4.3 Intensive Stream Biosurvey

The Intensive Stream Biosurvey is based on the habitat assessment and macroinvertebrate sampling approach developed by EPA in its *Rapid Bioassessment Protocols for Streams and Rivers* (Protocol II) and adapted by volunteer monitoring programs such as Maryland Save Our Streams and River Watch Network.

Like the Stream Habitat Walk and Streamside Biosurvey, this approach includes a study of macroinvertebrates and habitat. However, the Intensive Stream Biosurvey approach is more rigorous; it requires substantial volunteer training in habitat and macroinvertebrate sampling methods and in macroinvertebrate identification. This approach also requires the involvement of a stream biologist to advise the program participants regarding everything from the selection of reference conditions to taxonomy and data analysis.

Because of the need for training and professional assistance, the Intensive Stream Biosurvey approach can be expensive and labor-intensive for the volunteer program. Its benefits, however, are equally clear: with proper quality control and volunteer training, the Intensive Stream Biosurvey can yield credible information on subtle stream impacts and water quality trends. Key features of the Intensive Stream Biosurvey are as follows:

- *It relies on comparing the results for the sampling site to regional or local reference conditions.* This type of study is used to determine how streams in a given area compare to the best possible conditions. The reference condition is a composite of the best attainable (minimally impaired) stream conditions within the region and should be determined by an experienced aquatic biologist familiar with the characteristics of the ecological region.
- *It includes a detailed habitat assessment that requires the volunteer to rate 10 parameters on a scale of 0 to 20.* The results of the habitat assessment are compared to the score received by the stream's reference condition, and a percent similarity score is calculated.
- *The methods for collecting macroinvertebrates are similar to those of the Streamside Biosurvey.* However, rather than being processed streamside, the entire sample of macroinvertebrates is preserved and returned to a laboratory. A portion, or subsample, of the total organisms collected at each location is randomly selected and identified to taxonomic family level in the lab. After identification, a series of indices (or metrics) are calculated to provide a broad range of information about the stream site. The subsample and the rest of the collected organisms are maintained as a voucher collection, which serves as a quality assurance component.
- *The Intensive Stream Biosurvey requires that volunteers be extensively trained before habitat assessment and macroinvertebrate sampling and before attempting macroinvertebrate identification in the laboratory.* An experienced aquatic biologist is needed to determine and evaluate the regional reference conditions; train volunteers in habitat characteristics; and supervise and train volunteers in the collection, processing, and identification of sample macroinvertebrates. A laboratory (with microscopes) and a macroinvertebrate sample storage facility are required.

Step 1—Prepare for the Intensive Stream Biosurvey field work

Preparing for the Intensive Stream Biosurvey might take several months from the initial planning stages to the time when actual sampling occurs. An aquatic biologist should be centrally involved in all aspects of technical program development.

Issues that should be considered in planning the program include the following:

- Availability of reference conditions for your area
- Appropriate dates to sample in each season
- Appropriate sampling gear
- Sampling station location
- Availability of laboratory facilities and trainers
- Sample storage
- Data management
- Appropriate taxonomic keys, metrics, or measurements for macroinvertebrate analysis
- Habitat assessment consistency

Some of the preparation work for this approach is similar to that of the Stream Habitat Walk (section 4.1) and Streamside Biosurvey (section 4.2). Refer back to those sections for relevant information on the following tasks:

- Obtaining a USGS topographical map
- Becoming familiar with safety procedures

TASK 1 **Select monitoring locations**

If possible, the program coordinator, in conjunction with technical advisor(s), should preselect sampling locations for each stream. This adds an element of quality control to the sampling process. You might want to consider sampling at a few locations that are also sampled by state or local professionals, as a way to compare your results to theirs. *Be sure to secure approval to do so, however, and coordinate your sampling so as not to affect professional results.*

Provide detailed hand-drawn maps of the locations selected to the monitors. Know the latitude and longitude of your monitoring locations. This is critical for mapping and for many data management programs. Latitude and longitude can be calculated manually (see Appendix C) or by using a hand-held Global Positioning System (GPS).

TASK 2 **Schedule the field portion of the biosurvey**

Schedule your Intensive Stream Biosurvey for a time of year for which reference conditions have been established. Reference conditions might vary by season. It is also essential that seasonal data be collected within the same index period, or window of time, each year. In other words, if you sample during the last two weeks of March this year, do the same next year.

Another factor to keep in mind is weather. It is best to wait at least a week after a heavy rain or snow event before sampling. Heavy rains can have a scouring effect on macroinvertebrates, washing them downstream. If this happens, samples collected will not accurately reflect biological conditions. However, if you are studying the possible impact of runoff from a particular source (such as a construction site), you might decide to sample within a short time after heavy precipitation.

TASK 3 **Gather tools and equipment for the Intensive Stream Biosurvey**

In addition to the basic sampling equipment listed for the Stream Habitat Walk, collect the following equipment needed for the macroinvertebrate collection and habitat assessment of the Intensive Stream Biosurvey:

- Jars (2, at least quart size), plastic, wide-mouth with tight cap; one should be empty and the other filled about two thirds full with 70 percent ethyl alcohol. (Jars can be purchased from a scientific supply company or you might try using large pickle, mayonnaise, or quart mason jars.)
- Hand lens, magnifying glass, or field microscope
- Fine-point forceps
- Heavy-duty rubber gloves (kitchen gloves will work fine)
- Plastic sugar scooper or ice-cream scooper
- Kick net (rocky bottom stream) or dip net (muddy bottom stream) (see Fig. 4.7, page 63)
- Buckets (2)
- String or twine (50 yards); tape measure
- Stakes (4)
- Orange (a stick, an apple, or a fish float may also be used in place of an orange) to measure velocity
- Reference maps indicating general information pertinent to the sampling area, including the surrounding roadways, as well as hand-drawn station map
- Station ID tags
- Spray water bottle
- Pencils (at least 2)

TASK 4 **Become familiar with field data sheets and instructions/ definitions for conducting the macroinvertebrate collection and Habitat Assessment portions of the Intensive Biosurvey**

Step 2—Conduct the Intensive Biosurvey field work

The method you use to collect macroinvertebrates using this approach depends on the type of stream you are sampling.

Rocky-bottom streams are defined as those with bottoms made up of gravel, cobbles, and boulders in any combination. They usually have definite riffle areas. Riffle areas are fairly well oxygenated and, therefore, are prime habitats for benthic macroinvertebrates. In these streams, use the Rocky-Bottom sampling method.

Muddy-bottom streams have muddy, silty, or sandy bottoms that lack riffles. Usually, these are slow-moving, low-gradient streams (i.e., streams that flow along flat terrain). In such streams, macroinvertebrates generally attach to overhanging plants, roots, logs, submerged vegetation, and stream substrate where organic particles are trapped. In these streams, use the Muddy Bottom sampling method.

Each method is detailed below. Regardless of which collection method is used, the process for counting, identifying, and analyzing the macroinvertebrate sample for the Intensive Stream Biosurvey is the same. Following the discussion of both approaches to macroinvertebrate collection and habitat assessment procedures is a section on analyzing the sample.

Sieve Buckets

Most professional biological monitoring programs employ sieve buckets as a holding container for composited samples. These buckets have a mesh bottom that allows water to drain out while the organisms and debris remain. This material can then be easily transferred to the alcohol-filled jars. However, sieve buckets can be expensive. Many volunteer programs employ alternative equipment, such as the two regular buckets described in this section. Regardless of the equipment, the process for compositing and transferring the sample is basically the same. The decision is one of cost and convenience.

Sieve buckets can be very expensive so WV Save Our Streams uses an EZ-strainer, which is a fine mesh straining device that fits into the top of a 3 1/2 to 5 gallon bucket. This device can be purchased in the appropriate mesh size for macroinvertebrate assessment.

Rocky-Bottom Streams Part 1: Macroinvertebrate Sampling Method

Use the following method of macroinvertebrate sampling in streams that have riffles and gravel/cobble substrates. You will collect three samples at each site and composite them to obtain one large total sample.

TASK 1 — Identify the sampling location

You should already have located your site on a map along with its latitude and longitude (see Task 3, page 45)

1. You are going to sample in three different spots within a 100-yard stream site. These spots may be three separate riffles; one large riffle with different current velocities; or, if no riffles are present, three run areas with gravel or cobble substrate. Combinations are also possible (if, for example, your site has only one small riffle and several run areas).

   Mark off your 100-yard stream site. If possible, it should begin at least 50 yards upstream of any human-made modification of the channel, such as a bridge, dam, or pipeline crossing, Avoid walking in the stream, since this might dislodge macroinvertebrates and alter your sampling results.

2. Sketch the 100-yard sampling area. Indicate the location of your three sampling spots on the sketch. Mark the most downstream site as Site 1, the middle site as Site 2, and the upstream site as Site 3. (See Fig. 4.8.)

TASK 2 — Get into place

1. **Always approach your sampling locations from the downstream end and sample the site farthest downstream first (Site 1).** This keeps you from biasing your second and third collections with dislodged sediment or macroinvertebrates. Always use a clean kick-seine, relatively free of mud and debris from previous uses. Fill a bucket about one third full with stream water and fill your spray bottle.

2. Select a 3-foot by 3-foot riffle area for sampling at Site 1. One member of the team, the net holder, should position the net at the downstream end of this sampling area. Hold the net handles at a 45 degree angle to the water's surface. Be sure that the bottom of the net fits tightly against the streambed so no macroinvertebrates escape under the net. You may use rocks from the sampling area to anchor the net against the stream bottom. Don't allow any water to flow over the net.

