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New Mexico Environment Department
Surface Water Quality Bureau

Standard Operating Procedure

for

Benthic Macroinvertebrate Sampling

Approval Signatures



Seva Joseph
Subject Matter Expert

5-1-13

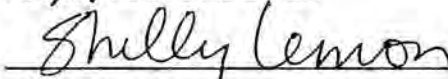
Date



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5-1-2013

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1.0 Purpose and Scope

The purpose of this document is to describe the sample collection techniques, preservation requirements, equipment, and quality control activities associated with benthic macroinvertebrate sampling.

2.0 Responsibilities

Personnel who conduct benthic macroinvertebrate sampling and data validation and verification activities or who supervise those who do are responsible for implementing this procedure.

3.0 Background and Precautions

SWQB utilizes a modified EPA EMAP macroinvertebrate sampling method for wadeable streams and rivers (Peck et al., 2006) by collecting a reach-wide composite sample from 9 equidistant transects along the stream reach. This protocol uses a 30 cm wide D-frame kick net with a mesh size of 500 μm and a sample area of 0.09 m^2 (1 ft^2) per individual sample (0.81 m^2 total sample area.). SWQB also utilizes the EMAP macroinvertebrate sample collection procedures for boatable rivers (Lazorchak et al., 2000).

Benthic macroinvertebrate samples should be collected at least 30 days after a bankfull flow event. Longer time may be required for the community to re-colonize if the flow event results in large amounts of sediment erosion and/or deposition.

Site conditions or project-specific data collection objectives may necessitate the use of alternative field procedures not included in this SOP. The use of field methods other than those presented in this SOP must be approved by the Program Manager and alternative methods must be accurately documented.

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4.0 Definitions

Benthic macroinvertebrates - animals without backbones, living in or on sediments or other substrates, of a size large enough to be seen by the unaided eye, and which can be retained by a US Standard No. 30 sieve (0.595-mm openings).

5.0 Equipment and Tools

- 30 cm wide D-frame kick net with a mesh size of 500 μ m
- sieve (500 μ m mesh size)
- bucket
- forceps
- 500-1000 mL sample container
- 95% ethanol (EtOH)

6.0 Sampling Process Description

Two different methods are used to sample benthic macroinvertebrates: (1) large river method and (2) reach wide multi-habitat method. If boatable EMAP monitoring is being conducted, then the large river method is used. Otherwise, a reach wide multi-habitat method is applied. There are two parts to each of these methods. The first step is laying out the reach to determine the location where the samples will be collected. The second step, is using the sampling technique appropriate for each location.

6.1 Wadeable Streams/Rivers

For each sampling point, record the habitat type (Table 1) and the dominant substrate type (Table 2) on the *HABITAT AND BIOTA FIELD WORK COVER SHEET*. Also note whether the sampling location is a “riffle/run” or a “pool/glide.” If there is sufficient current at the sampling point to fully extend the net, the habitat is classified as “riffle/run.” Any area where there is not sufficient current to extend the net is operationally defined as a “pool/glide” habitat. Use the appropriate sampling method for each habitat type to collect benthic macroinvertebrates (*Section 6.1.1 and Section 6.1.2*). All macroinvertebrate samples collected at a stream or river site are combined into a single composite sample to characterize the reach.

1. Establish 5 equidistant transects following protocols in Section 6.1.3.1 *Reach Layout* of SOP 5.0 - Physical Habitat Measurements. Figure 1 illustrates the reach layout for biological collections. For this modified EMAP method, only 9 samples are collected, one at each of the 5 transects (A-E) and one at the midpoints between transects (A.5-D.5). As you proceed upstream from transect to transect, combine all samples into a bucket or similar container, regardless of whether they were collected using the “riffle/run” or “pool/glide” procedure.
2. Using the appropriate sampling method for each habitat type encountered (riffle/run or pool/glide), start sampling on the LEFT side of Transect A (left when looking downstream) and follow the pattern of LEFT-CENTER-RIGHT as you move upstream to each consecutive sampling point. That is to say, collect a sample at 25% of the wetted width (LEFT) along Transect A, at 50% of the wetted width (CENTER) along Transect A.5, and at 75% of the wetted width (RIGHT) along Transect B. Repeat this pattern for the remaining 6 transects (B.5 – E). If you cannot collect a sample at the designated point because of deep water or unsafe conditions, relocate to another random point on the same transect. Record sample collection information on the *Field Form*.

