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ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM-SURFACE WATERS:

WESTERN PILOT STUDY
FIELD OPERATIONS MANUAL FOR
WADEABLE STREAMS

Edited by

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SECTION 11
BENTHIC MACROINVERTEBRATES

by
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Benthic invertebrates inhabit the sediment or live on the bottom substrates of streams. Benthic macroinvertebrate assemblages in streams reflect overall biological integrity of the benthic community. Monitoring these assemblages is useful in assessing the status of the water body and detecting trend in ecological condition. Benthic communities respond to a wide array of stressors in different ways so that it is often possible to determine the type of stress that has affected a macroinvertebrate community (e.g., Klemm et al., 1990). Because many macroinvertebrates have relatively long life cycles of a year or more and are relatively immobile, macroinvertebrate community structure is a function of present or past conditions.

The EMAP-SW benthic macroinvertebrate protocol is intended to evaluate the biological integrity of wadeable streams in the United States for the purpose of detecting stresses on community structure and assessing the relative severity of these stresses. It is based on the "Rapid Bioassessment Protocol III - Benthic Macroinvertebrates" published by the U.S. Environmental Protection Agency (Plafkin et al., 1989; Barbour et al., 1999) and adopted for use by many states. Modifications to the previously published protocol for EMAP-Surface Waters (Klemm et al., 1998) for the EMAP-SW Western Pilot Study are summarized in Table 11-1. The two man kick net procedure of the Rapid Bioassessment Protocol (RBP) is replaced in the EMAP-SW protocol with a D-frame kick net modified for use by one person (Figure 11-1). Note this net is modified from that used in previous EMAP and R-EMAP projects (Klemm et al., 1998), in terms of frame type, mesh size, and dimensions. The modified protocol still requires only one person and is the preferred macroinvertebrate collecting method for streams with flowing water (a second person is often used for water safety and to keep time and record information on the field forms).

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TABLE 11-1. SUMMARY OF BENTHIC MACROINVERTEBRATE PROTOCOL CHANGES FOR THE EMAP-SW WESTERN PILOT STUDY

Modifications from Klemm et al. (1998)

14. Two types of samples are collected, a "targeted riffle" sample and a "reach-wide" sample, replacing the "riffle/run" and "pool/glide" samples. The targeted riffle sample is focused on riffle areas only (i.e., if no riffle areas are present, the sample is not collected). The reach-wide sample is collected from transects spaced throughout the reach, as was described in the previous published protocol.

15. The number of kick samples in the targeted riffle sample is 8. The number of kick samples in the reach-wide sample is increased from 9 (transects B through J) to 11 (Transects A through K).

16. Each sample type is prepared as a single composite sample. For the reach-wide sample, all kick samples are combined into a single composite sample, replacing the "RIFFLE" composite and the "POOL" composite samples.

17. The sampling device is changed from a rectangular kick net to a D-Frame design. Mesh size is decreased from 595 Fm to 500 Fm. Net width is decreased from 18 in to 12 in (50 cm to 30 cm).

18. The area of each kick sample is reduced from 0.5 m^2 to 0.09 m^2 (1 ft^2).

19. The time for each kick sample is increased from 20 seconds to 30 seconds.

Modifications from Western Pilot Study Year 2000 Activities:

1. Clarified procedure for collecting at sampling points choked with vegetation.

2. Field form has been modified to record the microhabitat type (pool, glide, riffle, rapid) for each reachwide kicknet sample.

The "biomorphs" (refer to Figure 2-1) collect kick net samples for benthic macroinvertebrates at sampling points located on each cross-section transect (termed the "reach-wide" sample) and from riffle habitats located within the sampling reach (termed the "targeted riffle" sample). Kick net samples are collected at the same time as periphyton samples (Section 8). Samples collected as part of the "reach-wide" sample are combined into a single composite for the stream reach, while those collected for the "targeted riffle" sample are combined into a separate composite.