TASK 3 — Dislodge the macroinvertebrates

1. Approach the sample site from the downstream end.

2. Position the net at a 45° angle with the bottom tight against the substrate.

3. Dislodge macroinvertebrates by rubbing rocks thoroughly.

Rock rubbing is often done in the stream directly in front of the net. Only cobble size and larger rocks are rubbed.

4. Disturb the substrate thoroughly with your feet.

5. Remove the net with a forward scooping motion.

6. Flush out the net with clean stream water.

It is often more effective to rinse the net using water from another bucket instead of a spray bottle

1. Pick up any large rocks in the 3-foot by 3-foot sampling area and rub them thoroughly over the partially-filled bucket so that any macroinvertebrates clinging to the rocks will be dislodged into the bucket. Then place each cleaned rock outside of the sampling area. After sampling is completed, rocks can be returned to the stretch of stream they came from.
2. The member of the team designated as the "kicker" should thoroughly stir up the sampling area with their feet, starting at the upstream edge of the 3-foot by 3-foot sampling area and working downstream, moving toward the net. All dislodged organisms will be carried by the stream flow into the net. Be sure to disturb the first few inches of stream sediment to dislodge burrowing organisms. As a guide, disturb the sampling area for about 3 minutes, or until the area is thoroughly worked over.
3. Any large rocks used to anchor the net should be thoroughly rubbed into the bucket as above.

TASK 4 **Remove the net**

1. Next, remove the net without allowing any of the organisms it contains to wash away. While the net holder grabs the top of the net handles, the kicker grabs the bottom of the net handles and the net's bottom edge. Remove the net from the stream with a forward scooping motion.
2. Roll the kick net into a cylinder shape and place it vertically in the partially filled bucket. Pour or spray water down the net to flush its contents into the bucket. If necessary, pick debris and organisms from the net by hand. Release back into the stream any fish, amphibians, or reptiles caught in the net.

TASK 5 **Collect the second and third samples**

Once you have removed all the organisms from the net repeat these steps at Sites 2 and 3. Put the samples from all three sites into the same bucket. Combining the debris and organisms from all three sites into the same bucket is called *compositing*.

Hint: If your bucket is nearly full of water after you have washed the net clean, let the debris and organisms settle to the bottom of the bucket. Then cup the net over the bucket and pour the water through the net into a second bucket. Inspect the water in the second bucket to be sure no organisms came through.

TASK 6 **Preserve the sample**

1. After collecting and compositing all three samples, it is time to preserve the sample. All team members

should leave the stream and return to a relatively flat section of stream bank with all their equipment. The next step will be to remove large pieces of debris (leaves, twigs, and rocks) from the sample. Carefully remove the debris one piece at a time. While holding the material over the bucket, use the forceps, spray bottle, and your hands to pick, rub, and rinse the leaves, twigs, and rocks to remove any attached organisms. Use your magnifying lens and forceps to find and remove small organisms clinging to the debris. When you are satisfied that the material is clean, discard it back into the stream.

2. You will need to drain off the water before transferring material to the jar. This process will require two team members. Place the kick net over the second bucket, which has not yet been used and should be completely empty. One team member should push the center of the net into bucket #2, creating a small indentation or depression. Then, hold the sides of the net closely over the mouth of the bucket. The second person can now carefully pour the remaining contents of bucket #1 onto a small area of the net to drain the water and concentrate the organisms. Use care when pouring so that organisms are not lost over the side of the net (Fig. 4.16).

   Use your spray bottle, forceps, sugar scoop, and gloved hands to remove all the material from bucket #1 onto the net. When you are satisfied that bucket #1 is empty, use your hands and the sugar scoop to transfer all the material from the net into the empty jar.

   Bucket #2 captured the water and any organisms that might have fallen through the netting during pouring. As a final check, repeat the process above, but this time, pour bucket #2 over the net, into bucket #1. Transfer any organisms on the net into the jar.

3. Now, fill the jar (so that all material is submerged) with the alcohol from the second jar. Put the lid tightly back onto the jar and gently turn the jar upside down two or three times to distribute the alcohol and remove air bubbles.

4. Complete the Sampling Station ID tag. Be sure to use a pencil, not a pen, because the ink will run in the alcohol! The tag includes your station number, the stream, location (e.g., upstream from a road crossing), date, time, and the names of the members of the collecting crew. Place the ID tag into the sample container—writing side facing out, so that identification can be seen clearly.

Fig. 4.16

Pouring sample water through the net

Rocky-Bottom Streams
Part 2: Habitat Assessment Method

You will conduct a habitat assessment (which will include measuring general characteristics and local land use) in a 100-yard section of stream that includes the riffles from which organisms were collected.

TASK 1 — Delineate the habitat assessment boundaries

1. Begin by identifying the most downstream riffle that was sampled for macroinvertebrates. Using your tape measure or twine, mark off a 100-yard section extending 25 yards below the downstream riffle and about 75 yards upstream.
2. Complete the identifying information on your field data sheet for your habitat assessment site. On your stream sketch, be as detailed as possible, and be sure to note which riffles were sampled.

TASK 2 — Complete the General Characteristics and Local Land Use sections of the field sheet

For safety reasons as well as to protect the stream habitat, it is best to estimate these characteristics rather than actually wading into the stream to measure them.

General Characteristics

1. *Water appearance* can be a physical indicator of water pollution.
   - *Clear* - colorless, transparent
   - *Milky* - cloudy-white or grey, not transparent; might be natural or due to pollution
   - *Foamy* - might be natural or due to pollution, generally detergents or nutrients (foam that is several inches high and does not brush apart easily is generally due to pollution)
   - *Turbid* - cloudy brown due to suspended silt or organic material
   - *Dark brown* - might indicate that acids are being released into the stream due to decaying plants
   - *Oily sheen* -multicolored reflection might indicate oil floating in the stream, although some sheens are natural
   - *Orange* - might indicate acid drainage
   - *Green* - might indicate excess nutrients being released into the stream
2. *Water odor* can be a physical indicator of water pollution.
   - *None or natural smell*
   - *Sewage* - might indicate the release of human waste material
   - *Chlorine* - might indicate that a sewage treatment plant is over-chlorinating its effluent
   - *Fishy* - might indicate the presence of excessive algal growth or dead fish
   - *Rotten eggs* - might indicate sewage pollution (the presence of a natural gas)
3. *Water temperature* can be particularly important for determining whether the stream is suitable as habitat for some species of fish and macroinvertebrates that have distinct temperature requirements. Temperature also has a direct effect on the amount of dissolved oxygen available to aquatic organisms. Measure temperature by submerging a thermometer for at least 2 minutes in a typical stream run. Repeat once and average the results.

4. The *width of the stream channel* can be determined by estimating the width of the streambed that is covered by water from bank to bank. If it varies widely along the stream, estimate an average width.

Local Land Use

5. *Local land use* refers to the part of the watershed within 1/4 mile upstream of and adjacent to the site. Note which land uses are present, as well as which ones seem to be having a negative impact on the stream. Base your observations on what you can see, what you passed on the way to the stream, and, if possible, what you notice as you leave the stream.

TASK 3 **Conduct the habitat assessment**

The following information describes the parameters you will evaluate for rocky-bottom habitats. Use these definitions when completing the habitat assessment field data sheet.

The first two parameters should be assessed directly at the riffle(s) or run(s) that were used for the macroinvertebrate sampling.

1. *Attachment sites for macroinvertebrates* are essentially the amount of living space or hard substrates (rocks, snags) available for aquatic insects and snails. Many insects begin their life underwater in streams and need to attach themselves to rocks, logs, branches, or other submerged substrates. The greater the variety and number of available living spaces or attachment sites, the greater the variety of insects in the stream. Optimally, cobble should predominate and boulders and gravel should be common. The availability of suitable living spaces for macroinvertebrates decreases as cobble becomes less abundant and boulders, gravel, or bedrock become more prevalent.
2. *Embeddedness* refers to the extent to which rocks (gravel, cobble, and boulders) are surrounded by, covered, or sunken into the silt, sand, or mud of the stream bottom. Generally, as rocks become embedded, fewer living spaces are available to macroinvertebrates and fish for shelter, spawning and egg incubation.

   To estimate the percent of embeddedness, observe the amount of silt or finer sediments overlying and surrounding the rocks. If kicking does not dislodge the rocks or cobbles, they might be greatly embedded.

The following eight parameters should be assessed in the entire 100-yard section of the stream.

3. *Shelter for fish* includes the relative quantity and variety of natural structures in the stream, such as fallen trees, logs, and branches; cobble and large rocks; and undercut banks that are available to fish for hiding, sleeping, or feeding. A wide variety of submerged structures in the stream provide fish with many living spaces; the more living spaces in a stream, the more types of fish the stream can support.
4. *Channel alteration* is basically a measure of large-scale changes in the shape of the stream channel. Many streams in urban and agricultural areas have been straightened, deepened (e.g., dredged), or diverted into concrete channels, often for flood control purposes. Such streams have far fewer natural habitats for fish, macroinvertebrates, and plants than do naturally meandering

streams. Channel alteration is present when the stream runs through a concrete channel; when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when the stream is very straight for significant distances; when dams, bridges, and flow-altering structures such as combined sewer overflow (CSO) pipes are present; when the stream is of uniform depth due to dredging; and when other such changes have occurred. Signs that indicate the occurrence of dredging include straightened, deepened, and otherwise uniform stream channels, as well as the removal of streamside vegetation to provide dredging equipment access to the stream.

5. *Sediment deposition* is a measure of the amount of sediment that has been deposited in the stream channel and the changes to the stream bottom that have occurred as a result of the deposition. High levels of sediment deposition create an unstable and continually changing environment that is unsuitable for many aquatic organisms.

