SAVE OUR STREAMS® IZAAK WALTON LEAGUE OF AMERICA

Biological Monitoring Instructions for Stream Monitors

Surveying stream macroinvertebrates provides information about the health of your stream. Many stream-dwelling organisms are sensitive to changes in water quality. Their presence or absence can serve as an indicator of environmental conditions.

Before selecting a site to monitor, please follow these rules:

- Check with state and county agencies to make sure you are not disturbing a survey area used by government agencies (over-monitoring may harm the stream).
- Contact local landowners before monitoring to make sure you are not trespassing.
- Ask for permission if you need to access private land. Most landowners will give permission for your study and may even want to help you conduct your survey.

Monitoring should be conducted at the same station (location) each time you sample during the year. If you want to monitor several stations on your stream, make sure the locations are no closer than one-quarter mile. This means, for example, that if you want to monitor a one-mile segment of a stream, you can have a maximum of four monitoring locations. If the stations are spaced more closely, the monitoring activity may become the main impact on water quality.

Carefully record the location of your monitoring station on your Biological Monitoring Data Form. Include roads, bridges, and significant landmarks. Use your smartphone's GPS functionality to determine your latitude and longitude.

THINGS TO CONSIDER

If you are monitoring more than one station, begin monitoring downstream and move upstream. This will prevent macroinvertebrates disturbed by the first test from washing downstream and being captured in your net a second time. Each survey should record only the organisms present at that particular location and time.

Biological monitoring should be conducted twice per year at each station. The best times to monitor are in the fall (September, October, or the beginning of November) and the spring (April, May, or the beginning of June). Fall and spring are when we see the best macroinvertebrate populations, which ensures that we are documenting an accurate representation of the stream's health. Monitoring during the heat of summer or dead of winter also poses a risk to volunteers, which is why we ask that you refrain from monitoring then.

When scheduling monitoring events, remember that excessive monitoring can become a major threat to stream health because each monitoring event disturbs the streambed and dislodges macroinvertebrates. In general, monitoring stations should have at least two months to recover from a monitoring event. It is crucial to the integrity of your data that you do not over-monitor your stations. There is some flexibility in this rule. For example, if an oil spill occurs, you might want to monitor your stream, even if you have done your two surveys for the year. The data you collect might be the only data available on the immediate impacts of the spill.

The methods described in these instructions are for use in wadable streams. To be wadable, the water level in the stream must not exceed the height of your knees. When planning monitoring sessions for younger people, please keep in mind that knee height varies greatly between adults and children.

There are two sampling methods available to collect aquatic macroinvertebrates. Muddy Bottom Sampling is used in streams that do not have riffles, a streambed feature with cobble-sized stones between 2 to10 inches in diameter where the water bubbles over the rocks. If your stream has riffles, please refer to the Rocky Bottom Sampling section.

MUDDY BOTTOM SAMPLING

The Muddy Bottom Sampling method is intended for volunteers sampling streams that do not have rocky bottoms or riffles. Muddy bottom streams are composed of muddy or sandy substrate, overhanging bank vegetation, and submerged woody and organic debris. This method enables sampling of streams where kick-seining techniques do not yield the best representative sample of macroinvertebrates or allow easy collection from the most productive aquatic habitats.

Monitoring is conducted using an aquatic D-frame or dip net with 1/32-inch mesh and a four-foot pole. The dip net is used to sample a wide variety of habitats and collect many different kinds of organisms.

Before you begin monitoring, familiarize yourself with the four main habitats that can exist along muddy bottom streams: steep banks/vegetated margins, silty bottom with organic matter, woody debris with organic matter, and sand/rock/gravel substrate. Search for these habitats along a 100-yard section of stream at your monitoring station.

The following are simple descriptions of the habitat types and collection techniques for each habitat:

Steep banks/vegetated margins
The area along the bank and the edge of the water body consists of overhanging bank vegetation, plants living along the shoreline, and submerged root mats. Vegetated margins may be home to a diverse assemblage of dragonflies, damselflies, and other organisms. Move the dip net in a bottom-to-surface motion, jabbing at the bank to loosen organisms. Each scoop of the net should cover one foot of submerged area.

Silty bottom with organic matter
Silty substrates with organic matter can be found where the water is slow-moving and where there is overhanging vegetation or other sources of organic matter. The substrates harbor burrowing organisms such as dragonflies or burrowing mayflies. Collect samples by pushing the net upstream with a jabbing motion to dislodge the first few inches of organic layer.