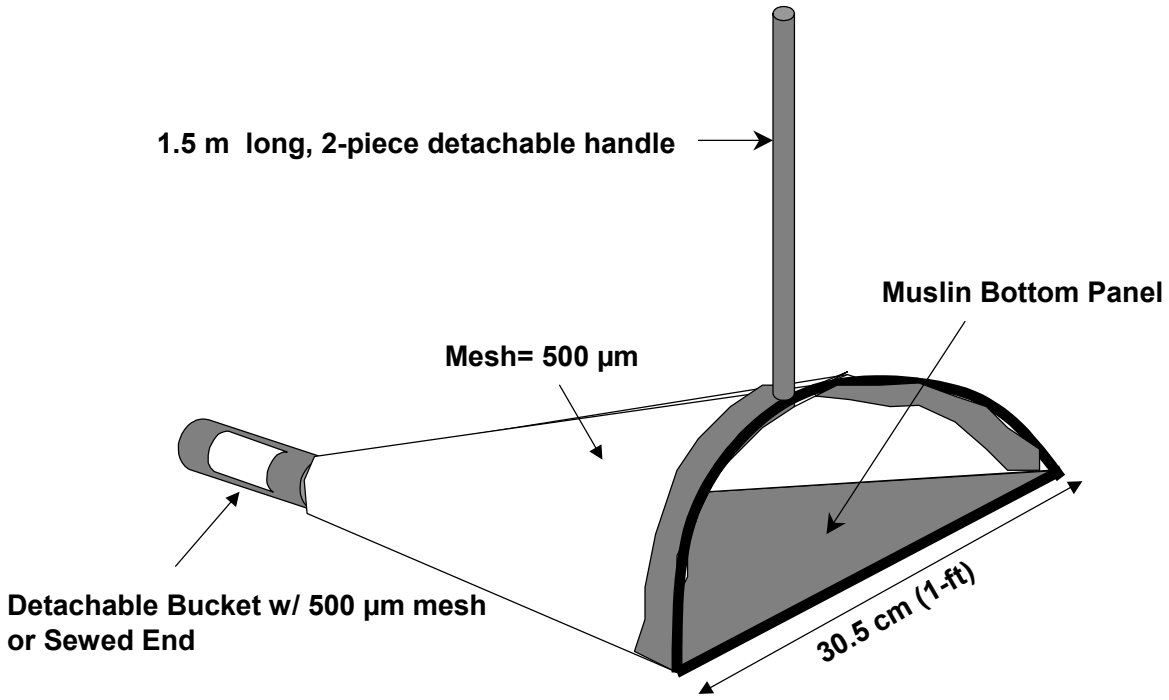


Figure 11-1. Modified D-frame kick net. (Not drawn to scale.)

11.1 SAMPLE COLLECTION

11.1.1 Reach-Wide Sample

The index sample design for collecting the reach-wide sample for benthic macroinvertebrates is shown in Figure 11-2. This design was used in the EMAP and R–EMAP stream studies in the mid-Atlantic region (refer to Section 1 for project descriptions).

A kick net sample is collected from each of the eleven cross-section transects (Transects “A” through “K”) at an assigned sampling point (Left, Center, or Right). These