   Sediments are naturally deposited in areas where the stream flow is reduced, such as pools and bends, or where flow is obstructed. These deposits can lead to the formation of islands, shoals, or point bars (sediments that build up in the stream, usually at the beginning of a meander) or can result in the complete filling of pools. To determine whether these sediment deposits are new, look for vegetation growing on them: new sediments will not yet have been colonized by vegetation.

6. *Stream velocity and depth combinations* are important to the maintenance of healthy aquatic communities. Fast water increases the amount of dissolved oxygen in the water; keeps pools from being filled with sediment; and helps food items like leaves, twigs, and algae move more quickly through the aquatic system. Slow water provides spawning areas for fish and shelters macroinvertebrates that might be washed downstream in higher stream velocities. Similarly, shallow water tends to be more easily aerated (i.e., it holds more oxygen), but deeper water stays cooler longer. Thus the best stream habitat includes all of the following velocity/depth combinations and can maintain a wide variety of organisms.

   slow (<1 ft/sec), shallow (<1.5 ft)
   slow, deep
   fast, deep
   fast, shallow

   Measure stream velocity by marking off a 10-foot section of stream run and measuring the time it takes a stick, orange, or other floating biodegradable object to float the 10 feet. Repeat 5 times, in the same 10-foot section, and determine the average time. Divide the distance (10 feet) by the average time (seconds) to determine the velocity in feet per second.

   Measure the stream depth by using a stick of known length and taking readings at various points within your stream site, including riffles, runs, and pools. Compare velocity and depth at various points within the 100-yard site to see how many of the combinations are present.

7. *Channel flow status* is the percent of the existing channel that is filled with water. The flow status changes as the channel enlarges or as flow decreases

as a result of dams and other obstructions, diversions for irrigation, or drought. When water does not cover much of the streambed, the living area for aquatic organisms is limited.

For the last three parameters, evaluate the condition of the right and left stream banks separately. Define the " left" and "right" banks by standing at the downstream end of your study stretch and looking upstream. Each bank is evaluated on a scale of 0-10.

8. *Bank vegetative protection* measures the amount of the stream bank that is covered by natural (i.e., growing wild and not obviously planted) vegetation. The root systems of plants growing on stream banks help hold soil in place, reducing erosion. Vegetation on banks provides shade for fish and macroinvertebrates and serves as a food source by dropping leaves and other organic matter into the stream. Ideally, a variety of vegetation should be present, including trees, shrubs, and grasses. Vegetative disruption can occur when the grasses and plants on the stream banks are mowed or grazed, or when the trees and shrubs are cut back or cleared.
9. *Condition of banks* measures erosion potential and whether the stream banks are eroded. Steep banks are more likely to collapse and suffer from erosion than are gently sloping banks and are therefore considered to have a high erosion potential. Signs of erosion include crumbling, unvegetated banks, exposed tree roots, and exposed soil.
10. The *riparian vegetative zone width* is defined here as the width of natural vegetation from the edge of the stream bank. The riparian vegetative zone is a buffer zone to pollutants entering a stream from runoff. It also controls erosion and provides stream habitat and nutrient input into the stream.

    A wide, relatively undisturbed riparian vegetative zone reflects a healthy stream system; narrow, far less useful riparian zones occur when roads, parking lots, fields, lawns, and other artificially cultivated areas, bare soil, rocks, or buildings are near the stream bank. The presence of "old fields" (i.e., previously developed agricultural fields allowed to revert to natural conditions) should rate higher than fields in continuous or periodic use. In arid areas, the riparian vegetative zone can be measured by observing the width of the area dominated by riparian or water-loving plants, such as willows, marsh grasses, and cottonwood trees.

Note: Instructions on sample processing, macroinvertebrate identification, and data analysis follow the sections on muddy-bottom macroinvertebrate sampling and habitat assessment. (See Step 3, page 101)

Muddy-Bottom Sampling
Part 1: Macroinvertebrate Sampling

In muddy-bottom streams, as in rocky-bottom streams, the goal is to sample the most productive habitat available and look for the widest variety of organisms. The most productive habitat is the one that harbors a diverse population of pollution-sensitive macroinvertebrates. Volunteers should sample by using a D-frame net to jab at the habitat and scoop up the organisms that are dislodged. The idea is to collect a total sample that consists of 20 jabs taken from a variety of habitats.

TASK 1 **Determine which habitats are present**

Muddy-bottom streams usually have four habitats (Fig. 4.17). It is generally best to concentrate sampling efforts on the most productive habitat available, yet to sample other principal habitats if they are present. This ensures that you will secure as wide a variety of organisms as possible. Not all habitats are present in all streams or present in significant amounts. If your sampling areas have not been preselected, try to determine which of the following habitats are present. (Avoid standing in the stream while making your habitat determinations.)

- *Vegetated bank margins* consist of overhanging bank vegetation and submerged root mats attached to banks. The bank margins may also contain submerged, decomposing leaf packs trapped in root wads or lining the streambanks. This is generally a highly productive habitat in a muddy–bottom stream, and it is often the most abundant type of habitat.
- *Snags and logs* consist of submerged wood, primarily dead trees, logs, branches, roots, cypress knees and leaf packs lodged between rocks or logs. This is also a very productive muddy-bottom stream habitat.
- *Aquatic vegetation beds and decaying organic matter* consist of beds of submerged, green/leafy plants that are attached to the stream bottom. This habitat can be as productive as vegetated bank margins, and snags and logs.
- *Silt/sand/gravel substrate* includes sandy, silty, or muddy stream bottoms; rocks along the stream bottom; and/or wetted gravel bars. This habitat may also contains algae-covered rocks (sometimes called Aufwuchs). This is the least productive of the four muddy-bottom stream habitats, and it is always present in one form or another (e.g., silt, sand, mud, or gravel might predominate).

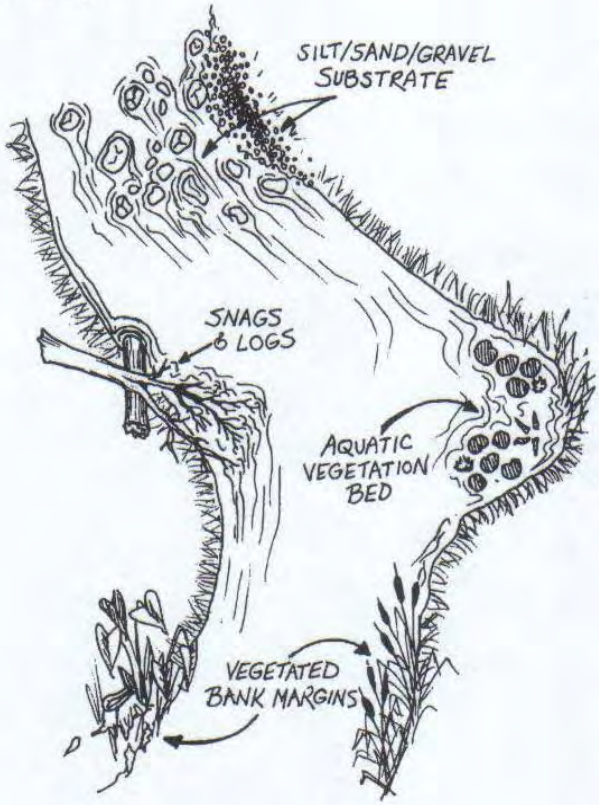


Figure 4.17

Four habitats found in muddy-bottom streams

Volunteers will likely find the most macroinvertebrates in vegetated habitats and snags and logs.

TASK 2 **Determine how many times to jab in each habitat type**

Your goal is to jab a total of 20 times. The D-frame net is 1 foot wide, and a jab should be approximately 1 foot in length. Thus, 20 jabs equals 20 square feet of combined habitat.

- If all four habitats are present in plentiful amounts, jab the vegetated banks 10 times and divide the remaining 10 jabs among the remaining 3 habitats.

- If three habitats are present in plentiful amounts and one is absent, jab the silt/sand/gravel substrate—the least productive habitat—5 times and divide the remaining 15 jabs among the other two more productive habitats.
- If only two habitats are present in plentiful amounts, the silt/sand/gravel substrate will most likely be one of those habitats. Jab the silt/sand/gravel substrate 5 times and the more productive habitat 15 times.
- If some habitats are plentiful and others are sparse, sample the sparse habitats to the extent possible, even if you can take only one or two jabs. Take the remaining jabs from the plentiful habitat(s). This rule also applies if you cannot reach a habitat because of unsafe stream conditions. Jab a total of 20 times.

Because you might need to make an educated guess to decide how many jabs to take in each habitat type, it is critical that you note, on the field data sheet, how many jabs you took in each habitat. This information can be used to help characterize your findings.

TASK 3 Get into place

Outside and downstream of your first sampling location (1st habitat), rinse the dip net and check to make sure it does not contain any macroinvertebrates or debris from the last time it was used. Fill a bucket approximately one-third full with clean stream water. Also, fill the spray bottle with clean stream water. This bottle will be used to wash down the net between jabs and after sampling is completed.

This method of sampling requires only one person to disturb the stream habitats. While one person is sampling, a second person should stand outside the sampling area, holding the bucket and spray bottle. After every few jabs, the sampler should hand the net to the second person, who then can rinse the contents of the net into the bucket.

