

Nature Forward (*formerly* Audubon Naturalist Society)
Data Collection Protocol for Piedmont Streams

A. Preliminary tasks

1. **Reach.** We sample a pre-determined 75-meter reach. We return to sample this during each monitoring period (April, July, October, and optionally in the winter). We sample benthic macroinvertebrates, collect water and air temperature, and conduct habitat surveys. The Habitat Assessment Field Data Sheet is filled out during every monitoring visit. The Spring and Summer Habitat Data Sheets are filled out only during those seasons.

2. **Sampleability.** Before sampling benthic macroinvertebrates, determine if the site can be sampled safely and effectively. Sampling can only be conducted safely if the stream is wadeable. If the depth or current velocity makes wading unsafe, the site should not be sampled. Often after a heavy rain, a site cannot be sampled for 24 hours. In addition, benthic macroinvertebrates may be hard to find in fast-flowing water. To determine whether the site can be sampled safely and well, visit the site, or consult relevant USGS stream gages at <https://waterdata.usgs.gov/md/nwis/rt>.

Although turbidity or darkly stained water should not prohibit benthic macroinvertebrate sampling, if the stream is so turbid that benthic macroinvertebrate habitat cannot be seen at all, do not sample it. Other situations that may preclude sampling include dry streams, impoundments such as those produced by beaver dams that are too deep to sample, construction for stream restoration, and areas where landowner permission is denied. Permits for sampling in Montgomery County parks are attached to the water quality clipboards.

B. Benthic macroinvertebrate sampling protocol

1. Survey the site. Before sampling, survey the site to locate the most productive habitats for benthic macroinvertebrates and to develop a plan for where to sample within the entire 75-meter reach. Sampling should be conducted at a combination of habitats that support the most diverse macroinvertebrate community within a site. The best available habitat is riffles. Other supportive habitats, in order of preference, are root wads, root mats and woody debris, and associated snag habitat; leaf packs; submerged macrophytes (plants growing in the water) and associated substrate. Other less preferred habitats include undercut banks and gravel runs. Note that, among all the habitats listed above, those that are most stable and in moving water are preferred to those that are unstable and in still water. Also try to sample in different microhabitats within habitat types. The most productive habitats should be sampled in proportion to the availability of each habitat type in the site, while ensuring that all potentially productive habitats are represented in the sample.

2. Collect the sample. 20 one-foot-square samples should be collected with a D-net. The material is pooled into white collecting pans. Conveniently, the standard D-net is about one foot wide, allowing for easy estimation of one square foot for each sample.

- Sampling riffles. Choose the one-foot square area to be sampled. Position the D-net immediately downstream of it, making sure the water current is sufficient to carry dislodged material into the net; that the flat side of the net is flush with the stream bottom; and that the skirt is open to allow water to flow through. Select one team member to hold the net and one or more other team members to sample. Take each rock within the one-foot square area, place it inside the net, and carefully and gently rub it on all sides to dislodge all benthic macroinvertebrates from it. As each rock is cleaned, place it outside the sample area. After all rocks in the sample area have been rubbed, carefully massage the streambed under the rocks to a depth of 3” to dislodge any macroinvertebrates that may be there. Draw your hands away from the net as you do this to make sure you do not push sand and silt into the net. If the streambed appears to be littered with glass or other sharp objects, use your feet to do this instead of your hands.

Repeat this process in other parts of the riffle. Samples should be taken in the range of substrate types and velocities found within the riffle to best represent the community of benthic macroinvertebrates living there.

- Sampling root wads. Hold the net downstream of the root wad and place the root wad inside the net. Gently rub the root wad from top to bottom – as if you are combing your fingers through the root tendrils. Be sure to keep the root wad inside the net. Remove the root wad carefully, making sure the contents of the net do not spill out. Alternatively, use the D-net in a jabbing or sweeping motion to dislodge organisms, and then place it downstream to catch them as they drift in the current.
- Sampling logs and snags. These should be rubbed by hand or with a small brush. Position the D-net just downstream of the log or snag so that the stream current carries the dislodged macroinvertebrates into the net as the log or snag is rubbed.
- Sampling leaf packs. Place the leaf pack inside the net and rub the leaves together to dislodge macroinvertebrates.
- Sampling macrophytes. Hold the D-net just downstream of the area to be sampled and run your fingers through the plants.
- Sampling undercut banks (not preferred habitat; if you sample a rootwad in an undercut bank, count it as a rootwad). Use the D-net in a jabbing or sweeping motion to dislodge organisms. Kicking the habitat prior to jabbing may also be done as needed. In soft substrates the net motion should be gentler to minimize the collection of detritus. In all cases the D-net should be placed downstream of the sampled substrate following jabbing and sweeping to make sure that dislodged organisms are carried into the net.

