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**ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM-
SURFACE WATERS:**

**FIELD OPERATIONS AND METHODS FOR MEASURING THE
ECOLOGICAL CONDITION OF WADEABLE STREAMS**

Edited by

James M. Lazorchak¹, Donald J. Klemm¹, and David V. Peck²

¹ U.S. Environmental Protection Agency
Ecosystems Research Branch
Ecological Exposure Research Division
National Exposure Research Laboratory
Cincinnati, OH 45268

² U.S. Environmental Protection Agency
Regional Ecology Branch
Western Ecology Division
National Health and Environmental Effects Research Laboratory
Corvallis, OR 97333

NATIONAL EXPOSURE RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

APPENDIX E

MODIFIED PROTOCOL FOR COLLECTING BENTHIC MACROINVERTEBRATES

by
Donald J. Klemm¹ and David V. Peck²

Field procedures described here are modified from those developed by the Oregon Department of Environmental Quality (Oregon Dept. of Environmental Quality, 1997), and the Washington Department of Ecology (Washington Dept. of Ecology, 1997). These procedures were implemented in an EMAP study of wadeable streams in Oregon in 1997, and the EPA Region 10 R-EMAP study in 1996-1997. Modifications to the basic EMAP protocol (Section 11 of the EMAP field operations manual for streams) were desired to maximize the comparability of EMAP results with both the R-EMAP project results, and with other data both State agencies routinely collect as part of their respective monitoring programs.

Within the defined sampling reach of 150 to 500 m, benthic invertebrate samples are collected from two principal macrohabitat types, erosional (operationally termed "riffle") and depositional (operationally termed "pool"). Riffle macrohabitats include low-gradient areas that are generally more shallow than pools. Many riffles exhibit surface turbulence associated with increased velocity and shallow water depth over gravel or cobble beds. However, the riffle classification also includes shallow areas without surface turbulence such as glides. Pool macrohabitats include areas of slow, deep water with low gradient. They are typically created by scour adjacent to obstructions or impoundments of water behind channel blockages and hydraulic controls such as logjams, bedforms, or beaver dams.

Individual kick net samples are collected from up to five points within each macrohabitat type, spaced throughout the sampling reach. Individual kick net samples collected from each macrohabitat type are processed and composited into a single sample for the stream. Thus for each stream, there will be two composite samples, one for riffles and one

¹ U.S. EPA, National Exposure Research Laboratory, Ecological Exposure Research Division, 26 W. Martin L. King Dr., Cincinnati, OH 45268.

² U.S. EPA, National Health and Environmental Effects Research Laboratory, Western Ecology Division, 200 SW 35th St., Corvallis, OR 97333.

for pools. Each composite sample is contained in a 500-mL or 1-L plastic screw-top jar, and preserved with 95% ethanol to a final concentration of 70% ethanol.

The sampling protocols described here differ from those presented in Section 11 of the EMAP streams field operations manual in that sampling points are allocated evenly across the two major macrohabitat types, rather than by sampling at predefined points located on the transects established for physical habitat characterization. Differences from R-EMAP protocols used by Oregon DEQ (1997) and Washington Department of Ecology (1997) include not determining the depth at each sampling point and not determining the substrate particle distribution at each sampling points.

E.1 SAMPLE COLLECTION

E.1.1 Selection of Sampling Points

Table E-1 presents the procedure for selecting individual sampling points within the two major macrohabitat types (riffle and pool). Note that in some stream reaches, one macrohabitat type will predominate to the extent that the other type is not sampled. There may also be stream reaches where two kick net samples are collected from a single macrohabitat unit. It is also permissible to sample a short distance beyond the upstream end of the sample reach in order to obtain the desired number (5) of macrohabitat units of each type.

