

FIELD PROCEDURE: BENTHIC MACROINVERTEBRATE SAMPLING

Benthic macroinvertebrates are animals without backbones, such as insects, worms, scuds, and mollusks, which live at the bottom of streams. Stream teams collect them once a year, during the regular fall monitoring session, Sept. 15–Oct. 15. Once collected, they are professionally identified and tallied. However, volunteers are welcome to examine the invertebrates before or after preserving them—see last part of this protocol—and can volunteer to help sort samples.

EQUIPMENT NEEDED (items with an * are in the regular bags rather than the “bug bag”):

- 1 complete Surber sampler (square frame with conical net and collection cup)
- 2 buckets, marked “clean” and “dirty”
- 2 500-micron sieves, also “clean” and “dirty”
- 4 rubber dishpans
- strong weeding fork to disturb substrate
- “clean water” decanter with handle
- 2 angled-spout wash bottles (one for water, one for alcohol)
- 2 spray bottles (one water, one alcohol)
- macroinvertebrate handling tools:
 - ◆ plastic spatula
 - ◆ flexible forceps (tweezers)
 - ◆ one rigid forceps
 - ◆ magnifying glasses
 - ◆ lighted magnifier
 - ◆ head magnifier
 - ◆ spoons
 - ◆ eye droppers
 - ◆ paintbrushes
- wide-mouth sample jars with tight lids
- foam holder for sample jars
- Leakproof containers for jars (not supplied; you’d have to bring from home)
- 95% denatured ethanol (*remember to get this from the storage cabinet outside the office before taking the field kit home*)
- electrical tape
- macroinvertebrate ID field keys
- pre-printed labels
- ziplock bags—small and large
- 3 washers with flagging tape attached
- permanent marker
- *reach map or site sketch for each reach
- *100’ tape
- *timepiece with second hand
- *camera
- *rubber gloves
- *tarp

- *data sheet, clipboard, pencil

WHY SAMPLE MACROINVERTEBRATES?

The best way to assess the health of a watershed for living things is to look at those living things. Undisturbed watersheds in the Pacific Northwest contain a marvelous variety of benthic macroinvertebrates, representing a smorgasbord of shapes, sizes, survival strategies, and adaptations. Human activities that interfere with natural processes in a watershed have a definite and predictable impact on the types and numbers of invertebrates that live there.

Many invertebrates are just as sensitive to changes in their environment as salmon. We tend to be more interested in fish than invertebrates, but there are several good reasons to sample invertebrates rather than or in addition to fish:

- It is easier and less intrusive to the environment to sample invertebrates.
- Whereas anadromous fish are impacted by a variety of factors such as ocean conditions and fishing pressure, stream invertebrates are primarily impacted by activities within their watershed.
- Invertebrates have a wide geographic distribution, making it fairly easy to predict which types should be present where.
- Since invertebrates are an important food source for fish (and other wildlife), sampling them measures an environmental component with a direct impact on fish.
- Undisturbed streams have such a great variety of invertebrates that sampling can reveal subtle disturbances over space and time.

NOTE: If possible, take a photo of the team while doing the collection.

HOW DO MACROINVERTEBRATES PROVIDE A “BLOOD TEST” OF A WATERSHED’S HEALTH?

We can assess the biological health of a stream by looking at the types of invertebrates that either thrive or do not thrive in it. If only a few types of invertebrates live there, or if the invertebrates are primarily ones that adapt well to disturbed streams, there is some kind of problem present.

A group of teachers and students at the University of Washington (later spun off as the nonprofit group SalmonWeb) developed a means to sample invertebrates in a uniform way, identify and count them, and then perform calculations to assess stream health. The calculations measure such things as:

- The total variety and balance of life forms present (a.k.a. biodiversity)
- The variety of types of mayflies, stoneflies, and caddisflies—invertebrates that form an important part of the diet of salmon and trout, and that respond in complex ways to different human disturbances
- The variety or number of invertebrates that are known to adapt very well or very poorly to streams that are unnaturally warm, cloudy, or de-oxygenated
- The variety of invertebrates that need clear spaces between rocks or stable habitat for a long time span.

