0	5 3 1
Caddisfly Rich.		>4	2-4	<2	5 3 1
% Chironomidae		<15	15-30	> 30	5 3 1
% Dominance (Top 3 Taxa)		<30	30-50	>50	5 3 1

Taxa Richness: This is the total number of invertebrate families identified from the sample.

Mayfly Richness: This is the total number of mayfly families identified from the sample.

Stonefly Richness: This is the total number of stonefly families identified from the sample.

Caddisfly Richness: This is the total number of caddisfly families identified from the sample.

% Chironomidae: This is the total number of chironomids in the sample divided by the total number of organisms sorted from the sample, multiplied by 100.

% Dominance (top 3 taxa): This is the total number of the three most abundant organisms divided by the total number sorted from the sample, multiplied by 100.

Add up the scores for each metric to determine the total site score or biotic index. The total scores are used to determine three levels of impairment as indicated below.

Score Range Stream Condition

> 23 No impairment: Passes level 2 assessment. Indicates good diversity of invertebrates and stream conditions with little disturbance.

17 – 23 Moderate Impairment: Evidence of some impairment exists. Requires further study and more detailed analysis.

< 17 Severe Impairment: Fails level 2 assessment. Evidence of stream disturbance exists. Further study may be warranted to confirm level of impairment and potential causes.

LEVEL III ASSESSMENTS:

Level 3 assessments are based on genus/species level identifications, which provides a more sensitive measure of the invertebrate community’s condition. Two analysis approaches can be used for level 3

assessments: multimetric analysis, or multivariate analysis.

To make accurate assessments between sites, using either multimetric or multivariate analysis techniques, **the same level of identification must be used for each taxonomic group for all sites being compared.** Because levels of identification can vary between taxonomists or between sites due to maturity of specimens or preservation quality, each data set should be checked by a taxonomist for identification consistency.

Multimetric analysis:

This approach is the same as that used for level 2, except more metrics are incorporated into the analysis. The metrics and associated scoring criteria for level 3 metric assessments are listed below.

Metric	Raw Value	Scoring Criteria			Score (circle)
		5	3	1	
Taxa Richness		>35	19-35	<19	5 3 1
Mayfly Richness		>8	4-8	<4	5 3 1
Stonefly Rich.		>5	3-5	3	5 3 1
Caddisfly Rich.		>8	4-8	<2	5 3 1
Sensitive Taxa		>4	2-4	<2	5 3 1
Sed. Sen. Taxa		>2	1	0	5 3 1
Modified HBI		<4.0	4-5	>5.0	5 3 1
% Tol. Taxa		<15	15-45	>45	5 3 1
% Sed Tol Taxa		<10	10-25	>25	5 3 1
% Dominant (single taxa)		<20	20-40	>40	5 3 1

Taxa Richness: This is the total number of invertebrate taxa identified from the sample.

Mayfly Richness: This is the total number of mayfly taxa identified from the sample.

Stonefly Richness: This is the total number of stonefly taxa identified from the sample.

Caddisfly Richness: This is the total number of caddisfly taxa identified from the sample.

Sensitive Taxa: This is the number of taxa identified that are known to be very sensitive to stream disturbance. The list of taxa that qualify as “sensitive” are listed in appendix ?.

Sediment Sensitive Taxa: These are taxa known to be very sensitive to inputs of fine sediment. The presence of one or more of these taxa indicate that fine sediments are probably not a major concern.

Modified HBI: “HBI” stands for Hilsenhof Biotic Index. This is an index of a taxa’s sensitivity to organic enrichment that typically occurs as a result of excessive nutrient inputs. Index values for individual taxa range from 1 to 10. Low scores indicate high sensitivity (found only in waters with low organic enrichment). High scores indicate low sensitivity (tolerant of waters with high organic enrichment). HBI index values for each taxa are listed in the taxa list for Oregon streams in appendix ?

% Tolerant Taxa: This is the percent of the invertebrate community made up of taxa tolerant to disturbance. Taxa counted as “tolerant” taxa are listed in appendix ?. Divide the abundance of tolerant taxa by the total number of organisms sorted from the sample, and multiply by 100.

% Sediment Tolerant Taxa: This is the percent of the invertebrate community made up of taxa tolerant to fine sediments (see appendix ?). Divide the abundance of sediment tolerant taxa by the total number of organisms sorted from the sample, and multiply by 100.