NOTE: If the net cannot be used, hand-pick a sample for 30 seconds from about 1 square foot (ft²) of substrate at the sampling point. For vegetation-choked sampling points, sweep the net through the

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vegetation within a 1-ft² quadrat for 30 seconds. Place this hand-picked sample directly into the sample container. Note which transect(s) required the modified collection procedure in the comments section of the Field Form and in NMEDAS.

Table 1. Sampling habitat types

HABITAT	CHARACTERISTICS
<u>P</u>ool	Still water; low velocity; smooth, glassy surface; usually deep compared to other parts of the channel
<u>G</u>Lide	Water moving slowly, with smooth, unbroken surface; low turbulence
<u>R</u>iffle	Water moving, with small ripples, waves, and eddies; waves not breaking and surface tension is not broken; “babbling” or “gurgling” sound
<u>R</u>apid	Water movement is rapid and turbulent; surface with intermittent “white water” with breaking waves; continuous rushing sound

Table 2. Substrate types

SUBSTRATE	CHARACTERISTICS
<u>F</u>ine/sand	Non-gritty to gritty particles (silt/clay/muck up to ladybug sized;<2 mm diameter)
<u>G</u>ravel	Fine to coarse gravel (ladybug to tennis ball sized; 2 mm to 64 mm diameter)
<u>C</u>oarse	Cobble to boulder (tennis ball to car sized; 64 mm to 4000 mm diameter)
<u>O</u>ther	Bedrock (larger than car sized; > 4000 mm diameter); hardpan (firm, consolidated fine substrate); wood of any size; aquatic vegetation, etc. Note the type of “other” substrate in comments section of field form.

