National Aquatic Monitoring Center (NAMC): Protocol for the Collection of Aquatic Macroinvertebrate Samples

Overview

The described sampling protocol was designed to generate data sufficient to characterize the condition and trend of aquatic macroinvertebrate assemblages including quantifying the effects of anthropogenic disturbances and/or restoration actions. This protocol is only applicable to ***wadeable, perennial streams***; individuals seeking to sample intermittent, wetland, spring, or other systems should consult the NAMC Director.

This protocol is compatible with most aquatic macroinvertebrate monitoring programs targeting wadeable streams throughout the Western United States (e.g., State environmental quality programs [OR, WA, UT, CO], USDA Forest Service Aquatic and Riparian Effectiveness Monitoring Program [AREMP], US EPA's National Rivers and Streams Assessment [NRSA]), thus slight variations to the quantitative protocol are permissible.

Sampling Procedure	**Recommendation**
When to sample	July 1st – October 15th; Earlier for extremely arid regions (May 1st)
Sampler type	Surber, kick net (D-frame), or Hess sampler
Mesh size	500 µm
Sample reach length	40 × wetted width or 20 x bankfull (minimum of 150 m)
Target habitat	Fast-water (riffle) habitats
Number of fixed area samples to composite	Total of eight 1 ft^2 (8 ft^2) fixed-area samples: • Riffle/fast-water: one to two (1 ft^2) samples collected from 4 to 8 different fast-water habitats • No riffle/fast-water habitats: one (1 ft^2) sample collected from eleven evenly spaced transects
Placement of sampling device	All sampling locations must be randomly determined to avoid bias: • Riffle/fast-water: generate eight random four digit numbers between 0 and 9999. The first two numbers represent the percent upstream along the habitat unit's length. The second two numbers represent the percent of the stream's width from the left bank. Sample where the length and width intersect. • Transect approach (no riffle/fast-water habitats): Take one sample (1 ft^2) from each of eleven transects. Collect samples alternately from the left quarter, center, and right quarter of each transect, with the first location randomly selected.
Collecting the sample	At each of the eight or eleven sample locations, orient the mouth of the sampler into (perpendicular) the flow. Collect invertebrates from within the area delineated by the net frame. If using a kick net, carefully delineate the 1 ft^2 sample area. Thoroughly wash all rocks, fine sediment, and organic debris to

	a depth of ~ 10 cm.
Field processing	Place all contents in a bucket filled with water and decant invertebrates and organic matter into a 500 µm sieve or net (optional step). Repeat this process until only sand and gravel remains in the bucket. Inspect the remaining gravel for cased caddisflies, snails, or other invertebrates.
Sample preservation	75-95% ethanol; 3:1 preservative to sample by volume.
Sample submission	http://www.usu.edu/buglab/SampleProcessing/sendSamples.cfm

Field Equipment (*required)
0.09 m² Surber, kick net, or Hess sampler with 500 µm mesh net*
Buckets (2), plastic, 8-10 qt. capacity
Sieve with 500 µm mesh
Squirt bottle (2), 0.5 – 1 L capacity*
White plastic wash tub*
500 mL HDPE plastic with screw caps sample jars*
Small spatula, scoop or spoon to transfer sample
Forceps
Funnel with large bore spout
95% ethanol*
Rubber gloves
Cooler
Labeling materials*
GPS

Sample Design
This protocol focuses exclusively on field procedures for benthic macroinvertebrate collection and does not explicitly address study design or data analysis. In general, stream reaches for benthic macroinvertebrate monitoring are selected using either a targeted or a probabilistic design depending on monitoring objectives and associated scope of inference. Sites selected using a targeted design generate data that is relevant for measuring impacts from a known source or answering other *site specific questions*. Sites selected using a probabilistic design provide information on the *overall condition or trend of the watershed, basin, or region*. The number of sampling locations and sampling frequency are two other important considerations that should be directly tied to explicit monitoring objectives. The described protocol applies to both targeted and probabilistic sampling designs.

Recommended sampling timeframe
Macroinvertebrate sampling should be conducted between July 1st and October 15th, although sampling in the southern, arid regions of CO, UT, NV, AZ, NM, and CA may begin as early as May 1st. This sampling timeframe is designed to minimize sampling variability related to season and macroinvertebrate phenology (i.e., developmental timing). Furthermore, this time period is considered optimal because the stream benthos has stabilized following spring runoff events, many macroinvertebrates have attained body sizes that can be readily identified, and macroinvertebrate species richness is generally maximized.

Type of Sampler

A variety of samplers exist for collecting quantitative, benthic macroinvertebrate samples. The two most important considerations for choosing a sampler are the ability to collect a fixed-area sample (i.e., standardize area of stream bottom to be sampled) and mesh size. Both Surber (1 ft x 1 ft / 0.30 x 0.30 m) and Hess (0.6 ft / 0.18 m radius) samplers are optimal because the net's metal frame delineates and standardizes the area to be sampled. In contrast, D-frame or kick nets require the user to carefully standardize the area to be sampled; depending on the area of the kick net, this will be either 1 ft^2 (0.093 m^2) or 1ft x 2ft (0.6 x 0.61). While Hess and kick nets are all compatible with this protocol, the use of a Surber sampler is recommended.

Mesh size refers to the size of the openings in the net of the sampling device. A 500 µm mesh size is recommended herein, regardless of sampler type, as this mesh size is consistently used by a majority of state and federal biological assessment programs. If a sieve is used to clean samples, ensure the sieve mesh sizes matches that of the sampler.