Woody debris with organic matter
Woody debris consists of dead or living trees, roots, limbs, sticks, and other submerged organic matter. It is a very important habitat in slow-moving rivers and streams. The wood traps organic particles that serve as food for the organisms and provides shelter from fish and other predators.

To collect woody debris, approach the area from downstream and hold the net under the section of wood you wish to sample, such as a submerged log. Rub the bottom of the net frame along the surface of the log for a total surface area of one foot. It also is good to dislodge some of the bark, as organisms may be hiding underneath. You can also collect sticks and leaf litter and rub roots attached to submerged logs. Be sure to thoroughly examine any small sticks or leaves you collect with your net before discarding them. There may be caddisflies, stoneflies, riffle beetles, and midges attached to the bark.

Sand/rock/gravel substrate
In slow-moving streams, bottoms are generally composed of only sand or mud because the water is not fast enough to transport large rocks. Sometimes you may find a gravel bar located at a bend in the river. The bottom can be sampled by pushing the net upstream with a jabbing motion to dislodge the first few inches of gravel, sand, or rocks. You may want to gently wash the gravel in a screen-bottom bucket and then discard the gravel into the stream.

If you have large rocks (greater than two inches in diameter), it is important to dislodge any burrowing organisms. To do this, hold the net on the downstream side of the rocks. In a one-square-foot area in front of the net, gently kick up the rocks with your toes or push them free with your fingers. This should dislodge burrowing organisms and allow them to wash into your net.

MUDDY BOTTOM SAMPLING EQUIPMENT

- D-frame aquatic dip net with 1/32-inch mesh
- SOS Biological Monitoring Data Form
- Macroinvertebrate identification resources, including:
  - IWLA: *A Guide to Aquatic Insects and Crustaceans*
  - Voshell: *A Guide to Common Freshwater Invertebrates of North America*
  - Creek Critters app
- Aquatic thermometer
- White plastic tablecloth
- Shallow light-colored pan
- Specimen jars or ice cube trays for sorting organisms
- Magnification – hand lens, microscope, etc.
- Sorting utensils – spoons, tweezers, pipettes, etc.
- Spray bottle full of stream water
- Sieve bucket with 1/32-inch mesh (optional)
- Clipboard (optional)
- Boots, sneakers, close-toed sandals that secure to your feet. Waders may be preferred in cold weather or for additional leg protection when water is cloudy.

To provide for accuracy of collection and comparability of data from one station to another, take a total of 20 scoops from the different habitats. Ideally, you should identify locations for all four habitat types and collect the following number of scoops from each:

- 10 scoops from steep banks/vegetated margins
- 3 scoops from silty bottom with organic matter
- 4 scoops from woody debris with organic matter
- 3 scoops from sand/rock/gravel substrate

If one of the habitat types is not present, divide the number of assigned scoops from that habitat between the other habitat types that are present. For example, if the stream does not have sand/rock/gravel substrate, take one extra scoop from each of the other three habitat types. The most important thing is to have a total of 20 scoops and to make sure all habitat types that are present are represented. The D-frame net is one foot wide, so one scoop equals one square foot being monitored.

After collecting some samples, dump the net into a shallow light-colored pan filled with a few inches of water. Each time you sample, sweep the mesh bottom of the D-frame net back and forth through the water (not allowing water to run over the top of the net) to rinse fine silt from the net. This will avoid a large amount of sediment and silt from collecting in the pan and clouding the water.

Collect organisms from the net or pan and place them in similar groups as you go through the sample. This will make your identification quicker when you are ready to record results on your survey form. Plastic ice cube trays are helpful when sorting the sample. For example, put all organisms with two tails in one section and all organisms with three tails in another section. See the "Identification" section for details on identifying the organisms in your sample.

ROCKY BOTTOM SAMPLING

The Rocky Bottom Sampling method is intended for volunteers sampling streams that have rocky bottoms or riffles. A kick-seine net - a finely meshed net with support poles on each side - is the best tool to use for collecting macroinvertebrates in rocky bottom streams. The League's Rocky Bottom Sampling method recommends using a kick-seine net that is 3-feet square with 1/32-inch mesh. The 1/32-inch mesh net will provide you with a large sample because it captures younger, and therefore smaller, organisms of each species, and some state and local government agencies require use of the 1/32-inch mesh.