% Dominant (single taxa): This is the total abundance of the single most abundant taxon in the sample divided by the total number of organisms sorted from the sample, multiplied by 100. A high percent of a single taxon indicates some disturbance has likely occurred to the invertebrate community.

After calculating each individual metric score add them together for the total score or biotic index. Stream condition levels are based on the ranges of total scores listed below.

<u>Score Range</u>	<u>Stream Condition</u>
> 39	No Impairment: Passes level 3 assessment. Indicates good diversity of invertebrates and stream conditions with little or no disturbance.
30 – 39	Slight Impairment: Evidence of some impairment exists.
20 – 29	Moderate Impairment. Clear evidence of disturbance exists.
< 20	Severe Impairment. Conditions indicate a high level of disturbance.

Multivariate Analysis:

Level 3 assessments can also be analyzed using multivariate analysis techniques. In this approach, reference sites (high quality, least disturbed sites) are used as a benchmark against which sites of interest (test sites), are compared. The method has two basic elements: the development of a relatively sophisticated predictive model based upon reference conditions; and, direct comparison of the

stream taxa collected at a test site against model predictions.

Reference sites are first grouped together to generate “reference community types”. This is done using a cluster analysis. These reference groups are then categorized by their geographic and geomorphologic characteristics such as latitude, longitude, elevation and gradient. Discriminant function analysis is a common way to achieve this. A test site at a particular geographic location and with a particular geomorphology is compared directly against its appropriate reference community group. The model generates a list of expected taxa for each test site. A determination of impairment is made based upon the number of predicted taxa that were actually collected at the test site.

Although the final rating of the test site is a simple arithmetic ratio of observed taxa over expected taxa, the modeling process itself requires appropriate computer software. An example of how a rating is derived is given below. In this example the test site has a rating of 81 % of the target reference condition.

Use both multimetric and multivariate analysis techniques when possible. This provides the most robust assessment of a site’s condition.

Test Site Assessment against Reference Condition using a Multivariate Model

Predicted Taxa (from Reference Model)	Taxa Collected at Test Site
Baetis tricaudatus	Yes
Dipheter hageni	Yes
Paraleptophlebia	Yes
Drunella doddsi	
Epeorus grandis	
Sweltsa	Yes
Calineuria californica	Yes
Zapada cinctipes	Yes
Isoperla	
Moselia infuscata	
Wormaldia	Yes
Rhyacophila	
Hydropsyche	Yes
Chironomidae	Yes
Hexatoma	Yes
Zaitzevia	Yes
Optioservus	Yes
Hydracarina	Yes

No. Taxa Predicted by Model: 18
 No. Expected Taxa from Probability Weighting (E): 16
 No. Taxa Observed at Test Site (O): 13
 Observed/Expected (O/E): 81%

QUALITY ASSURANCE

Overview:

Quality assurance procedures (QA) assess the environmental variability, sampling procedures validity, repeatability of the sample methods, and identification quality. The quality assurance procedures involves a system of following standard methods and protocols, duplicate sampling, and identification reviews.

Field QA sample:

Ten percent of all stream sites sampled, or one sample per survey, which ever is greater, should have a duplicate set of field samples collected. The duplicate sample is from the same sample reach. This is called a field quality assurance sample (FQA).

Field QA samples look at the natural variability within a riffle and insures that the field sampling method is repeatable. This sample is sorted and identified the same as any other sample.

Laboratory QA samples:

Ten percent of all composite samples collected, or one sample per survey, which ever is greater, is resorted for an additional 300 specimen sub-sample from the original preserved composite sample. The result is a duplicate sample from the same composite. This is a laboratory quality assurance sample (LQA).

Lab QA samples look at the variability inherent in the sub-sampling procedure and insures that the sub-sampling method is repeatable and within an acceptable range of variability .

Type collection:

It is useful to maintain a macroinvertebrate type collection for each major basin, watershed, or ecoregion studied. This collection has a representative of each taxon identified and serves as a basin record, and as a reference for checking identifications.

Identification review:

For level 3 assessments data should be reviewed by an experienced taxonomist for anomalous identifications. Randomly selected samples should also be identified by an experienced entomologist independently of the first identification. Finally, specimens entered into the type collection should be checked by an experienced entomologist for accurate identification.

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