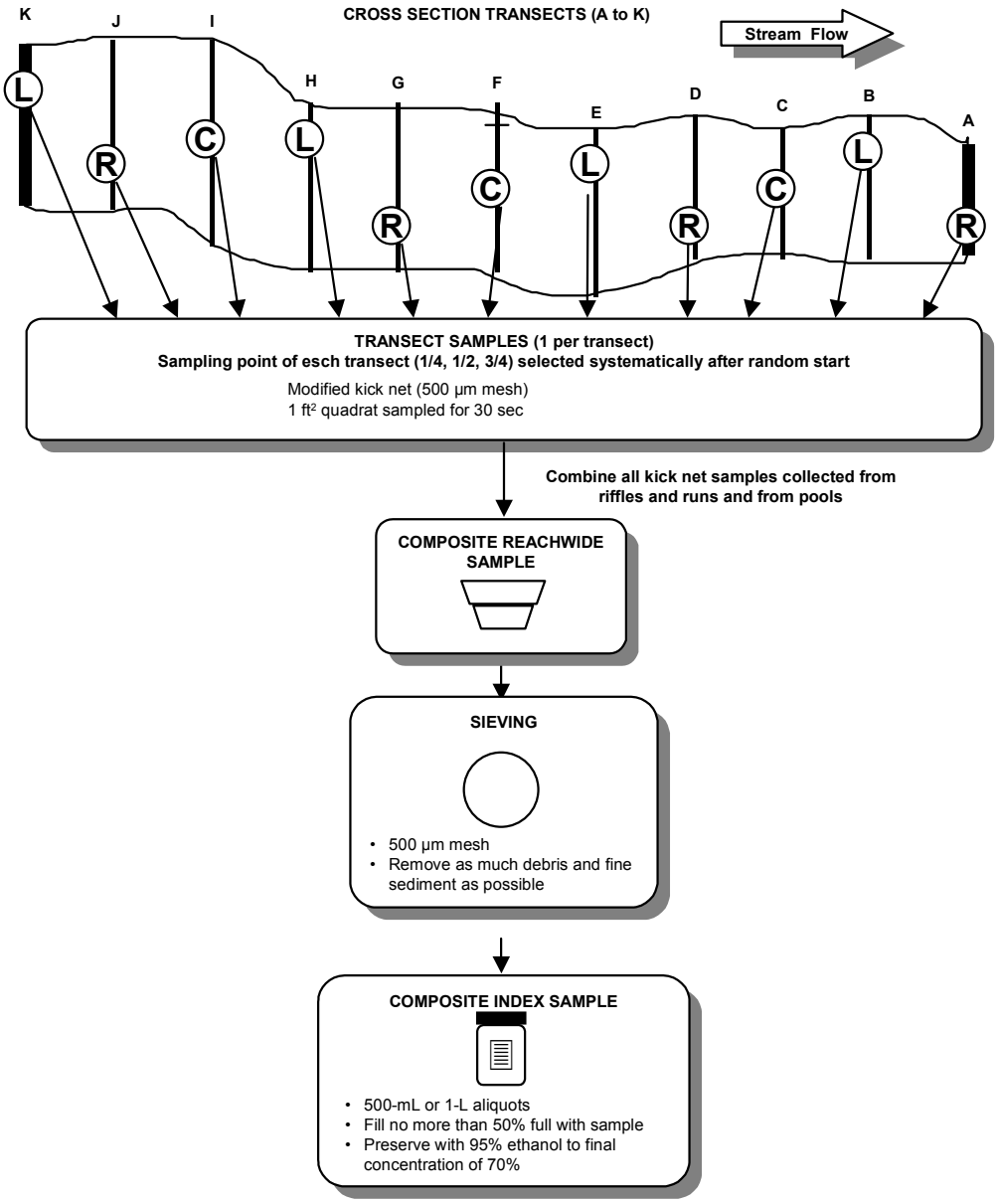


Figure 11-2. Index sampling design for benthic macroinvertebrate reachwide sample.

points may have been assigned when the sampling reach was laid out (Figure 11-2; refer also to Section 4; Table 4-3). If not, the sampling point at Transect "A" is assigned at random using a die or other suitable means (e.g., digital watch). Once the first sampling point is determined, points at successive transects are assigned in order (Left, Center, Right). These are the same sampling points as those used for periphyton samples (Section 8). At transects assigned a "Center" sampling point where the stream width is between one and two net widths wide, pick either the "Left" or "Right" sampling point instead. If the stream is only one net wide at a transect, place the net across the entire stream width and consider the sampling point to be "Center". If a sampling point is located in water that is too deep or otherwise unsafe to wade, select an alternate sampling point on the transect at random.

The procedure for collecting a kick net sample at each transect is described in Table 11-2. At each sampling point, determine if the habitat is a "riffle/run" or a "pool/glide". Any area where there is not sufficient current to extend the net is operationally defined as a pool/glide habitat. Record the dominant substrate type (fine/sand, gravel, coarse substrate (coarse gravel or larger) or other (e.g., bedrock, hardpan, wood, aquatic vegetation, etc.) and the habitat type (pool, glide, riffle, or rapid) for each kick net sample collected on the Sample Collection Form as shown in Figure 11-3. As you proceed upstream from transect to transect, combine all kick net samples into a bucket or similar container labeled "REACH-WIDE", regardless of whether they were collected using the "riffle/run" or "pool/glide" procedure.