Select a riffle that is a shallow, fast-moving area of water with a depth of 3 to 12 inches and cobble-sized stones (2 to 10 inches) or larger. Before taking your sample, record observations about riffle composition on the back of the Biological Monitoring Data Form.

Place the kick-seine net at the downstream edge of the riffle. Kick net should be placed perpendicular to water flow and held at approximately a 45° angle. Be sure that water is flowing through the net, and not over the top. Spread net as widely as possible and allow direct flow of water into the center of the net. Place rocks from within the sample area on the bottom edge of the net to secure it tightly against the streambed so that no organisms escape under the net.

Monitor the streambed for a distance of three feet upstream of the kick-seine and across the width of the net. Firmly and thoroughly rub your hands over all rock surfaces to dislodge any attached insects for 40 seconds. After you have rubbed off any macroinvertebrates, carefully place each large rock outside of your three-foot sampling area. Stir up the bed with your hands and feet until the entire area has been searched. All exposed and detached organisms will be carried into the net. Then, for 20 seconds, use the toe of your shoe to jab the streambed with a shuffling motion, moving towards the net. Disturb the first few inches of sediment to dislodge burrowing organisms.

Before removing the net, rub any rocks that you used to anchor the net to the stream bottom and remove the rocks from the bottom. Firmly grab the bottom of the net so that your sample does not fall from the net, and then remove it from the water with a forward-scooping motion. The idea is to remove the net without allowing any insects to be washed under or off it. Once you've finished taking your sample, carefully return all rocks to the riffle that were removed in the sampling process.

Placing a white plastic tablecloth under the net before sorting the sample will catch any tiny organisms that may crawl through the net. Use a spray bottle to periodically mist your net. The organisms will stop moving as the net dries out. Occasionally wetting the net will cause the organisms to move, making them easier to spot. Watering the net is especially important on hot, dry days.

Place the net on a flat, bright area, out of direct sunlight. Using tweezers or your fingers, separate all the organisms from the net and place them in your collecting container, which should be half full of water from the stream. Sort

ROCKY BOTTOM SAMPLING EQUIPMENT

- 3×3 ft^2 Kick-seine Net (1/32-inch mesh)
- 2 net poles (wooden dowels, 1.25"x48")
- SOS Biological Monitoring Data Form
- Macroinvertebrate identification resources, including:
  - IWLA: *A Guide to Aquatic Insects and Crustaceans*
  - Voshell: *A Guide to Common Freshwater Invertebrates of North America*
  - Creek Critters app
- Aquatic thermometer
- White plastic tablecloth
- Specimen jars or ice cube trays for sorting organisms
- Magnification – hand lens, microscope, etc.
- Sorting utensils – spoons, tweezers, pipettes, etc.
- Spray bottle full of stream water
- Clipboard (optional)
- Boots, sneakers, close-toed sandals that secure to your feet. Waders may be preferred in cold weather or for additional leg protection when water is cloudy.

organisms into similar groups as you separate your sample. This will make your identification quicker when you are ready to record results. Plastic ice cube trays are helpful when sorting the sample. For example, put all organisms with legs in one section and all organisms with no legs in another section. Any organism that moves, even if it looks like a worm, is part of the sample. Look closely, since most aquatic macroinvertebrates are only a fraction of an inch long.

Count the total number of macroinvertebrates to determine if you have collected at least 100 individual macroinvertebrates. If not, complete a second sampling. You should only take a maximum of 2 samples in an attempt to get 100 macroinvertebrates.

IDENTIFICATION

Once organisms are collected through either the Rocky Bottom or Muddy Bottom Sampling methods, they are sorted and identified. We recommend using IWLA's *A Guide to Aquatic Insects and Crustaceans* or Reese Voshell's *A Guide to Common Freshwater Invertebrates of North America*. Links to purchase these resources can be found at *iwla.org/water/resources-for-monitors*. The League's free Creek Critters app provides easy-to-follow instructions to help you identify your macroinvertebrates. Search for it in the Apple Store and Google Play Store.

Izaak Walton League macroinvertebrate guides provide a general overview of the macroinvertebrate types found across the United States. The composition of macroinvertebrate populations varies depending on local geography and geology. Try contacting your local environmental protection agency or universities for more information about local macroinvertebrates. Local experts might be able to share additional field guides that are specifically designed for your area.