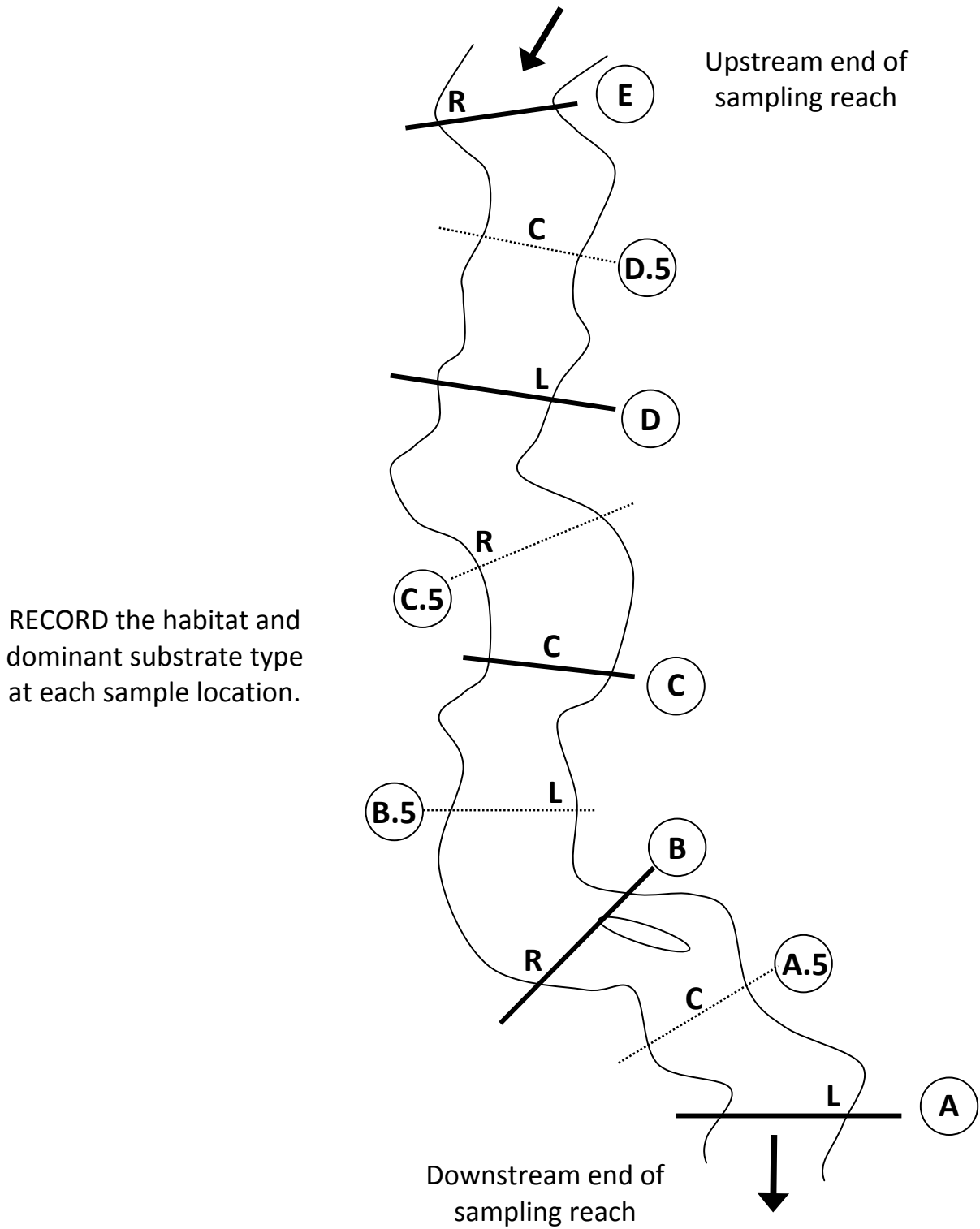


Figure 1. Reach layout for biological collections in wadeable streams

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6.1.1 Riffle/Run Habitats

1. Position the net 1 m downstream of each transect with the opening facing upstream, quickly position the net securely on the stream bottom and eliminate gaps under the frame. Avoid large rocks that prevent the net from seating properly on the stream bottom.
2. Holding the net in position on the substrate, visually define a quadrat that is one foot wide and one foot long immediately upstream of the net opening. The area within this quadrat is 1 square foot.
3. Check the quadrat for heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the net. Pick up loose rocks or other larger substrate particles in the quadrat. Use your hands or a scrub brush to dislodge organisms and wash them into the net. Scrub all rocks that are golf ball-sized or larger and which are at least 50% into the quadrat. After scrubbing, place the substrate particles outside of the quadrat.
4. Hold the D-net securely in position. Starting at the upstream end of the quadrat, vigorously kick the remaining finer substrate within the quadrat for 30 seconds (use a stopwatch).

NOTE: If there is insufficient water to collect the sample with the D-net, randomly pick up 10 rocks from the riffle and wash the organisms off into a bucket which is half full of water.

NOTE: For samples located within dense beds of long, filamentous aquatic vegetation (e.g., algae or moss), kicking within the quadrat may not be sufficient to dislodge organisms in the vegetation. Usually these types of vegetation are lying flat against the substrate due to current. Use a knife or scissors to remove only the vegetation that lies within the quadrat (i.e., not entire strands that are rooted within the quadrat) and place it into the net.

5. Pull the net up out of the water. Immerse the net in the stream flow (being careful not to disturb the stream bottom) several times to remove fine sediments and to concentrate organisms at the end of the net. Avoid having any water, detritus, or substrate enter the mouth of the net during this step.
6. Process the composite sample as described in Section 6.3 Sample Processing.

6.1.2 Pool/Glide Habitats

1. Start at 1 m downstream of each transect, visually define a quadrat that is one foot wide and one foot long at the sampling point. The area within this quadrat is 1 square foot.
2. Check the quadrat for heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the net. Pick up loose rocks or other larger substrate particles in the quadrat. Use your hands or a scrub brush to dislodge organisms and wash them into the net. Scrub all rocks that are golf ball-sized or larger and which are at least 50% into the quadrat. After scrubbing, place the substrate particles outside of the quadrat.
3. Vigorously kick the remaining finer substrate within the quadrat with your feet while dragging the net repeatedly through the disturbed area just above the bottom. Keep moving the net at all times so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds (use a stopwatch).

NOTE: If there is insufficient water to use the kick net, stir up the substrate with your gloved hands and use a sieve with 500 μ m mesh size to collect the organisms from the water in the same way

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the net is used in larger pools. For vegetation-choked sampling points, sweep the net through the vegetation for 30 seconds.

4. After 30 seconds, remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net.
5. Process the composite sample as described in Section 6.3 Sample Processing.