TASK 4 Dislodge the macroinvertebrates

Approach the first sample site from downstream, and sample as you walk upstream. Here is how to sample in the four habitat types:

- Sample vegetated bank margins by jabbing vigorously, with an upward motion, brushing the net against vegetation and roots along the bank. The entire jab motion should occur underwater.
- To sample snags and logs, hold the net with one hand under the section of submerged wood you are sampling (Fig. 4.18). With the other hand (which should be gloved), rub about 1 square foot of area on the snag or log. Scoop organisms, bark, twigs, or other organic matter you dislodge into your net. Each combination of log rubbing and net scooping is one jab.
- To sample aquatic vegetation beds, jab vigorously, with an upward motion, against or through the plant bed. The entire jab motion should occur underwater.
- To sample a silt/sand/gravel substrate, place the net with one edge against the stream bottom and push it forward about a foot (in an upstream direction) to dislodge the first few inches of silt, sand, gravel, or rocks. To avoid gathering a netful of mud, periodically sweep the mesh bottom of the net back and forth in the water, making sure that water does not run over the top of the net. This will allow fine silt to rinse out of the net. When you have com-

Figure 4.18

Collecting a sample from a log

Volunteer rubs the log with one hand and catches dislodged organisms and other material in the net.

pleted all 20 jabs, rinse the net thoroughly into the bucket. If necessary, pick any clinging organisms from the net by hand and put them in the bucket.

TASK 5 **Preserve the sample**

1. Look through the material in the bucket and immediately return any fish, amphibians, or reptiles to the stream. Carefully remove large pieces of debris (leaves, twigs, and rocks) from the sample. While holding the material over the bucket, use the forceps, spray bottle, and your hands to pick, rub, and rinse the leaves, twigs, and rocks to remove any attached organisms. Use your magnifying lens and forceps to find and remove small organisms clinging to the debris. When you are satisfied that the material is clean, discard it back into the stream.
2. You will need to drain off the water before transferring material to the jar. This process will require two team members. One person should place the net into the second bucket, like a sieve (this bucket, which has not yet been used, should be completely empty) and hold it securely. The second person can now carefully pour the remaining contents of bucket #1 onto the center of the net to drain the water and concentrate the organisms.

   Use care when pouring so that organisms are not lost over the side of the net. Use your spray bottle, forceps, sugar scoop, and gloved hands to remove all the material from bucket #1 onto the net. When you are satisfied that bucket #1 is empty, use your hands and the sugar scoop to transfer all the material from the net into the empty jar. You can also try to carefully empty the contents of the net directly into the jar by turning the net inside out into the jar.

   Bucket #2 captured the water and any organisms that might have fallen through the netting. As a final check, repeat the process above, but this time, pour bucket #2 over the net, into bucket #1. Transfer any organisms on the net into the jar.
3. Fill the jar (so that all material is submerged) with alcohol. Put the lid tightly back onto the jar and gently turn the jar upside down two or three times to distribute the alcohol and remove air bubbles.
4. Complete the sampling station ID tag. Be sure to use a pencil, not a pen, because the ink will run in the alcohol. The tag should include your station number, the stream, location (e.g., upstream from a road crossing), date, time, and the names of the members of the collecting crew. Place the ID tag into the sample container, writing side facing out, so that identification can be seen clearly (Fig. 4.19).

Muddy-Bottom Streams
Part 2: Habitat Assessment

You will conduct a habitat assessment (which will include measuring general characteristics and local land use) in a 100-yard section of the stream that includes the habitat areas from which organisms were collected.

TASK 1 — Delineate the habitat assessment boundaries

1. Begin by identifying the most downstream point that was sampled for macroinvertebrates. Using your tape measure or twine, mark off a 100-yard section extending 25 yards below the downstream sampling point and about 75 yards upstream.
2. Complete the identifying information on your field data sheet for your habitat assessment site. On your stream sketch, be as detailed as possible, and be sure to note which habitats were sampled.

TASK 2 — Complete the General Characteristics and Local Land Use sections of the field sheet

For safety reasons as well as to protect the stream habitat, it is best to estimate these characteristics rather than actually wading into the stream to measure them. For instructions on completing these sections of the field data sheet, see the rocky-bottom habitat assessment instructions.

TASK 3 — Conduct the habitat assessment

The following information describes the parameters you will evaluate for muddy-bottom habitats. Use these definitions when completing the habitat assessment field data sheet.

STATION ID TAG

Station #: _____

Stream: _____

Location: _____

Date/Time: _____

Team members:

_____

_____

_____

Figure 4.19

Example of a Station ID tag

To prevent samples from being mixed up, volunteers should place the ID tag *inside* the sample jar.

1. *Shelter for fish and attachment sites for macroinvertebrates* are essentially the amount of living space and shelter (rocks, snags, and undercut banks) available for fish, insects, and snails. Many insects attach themselves to rocks, logs, branches, or other submerged substrates. Fish can hide or feed in these areas. The greater the variety and number of available shelter sites or attachment sites, the greater the variety of fish and insects in the stream.

   Many of the attachment sites result from debris falling into the stream from the surrounding vegetation. When debris first falls into the water, it is termed new fall and it has not yet been "broken down" by microbes (conditioned) for macroinvertebrate colonization. Leaf material or debris that is conditioned is called old fall. Leaves that have been in the stream for some time lose their color, turn brown or dull yellow, become soft and supple with

age, and might be slimy to the touch. Woody debris becomes blackened or dark in color; smooth bark becomes coarse and partially disintegrated, creating holes and crevices. It might also be slimy to the touch.

2. *Pool substrate characterization* evaluates the type and condition of bottom substrates found in pools. Pools with firmer sediment types (e.g., gravel, sand) and rooted aquatic plants support a wider variety of organisms than do pools with substrates dominated by mud or bedrock and no plants. In addition, a pool with one uniform substrate type will support far fewer types of organisms than will a pool with a wide variety of substrate types.
3. *Pool variability* rates the overall mixture of pool types found in the stream according to size and depth. The four basic types of pools are large-shallow, large-deep, small-shallow, and small-deep. A stream with many pool types will support a wide variety of aquatic species. Rivers with low sinuosity (few bends) and monotonous pool characteristics do not have sufficient quantities and types of habitats to support a diverse aquatic community.
4. *Channel alteration* (See description in habitat assessment for rocky-bottom streams.)
5. *Sediment deposition* (See description for rocky-bottom streams.)
6. *Channel sinuosity* evaluates the sinuosity or meandering of the stream. Streams that meander provide a variety of habitats (such as pools and runs) and stream velocities and reduce the energy from current surges during storm events. Straight stream segments are characterized by even stream depth and unvarying velocity, and they are prone to flooding. To evaluate this parameter, imagine how much longer the stream would be if it were straightened out.
7. *Channel flow status* (See description in habitat assessment for rocky-bottom streams.)
8. *Bank vegetative protection* (See description for rocky-bottom streams.)
9. *Condition of banks* (See description for rocky-bottom streams.)
10. The *riparian vegetative zone width* (See description for rocky-bottom streams.)

Reference Collection

A reference collection is a sample of locally-found macroinvertebrates that have been identified, labelled, and preserved in alcohol. The program advisor, along with a professional biologist/entomologist, should assemble the reference collection, properly identify all samples, preserve them in vials, and label them. This collection may then be used as a training tool and, in the field, as an aid in macroinvertebrate identification.

Step 3—Leave the field, complete data forms, clean the site, and return material

After completing the stream characterization and habitat assessment, make sure that all of the field data sheets have been completed properly and that the information is legible. Be sure to include the site's identifying name and the sampling date on each sheet. These will function as a quality control element. If you can't determine how to answer a question on the field data sheet, just leave the space blank.

Before you leave the stream location, make sure that all your equipment has been collected and rinsed properly. Double-check to see that sample jars are tightly closed and properly identified. All samples, field sheets, and equipment should be returned to the coordinator at this point. You might want to keep a copy of the field data sheet for comparison with future monitoring trips and for personal records.

Step 4—Prepare for macroinvertebrate laboratory work

This step includes all the work needed to set up a laboratory for processing samples into subsamples and identifying macroinvertebrates to the family level. A professional biologist/entomologist or the program advisor should supervise the identification procedure. All interested volunteers should be encouraged to participate. In general it is a good idea to train volunteers in identification procedures before each lab session and to start new volunteers with less diverse samples. Refresher workshops for experienced volunteers are strongly encouraged.

TASK 1 **Gather tools and equipment for the laboratory**

The following lab equipment is recommended for the macroinvertebrate identification process. Enough of each will need to be provided for each volunteer work station:

- Reference collection and taxonomic keys
- Fine-point forceps
- Petri dishes or small, shallow, clear container
- Alcohol preservative (used in field and lab): 70 percent ethyl alcohol, denatured; no other preservatives used
- Microscope, dissecting microscope, and magnifying glass, or hands lens
- Sample containers, preferably shatterproof with poly-seal caps that prevent evaporation of the preservative (jars or vials are used in field and lab). Shatterproof vials with poly-seal caps are available from scientific supply houses.
- Wash bottles or spray bottles
- Shallow, rectangular white pans (large enough to hold entire macroinvertebrate sample)
- Additional shallow white containers (heavy duty plastic plates with a rim, white pans, or cafeteria trays are all possible choices).
- Plastic spoons or unslotted spatulas
- Sieve, purchased from scientific supply company (#30) or homemade (with same mesh size as sampling net)
- Permanent ink markers
- Ruler
- Macroinvertebrate assessment worksheet
- Pencils
- Note paper for counting

TASK 2 **Create gridded subsampling pans**

Using the ruler, measure the inside width and length of the large rectangular white pan. Draw a grid of evenly sized squares on the inside of the pan, using permanent ink. The grid should fill the entire inside of the pan. Number each square. One pan will be needed for each work station. Volunteers will use these pans for randomizing the sample and selecting a subsample of organisms.