Not all organisms in your stream are listed in the guides or included in the SOS biological survey. For instance, macroinvertebrates such as whirligig beetles, water striders, and predaceous diving beetles are not included on the survey sheet. They are surface breathers and do not provide any indication of water quality. If you encounter aquatic macroinvertebrates that are not part of the survey, you may make a note of them in the comments section.

When beginning your identification, ask yourself the following questions:

- How large is the organism?
- Is the body long and slender, round, or curved?
- Does the organism have any tails? How many?
- Does the organism have any antennae?
- Does the organism have legs? How many? Where?
- Is the body smooth and all one section, or is it segmented (two or more distinct sections)?
- Does the organism have any gills (fluffy or plate-like appendages)?
- Where are the gills located? Sides, back, underside, under its legs?
- Does it have pinching jaws like a beetle larvae?
- Are any legs or antennae missing because they were broken off in the net?
- What color is the organism?
- Does the organism swim underwater or remain on the surface?

When using the macroinvertebrate guides, read the descriptions for each organism. Sizes are provided for reference. However, if you catch a young macroinvertebrate that has just hatched and has not yet reached full size, it may be smaller than indicated in the guides. Using magnification tools (hand lenses, microscope, etc.) may ease identification.

During identification of macroinvertebrates, tally/record your results on the biological monitoring form. Once all collected macroinvertebrates have been identified and counted, check off each species in the Biological Monitoring Data Form table and add the total of each species found. Add the number of check marks in each column — Sensitive, Less Sensitive, and Tolerant — and multiply by the index value at the bottom of that column. Add the subtotal for each column to arrive at your total index value, which provides the final water quality rating. Return the organisms to the stream after sampling is completed.

You will notice that neither the number of each macroinvertebrate identified nor the total number of macroinvertebrates found affects the final water quality rating. This is because the stream health survey is based primarily on the diversity, not the numbers, of individual organisms found. However, changes in the numbers of each macroinvertebrate can indicate changes in stream health over time, which is why it is important to monitor a site multiple times.

The Izaak Walton League updates the sensitivity rankings for macroinvertebrates based on the most recent scientific research. To download a copy of the latest data form, please visit *iwla.org/water/resources-for-monitors*.

WATERSHED CONDITIONS

The Biological Monitoring Data Form also includes questions about the land and vegetation surrounding the stream. These questions help characterize the quality of stream habitat and its ability to support a healthy population of stream organisms. The land use information also paints a picture of the stream for other people who might review your data. Guidelines for correctly answering these questions are given below. Record the answers based on the area that is upstream from your monitoring site; generally, you should record the data for the area you can see. For land use information, include uses for one mile upstream from your site or the section of stream you have adopted. If necessary, take a walk or consult a map for this information.

Fish populations: Different fish have different tolerances to pollution. The type of fish present may indicate the type of water quality expected. If you encounter fish but don't recognize the type, write a description of the fish on the data form or take a picture to use for later reference. You can find fish identification charts or experts to help with fish identification at local schools, agencies, libraries, or online.

Barriers to fish movement: The absence of certain fish types may be due to a dam or other large obstacle, not because of water quality. Note on your survey form if the dam is upstream or downstream from your monitoring site and how far away. Waterfalls should only be recorded if they are large enough that a fish could not reasonably jump over them or swim around them. Usually, waterfalls of a few feet or less are not impediments to the upstream movement of fish.

Surface water appearance: You may check more than one of the colors listed but not all of them. Note if strange colors are present throughout the stream or only in one section, such as immediately below a discharge pipe or highway culvert.

Streambed deposit (bottom): Record the overall appearance of the stream bottom. If the streambed does not have any apparent coating, you may note it as "other" and write in "normal."

Odor: Note any unusual odors. Odors may come from natural processes or may indicate potential water quality problems.

Stability of streambed: An unstable streambed can mean that soil is eroding from the bottom of the stream and may indicate water quality problems. When standing in the stream, determine how frequently the bed sinks beneath your feet.

Stream channel shade: Over the course of the day, estimate what percentage of the stream channel is shaded by streamside trees, shrubs, and grasses. Shading helps keep water cool and can be beneficial for aquatic life.

Streambank composition: Remember to look at both sides of the stream's banks. When questions ask for a percentage, use the information for both the left and right bank and combine values. For instance, if one side of the bank is completely bare due to erosion while the other side is well vegetated, you should record the percent of bank coverage as 50 percent.