If it is impossible to sample at the sampling point with the modified kick net following either procedure, spend about 30 seconds hand picking a sample from about 0.09 m^2 (1 ft^2) of substrate at the sampling point. For vegetation-choked sampling points, sweep the net through the vegetation for 30 seconds. Place the contents of this hand-picked sample into the "REACH-WIDE" sampling container.

11.1.2 Targeted Riffle Sample

Figure 11-4 illustrates the sampling design for the targeted riffle sample. Table 11-3 presents the procedure for selecting individual sampling points within the available riffle macrohabitat units located within the sampling reach. Note that if the total available area of riffle habitat is less than 8 ft^2 (i.e., such that 8 non-overlapping kick net samples cannot be collected), do not collect a targeted riffle sample. There may be stream reaches where more than one 1 ft^2 kick net sample is collected from a single riffle unit. The objective for

TABLE 11-2. PROCEDURE TO COLLECT KICK NET SAMPLES FOR THE REACH-WIDE COMPOSITE SAMPLE

1. At each cross-section transect, beginning with Transect "A", locate the assigned sampling point (Left, Center, or Right as you face downstream) as 25%, 50%, and 75% of the wetted width, respectively. If you cannot collect a sample at the designated point because of deep water or unsafe conditions, relocate the point on the transect nearby.

2. Attach the 4-ft handle to the kick net. Make sure that the handle is on tight or the net may become twisted in a strong current, causing the loss of part of the sample.

3. Determine if there is sufficient current in the area at the sampling point to fully extend the net. If so, classify the habitat as "riffle/run" and proceed to Step 4. If not, use the sampling procedure described for "pool/glide" habitats (Step 9).

   NOTE: If the net cannot be used, spend 30 seconds hand picking a sample from about 0.09 m^2 (1 ft^2) of substrate at the sampling point. For vegetation-choked sampling points, sweep the net through the vegetation within a 0.09 m^2 (1 ft^2) quadrat for 30 seconds. Place the contents of this hand-picked sample into the "REACH-WIDE" sampling container. Go to Step 15.

Riffle/Run Habitats:

4. With the net opening facing upstream, position the net quickly and securely on the stream bottom to eliminate gaps under the frame. Avoid large rocks that prevent the sampler from seating properly on the stream bottom.

   NOTE: If there is too little water to collect the sample with the kick net, randomly pick up 10 rocks from the riffle and pick and wash the organisms off them into a bucket labeled "REACH-WIDE" which is half-full of water.

5. Holding the net in position on the substrate, visually define a rectangular quadrat that is one net width wide and one net width long upstream of the net opening. The area within this quadrat is ~0.09 m^2 (1 ft^2). Alternatively, place a wire frame of the correct dimensions in front of the net to help delineate the quadrat to be sampled.

6. Hold the net in place with your knees. Check the quadrat for heavy organisms, such as mussels and snails. Remove these organisms from the substrate by hand and place them into the net. Pick up any loose rocks or other larger substrate particles in the quadrat. Use your hands or a small scrub brush to dislodge organisms so that they are washed 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. After scrubbing, place the substrate particles outside of the quadrat.

7. Keep holding the sampler securely in position. Start at the upstream end of the quadrat, vigorously kick the remaining finer substrate within the quadrat for 30 seconds (use a stopwatch).

8. 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.

9. Go to Step 14.

(continued)

TABLE 11-2. (Continued)

Pool/Glide habitats:

10. Visually define a rectangular quadrat that is one net width wide and one net width long at the sampling point. The area within this quadrat is ~0.09 m^2 (1 ft^2). Alternatively, lay a wire frame of the correct dimensions in front of the net at the sampling point to help delineate the quadrat.

11. Inspect the stream bottom within the quadrat for any heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the net or into a bucket labeled "REACH-WIDE". Pick up any loose rocks or other larger substrate particles within the quadrat and hold them in front of the net. Use your hands (or a scrub brush) to rub any clinging organisms off of rocks or other pieces of larger substrate (especially those covered with algae or other debris) into the net. After scrubbing, place the larger substrate particles outside of the quadrat.

12. 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 all the time so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds. NOTE: If there is too little water to use the kick net, stir up the substrate with your gloved hands and use a sieve with 500 Fm mesh size to collect the organisms from the water in the same way the net is used in larger pools.

