

Standard Operating Procedure 3: Sampling Site Establishment and Benthic Macroinvertebrate Sample Collection (Version 1.0)

Summary

This SOP describes procedures for benthic macroinvertebrate (BMI) sampling reach delineation and sample collection. These procedures have been slightly modified from protocols developed for the USGS National Water-Quality Assessment Program (Moulton et al., 2002). The primary difference between this SOP and USGS methods is that ERMN uses ethanol, instead of formalin, as a preservative because it poses lower human and environmental health risk. Otherwise, this SOP is a streamlined version of the USGS approach – methods (e.g., fine sediment sampling) that are described in the USGS protocol that are not used by ERMN are not included in this document.

Revision Log

Original Version #	Date of Revision	Revised By	Changes	Justification	New Version #
NA	2/23/2016	C. Tzilkowski	NA	Protocol Implementation Plan publication	1.0

Field Operations Overview

Prior to each sampling season, typically in February, sampling equipment and supplies (Table 1) should be inventoried so that enough time is allowed for the Aquatic Ecologist (AE) to order replacements.

Table 1. Benthic macroinvertebrate (BMI) sampling equipment and supplies.

Sample collection supplies
Slack sampler with 500- μ m mesh, PVC template, BMI Sampling Forms, Habitat Assessment Forms, White plastic buckets (19 L), Wire mesh sieves (20 cm and 30 cm diameter, 500 μ m mesh), Knives (pocket and putty), Scissors, White plastic trays, Hand rakes, Squirt bottles, Wide-mouth sample jars (500 mL and 1 L) with waterproof labels on inside and outside, Clipboards, Waterproof pens, Pencils, Waterproof paper, Field data sheets (including extra sheets) on waterproof paper
Electronics and accessories
Global positioning system unit with preloaded sampling location waypoints, Digital camera, Calibrated current velocity meter, Eureka Sub 2 (or similar) handheld meter to measure core water quality parameters, Batteries (various sizes), Cellular phone and charger, Radio tuned to appropriate park frequencies, LED headlamp and flashlight (per person), "spot" beacon
Miscellaneous Supplies
Safety SOP/emergency phone numbers, Stocked first aid kit, Backpacks (2), Wader repair kit, Ruler and tape measure, Braided 1.9-cm nylon rope, Tool set, Paper towels, Heavy-duty nylon thread/sewing needles, Handsaw or lopping shears, Re-sealable plastic bags (various sizes), Plastic trash bags, Duct tape
Personal supplies (per person and extra pair)
Studded wading boots, breathable chest waders, Rain gear, Polarized sunglasses, Shoulder-length PVC gloves
Liquids
95% ethanol, distilled/deionized water, calibration standard solutions for calibrating specific conductance (10, 100, and 1000 μ S/cm) and pH (4.0, 7.0, and 10.0), 10% GS256 disinfectant solution, Insect repellent, Sun screen, Antibiotic liquid soap, drinking water

Sampling During or After Rain Events

Although sampling cannot be restricted to only baseflow conditions, sampling during high flow events should be avoided. High stream flows can be unsafe; moreover, biological (and chemical) conditions during these episodes are often quite different from those during baseflow. Collecting erroneous data due to incomparable physical conditions among years will be counterproductive by introducing uncertainty into long-term datasets. Decisions regarding whether streams should be sampled must be made based on professional judgment; however, monitoring weather forecasts and USGS stream flow gages can greatly inform when optimal sampling conditions are available. A general rule is that if a reach appears unduly influenced (e.g., greater than typical turbidity) by a recent storm event, do not sample the reach that day.

Sample Reach Establishment

Benthic macroinvertebrate (BMI) sampling is conducted by a two-person field crew (Table 2). Typically a maximum of five reaches can be sampled per day depending on driving and hiking distance between reaches. Once a reach has been established or located, sampling activities are typically conducted by the AE and HT in the following order:

- 1) Prepare Macroinvertebrate Sample Field Data Sheet (SOP1), organize sampling gear, and measure core water quality parameters (SOP 5),
- 2) Collect five discrete BMI samples in riffles throughout reach and record microhabitat characteristics at each sampling location,
- 3) Composite and elutriate BMI sample,
- 4) Complete Rapid Habitat Assessment datasheet (SOP 6),
- 5) Preserve BMI sample,
- 6) Decontaminate sampling gear (SOP 7), and
- 7) While near the upstream and downstream reach bounds, take digital photographs of both reach ends (SOP 4).