3. Prepare the sample for sorting. You may empty the contents of the net into a white pan after collecting each sample or wait until after collecting a few samples, depending on how full the net is and whether the macroinvertebrates are likely to escape.

Before emptying the collected materials into the pan, pour stream water through the net and its contents until the water runs clear. You may need to do this many times. This is an important step to make sorting easier. When the water is murky, macroinvertebrates are very hard to find!

Empty the contents of the net into the white pans; then invert the net and wash it with stream water, letting the water run through the net into the collection pan to dislodge any macroinvertebrates that may have attached to the net. Check the net for macroinvertebrates that may still be attached; remove them gently and place them in the collection pan.

4. Sort the sample. Inspect all rocks, leaves, and sticks for macroinvertebrates that might be attached. Swish these materials around in the water to dislodge any hangers-on and place them in a separate pan of stream water in case some have succeeded in avoiding detection. (Their lives depend on hiding, and they are very good at it!) After a while, check to see if any macroinvertebrates have emerged.

Remove all macroinvertebrates from the collecting pans and place them at random into cells of the sorter box. Do not put all like types together. If the sorter box is out-of-reach, sort them into a container of water and later add them at random to the sorter box. Place all predators in separate containers so that they do not eat others in the sample. You may use ice cube trays for this so long as the macroinvertebrates do not try to escape. You may also have to place wanderers like crane flies and larger macroinvertebrates like crayfish in separate larger containers.

5. Identify the sample. Identify a subset of 100 organisms by subsampling using a sorter box. Record your findings on the benthic taxonomic identification sheet.

The sorter box contains numbered cells from 1-27. To determine the order in which cells are chosen for counting, create a randomized list by using an online random sequence generator, such as the one at www.random.org, or by choosing numbers out of a bag or a hat. This will be the order in which you count the macroinvertebrates. To include in your count predators, wanderers, and larger organisms that are being held outside the sorter box, choose a number from 1-27 for each of them. Make a note next to that number on your list to include them when that cell is counted.

Follow the order of your randomized list to count the macroinvertebrates. Identify all macroinvertebrates within a given cell before going on to the next cell. Put macroinvertebrates that have been counted in a separate container to go back to the stream. When you reach 100 organisms, finish identifying all macroinvertebrates in that cell, but then stop. Do not count the macroinvertebrates in the other cells, but check the uncounted cells to see if there are any families that have not been counted. If so, add a count of 1 for each of these families. Record your findings on the benthic taxonomic identification sheet and note that you subsampled.

C. Collecting air and water temperature

1. To take the ambient air temperature:
 - a) Hold or hang the thermometer in a shaded area approximately 3 feet above the ground.
 - b) Wait at least 3 minutes before reading the thermometer.
 - c) Record the air temperature and time of day on the data collection sheet

2. To take the water temperature:
 - a) Place the thermometer in a pint jar and lodge it between some rocks in the stream, making sure that the opening is pointing upstream and the jar is filled with water.
 - b) Wait at least 3 minutes. Remove the water-filled jar from the stream, keeping the bulb of the thermometer in the water, and read the temperature as quickly as possible.
 - c) Repeat two more times in different areas of the reach, then record the average of all three temperatures on the data collection sheet.

D. Collecting pH

Use the pH test kit from the equipment closet. Rinse the tube first with stream water. Follow the instructions accompanying the pH kit and record the reading on the data cover sheet. Dispose of the solution on land away from the stream, and then rinse.

E. Habitat assessment forms

Fill out the appropriate habitat assessment forms for the time of year. Instructions are provided for the Spring and Summer Habitat Data Sheets. The 10-question Habitat Assessment Field Data Sheet is filled out during every monitoring visit.

E. Monitoring equipment

- ___ data collection sheets
- ___ clipboard
- ___ 2 D-nets
- ___ 4 collecting pans
- ___ several plastic cups/bowls
- ___ 1 pint jar
- ___ 1 thermometer
- ___ pH kit
- ___ 2 field microscopes
- ___ 2 forceps
- ___ pipettes (1/team member)
- ___ plastic spoons (1/team member)
- ___ 2 pencils
- ___ 1 vial of alcohol
- ___ Sorter kit with subsampling box and numbers
- ___ 4 petri or “scope” dishes
- ___ 1 hand lens
- ___ ice cube trays (optional; use for isolating predators during counting)
- ___ yellow rope (for measuring in Spring and Summer monitoring)

**Thank you for returning the equipment and your data collection sheets as soon as possible!
There are not enough sets of equipment for every team**