13. After 30 seconds, remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net.

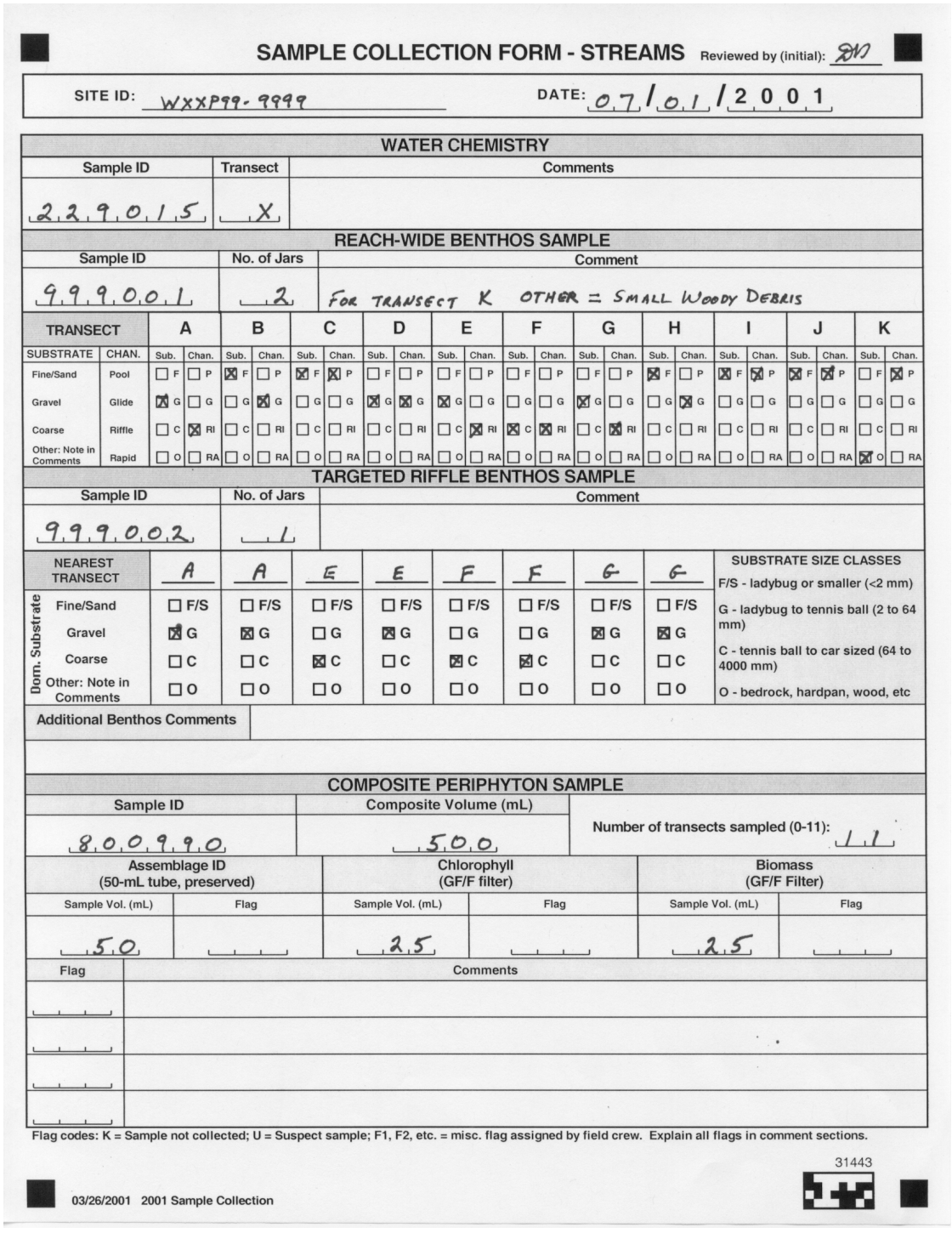
All samples:

14. Invert the net into a plastic bucket marked "REACH-WIDE" and transfer the sample. Inspect the net for any residual organisms clinging to the net and deposit them into the "REACH-WIDE" bucket. Use watchmakers' forceps if necessary to remove organisms from the net. Carefully inspect any large objects (such as rocks, sticks, and leaves) in the bucket and wash any organisms found off of the objects and into the bucket before discarding the object. Remove as much detritus as possible without losing any organisms.