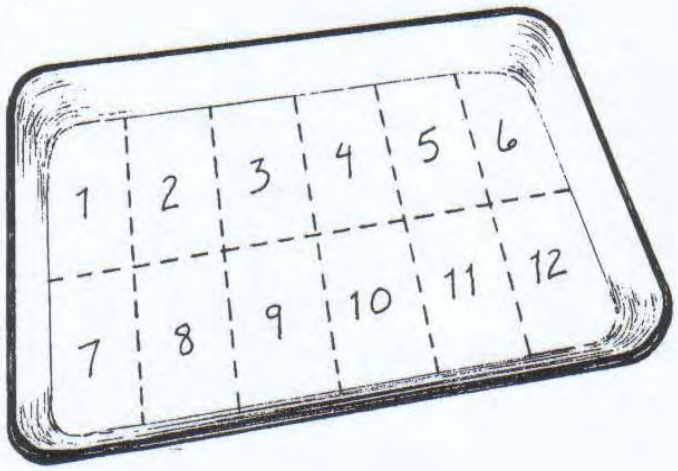
TASK 3 **Prepare the lab and the individual work stations**

Before volunteers enter the lab, the program manager will need to prepare work stations. Make sure that all microscopes are functioning properly and that each station has access to all other equipment. The reference collection should be centrally located as should any other visual training displays. The lab itself should be well lit and well ventilated. A copy of lab safety instructions should be visible to all volunteers.

Figure 4.20

A gridded subsampling pan

Volunteers collect a subsample of organisms by picking them from randomly selected grid squares.



Step 5—Conduct macroinvertebrate processing and identification

If possible, before beginning the subsampling and identification processes, all volunteers should become familiar with the lab equipment, microscope(s), the reference collection, and the taxonomic key chosen by the advisor.

Processing a subsample and identifying the organisms are two separate activities. Some programs might prefer to split these tasks into separate lab sessions.

Session 1: Picking a subsample of aquatic organisms

TASK 1 **Prepare the sample**

1. Carefully remove the station ID tag from the sample container and put it aside. You will need it later.
2. Cover the bottom of the gridded pan with about 1/4 inch of clean water.
3. Pour the preserved sample (alcohol and debris) into the sieve and wash off preservative over a sink, using a spray or wash bottle filled with water.
4. Transfer the sample to the white gridded pan by turning the sieve upside down over the pan. Tap it several times to empty the contents onto the pan. Squirt a small amount of water over the bottom of the sieve to flush the organisms into the pan.
5. With your hands and by gently shaking the pan, evenly disperse the sample over the entire bottom of the pan, making sure that even the corners are covered. The water will help in distributing the sample throughout the pan. This is called randomizing the sample.

TASK 2 **Randomly select a square for the subsample**

1. Randomly choose a square to start sorting organisms. You may use a random numbers table, draw numbers from a hat, or roll a pair of dice. The most important thing to remember is that the grid selection should be random. Indicate the square number selected on the lab sheet.
2. Using a plastic spoon or unslotted spatula, remove *all* the material from the square and transfer it to another container (another pan, tray, or plate) for sorting. The organisms in this container will become your subsample.

TASK 3 **Pick the subsample**

1. Prepare a container to house the subsample by filling a vial or jar one-half full of alcohol. Place the new label into the vial, writing side out. Keep the vial on a flat, stable area.
2. Using forceps, carefully and systematically remove all organisms from the pan or tray and place them one by one into the prepared subsample vial. Examine all debris such as leaves or sticks for clinging organisms. Count each organism as it is transferred. Keep a written count of the number of organisms you have transferred. The objective is have at least 100 individual organisms in your subsample. If you reach 100 and there are still organisms remaining in your subsample plate or tray, continue picking until *all* the organisms are removed even though you might end up with more than 100.

   When you think all the organisms have been transferred from the plate or tray to the subsample vial, have a second volunteer check to confirm that all organisms have been removed. On your lab sheet, record how many organisms are in the subsample.
3. If you finish picking the contents of the first square selected and have fewer than 100 organisms, randomly select another square and repeat the process of removing the contents of the square to the subsample plate or tray; picking organisms with the forceps and transferring them to the vial (all organisms that will be part of the subsample should be transferred to the same vial). Record the number of organisms you obtain from the second square. Repeat this process until at least 100 organisms have been placed into the vial or until the entire sample in the gridded pan has been picked clean. Remember, any square started must be picked clean.

   If, after picking the entire gridded pan clean, you have fewer than 100 organisms, and your reference site

SUBSAMPLE ID TAG

Station #: _____

Stream: _____

Location: _____

Date/Time: _____

Subsample team members:

_____

_____

_____

Figure 4.21

Example of a Subsample ID tag

To prevent subsamples from being mixed up, volunteers should place the ID tag *inside* the subsample jar.

produced 100 or more organisms, either your site is impaired or your sampling technique is flawed. It is also possible that recent heavy rains might have washed many organisms downstream. If you do not find 100 organisms in the entire sample, be sure to note the potential cause for such a problem on the Habitat Assessment Data Sheet.

TASK 4 Label and store the subsample

Fill out a new Subsample ID Tag (Fig. 4.21) for the subsample. Remember to use pencil because ink will run in the alcohol. The vial housing the subsample must be labeled with the same station number, stream name, location, and date found on the original sample ID tag. The vial tag should also include information on when the subsample was picked (i.e., 100 or more organisms counted) and by whom. Place the tag in the vial with the writing side out. Make sure the vial is tightly closed before giving the subsample in the vial to the program coordinator.

TASK 5 Replace remainder of original sample back into the sample jar

Place the remaining sample back into the original container. Be sure that the original station ID tag is included, writing side out. Fill the jar with 70 percent alcohol. This sample will be retained as part of a voucher collection. Make sure the jar is tightly closed before returning it to the program coordinator.

Session 2: Identifying the subsample to family level

TASK 1 Prepare for the ID

1. Make sure that you have several petri dishes, fresh alcohol, and fresh water close at hand. Also have your taxonomic keys handy for all stages of the ID process. Check to make sure that your microscope is working properly.
2. Carefully remove the station ID tag from the subsample vial and put it aside. You will need it later. Be sure no organisms are clinging to it. If they are, remove them with forceps.
3. Using the information on the station ID tag, complete the first section of the Macroinvertebrate Assessment Sheet with your name, date, the stream name, station number, and any other information requested.

TASK 2 Identify the sample to order level

1. Place a few of the macroinvertebrates in a petri dish (or other small, shallow container) and examine them under the microscope. Include some ethyl alcohol in the dish to ensure that the organisms do not dry out. Compare the organisms in the dish to those in the taxonomic key and/or reference collection.
2. Roughly sort organisms by taxonomic order into petri dishes. Many volunteers find it helpful to use one dish for every major taxonomic order found in the subsample. Place any organism that you cannot identify into another dish for the biological advisor to examine.

TASK 3 **Identify the organisms within each order to family level**

1. Starting with one order, and using the taxonomic keys, reference collection, and assistance of the biological advisor, identify each individual to family level.
2. Keep a running count of how many individuals there are in each family on a piece of scratch paper.
3. Place any organisms that you cannot identify into a separate container. Make sure that the biological advisor sees them and assists you with the ID.
4. After all organisms have been identified, note the total number of organisms in each family on the Macroinvertebrate Assessment Sheet. Write in pencil and make sure your writing is legible. These lab sheets will be the basis for the data analysis. It is important that they are accurate and easy to read.

TASK 4 **Return the organisms to the vial**

1. After you have identified and counted all organisms in the subsample, return them to the subsample vial and replace the subsample ID Tag, writing side out.
2. Refill the subsample vial with 70 percent ethyl alcohol (new or recycled). Be sure to secure the caps on the vial tightly to prevent the organisms from drying out.
3. Return the subsample vial and the assessment worksheet to the program manager.

Voucher Collection

Maintaining a voucher collection adds another layer of credibility to the program by documenting the accuracy of the volunteer identifications. It substantiates and provides evidence to support the analysis of the data—a powerful quality control element. However, an important issue to consider is how long to keep the samples. Program managers, in collaboration with technical advisors, will have to consider the following in keeping a voucher collection.

- *Sample maintenance.* Even jars and vials with tight fitting lids require maintenance on a regular basis (every 2-3 months) to ensure that alcohol levels are adequate.
- *Fire safety.* When you are dealing with alcohol, you will need to consider fire safety and ventilation issues to make sure that you are in line with local codes.
- *Availability of storage space.* In addition to needing well-ventilated and fire-proof storage cabinet, you will need a well-ventilated room to store samples. Samples should not be stored in someone's office for any length of time.
- *Length of storage.* How long samples should be maintained is an issue determined by program goals. Data collected for regulatory purposes will probably require longer storage than other samples. Generally, 1-5 years is recommended for storage.

Step 6—Performing habitat assessment data analysis

To evaluate the condition of your stream site properly, you should compare it to an optimal or best condition found in the region. This is called a reference condition. In an ideal world, the reference condition would reflect the water quality, habitat, and aquatic life characteristics of pristine sites in the same ecological region as your stream. In real life, however, few pristine sites remain. The reference condition is, therefore, a composite of sites that reflect the best physical, chemical, and biological conditions existing in your ecological region. State water quality or natural resource agencies might have already established reference conditions for the ecological regions in your state.

Table 4.5

Reference scores for sampling site comparison

If a score falls at or near the break between categories, use your best judgement to determine the appropriate score.

% Similarity to Reference Score	Habitat Quality Category	Attributes
> 90 %	Excellent	Comparable to the best situation to be expected within an ecoregion. Excellent overall habitat structure conducive to supporting healthy biological community.
75 - 88%	Good	Habitat structure slightly impaired. Generally, diverse instream habitat well-developed; some degradation of riparian zone and banks; a small amount of channel alteration may be present.
60 - 73%	Fair	Loss of habitat compared to reference. Habitat is a major limiting factor to supporting a healthy biological community.
< 58%	Poor	Severe habitat alteration at all levels.