E.1.2 Collection of Kick Net Samples

The kick net is designed to obtain a qualitative and semi-quantitative sample of benthic macroinvertebrates from a variety of substrates in streams. A modified USGS kick net (Wildco # 425-J50-595) is used. This is the same net as is described in Section 10 of the EMAP field operations manual for streams for EMAP. Modifications from the standard configuration include the net mesh size (600 μ m), the length of the net (61 cm or 24 in.) and the type of bag (tapering closed bag). The frame dimensions of the net are 30.48 cm (12 in.) high and 50.8 cm (20 in.) wide. The style and dimensions of this net differ from that used by Oregon DEQ and Washington Department of Ecology, who use a smaller net of a D-frame configuration. However, mesh sizes are the same.

Procedures for collecting a point sample using the kick net from riffle and pool macrohabitat units are presented in Tables E-2 and E-3, respectively. At each sampling

**TABLE E-1. LOCATING SAMPLING POINTS FOR KICK NET SAMPLES:
WADEABLE STREAMS**

1. Before sampling, survey the stream reach to visually estimate the number of pool and riffle macrohabitat "units" contained in the defined stream reach. To be considered as a unit, the length of a stream occupied by a particular macrohabitat type unit should be at least equal to the stream's average wetted width estimate used to define the length of stream reach.
 - A. Do not sample poorly represented habitats. If the reach contains < 2 macrohabitat units of a given type, then do not sample that macrohabitat type. If only one macrohabitat unit occurs in the defined reach but, more are present within 100 meters upstream, sample those as they were part of the reach.
 - B. If the reach contains 3 or 4 macrohabitat units of a given type, then randomly select those macrohabitat unit(s) from which to collect a second kick net sample to bring the total number of kick net samples for the macrohabitat type to five.
 - C. If the number of units is greater than five of either, skip one or more habitat units at random as you work upstream.
2. Begin sampling at the most downstream unit, and sample units as they are encountered to minimize instream disturbance. This will require separate containers for pool and riffle samples.
3. At each unit, exclude "margin" habitats by constraining the potential sampling area. Margin habitats are edges, along the channel margins or upstream or downstream edges of the macrohabitat unit. Define a core area for each unit as the central portion, visually estimating a "buffer" strip circumscribing the identified unit. In some cases, the macrohabitat unit may be so small that it will not be feasible to define a core area and avoid an edge.
4. Visually lay out the core area of the unit sampled into 9 equal quadrats (i.e., a 3 × 3 grid). For each macrohabitat type, select a quadrat for sampling as follows:
 - First unit: Lower right quadrat
 - Second unit: Center quadrat
 - Third unit: Upper left quadrat
 - Fourth unit: Lower left quadrat
 - Fifth unit: Upper right quadrat.
5. Collect the kick sample in the center of the selected quadrat, following the protocol for the type of macrohabitat unit.
6. If a second sample is required from a single macrohabitat unit, select a new quadrat.

**TABLE E-2. COLLECTING A KICK NET SAMPLE FROM WADEABLE STREAMS:
RIFFLER MACROHABITATS**

1. Locate the sampling point within the macrohabitat unit as described in Table 1.
 2. Position the kick net quickly and securely on the stream bottom so as to eliminate gaps between the frame and the stream bottom. If necessary, rotate the net so the narrower side is against the bottom.
 3. Hold the sampler firmly in position on the substrate. Define a quadrat immediately upstream from the mouth of the net having a width equal to the width of the net frame and a total area = 0.5 m². If the kick net is oriented normally, the length of the quadrat = 1 m (approx. equal to 2 times the width of the net [0.5 m]). If the net is rotated so the short side is against the substrate, the length of the quadrat = 1.67 m.
 4. Lightly kick the substrate throughout the quadrat. Start at the upstream end and work toward the net.
 5. Hold the net in place with the knees and pick up any loose rocks in the quadrat and rub off organisms so that they are washed into the net. With a small brush dislodge organisms from the rocks into the net. Scrub all rocks that are golf ball-sized or larger and which are over halfway into the quadrat. Large rocks that are less than halfway into the sampling area are pushed aside.
 6. Keep holding the sampler securely in position and kick through the quadrat again, this time vigorously, for 20 seconds.
 7. Pull the net up out of the water. Immerse the net in the stream several times to remove fine sediments and to concentrate organisms at the end of the net. Avoid having any water or material enter the mouth of the net during this operation.
 8. Invert the net into a plastic bucket marked "riffle" and transfer the sample. Inspect the net for any residual organisms clinging to the net and deposit them into the "riffle" bucket. Use watchmakers' forceps if necessary to remove organisms from the net.
 9. Thoroughly rinse the net before proceeding to the next macrohabitat unit.
 10. Repeat steps 1-9 at subsequent riffle macrohabitat units until 5 kick samples have been collected and placed into the "riffle" bucket.
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**TABLE E-3. COLLECTING A KICK NET SAMPLE FROM WADEABLE STREAMS:
POOL MACROHABITATS**