Sample Reach Length

Sample reaches need to be long enough to incorporate local habitat-scale variation and thus represent average conditions. In meandering alluvial channels, pool-riffle sequences generally alternate every 5 – 6 times bankfull width. Therefore, to incorporate multiple riffle habitat units sample reaches should be scaled in proportion to stream size, with the sample reach encompassing forty (40) times the low flow wetted width or twenty times bankdull width and a minimum reach length of 500 ft (150 m). Note that other monitoring parameters such as physical habitat or fish assemblages may influence the length of the sample reach. At a minimum, four (4) different fast-water habitats, if present, should be sampled.

Target sampling habitats

Macroinvertebrate samples should be collected from fast-water habitats (i.e., riffles), if available. Riffles are characterized by relatively fast currents, moderate to shallow depth, cobble/gravel substrates, and generally have the most diverse macroinvertebrate assemblages. Furthermore, standardizing sampling to a fixed habitat type simplifies sampling methodologies and facilitates comparisons among sites. See instructions below for sampling reaches with no fast-water habitats.

Compositing and number of samples to composite

Due to the patchy distribution of benthic macroinvertebrates, multiple samples should be collected and composited into a single sample. Specifically, sampling a minimum of eight (8) replicates (total of 8 ft^2 [0.74 m^2] if using a Surber sampler) is recommended. Given the sampling area of the samplers described above and the number of samples needed to adequately characterize benthic heterogeneity, macroinvertebrate samples should be taken from either:

- *4 different fast-water habitats:* Two separate 1 ft^2 fixed-area samples taken from each of 4 fast-water units for a total of 8 samples (8 ft^2 of stream bottom sampled); or
- *8 different fast-water habitats:* One (1 ft^2) fixed-area sample taken from 8 fast-water habitats for a total of 8 samples.

If no fast-water habitats are present, locate eleven equally spaced transects (perpendicular to the thalweg) along the study reach. Take one 1 ft^2 sample from each of the eight transects. Samples

should be alternately taken from the left quarter, center, and right quarter of each transect, with the first location being randomly selected. For example, if the first sample was collected from the middle of the first transect, subsequent samples would be collected from the right quarter of the second transect, the left quarter of third transect, the middle of the fourth transect and so on.

Placement of sampling device

Once the target stream reach has been delineated and the riffle/fast-water habitats identified, the location of the eight individual samples needs to be determined. To avoid bias, individual samples should be located such that each square foot of riffle habitat has an equal probability of being selected. Net placement is most easily determined by generating eight (8) random four digit numbers between 0 and 9999. The first two numbers represents the percent upstream along the habitat unit's length. The second two numbers represents the percent of the stream's width from the left bank. Take samples where the length and width distances intersect (estimate by eye). If it is not possible to take a sample at the locations (e.g., log in the way, too deep, etc.), draw additional random numbers until you can. Be sure to first sort the eight, four digit numbers from lowest to highest to facilitate working from downstream to upstream.

Collecting the sample

At each of the eight randomly located sample locations, place the sampler so the mouth of the net is facing into and perpendicular to the flow. Collect invertebrates from the area delineated by the net frame, if using a kick net, carefully delineate the $1ft^2$ or $2ft^2$ area to be sampled. Work from the upstream edge of the sampling plot backward and carefully pick up and rub stones directly in front of the net to remove attached animals. Quickly inspect each stone to make sure you have dislodged everything and then set it aside. If a rock is lodged in the stream bottom, rub it a few times concentrating on any cracks or indentations. After removing all large stones, disturb small substrates (i.e., sand or gravel) to a depth of about 10 cm by raking and stirring with your hands. Continue this process until you can see no additional animals or organic matter being washed into the net. After completing the sample, hold the net vertically, with the mouth up, and rinse material to the bottom of the net. If a substantial amount of material is in the net, empty the net into the bucket for processing before continuing to the next sample location. Otherwise, move to the next sample location and repeat the above procedure.

Field processing

After taking the eight samples, empty the net contents into a plastic bucket. Add water to the bucket with the sample, swirl, and decant invertebrates and organic matter from the sample by mixing the contents of the bucket with your hand and then pouring suspended material back into the Surber net or a 500 μm sieve (optional step). Repeat this process until no additional material can be decanted (i.e., only sand and gravel is left in the bucket). Transfer the material in the net (invertebrates and organic matter) into the sample jar and then wash any remaining material in the sieve into the jar with a squirt bottle. Inspect the gravel on the bottom of the bucket for any cased caddisflies, snails, or other animals that might remain. Remove any remaining animals by hand and place in the sample jar.

Once the sample has been processed, add 95% ethanol to fill the jar or vial. Be careful not to add too much organic matter relative to ethanol – recommended dilution is three (3) parts of preservative to one (1) part sample by volume. Immediately label the jars BOTH INSIDE AND

OUT with the date, stream name, county, and state. Write the label for the outside of the jar on tape and affix it to the lid of the jar.

Submitting samples to NAMC

See the NAMC website, sample processing section for details regarding sample submission. (http://www.usu.edu/buglab/SampleProcessing/sendSamples.cfm)

At a minimum, the following information must be included for each unique sample:

- Station Name
- Collection Date
- State
- County
- Latitude and Longitude
- Sample device and mesh size
- Total area sampled