Your program's consulting biologist should work in cooperation with the state agency to identify the reference condition(s) you will need to conduct an Intensive Stream Biosurvey. The biologist will use the reference condition to establish a water quality rating system against which to rank your monitored stream sites.

To perform the habitat assessment data analysis for the Intensive Stream Biosurvey, perform the following tasks.

TASK 1 **Determine the habitat index score**

Add together the scores of all 10 habitat parameters. This sum is the habitat index score for the study stretch.

TASK 2 **Determine the percent similarity to the reference score**

Divide the habitat index score by the reference index score and then multiply the result by 100. This number is the percent similarity to the reference score.

TASK 3 **Determine the stream habitat quality rating**

Compare the percent similarity of your results with the range of percent similarity numbers in the stream habitat rating table to obtain the habitat quality category for your site(s) (Table 4.5). Enter the appropriate descriptive rating (excellent, good, fair, or poor) on the field data sheet. If your score falls at or near the break between habitat quality categories, use your best judgment to determine an appropriate rating.

Step 7—Conduct macroinvertebrate data analysis

In general, the program's biological advisor, rather than the volunteers, should analyze the results of the Intensive Stream Biosurvey's macroinvertebrate identification. The advisor's knowledge of local ecological conditions will help in the interpretation of the data findings and will lend additional credibility to the sampling effort. Volunteers can contribute significantly to the advisor's data analysis by interpreting field notes, assisting with

macroinvertebrate identification, and counting organisms on the aquatic macroinvertebrate assessment worksheet. Relay the results of the data analysis to the volunteers as soon after the sampling date as possible.

TASK 1 **Determine which metrics or measurements are appropriate**

A number of metrics (or measures) can be used to calculate stream health using benthic macroinvertebrates. These metrics should be calculated for both the sample site and the reference condition. By comparing the two, the program advisor can reach a clear understanding of the biological health of the sampling site.

The Intensive Stream Biosurvey recommends the use of four basic metrics (taxa richness, number of EPT taxa, percent abundance of EPT, and sensitive taxa index) plus two optional metrics (percent abundance of scrapers and percent abundance of shredders). These metrics are discussed briefly below. Refer to the reference list for more information.

The term *taxa* (plural for taxon), used below, refers to the specific taxonomic groupings to which organisms have been identified. For the Intensive Stream Biosurvey, organisms are identified to the taxon of family. Your volunteer monitoring program should identify organisms to a specific taxonomic grouping if it is to compare results over time and between sites. The following metrics are generally applicable throughout the country (but confirm this with a local biologist).

1. *Number of taxa (taxa richness)*—this measure is a count of the number of taxa (e.g., families) found in the sample. A high diversity or variety is good.
2. *Number of EPT taxa (EPT richness)*—this measure is a count of the number of taxa in each of three generally pollution-sensitive orders: Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). A high diversity or variety is good.
3. *Percent dominance*—this measure is the percent composition of the most abundant family from your station. It indicates how dominant a single taxon is at a particular site. A high percent dominance is not good.
4. *Sensitive taxa index (modified Hilsenhoff index)*—this measure is calculated by multiplying the number of organisms in each taxon by the pollution tolerance value assigned to each taxon, adding these for all taxa represented in the sample, and dividing by the total number of taxa in the sample. A high index number is not good.

$$\text{Sensitive taxa index} = \frac{\sum (X_i\, t)}{n}$$

where:

- $\sum$ = the summation of $X_i t$
- X_i = the number of individuals in each taxon
- t = tolerance value for each taxon in the sample
- n = number of individuals in the sample

The following optional metrics can be used in rocky-bottom streams if at least 10 scraper and shredder organisms are collected.

5. *Percent abundance of scrapers*—in the majority of rocky-bottom streams, the basic food source for many aquatic organisms is algae covering the rocks in the stream.

   Macroinvertebrates that "scrape" or graze on these algae are known as scrapers. To compute the percent

Selecting Metrics to Determine Stream Health

Metrics are used to analyze and interpret biological data by condensing lists of organisms into relevant biological information. In order to be useful, metrics must be proven to respond in predictable ways to various types and intensities of stream impacts. This manual recommends using a multimetric approach that combines several metrics into a total Biosurvey Score. The four primary and two optional metrics discussed in this chapter have been tested extensively in the mid-Atlantic region and have been shown to respond in predictable ways to stream impacts. In other parts of the country, other metrics and scoring systems may be more appropriate. For example, the Benthic Index of Biotic Integrity (B-IBI), developed by Dr. James Karr, is another multimetric approach, using different metrics, that has been tested in the Tennessee Valley, the Midwest, and the northwest. The River Watch Network suggests that, while you should always use multiple metrics to summarize your data, you shouldn't rely solely on an overall score to interpret your data; individual metrics can also provide a wealth of information. In any case you will need to select metrics that have been proven to respond predictably to various impacts. As always, consult with your program's biological advisor for help in selecting appropriate metrics for your region and for determining whether an overall biosurvey score is recommended.

Below are metrics that are commonly used in rocky bottom streams. This is only a partial list of the dozens of metrics used by monitoring programs throughout the country. These metrics fall under four general categories: 1) taxa richness and composition, 2) pollution tolerance and intolerance, 3) feeding ecology, and 4) population attributes. Metrics marked with a (*) are included in the recommended suite of metrics in this manual. The River Watch Network's *Benthic Macroinvertebrate Monitoring Manual* contains detailed guidance on selecting, calculating, aggregating, and interpreting the metrics discussed below. (See Dates, G. and J. Byrne in References and Further Reading)

Taxa Richness and Composition Metrics

- *Total Number of Taxa* *: the total number of taxa found in the sample.
- *Number of EPT Taxa* *: the combined number of mayfly (E), stonefly (P) and caddisfly (T) taxa found in the sample. The number of taxa in each of these macroinvertebrate orders can also be reported separately since each order may respond differently to various impacts.
- *Number of Long-Lived Taxa:* the number of organism families found in the sample (such as giant stoneflies and dobson flies) that live more than one season.
- *Percent Abundance of the Major Groups* *: the percent of the sample that is comprised of individuals in each of the selected major groups (mostly orders).
- *Percent Model Affinity* (Bode, 1991): used in conjunction with *Percent Composition of the Major Groups*, this metric measures the similarity of the sample to a model "nonimpacted" community of organisms (adjusted for ecoregional conditions) based on the percent composition of the major groups.
- *Quantitative Similarity Index* (from Shackleford, 1988): used in conjunction with *Percent Composition of the Major Groups*, this metric shows the percent similarity between two sites based on the percent of the sample in each of the major groups.
- *Dominants in Common* (from Shackleford, 1988): the number of dominant (5 most abundant families) families common to two sites.

Tolerance and Intolerance Metrics

- *Number of Intolerant Taxa:* the number of taxa in the sample that are in the 10-15% of the least tolerant taxa in a region or that have a pollution tolerance value of 1 (based on the Hilsenhoff scale of 0-10).
- *Percent of Individuals in Tolerant Taxa:* the number of taxa in the sample that are in the 10-15% of the most tolerant taxa in a region or that have a pollution tolerance value of 10 (based on the Hilsenhoff scale of 0-10).
- *Number of Clinger Taxa:* the number of families in the sample that live by clinging to the bottom of the stream.
- *Sensitive Taxa Index* *: the pollution tolerance values (based on the Hilsenhoff scale of 0-10) assigned to each family aggregated into an overall pollution tolerance value for the sample.

Feeding Ecology Metrics

- *Percent Composition of Functional Feeding Groups:* the percentage of the total number of individuals in the sample that belong to each of the five functional feeding groups (scrapers, shredders, filtering collectors, gathering collectors, and predators).
- *Percent Abundance of Scrapers* *: the percent of the total number of individuals in the sample that use bottom-growing algae as their primary food source.
- *Percent Abundance of Shredders* *: the percent of the total number of individuals in the sample that use leaves and other plant debris as their primary food source.
- *Percent Abundance of Predators:* the percent of the total number of individuals in the sample that eat other animals as their primary food source.

Population Attributes Metrics

- *Percent Dominance (of the most abundant family)* *: the percentage of the total number of individuals in the sample that are in the sample's most abundant family.
- *Percent Dominance (of the three most abundant families):* the percentage of the total number of individuals in the sample that are in the sample's three most abundant families.
- *Organism Density Per Sample (total abundance):* the total number of individuals in the sample (calculated if a subsample is used).

abundance of the scrapers in the macroinvertebrate community, divide the number of organisms classified as grazers or scrapers by the total number of organisms in the sample. A high percent abundance of scrapers is good.

6. *Percent abundance of shredders*—leaf litter and other plant debris are broken down and processed by organisms called shredders. To compute the percent abundance of shredders in the macroinvertebrate community, divide the number of organisms classified as shredders by the total number of organisms in the sample. A high percent abundance of shredders is good.

The following optional metrics can be used in muddy-bottom streams as additional metrics to provide more information about the condition of the macroinvertebrate assemblage.

7. *Percent abundance of EPT*—this measure compares the number of organisms in the EPT orders to the total number of organisms in the sample. (The number of organisms in the EPT orders is divided by the total number of organisms in the sample to calculate a percent abundance.) A high percent abundance of EPT orders is good.
8. *Percent abundance of midge larvae*—this measure compares the number of midges to the total number of organisms in the sample. (The number of organisms in the chironomidae family is divided by the total number of organisms in the sample to calculate a percent composition.) A low percent abundance of midge larvae is good.