When recording total percentages of shrubs, grasses, and trees, you should also look at both sides of the bank. However, if one side has artificial structures such as rock riprap or concrete, you will have to account for such ground cover. For instance, if the left side of the bank is not vegetated, you cannot have more than 50 percent of shrubs, grasses, and trees total when those values are added together.

Streambank erosion: Again, look at both sides of the bank to determine the percentage of soil erosion.

Riffle composition: This question refers to the 3x3-foot area of the stream sampled for Rocky Bottom Sampling techniques with a kick-seine net. Do not fill out this question when using the Muddy Bottom sampling technique.

If you used a kick-seine to conduct the Rocky Bottom sampling method, answer this question before you disturb the site. The organisms you collect are most abundant in riffles composed of predominantly cobble-sized stones (more than 70 percent cobbles is a good riffle habitat). Start with the largest rocks first when recording bed composition. If you don't have any boulders (rocks larger than 10 inches), record cobble-sized stones and continue until your percentages equal 100 percent. A typical riffle in a medium-gradient stream might be recorded as 5 percent boulders, 65 percent cobbles, 15 percent gravel, 10 percent sand, and 5 percent silt. Ranges are given on the survey form for the rock sizes. For the smaller rock sizes, remember that silt feels like talcum powder and sand feels gritty. If your riffle had 40 percent silt, 10 percent gravel, and no cobbles, you should either find another station to monitor or switch to the Muddy Bottom sampling method.

LAND USES IN THE WATERSHED

The survey form asks if land use impacts within a one-mile radius of your sampling site are high (H), moderate (M), slight (S), or none (N). Although these questions are somewhat subjective, determining the impact is easy and straightforward.

- Note "H" for a land use if it:
  - Comprises the majority of land in the watershed and is polluting the stream, such as a stream traveling through land that is being strip mined for coal.
  - Has a severe impact on stream quality even though the land use does not utilize a great deal of land, such as a construction site that has caused the stream to be full of silt.
- Note "M" if the land use is definitely contributing to stream degradation but is not the major cause for degradation (or is one of many causes). For example, parking lot runoff and trash from a shopping mall may contribute significantly to stream pollution, but they may not be the only causes of stream degradation.
- Note "S" for a land use if its impacts only slightly pollute the stream. For example, although a farm may be present, good farming practices and conservation measures may mean the pollution impact is negligible.
- Note "N" if the land use is present but causing no pollution.
- If the land use is not present, do not write anything.

Take the time to drive or walk through your watershed before filling out this section to determine if these land uses are present and impacting the stream.

When considering land use as the controlling factor in stream quality, look not just at the area visible from the stream but at all the land draining into the stream – the watershed. If the stream collects water from an intensely developed or agricultural area, do not be surprised if no organisms are found. Should this be the case, consider visiting a forested stream of the same size in the same watershed for sampling comparison. You might be surprised by the different types of organisms you find.

You can identify a pollution source by sampling the stream at quarter-mile intervals upstream from the initial sampling point (where a pollution impact is suspected) until quality improves. The pollution sources should be identified somewhere between the point where degraded conditions were first found and the point where water quality improves.

Comments: Use this space to record observations that are not noted elsewhere on the data form. This may include current and potential future threats to the stream's health.

STREAM PROBLEMS AND THEIR EFFECTS ON STREAM ORGANISMS

1. **Physical Problems** may include excessive sediment from erosion, street runoff, or discharge pipes. Sediment can create poor riffle characteristics, contribute to excessive flooding, reduce flow, change water temperature, and smother aquatic life. The result is usually a reduction in the number of macroinvertebrates in the study area.

2. **Organic Pollution** is from excessive human or livestock wastes or high nutrient enrichment from farm or yard runoff. The result is usually a reduction in the diversity of invertebrates.

3. **Toxic Pollution** includes chemical pollutants such as chlorine, acids, metals, pesticides, and oil. The result is usually a reduction in the number of invertebrates.

MACROINVERTEBRATE COUNT AND WATER QUALITY

Observation	Analysis
• High diversity, high numbers; many sensitive species such as stoneflies, caddisflies, and mayflies	• No problem; good water quality
• High diversity, low numbers	• Possibly due to poor habitat conditions
• Low diversity, high numbers	• Organic pollution (nutrient enrichment) or sedimentation; excessive algae growth from nutrient enrichment
• Low diversity, low numbers; or no bugs found but the stream appears clean	• Toxic pollution (e.g. chlorine, acids, heavy metals, oil, herbicides, insecticides)