Be sure to completely and legibly fill out the Macroinvertebrate Sample Field Data Sheet and Rapid Habitat Assessment and take detailed notes that will help interpret monitoring results and provide consistency among sampling events. Notes should include descriptions of where samples were taken throughout the reach, observations of aquatic or semi-aquatic vertebrates, and reach characteristics, particularly if reach conditions are considerably different from previous years.

Table 2. General work flow and estimated time requirements for benthic macroinvertebrate sampling activities at each sampling reach. AE = Aquatic Ecologist; HT = Hydrologic Technician.

Activity	Personnel Responsible	Estimated Time Required
Navigate to and survey the sampling reach for five optimal BMI sampling locations.	AE, HT	0.50 hr
Core water quality parameter measurements	HT	
Sampling equipment preparation, taking photos at up- and downstream ends of sampling reaches, and recording of sampling notes	AE	0.25 hr
Benthic macroinvertebrate sampling and microhabitat measurements	AE, HT	1.00 hr
Elutriating and processing benthic macroinvertebrate sample	AE	
Reach-scale rapid habitat assessment	HT	0.25 hr
Take reach photos/collection of Global Positioning waypoints	AE	0.25 hr
Sample preservation and packing, equipment decontamination	AE, HT	0.25 hr
Total		2.50 hr

An effort has been made to eliminate non-sampleable (i.e., intermittent) streams from BMI monitoring; however, environmental conditions may occasionally prevent sampling. Reaches will be classified as “sampleable” and “unsampleable.” After the reach has been classified as sampleable or non-sampleable, it will be further defined as: Dry, No Flow, Low, Normal, Above Normal, or Flood. Flow will be evaluated visually using Water Resource Division descriptions (Table 3) and because BMI sampling during March and April and not conducted immediately following heavy or prolonged precipitation events, flow is almost always characterized as “Normal”.

Table 3. Flow level descriptions as developed by the Water Resource Division (WRD).

Flow Level	Description as defined by WRD
Dry	No visible water in stream (typical of dry period for an ephemeral/intermittent stream)
No Flow	Discrete pools of water with no apparent connecting flow (at surface)
Low	Stream flow within roughly 10% to 20% of base flow condition
Normal	When stream flow is considered normal (greatest time that stream is characterized by this in terms of flow quantity, level, or general range of flow during a falling or rising hydroperiod, but above base flow).
Above Normal	Bank full flow or approaching bank full (generally within upper 20% of bank full conditions)
Flood	Flow extends outside normal bank full conditions or spreads across floodplain

Sample Collection

Benthic macroinvertebrate sampling conducted by the ERMN is based on methods developed by Moulton et al. (2002) and is termed 'semi-quantitative richest-targeted habitat (RTH) sampling'. A RTH is a habitat (usually a riffle) where the faunistically richest BMI communities typically occur. Riffles are considered the optimal RTH and, as intended by the ERMN sampling design, are available at all sampling reaches; consequently, procedures for sampling riffles are included in this protocol. Moulton et al. (2002) describe a method for sampling woody snags, and should woody snag sampling (or other habitat) become necessary (e.g., if reaches without riffles need to be sampled), the methods described by Moulton et al. (2002) for sampling that habitat type will be followed.

The riffle sample consists of discrete collections (i.e., five Slack samples) that are processed and combined into a single composited sample. Generally, BMI diversity and abundance increase with increasing particle size and then decline with particle sizes greater than the size of cobble; therefore, cobble is considered the optimal substrate (Table 4) for collecting BMI samples in the ERMN. The optimal RTH type in ERMN reaches is 'natural bed' in 'riffles' found in the 'main channel'.

The five discrete BMI collections are made at each sampling site as follows:

1. Select riffles throughout the reach from which five discrete collections can be taken.
 - a. All sampling locations should be as similar as possible with respect to dominant substrate type (typically cobble, Table 4), current velocity, depth, and debris accumulation.
 - b. Avoid areas adjacent to other instream habitats (e.g., woody snags), that are directly below obstructions, or are normally outside of the main channel.
 - c. Collect samples beginning at the downstream end of the reach and proceed upstream.
 - d. Be sure to sample riffles throughout the reach when possible. Walk the length of the reach along the bank to survey where optimal riffle sampling locations are located. If riffles are not available throughout the reach, spread the samples as far from each other as possible.