TASK 2 **Calculate a score for the site**

The metric worksheets Tables 4.6 and 4.7 are designed to help calculate a total score for the monitored site. Table 4.8 provides an example of a sample metric worksheet for the fictional Volunteer Creek (rocky-bottom stream). This score should be compared to reference conditions to determine the biological condition of the stream at that site. You should also note that these worksheets were developed for use in mid-Atlantic states; they might need to be modified to reflect local conditions.

To calculate a score for your stream site using one of these worksheets, enter the metric values at the monitored site in the (M) column. Compare each metric value from your monitored site to the value ranges presented in the biosurvey score columns. Choose the matching range and circle it; this gives you the corresponding score (6, 3, or 0) for your metric value. Add the metric scores to obtain the total biosurvey score (see instructions in Tables 4.6 and 4.7).

TASK 3 **Determine the biological condition**

To determine the biological condition of the site, refer to Table 4.9, Biosurvey Scoring Guide.

TASK 4 **Return the lab sheets and metric worksheets to the program coordinator**

All remaining worksheets should be returned to the program coordinator once the site's final score has been determined. The program coordinator will determine how to proceed with the findings of the biological assessment (e.g., the data may be entered into a database or shared with a state or local agency). It is important that the biological advisor include documentation of any problems encountered in the process of monitoring, identifying macroinvertebrates, or analyzing the data.

An example of WV Save Our Streams' level-two survey data sheet is provided beginning on the next page.

Save Our Streams
A citizens volunteer stream monitoring program

(**1**) Determine your stream-reach boundary; this is a stream length up to 100-meters, which may be more or less under certain circumstances. (**2**) Near the lower end of the reach (in the deepest portion of the run), collect water samples and analyze using the chemical tests you have available. You may use your collection container to observe watercolor and clarity and to determine water odors. (**3**) Measure the width-depth and velocity, and estimate the water level. (**4**) If you use a two-pole **kick-net**, collect a minimum of three benthic macro-invertebrate samples from the best riffle or runs within your stream reach. Use the table on page five to record information about your collections. (**5**) Evaluate the physical and habitat conditions; record information about known land use activities. (**6**) Sketch your reach or submit photographs with the survey, and add any other comments that you feel are important for evaluating the conditions of your stream study site.

Stream name _____ Survey date _____
Watershed _____ Station code _____
Latitude _____ Longitude _____ Directions to site _____

Survey completed by _____
Current weather conditions _____
Past weather conditions (last 3-days) _____
Affiliation _____ Email _____
Mailing address _____ Phone number _____

Water chemistry: Use the spaces below to record the results of your water chemistry analysis; attach additional sheets if necessary.

	Result	units		Result	units		Result	units
Temperature (C/F)			Conductivity			Alkalinity		
Dissolved oxygen			Nitrate/Nitrite			Metals (describe)		
pH			Phosphate			Fecal/E-coli		

Additional tests (describe and record results) _____

Physical conditions: Use the check boxes below to describe the conditions that closely resemble those of your stream. The extra lines are provided to write in any additional comments. You may see more than one type of condition; if so, be sure to indicate these on your survey (check all that apply). If multiple conditions are observed, always indicate the most dominant condition. If the condition you observe is not listed, describe it in the comment section.

Water clarity		Water color		Water odor		Surface foam	
Clear		None		None		None	
Murky		Brown		Fishy		Slight	
Milky		Black		Musky		Moderate	
Muddy		Orange/red		Rotten egg		High	
Other (describe)		Gray/White		Sewage			
		Green		Chemical			

Algae color		Algae abundance		Algae growth habit		Streambed color	
Light green		None		Even coating		Brown	
Dark green		Scattered		Hairy		Black	
Brown		Moderate		Matted		Green	
Other (describe)		Heavy		Floating		White/gray	
						Orange/red	

Physical condition comments: _____

Station _____ Date(s) _____

Estimate the percentage of your reach that is shaded.

> 80	80-60	60-40	< 40
Excellent	Good	Marginal	Poor

Circle your estimate

Width and depth: Record the wetted width and depth of the channel's habitats (riffles, runs or pools). Choose one or more features to measure. Record the average depth from a minimum of four measure-ments (one of these should be from the deepest part of the habitat). The width should be measured from the widest section of the feature. Be sure to indicate the type(s) of habitat that you have chosen. **It is best to measure the width and depth when you determine the discharge**.

				Riffle	Run	Pool
1. Width (feet)	_____	Depth (feet)	_____			
2. Width (feet)	_____	Depth (feet)	_____			

Channel profiles: Width and depth measurements can be used to create a cross section profile within your reach. Choose a location in your reach across one of the channel types above. Stretch a tape from bank to bank and anchor it at both ends. Move from left to right facing in an upstream direction; measure the distance from the stream bottom to the top of the tape at selected intervals (i.e. every foot). Record your measurements in the table below. The table provides enough spaces for 20 measurements; if more are necessary you can create your own table on a separate piece of paper. Your tape measure will probably not start at zero so make sure to record the actual position of the tape as you measure across the channel.

Width intervals

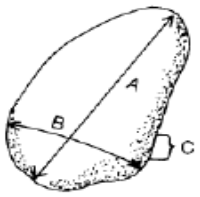
1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20

Depth measurements

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20

Pebble count: Collect a minimum of 100-particles from your reach using a Zigzag method, percent habitat method or specific transects (e.g. every 10-meter). If you do not complete a pebble count, **always estimate** streambed composition from the riffles/runs chosen for your macroinvertebrate sample collections.

Indicate your method from the choices below.	Size Classes (Intermediate axis in millimeters)						
	Silt/clay < 0.06	Sand 0.06 – 2	Fine Gravel 2 – 24	Coarse Gravel 25 – 64	Cobble 65 – 255	Boulder 256 – 1096	Bedrock > 1096
Zigzag							
% Habitat							
10-m Transects							
Woody Debris (Includes sticks, roots, leaves etc.)							
Totals							

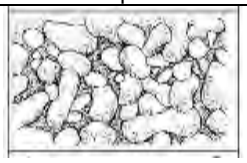
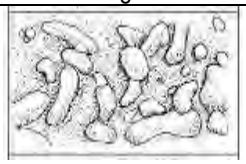


(A) Long axis (**Length**)
(B) Intermediate axis (**Width**)
(C) Short axis (**Height**)

Pebble counts require two people, one in the stream and one on shore. The person in the stream slowly walks upstream from bank to bank using one of the methods above. After each step the person reaches down without looking, picks up the first particle touched, and measures the intermediate axis with a ruler. The on-shore partner records the measurement. The process continues until 100 pebbles have been measured or the reach has been walked.

Station _____ Date(s) _____

Habitat conditions: Score each habitat condition using the scales provided. Add all of the scores to determine your overall habitat score and integrity rating. Feel free to describe additional features that you feel are important. See the next page for more information about sediment deposition.

Point values	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Sediment deposition	Little or no formation of depositional features; < 20% of the reach affected.	Some increase in depositional features; 20-40% of the reach affected.	Moderate amounts of depositional features; 40-60% of the reach affected.	Heavy amounts of deposition; > 60% of the reach affected.
Rating	**O**ptimal	**S**uboptimal	**M**arginal	**P**oor
Embeddedness	 Fine sediments surrounds <10% of the spaces between the gravel, cobble and boulders.	 Fine sediment surrounds 10-30% of the spaces between the gravel, cobble and boulders.	 Fine sediment surrounds 30-60% of the spaces between the gravel, cobble and boulders.	 Fine sediment surrounds > 60% of the spaces between the gravel, cobble and boulders.
Rating	**O**ptimal	**S**uboptimal	**M**arginal	**P**oor

Embeddedness should be evaluated in riffles, prior to or during your macroinvertebrate collections.

Point values	10 9	8 7 6	5 4	3 2 1
Bank vegetative protection	> 90% of the banks are covered by natural vegetation; all levels (trees, shrubs and herbs) represented; disruption from grazing, mowing etc. minimal or absent; all plants allowed to grow naturally.	70-90% of the banks covered by natural vegetation; one level of plants may be missing or not well represented; some disruption of vegetation evident; > 50% of the potential plant height remains.	50-70% of the banks covered by natural vegetation; patches of bare soil may be present and closely cropped vegetation is common; < 50% of the potential plant heights remains.	< 50% of the banks covered by natural vegetation; disruption is high; vegetation has been removed or the potential plant heights are greatly reduced.
Left / Right	**O**ptimal	**S**uboptimal	**M**arginal	**P**oor
Bank stability	Banks are stable; no evidence of erosion or bank failure; little or no potential for future problems.	Banks are moderately stable; infrequent areas of erosion occur, mostly shown by banks healed over.	Banks are moderately unstable; 60% of the reach has some areas of erosion; high potential for erosion during flooding events.	Banks are unstable; many have eroded areas (bare soils) along straight sections or bends; obvious bank collapse or failure; > 60% of the reach has erosion scars.
Left / Right	**O**ptimal	**S**uboptimal	**M**arginal	**P**oor
Riparian buffer width	Mainly undisturbed vegetation > 60 ft; no evidence of human impacts such as parking lots, road beds, clear-cuts, mowed areas, crops, lawns etc.	Zone of undisturbed vegetation 40-60 ft; some areas of disturbance evident.	Zone of undisturbed vegetation 20-40 ft; disturbed areas common throughout the reach.	Zone of undisturbed vegetation < 20 ft; disturbed areas common throughout the entire reach.
Left / Right	**O**ptimal	**S**uboptimal	**M**arginal	**P**oor

Totals	> 80	80 - 60	59 - 40	< 40
	Optimal	**S**uboptimal	**M**arginal	**P**oor

Habitat comments: _____

Station _____ Date(s) _____

Sediment deposition may cause the formation of islands, point bars (areas of increased deposition usually at the beginning of a meander that increase in size as the channel is diverted toward the outer bank) or shoals, or result in the filling of runs and pools. Usually deposition is evident in areas that are obstructed by natural or manmade debris and areas where the stream flow decreases, such as bends.