By sampling at many sites, both disturbed and undisturbed, these scientists were able to develop a set of calculations that give a good indication of the biological health of streams and their surrounding watersheds. These metrics are known as the Benthic Index of Biological Integrity (B-IBI) for the Puget Sound Lowlands.

The macroinvertebrate samples you collect will be professionally identified, and the counts will be entered into a database that calculates the B-IBI for each sampled reach. The B-IBI yields a single number on a scale (much like a test score), along with a description of that number, ranging from Healthy to Critically

Impaired (much like a grade on a report card). Thus, the B-IBI enables us to transform information about invertebrate populations into a generalization about stream health. It is a powerful analytical tool.

A wealth of information about the B-IBI is available on the Streamkeepers web site at http://www.clallam.net/streamkeepers/html/biological_monitoring.html.

WHEN TO SAMPLE

In order to provide comparable information, macroinvertebrate sampling must occur between September 15 – October 15 each year. In some circumstances, it may be possible to sample earlier or later, depending on flow and water levels; check with staff. Also, check weather reports beforehand: if riffles are more than a foot deep, you can't see under the water, or conditions are unsafe, don't sample that day—but you might be able to do your regular water-quality monitoring instead. Generally, you're less likely to get rained out earlier in the sampling month, so try to schedule early if possible. If you do sample when water levels are up, avoid sampling a riffle that would have been dry before the rain.

WHERE TO SAMPLE

- Try to sample at least 165' upstream or 660' downstream of a bridge or other large human-made structure (unless you are trying to measure the structure's impacts).
- Find the best riffle (fast, turbulent water moving over gravel or cobble substrate) where the stream is straight and fairly uniform (depth, velocity, and rock size) all across the channel. If there is no riffle anywhere in the vicinity, sample in an area with shallow water and appropriate substrate (see below). The water should be 4-12" deep, though shallower is possible (see below).
- The best substrate would be 2-4" rocks, with smaller pebbles underneath. Do not sample in rocks larger than 12" on the surface-layer or larger than 4" below that.

- Sample midstream and avoid riffle-edges if possible. (If there is only one short, wide riffle available, you can sample across its width, but note that on your data sheet.)
- Find the area with the best overhead canopy and riparian vegetation.
- If possible, avoid sites with filamentous algae on the rocks.

To try to meet the above conditions, you can range fairly widely from your normal water-quality monitoring location, so long as you:

- Stay in an area that has similar gradient, valley shape, and riparian land-cover.
- Don't cross any inputs to or outflows from the stream.
- Document your location (explained below).

Note that in many cases, you are being asked to sample a site because of its disturbances, so there may, for example, be no riffles or only fine sediment. In that case, your job is to find the best sampling spot you can there.

NOTE: If your site is less than ideal or simply not appropriate for collecting a sample, explain the situation in the "Comments" section of your data sheet, and take photos illustrating your points.

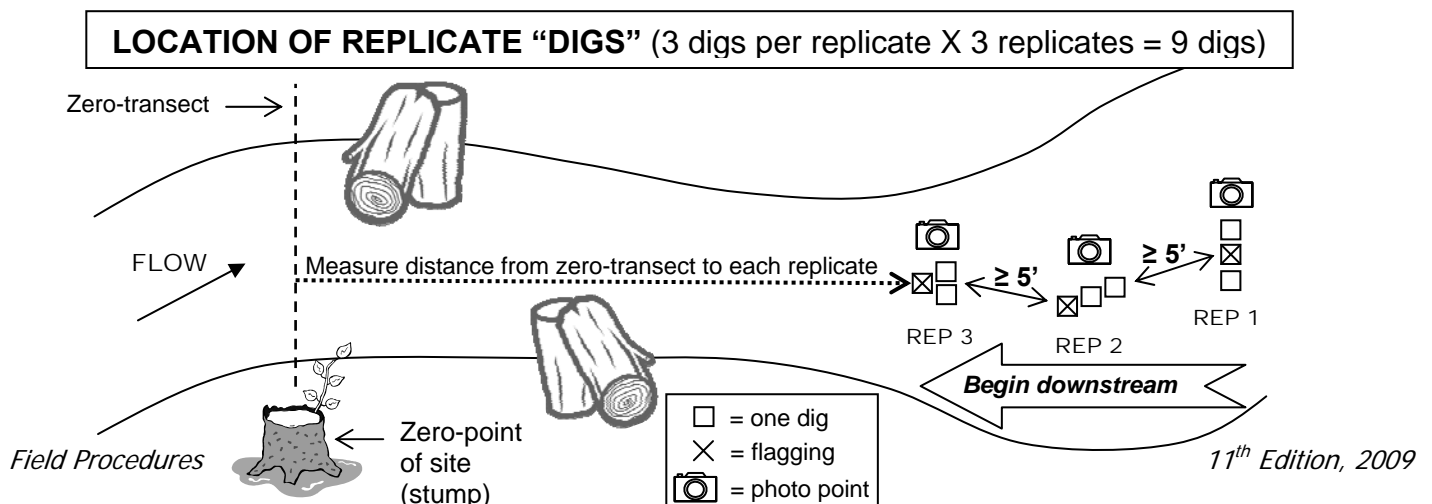
FIELD PROCEDURE:

- 1) **Overview: Beginning downstream and moving upstream**, you will collect **three replicates**, and for **each** replicate, you will dig with the Surber sampler **three** times. Therefore, at each monitoring reach, you will dig **nine times**—see

diagram below. (*This modification of the B-IBI sampling protocol for the Olympic Peninsula comes from Fore, 2001.*) The three placements of the sampler for each replicate should be close together, but the different replicates should each be at least 5' apart. If there is no single riffle large enough, you may need to sample from adjacent riffles or even go outside your regular monitoring reach. However, all 3 replicates must be collected from a single reach with similar characteristics of stream gradient and ravine or valley shape. If you only have a tiny amount of riffle in the entire reach area, you may have to squeeze your replicates closer than 5' apart; note this on your data sheet.

2) **Collect a replicate:**

- a) Avoid disturbing vegetation overhead or upstream of your sampling site.
- b) Inspect the Surber sampler netting and collection cup for tears, holes, or critters. Apply duct tape if needed and note problems on data sheet.
- c) Frame out your Surber sampler, and place it on the selected spot with the opening of the nylon net facing upstream and the collection cup stretched out behind. Hold the frame firmly on the stream bottom. The current should move directly into the net. If the current is very slow or the water very shallow, take extra precautions to make sure the water is moving all the way down the net and into the cup. You may need to



BENTHIC MACROINVERTEBRATE SAMPLING

remove rocks or even excavate a little channel downstream of the frame.

- d) Lift the larger rocks resting within or beneath the frame and, holding them in the water in front of the net, brush off any crawling or loosely attached organisms so that they drift into the net. After “cleaning” the rocks, place them in a dishpan. Once these rocks have been removed, the frame should be squarely on the stream bottom. At this point, note the water depth in inches in the margin of your data sheet, using the marked notches in the Surber’s frame.
- e) Once the larger rocks are removed, disturb the substrate vigorously with the weeding fork for 60 seconds, to a depth of about 4”. (You should dig vigorously enough to be breathing hard). Organisms and detritus should wash into the net. Drag the fork toward the net as you dig, to keep material from going around the side.
- f) If you hit more big rocks while digging, take a “time out” from the 60 seconds to pry them out, wash them in front of the net, and put them in the dishpan. If you can’t pry a big rock out, you’ll have to decide whether to “abort” that dig or not; note your decision on the data sheet.
- g) If you’re sampling on a high-water day, make a special effort to dig as deeply as you can, because critters go down when the water goes up.
- h) At 60 seconds, lift the sampler out of the water, keeping the open end pointing upstream and tilting it up out of the water, to help wash organisms into the collection cup.
- i) ***Without emptying the cup***, repeat the sampling procedure twice more at nearby spots. (Remember to record water depths of the 2nd & 3rd spots.) These three sampling efforts, combined into the collection cup,

constitute a ***single*** replicate.