Table 4. Median diameter and descriptions of substrate classes. Based on USEPA (2007).

Substrate	Size Class Size Range (mm) Description
Bedrock (Smooth)	>4000 - Smooth surface rock bigger than a car
Bedrock (Rough)	>4000 - Rough surface rock bigger than a car
Hardpan	>4000 - Firm, consolidated fine substrate
Boulders (large)	>1000 to 4000 - Yard/meter stick to car size
Boulders (small)	>250 to 1000 - Basketball to yard/meter stick size
Cobbles	>64 to 250 - Tennis ball to basketball size
Gravel (Coarse)	>16 to 64 - Marble to tennis ball size
Gravel (Fine)	> 2 to 16 - Ladybug to marble size
Sand	>0.06 to 2 - Smaller than ladybug size - gritty between fingers
Fines	≤0.06 - Silt Clay Muck (not gritty between fingers)
Wood	Regardless of Size - Wood & other organic particles
Concrete,	Regardless of size - Record size class in comment field
Other,	Regardless of Size - Metal, tires, car bodies etc. (describe in comments)

2. Make each discrete collection by positioning the Slack sampler perpendicular to the stream flow direction, directly downstream of the template (Figure 1).
 - a. The five combined collections yield a total sampled area of 1.25 m².
 - b. Be careful to not walk or stand in front of the net. Otherwise, organisms and material from outside of the template could drift into the net.
 - c. Prior to disturbing substrate within the area template, measure: 1) water depth and velocity and 2) dominant and codominant substrate types, for each discrete collection. Record this information on the Macroinvertebrate Sample Field Data Sheet.
 - d. Press the net tightly against the stream bottom and ensure that water depth does not exceed the height of the sampler frame.
 - e. Carefully discard large debris (e.g., large cobble, wood) from the sampling area if <50 % of its surface area is within the sampling area.
3. Inspect substrate/debris within the sampling template for attached BMI.
 - f. Gently swirl large substrate (e.g., cobble) in the stream directly upstream and near the middle of the net, which should dislodge most BMI; subsequently, gently hand scrub or hand pick attached BMI from the debris directly upstream of the Slack sampler opening. Typically, one crewmember holds the net while scrubbing substrate with their free hand whereas the other crewmember scrubs larger substrate and debris with both hands.
4. After medium and large debris (i.e., larger than small cobble) have been removed from the sampling template, disturb the sampling area by digging to a depth of approximately 10 cm (or as deeply as practical) with a hand rake.

- g. Minimize collecting excessive amounts of sand and pebbles by raking in an upstream direction, which allows most of the inorganic (i.e., mineral) material to settle out into the sampling area and not into the net.
 - h. Begin raking at one side of the area template and make two passes across the template in a downstream-to-upstream direction.
 - i. Avoid disturbing the substrate by kicking because it often results in extreme damage to organisms, forces excessive debris into the collection net, and causes areas outside of the sampling area to be disturbed.
- 5. Retrieve the net by lifting it out of the water with a slight forward motion to ensure that none of the sample is lost.
- 6. Inspect the net for large, typically predaceous organisms (e.g., crayfish, hellgrammites) and place them directly into the composite sample jar.
- 7. Rinse the collected material into the cod-end of the net by splashing water onto the outside of the net.
- 8. Sequentially transfer each discrete collection into one 19 L (5 gal) white plastic bucket.
 - j. Inspect the inside (not outside) of the collection net for BMI and place any organisms found into the bucket.
 - k. Inspect the plastic bucket for large predaceous organisms after each discrete collection is transferred; place these organisms directly into a composite sample jar.



Figure 1. Correct positioning of the Slack Sampler and 0.25 m² area template relative to stream flow direction.

Sample Processing Procedures in the Field

Processing samples in the field can greatly improve the quality and consistency of macroinvertebrate samples for two reasons: 1) it reduces the sample volume so that less preservative is required to preserve BMI and 2) it minimizes the quantity of hard, heavy, and abrasive materials (e.g., gravel) that can damage fragile BMI during transport.