Land use: Indicate the land uses that you believe may be having an impact on your stream station. Use the letters (**S**) streamside, (**M**) within ¼ mile and (**W**) somewhere in the watershed, to indicate the approximate location of the disturbance and the numbers (**1**) slight, (**2**) moderate or (**3**) high, to represent the level of disturbance.

Active Construction			Pastureland			Single-family residences		
Mountaintop mining			Cropland			Sub-urban developments		
Deep mining			Intensive feedlots			Parking lots, strip-malls etc.		
Abandoned mining			Unpaved Roads			Paved Roads		
Logging			Trash dumps			Bridges		
Oil and gas wells			Landfills			Other (describe)		
Recreation (parks, trails etc.)			Industrial areas					

Land use comments: _____ Pipes? Yes No

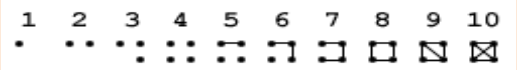
Describe the types of pipes observed and indicate if there is any discharge from the pipes. Also describe the colors and odors of the discharge. _____

Photograph and **sketch your reach**: Use the space below or a separate piece of paper to draw your study reach. Indicate the direction of flow, north, sample locations and important features of the reach. Photographs are an excellent method for tracking changes, especially changes related to the condition of the habitat. Choose a minimum of two permanent locations from which to take your photos. Submit your photos with your survey data sheet.

Benthic macroinvertebrates: Assess your macroinvertebrate collections by counting and identifying to the family-level if possible. Use the table on the **next two pages** to record your collections data.

Note: Although streamside identification is possible, WV Save Our Streams Coordinator recommends preserving your samples using a full count or standard sub-sampling procedure in a well-lit and more comfortable setting.

The dot-dash tally method is a convenient way to record your data. Each dot or dash represents one tally.



Station _____ Date(s) _____

Insect Groups

Patterned stoneflies Taxa ____ Total ____	Winter stoneflies Taxa ____ Total ____	Roach-like stonefly Total ____
Giant stonefly Total ____	Brown stonefly Total ____	Spiny crawler mayfly Total ____
Square-gilled mayfly Total ____	Minnow mayflies Taxa ____ Total ____	Flatheaded mayfly Total ____
Brush-legged mayfly Total ____	Burrowing mayflies Taxa ____ Total ____	Net-spinning caddisflies Taxa ____ Total ____
Case-building caddisflies Taxa ____ Total ____	Free-living caddisfly Total ____	Common netspinner Total ____
Dragonflies Taxa ____ Total ____	Damselflies Taxa ____ Total ____	Riffle beetle Total ____
Long-toed beetle Total ____	Water penny Total ____	Other beetles (true bugs) Taxa ____ Total ____
Hellgrammite/Fishfly Total ____	Alderfly Total ____	Aquatic moth Total ____
Non-biting midge Total ____	Black fly Total ____	Crane fly Total ____
Watersnipe fly Total ____	Dance fly Total ____	Dixid midge Total ____

Station _____ Date(s) _____

Net-wing midge Total	Horse fly Total	Other fly larva Taxa Total

Non-Insect Groups

Crayfish Total	Scud/Sideswimmer Total	Aquatic sowbug Total
Water mite Total	Operculate snails Taxa Total	Non-operculate snails Taxa Total
Pea clam Total	Asian clam Total	Mussel Total
Flatworms Total	Aquatic worms Total	Leeches Total
Other aquatic invertebrates Taxa Total	Comments:	

Total Taxa	Total Number

Describe other aquatic life (e.g. fish, amphibians) collected or observed, as well as other indications that the reach is being used by other animals (i.e. birds, mammals, reptiles).

Discharge

Determine the discharge by using a flow meter (if available) or other methods such as the **float method** or a **velocity head rod** (VHR). Discharge should be measured from a run (area of the channel with fast moving water with no breaks in the surface such as protruding rocks). The more measurements collected the more accurate your discharge results will be. To convert inches into feet divide by 12. For example, if your depth measurement was 6-inches the result in feet would be 0.5. Indicate the methods chosen to measure the discharge and use the tables to record your results. Use the table on the next page to record your measurements.

Discharge method used

Float | Velocity Head Rod | Flow meter

Water Level

Low | Normal | High | Dry

Channel width _____ feet

Use the table on the next page to record your velocity data

Station _____ Date(s) _____

Level-two survey data sheet

Distance (ft)	Depth (ft)	Velocity (ft/sec)	VHR (Rise-inches)	Float (sec)	Discharge (cfs)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Average Depth _____ feet

Cross Sectional Area (CSA) _____ ft^2
(CSA = Average Depth x Width)

Discharge = CSA x Velocity
= _____ x _____
= _____ cfs (ft^3/sec)

If you use a float record your distance below and the number of seconds it took to travel the distance in the column indicated.

Float distance (feet) _____

Use the table below to determine **VHR velocity** from the rises recorded above. The rises below are in inches.

Rise (R)	Velocity	Rise (R)	Velocity
¼	1.2	3 ¼	4.2
½	1.6	3 ½	4.3
¾	2.0	3 ¾	4.5
1	2.3	4	4.6
1 ¼	2.6	4 ¼	4.8
1 ½	2.8	4 ½	4.9
1 ¾	3.1	4 ¾	5.0
2	3.3	5	5.2
2 ¼	3.5	5 ¼	5.3
2 ½	3.7	5 ½	5.4
2 ¾	3.8	5 ¾	5.5
3	4.0	6	5.7

Additional comments: _____

Submit an original or clear copy of your survey to the coordinator at the address provided below.

WV Department of Environmental Protection
Save Our Streams Program
601 57th Street, SE
Charleston, WV 25304

Office: (304) 926-0499 (1040); Mobile: (304) 289-7630
E-mail: timothy.d.craddock@wv.gov
Web page: http://www.dep.wv.gov/sos

Station _____ Date(s) _____

Level-two assessment

The **light blue** shaded boxes indicate that multiple families (**kinds**) are possible; tolerance values are provided.

Macroinvertebrates		Totals	Tolerance score	Number of kinds	Macroinvertebrates		Totals	Tolerance score	Number of kinds
1	Patterned stoneflies				6	Aquatic moth			
2	Winter stoneflies				4	Riffle beetle			
1	Roach-like stonefly				5	Long-toed beetle			
1	Giant stonefly				3	Water penny			
2	Little brown stonefly				5	Whirligig beetle			
3	Spiny crawler mayfly				7	Other beetles/bugs			
5	Square-gilled mayflies				3	Hellgrammite/Fishfly			
4	Minnow mayflies				6	Alderfly			
3	Flatheaded mayfly				8	Non-biting midge			
3	Brush-legged mayfly				6	Black fly			
5	Burrowing mayflies				4	Crane fly			
4	Net-spinning caddisflies				3	Watersnipe fly			
3	Case-building caddisflies				6	Dance fly			
5	Common netspinner				5	Dixid midge			
3	Free-living caddisfly				2	Net-wing midge			
4	Dragonflies				7	Horse fly			
7	Damselflies				7	Other fly larva			
Non-Insect Groups									
5	Crayfish				5	Pea clam			
5	Scud/Sideswimmer				6	Asian clam			
7	Aquatic sowbug				4	Mussel			
6	Water mite				5	Operculate snails			
10	Aquatic worms				7	Non-operculate snails			
10	Leeches				Other invertebrates (**Describe**)				
7	Flatworms								

Complete your calculations using the metrics below. These metrics are combined to determine your overall score and integrity rating.

Total Number	**Total Tolerance**	**Total Kinds**

Comments: _____

Metrics	Results	Points	**8**	**6**	**4**	**2**
1. Total Taxa			> 18	18 - 13	12 - 8	< 8
2. EPT Taxa			> 10	10 - 7	6 - 4	< 4
3. Biotic Index			< 4.0	4.0 – 5.0	5.1 – 6.0	> 6.0
4. % EPT Abundance			> 80	80 - 60	59.9 - 40	< 40
5. % Tolerant			< 2	2 - 10	10.1 - 30	> 30

Stream Score _____

Integrity Rating

> 32	32 - 24	23 - 16	< 16
Optimal	Suboptimal	Marginal	Poor

1. **Total Taxa** is simply the total number of families collected.
2. **EPT Taxa** is the total number of families within the orders of **Ephemeroptera**, **Plecoptera** and **Trichoptera**.
3. **Biotic Index** is calculated by multiplying the organism by its tolerance value to determine a tolerance score. The total tolerance is then divided by the total number of organisms collected.
4. **% EPT Abundance** is calculated by dividing total number of organisms within the orders Ephemeroptera, Plecoptera and Trichoptera by the total number collected. This result is multiplied by 100 to determine the percentage.
5. **% Tolerant** is calculated by dividing the number of tolerant organisms (≥ 7) by the total number collected. This result is multiplied by 100 to determine the percentage.

- The **Stream Score** is the sum of all five point values.

Station _____ Date(s) _____

Stream Name _____ Basin _____

Collection Date _____ Station ID _____ County _____

ANNELIDA	COUNT	TRICHOPTERA	COUNT
BIVALVIA			
GASTROPODA			
		PLECOPTERA	
CRUSTACEA			
MEGALOPTERA			
		ODONATA	
EPHEMEROPTERA			
		COLEOPTERA	
		DIPTERA	
MISCELLANEOUS			
TOTAL			
TOTAL FAMILIES			

ID DATE _____ ID BY: _____ LAT | | | | | LON | | | |