- j) Mark the area of this replicate’s sampling with one of the flagged washers. Among your three “digs,” mark the spot furthest upstream, and laterally at the middle of the 3 digs (see previous diagram).
- k) Put a little alcohol in a sample collection jar and put the jar in the foam holder, near the dishpan.
- l) Examine the large rocks collected in the dishpan, using a magnifying glass. Using a brush or forceps, gently move any organisms found into the sample jar. After examining each rock, wash it over the pan with “clean” water or spray it with alcohol, then set it on the bank.

TO MAKE “CLEAN” WATER FOR RINSING: Pour it from the “dirty” bucket, through the “dirty” sieve, into the “clean” bucket.

- m) When all rocks have been cleaned, pour the water from the dishpan through the ***clean*** sieve. Rinse the pan, agitate and pour again. This should filter out any invertebrates that washed off of the rocks. Then return the rocks to the stream in the area of the sampling site.

STRINGY ALGAE ON THE ROCKS? Scrape off as much as you can with something sharp and collect it all. You may have to get spaghetti jars from home—don’t fill any jar over halfway.

- n) Meanwhile, the other samplers should attend to the Surber sampler. Wash all objects caught on the inside of the net into the collection cup:
 - ***With the opening out of the water***, rotate the net around in the water so that most of the objects inside wash into the cup.
 - On the bank, finish rinsing the contents of the net into the cup. You may use the “dirty” bucket to pour unfiltered water into the net

- from the outside, or you may pour filtered ("clean") water down the sides of the net from the inside.
- o) When the net is clean, empty the contents of the collection cup into the 2nd dishpan. Clean the neck and collar of the sampler over the dishpan to collect any critters that may remain inside. Examine the net carefully and pick out any remaining invertebrates, then set it aside. Rinse the cup and empty again, continuing until you have emptied it completely. (To rinse, you may pour clean water inside the cup; or you may dip the cup into the stream, holding it upright, and let the stream water filter in through the mesh on the side of the cup.)
- p) **Pick out any bigger, predatory-looking insects** and put them in the sample jar to protect the other ones!
- q) **Pick out non-decayed large debris** (sticks and leaves) from the material in the dishpan. Using a magnifying glass and squirt bottle or tools, pick off any organisms and return them to the dishpan or sample jar before discarding these pieces. Decayed debris should be collected in the sample because it might hide organisms inside (e.g., if a leaf is limp, damaged, or "skeletonized").
- r) Pour some **clean** water into the dishpan and swirl the sample around in it. While the water is still agitated, pour it off into the **clean** sieve. Most of the organic matter should enter the sieve with the water, while the rocks stay at the bottom. Repeat this decanting procedure until the water is completely clear and there are no invertebrates still crawling around in the debris in the dishpan. If you've collected a mat of algae, shake this mat to loosen up any mud stuck to it; the mud will later escape through the sieve.
- s) Put a bit of water back in the dishpan and comb over it with the lighted magnifier (which will get critters to move) and rigid forceps (which will pick them up efficiently). Keep a keen eye out for caddisflies, which in their cases look very much like small rocks, and chironomid tubes, which look like globs of dirt or sand. If you are sure that only rocks and sand are left in the dishpan, discard the contents; if not...

Critters still crawling in the sand? Try an "alcohol float": Decant the water out of the dishpan, then put in enough alcohol to cover. Swirl and see if critters start releasing from the sand particles. If so, decant them into the sieve, catching the alcohol in another dishpan. If there are critters in that waste alcohol too, save that as well, marking it "Thru sieve from alcohol-float." If this doesn't work, collect both sand and critters.

- t) Transfer the remaining contents of the clean sieve into the sample jar, which the rock-pickers should have finished with by now. Your job is to get **everything** that's now in the sieve into the sample jar, a.s.a.p. Get the contents down at one end to make them easier to remove, by agitating the sieve at an angle in shallow clean water in one of the dishpans (like panning for gold). Use gentle forceps, a spatula, and/or a squirt bottle to move the remaining contents of the clean sieve into the sample jar. (One technique is to squirt alcohol from the back, washing the material along with the alcohol into the cup.) Fill the jar no more than halfway with "stream stuff," then fill to near the top with alcohol.
- u) Examine the sieve carefully to make sure there are no critters left; any still hanging onto or wrapped around the mesh can be removed with a bit of alcohol and the rigid forceps.
- v) Use a pencil to fill out one of the pre-