After collecting five discrete BMI collections and compositing them into one sample, process them as follows:

1. Remove and discard large debris (e.g. cobbles, twigs) from the composited sample after carefully inspecting it for attached BMI, especially small, cryptic BMI (e.g. microcaddisflies).
 - a. If necessary, large quantities of large debris (e.g. deciduous leaves) can be removed from the sample by using a dual bucket sieving and washing technique. Fill a 19 L (5 gal) bucket two-thirds full of water. Add large debris from the sample to the dunking bucket while it is inside the 19 L (5 gal) bucket. Carefully move the dunking bucket up and down to sieve the fine organic debris and BMI, leaving behind the rinsed large debris. Carefully pour the contents of the large bucket into a 500 μm sieve and discard the large debris retained by the dunking bucket after it has been inspected for BMI.
2. Remove BMI from filamentous algae and moss by gently agitating the algae or moss clumps for several seconds in a bucket filled approximately one-third full with water.
 - a. Periodically inspect the clumps to determine if more agitation is necessary, and when completed, pour the organisms remaining in the bucket onto the 500 μm sieve after elutriation.
3. Gently stir sample contents (with at least $\frac{1}{2}$ of the 5 gal bucket full of water) by hand to suspend as much of the sample material as possible.
4. While the sample material is still suspended, carefully decant it onto a 500 μm mesh sieve.
5. As decanting proceeds, watch the advancing inorganic sediment front to avoid pouring it onto the sieve. Stop decanting when the inorganic sediment front reaches near the lip of the bucket.
6. Repeat elutriation three to five times until the inorganic debris is free of detritus and organisms.
 - a. Heavy organisms (e.g. mollusks, case-building caddisflies) may settle too fast during decantation and will not be able to be elutriated. Inspect the elutriate sample component remaining in the bucket for BMI (Figure 2), particularly heavy organisms (e.g. mollusks) that often do not decant onto the sieve during elutriation.

- b. Place BMI found in the elutriate into the sample and reduce the volume of the sample if it exceeds 750 mL by using the sieve diameter splitting method.
7. If sample splitting is necessary, ensure that large debris and large-rare organisms have been removed from the sample.
 - a. Use a standard 20 cm or 30 cm diameter metal sieve marked with six equally spaced (30 degrees apart) diameters. The diameter markings are extended up the inside walls of the sieve and numbered 1 through 6 (Figure 3).
8. Evenly distribute the sample on the 500- μ m sieve by immersing the sieve in water and then carefully lifting the sieve level out of the water so the sample remains in place on the sieve. Roll a dice or otherwise randomly select which of the six diameters will be used to divide the sample in half. Divide the sample along the randomly selected diameter with a ruler and a knife. Flip a coin to determine which half becomes the new, reduced sample; the corresponding half is designated as the split sample component.
9. Repeat the sieve diameter splitting method on each new sample until a volume $\leq$ 750 mL is obtained. Discard each split sample component that is produced up to the final split. The final split-sample component may be discarded in the field or preserved and retained if the integrity of the sample is compromised during shipping or laboratory analysis.
10. Track and record the number of splits performed because this information is required to determine the total sample abundances for individual BMI taxa after laboratory analyses.
11. Place the final sample component into a single, appropriately sized wide-mouth plastic bottle that does not exceed a volume of 1 L. Ensure that sufficient space remains in the bottle for adding ethanol to preserve the sample.
12. Preserve the sample by adding enough 95% ethanol to bring the total volume to within 2 cm of the top of the bottle.
 - a. Cap the bottle and carefully invert it several times to mix the sample and 95% ethanol.
 - b. Open the bottle and top off with additional 95% ethanol. This step minimizes large void spaces that can cause increased agitation of the sample (especially during shipping) and potentially damage fragile BMI.
13. Place a sample label inside and on the outside of the main-body and split (if applicable) component bottles.
14. Record collection and sample information on the appropriate field data sheet corresponding to a particular sample type.



Figure 2. Mineral component of benthic macroinvertebrate (BMI) sample after elutriation to be examined for heavy BMIs (e.g., cased caddis flies, mollusks).



Figure 3. Metal sieve (30 cm diameter; 500-µm screen) used for benthic macroinvertebrate sample splitting. The sieve is marked with six equally spaced (30 degrees apart) diameter markings that are extended up the inside walls of the sieve and numbered 1 through 6.

Literature Cited

- Moulton, S. R., Kennen, J. G., Goldstein, R. M., and J. A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate and fish communities as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 02-150, 87 p.
- USEPA. 2007. National Rivers and Streams Assessment: Field Operations Manual. EPA-841-B-07-009. U.S. Environmental Protection Agency, Washington, DC.