1. Locate the sampling point within the macrohabitat unit as described in table 1.
2. Define a sampling area as a quadrat having a width equal to the width of the net frame and a total area = 0.5 m². If the kick net is oriented normally, the length of the quadrat = 1 m (approx. equal to 2 times the width of the net [0.5 m]). If the net is rotated so the short side is against the substrate, the length of the quadrat = 1.67 m.
3. Inspect the quadrat for heavy organisms such as mussels and snails. Hand pick any of these large organisms and place them into the sieve bucket or plastic bucket marked "pool".
4. Kick vigorously with the feet within the quadrat for 10 seconds. Then drag the net repeatedly through the disturbed area just above the bottom. Keep moving the net to prevent organisms from escaping. Continue this for 1 minute.
5. Pull the net up out of the water. Immerse the net into the stream several times to remove fine sediments and to concentrate organisms at the end of the net. Avoid having any water or material enter the mouth of the net during this operation.
6. Invert the net into the bucket marked "pool" and transfer the sample. Inspect the net for any residual organisms clinging to the net and deposit them into the "pool" sieve bucket. Use watchmakers' forceps if necessary to remove organisms from the net.
7. Thoroughly rinse the net before proceeding to the next macrohabitat unit to prevent cross-contamination of riffle and pool samples.
8. Repeat steps 1-7 at subsequent pool macrohabitat units until 5 kick samples have been collected and placed into the "pool" sieve bucket.

point, a quadrat having a total area of 0.5 m² is sampled. The dimensions of the quadrat will vary depending on how the kick net must be oriented against the substrate. In narrow streams, the net may have to be rotated so that the narrow side is against the stream bottom. Riffle and pool samples are kept in separate containers. Note that in pool units, the substrate is first disturbed, and the net is dragged through the disturbed area just above the substrate. Because units are sampled in the order they are encountered, it is very important to rinse the kick net thoroughly between samples to avoid carryover and possible cross-contamination of riffle and pool samples.

E.2 Sample Processing

The procedure for processing kick net samples is presented in Table E-4; the procedure is identical for riffle and pool samples. Process one sample at a time to avoid mixing riffle and pool samples in the same container. Reduce the amount of residue in each composite sample as much as possible without losing organisms. However, if there is a sizable quantity of material remaining, distribute the sample into additional containers to ensure proper preservation. A sample jar should not be more than half-full of material. Modified sample labels are shown in Figure E-1, and the modified Sample Collection Form is presented in Figure E-2.

E.3. LITERATURE CITED

Oregon Department of Environmental Quality. 1997. *Biological Assessment of Wadeable Streams of the Upper Deschutes River Basin: Quality Assurance Project Plan.*

Washington Department of Ecology. 1997. *Biological Assessment of Wadeable Streams of the Chehalis River Basin: Quality Assurance Project Plan.*