BENTHIC MACROINVERTEBRATE SAMPLING

printed labels, with the date, stream, reach number, replicate number, first initials and last names of samplers. Place inside the jar, ideally so that the writing can be seen from the outside. Close the jar tightly (users of “pop-top” jars pay close attention here!) and wrap the seam several times with electrical tape (if using a screw-cap jar, 3 times will suffice; a “pop-top” jar will need 4-5). Leave ½” at the end to form a pull-tab for the lab technician. On the lid (still using the pencil), write date, site name, and replicate number. (If the material will not fit in one jar, put it into two or more jars, and add “Jar 1 of 2,” etc. to the slips of paper inside the jars and the jar lids.) Place the jar(s) from a single replicate in a single *small* ziplock bag, labeled with the same information as the lid. (Larger bags are included in the field kit in case you need three or more jars to hold one replicate.)

SAMPLE JAR LID:
9/15/2000
Morse 1.1, Rep 1
Jar 1 of 2

3) **Collect two more replicates, following the same procedure as above.**

Remember to keep moving upstream.

4) You now have flagging at each of your 3 replicates. At each replicate location:

- a) Use the measuring tape to measure and record its **direction** (upstream or downstream) **and distance from the zero-transect or reference point of your reach** (to the nearest number of feet) in the appropriate boxes on your data sheet.
- b) Record the following information:
 - i) The average water **depth** of the 3 spots where you dug that replicate, to the nearest number of inches. (Look at the numbers you wrote in the margin of your data sheet.)
 - ii) The **width** and **length** of the riffle you dug in, to the nearest number

of feet.

- c) With the flagging and measuring tape still in place, **photograph** the replicate location as follows:
 - i) If all three reps were taken from the same riffle or riffle sequence, one set of photos will suffice.
 - ii) Reps taken from areas far apart or very different should have separate sets of photos. Use your judgment.
 - iii) A set of photos consists of:
 - The riffle area itself, ideally showing the measuring tape and some of the substrate. (Find the angle where the light gives the best view.)
 - The riparian corridor taken up- and downstream from the sampling area.
- d) Complete the photo log section of your datasheet (see sample below).
- e) Collect your flagging.

Site Name: Peabody 0.5 Date: 9/15/08 Visit ID#: _____ (entered in office)

STREAMKEEPERS OF CLALLAM COUNTY – NEW PHOTO LOG			
Photos taken at this site on this date?			Sampler's Initials:
Yes <input checked="" type="checkbox"/>			<u>JRK</u>
FOR SUMMER & WINTER PHOTOS: AT SITES WITHOUT ESTABLISHED CROSS-SECTION, USE CENTRAL SAMPLING POINT (A.K.A. "ZERO POINT") AS CENTRAL POINT FOR PHOTOS.			
Photo Log: <input type="checkbox"/> fill in the log below completely for each picture including where it was taken from			
Roll#	Photo#	Subject	Vantage Point
96	27	Macroinvert site, Rep 1	directly above
	28	" " " 2	" "
	29	Ren & Robin Schwell emptying the net	
	30	Coleman Byrnes digging Rep 3	
	31	Macroinvert Rep 3 site	directly above
	32	Riparian corridor looking u/s	Rep 2 site
	33	" " " d/s	" "
	34	Overview of sampling riffle	LB 20' d/s
	35	Sue Nattinger deconting	
	36	Lucille Schmitt with head magnifier	
102	1	Salamander caught in sample & released!	

- 5) In the “Sampler’s Initials” box on the data sheet, put all the initials of one sampler taking responsibility for this sample.
- 6) Clean and store the equipment. Make sure the net and sieves are clean—use the brush in the kit to clean them if needed.
- 7) If you would like to examine your samples, you may do so either after preserving them (transferring carefully from jar to dishpan and back again), or **briefly** before preserving them. To do this, empty the sieve contents into a dishpan and add a little clean water. But if you keep them in the tray too long, the big critters might

start eating the little ones, and you'll start losing your sample!