6.2 Large Rivers

If boatable EMAP monitoring is being conducted, then the large river method described below is used. Benthic macroinvertebrate sample locations are shown in Figure 2 and accessed by boat. Benthic macroinvertebrate samples are collected from the two most dominant habitat types that are found within a 10m x 15m randomly selected sampling station at 11 equidistant transects along the reach (Figure 2). Two 1 meter sweeps are collected from the dominant habitat and a third sweep is collected from the secondary habitat (i.e. 3 sweeps from the 10m x 15m station at each transect). Sampling should be conducted from upstream to downstream along the reach (from Transect A progressively down to Transect K). All macroinvertebrate samples are combined into a single composite sample to characterize the reach.

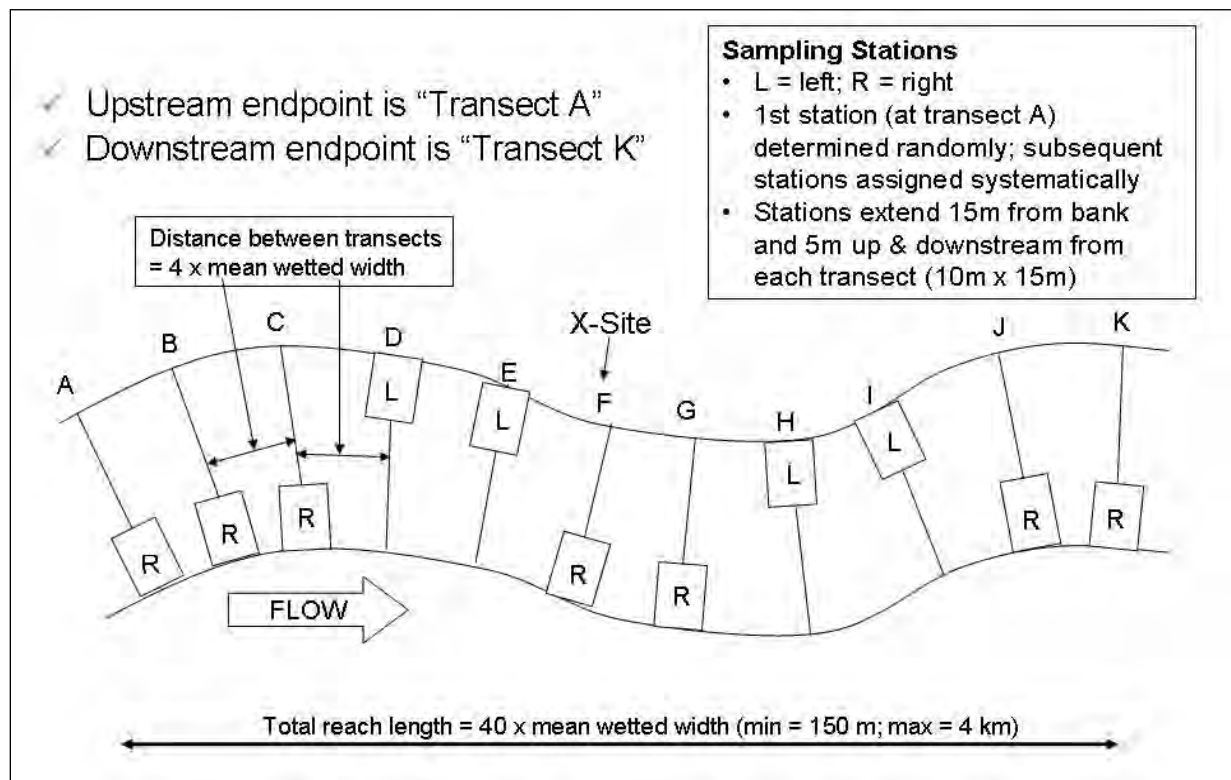


Figure 2. Sampling design for reach wide benthic macroinvertebrate collection in large rivers

1. After locating the sampling station site, identify the two dominant habitat types within the 10X15m station as described in Figure 2:

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- Rocky/cobble/gravel
 - Macrophyte beds
 - Organic fine mud or sand
 - Leaf Pack
2. Use the D-frame dip net to sweep through 1 linear meter of the most dominant habitat type at two locations within the 10m x 15m sampling station (i.e., take 2 sweeps of the dominant habitat type), making sure to disturb the substrate enough to dislodge the organisms.
 - If the dominant habitat is rocky/cobble/large or woody debris, it may be necessary to exit the boat and disturb the substrate (e.g., overturn rocks, logs) using your feet while sweeping the net through the disturbed area.
 - Because a dip-net is being used for sampling, the maximum depth for sampling will be approximately 0.5 m. In cases in which the depth of the river quickly drops off, it may be necessary to sample in the nearest several meters to the shore.
 3. After completing the first 1-meter sweep, remove organisms and debris from net and place them in a bucket or sample container.
 4. Conduct a sweep of the most dominant habitat at a second location; again, remove organisms and debris from net and place them in a bucket or sample container.
 5. Conduct a third and final sweep of the secondary habitat; again, remove organisms and debris from net and place them in a bucket or sample container. If only one habitat type is present, all 3 sweeps will be in that habitat.
 6. Record the sampled substrate types (Table 2) on the Sample Collection Form.
 7. Identify the channel habitat type (Table 1) where the sampling quadrat was located. Place an "X" in the appropriate channel habitat type box for the transect on the *Field Form*.
 8. Proceed to the next sampling station and repeat steps 1-7. The organisms and detritus collected at each station on the river should be combined to create a single composite sample. After sampling at all 11 stations is completed, process the composite sample in the bucket according to procedures described in Section 6.3 Sample Processing.

6.3 Sample Processing

After collecting all of the samples, prepare one reach wide composite sample. Place contents of the bucket into a sieve (500 μ m mesh size) and wash off and discard large objects. Depending on how much debris was collected, this may need to be done in several steps, being careful not to overfill the sieve. Repeated rinsing should be done until rinse water is clear. When large amounts of sediment are collected, thoroughly agitate the contents by swirling and pour off all organic debris into the sieve. Refill the bucket with stream water to 1/4 – 1/2 full. Once organic debris has been removed, remove any large pebbles, inspect for attached macroinvertebrates (scrub off all that are present), and discard. Agitate the bucket contents by swirling, and immediately pour off the water into the sieve before lighter items in the water column can settle out. Be careful not to pour out large amounts of sediment. Repeat several times until you are confident that all macroinvertebrates have been removed. Visually inspect any remaining sediment for snails, clams, mussels, and macroinvertebrate cases and tubes. Remove these items and place in the sieve or directly into the sample jar.

Using forceps and a rinse bottle, wash the contents of the sieve into the sample jar. Put as little water as possible into the sample container. Do not fill the sample container more than 1/2 full. Divide the sample into as many jars as needed and indicate the jar number on the sample label and the id of the jar (e.g., 1 of 3). Fill the jar with 95% ethanol (EtOH). Tighten the cap on each jar and slowly tip the jar to a horizontal position, gently rotating the jar to mix the preservative. Do not shake the jar.

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A sample label is required for all sample jars from a single composite sample. The label should be written in pencil and contain the site name and date that the sample was collected, as well as the jar number if multiple containers were used.

7.0 Quality Assurance/Quality Control

All staff conducting benthic macroinvertebrate sampling will attend an annual training section on the sampling and sample processing procedures. Before leaving the sampling site, all forms will be checked for completeness.

8.0 Forms

HABITAT AND BIOTA FIELD WORK COVER SHEET

- *Sample Collection Form – Boatable (PENDING)*
- *BUGS, HABITAT, and NUTRIENT Survey Equipment Checklist*

9.0 Revision History

Original.

REFERENCES

Lazorchak, J.M., B.H. Hill, D.K. Averill, D.V. Peck, and D.J. Klemm (editors). 2000. Environmental Monitoring and Assessment Program-Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Non-Wadeable Rivers and Streams. U.S. Environmental Protection Agency, Cincinnati OH.

Peck, D.V., A.T. Herlihy, B.H. Hill, R.M. Hughes, P.R. Kaufmann, D.J. Klemm, J.M. Lazorchak, F.H. McCormick, S.A. Peterson, P.L. Ringold, T. Magee, and M. Cappaert. 2006. Environmental Monitoring and Assessment Program -Surface Waters Western Pilot Study: Field Operations Manual for Wadeable Streams. EPA/620/R-06/003. U.S. Environmental Protection Agency, Office of Research and Development, Washington, D.C.