TABLE E-4. PROCESSING KICK NET SAMPLES: WADEABLE STREAMS

1. Fill out a sample label for the riffle composite samples. Attach a label to a 1-gallon plastic bag with a zipper-type closure. If the sample contains a large volume of material, complete a sample label for additional containers and attach it to a second bag. Make sure the barcode numbers on each label agree.
2. Hand pick large organisms from the bucket containing the composited riffle kick net samples and place them into the appropriately labeled plastic bag.
3. Hand pick large rocks and sticks remaining in the bucket. Use a small brush to scrub debris from them back into the bucket. Discard the rock or stick.
4. Empty the contents of the bucket into the labeled plastic bag. If necessary, distribute the sample among two or more labeled bags. Rinse residue from the bucket into the plastic bag using a wash bottle and a **small** volume of water.
5. Place each bag inside a second bag.
6. Add 95% ethanol to each labeled bag in a volume which is equal to the volume of the sample.
7. Rinse the bucket well to eliminate any residue.
8. Repeat Steps 1-7 for the pool composite sample.
9. Complete the Sample Collection Form. Record the barcode number of each composite sample (riffle and pool), and the habitat type from the sample label. If more than one container was required for a sample, record the number of containers on the collection form. Also, note any peculiarities associated with a particular sample by using a flag code and/or a written comment on the collection form.

COMPOSITE BENTHOS

SITE ID: O R S T 9 7 - _____

DATE: _____ / _____ / **97**

HABITAT: Riffle Pool


238200

Figure E-1. Modified sample labels.

Reviewed by (initial): _____

SAMPLE COLLECTION FORM - STREAMS											
SITE NAME: _____						DATE: / / 97 VISIT: ☐ 1 ☐ 2 _____					
SITE ID: O R S T 9 7 - _____						TEAM ID (X): ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8					
COMPOSITE BENTHOS SAMPLES											
SAMPLE ID (BARCODE)	HABITAT (X ONE)		No. OF JARS	FLAG	COMMENTS						
	R	P									
STATION	A	B	C	D	E	F	G	H	I	J	K
RIFFLER OR POOL - (X ONE) -		☐ R ☐ P	☐ R ☐ P	☐ R ☐ P	☐ R ☐ P	☐ R ☐ P	☐ R ☐ P	☐ R ☐ P	☐ R ☐ P	☐ R ☐ P	
LEFT, CENTER, OR RIGHT - (X ONE) -		☐ L	☐ L	☐ L	☐ L	☐ L	☐ L	☐ L	☐ L	☐ L	
		☐ C	☐ C	☐ C	☐ C	☐ C	☐ C	☐ C	☐ C	☐ C	
		☐ R	☐ R	☐ R	☐ R	☐ R	☐ R	☐ R	☐ R	☐ R	
COMPOSITE PERIPHYTON SAMPLES					HABITAT TYPE (X) → ☐ RIFFLE ☐ POOL ☐ OTHER						
SAMPLE ID (BARCODE) → _____					COMPOSITE VOLUME → _____ ML						
ASSEMBLAGE ID (50-ML TUBE)		CHLOROPHYLL (GF/F FILTER)		BIOMASS (TARED FILTER)				APA SAMPLE (50-ML TUBE)			
SUB. SAMPLE VOL.		VOL. FILTERED		FILTER NO.		VOL. FILTERED		SUB. SAMPLE VOL.			
_____ ML		_____ ML				_____ ML		_____ ML			
COMPOSITE PERIPHYTON SAMPLES					HABITAT TYPE (X) → ☐ RIFFLE ☐ POOL ☐ OTHER						
SAMPLE ID (BARCODE) → _____					COMPOSITE VOLUME → _____ ML						
ASSEMBLAGE ID (50-ML TUBE)		CHLOROPHYLL (GF/F FILTER)		BIOMASS (TARED FILTER)				APA SAMPLE (50-ML TUBE)			
SUB. SAMPLE VOL.		VOL. FILTERED		FILTER NO.		VOL. FILTERED		SUB. SAMPLE VOL.			
_____ ML		_____ ML				_____ ML		_____ ML			
COMMENTS: _____											

Flag codes: K= Sample not collected; U= Suspect sample; F1, F2, etc.= misc. flag assigned by field crew. Explain all flags in Comment sections.

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SAMPLE COLLECTION FORM - STREAMS - 1

Figure E-2. Modified Sample Collection Form.