15. Place an "X" in the appropriate substrate type box for the transect on the Sample Collection Form.

    Fine/sand: not gritty (silt/clay/muck < 0.06 mm diam.) to gritty, up to ladybug sized (2 mm diam.)
    Gravel: fine to coarse gravel (ladybug to tennis ball sized; 2 mm to 64 mm diam.)
    Coarse: Cobble to boulder (tennis ball to car sized; 64 mm to 4000 mm)
    Other: bedrock (larger than car sized; > 4000 mm), hardpan (firm, consolidated fine substrate), wood of any size, aquatic vegetation, etc.). Note type of "other" substrate in comments on field form.

16. Thoroughly rinse the net before proceeding to the next sampling location. Proceed upstream to the next transect (including Transect K, the upstream end of the sampling reach) and repeat Steps 1 through 9. Combine all kick net samples from riffle/run and pool/glide habitats into the "REACH-WIDE" bucket.



SAMPLE COLLECTION FORM - STREAMS Reviewed by (initial): BM

SITE ID: WXXP99-9999 DATE: 07/01/2001

WATER CHEMISTRY

Sample ID	Transect	Comments
229015	X	

REACH-WIDE BENTHOS SAMPLE

Sample ID	No. of Jars	Comment
999001	2	FOR TRANSECT K OTHER = SMALL WOODY DEBRIS

TRANSECT	A		B		C		D		E		F		G		H		I		J		K	
SUBSTRATE	CHAN.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.
Fine/Sand	Pool	☐ F	☐ P	☒ F	☐ P	☒ F	☒ P	☐ F	☐ P	☐ F	☐ P	☐ F	☐ P	☐ F	☐ P	☒ F	☐ P	☒ F	☒ P	☒ F	☒ P	☐ F	☒ P
Gravel	Glide	☒ G	☐ G	☐ G	☒ G	☐ G	☐ G	☒ G	☒ G	☒ G	☐ G	☐ G	☐ G	☒ G	☐ G	☐ G	☒ G	☐ G	☐ G	☐ G	☐ G	☐ G	☐ G
Coarse	Riffle	☐ C	☒ RI	☐ C	☐ RI	☐ C	☐ RI	☐ C	☐ RI	☐ C	☒ RI	☒ C	☒ RI	☐ C	☒ RI	☐ C	☐ RI	☐ C	☐ RI	☐ C	☐ RI	☐ C	☐ RI
Other: Note in Comments	Rapid	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☒ O	☐ RA

TARGETED RIFFLE BENTHOS SAMPLE

Sample ID	No. of Jars	Comment
999002	1	

NEAREST TRANSECT	A	A	E	E	F	F	G	G
Dom. Substrate: Fine/Sand	☐ F/S	☐ F/S	☐ F/S	☐ F/S	☐ F/S	☐ F/S	☐ F/S	☐ F/S
Gravel	☒ G	☒ G	☐ G	☒ G	☐ G	☐ G	☒ G	☒ G
Coarse	☐ C	☐ C	☒ C	☐ C	☒ C	☒ C	☐ C	☐ C
Other: Note in Comments	☐ O	☐ O	☐ O	☐ O	☐ O	☐ O	☐ O	☐ O

SUBSTRATE SIZE CLASSES
F/S - ladybug or smaller (<2 mm)
G - ladybug to tennis ball (2 to 64 mm)
C - tennis ball to car sized (64 to 4000 mm)
O - bedrock, hardpan, wood, etc

Additional Benthos Comments

COMPOSITE PERIPHYTON SAMPLE

Sample ID	Composite Volume (mL)	Number of transects sampled (0-11):
800990	500	11

Assemblage ID (50-mL tube, preserved)		Chlorophyll (GF/F filter)		Biomass (GF/F Filter)	
Sample Vol. (mL)	Flag	Sample Vol. (mL)	Flag	Sample Vol. (mL)	Flag
50		25		25	

Flag	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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03/26/2001 2001 Sample Collection

Figure 11-3. Sample Collection Form (page 1), showing information for the reach-wide and targeted riffle benthic macroinvertebrate samples.

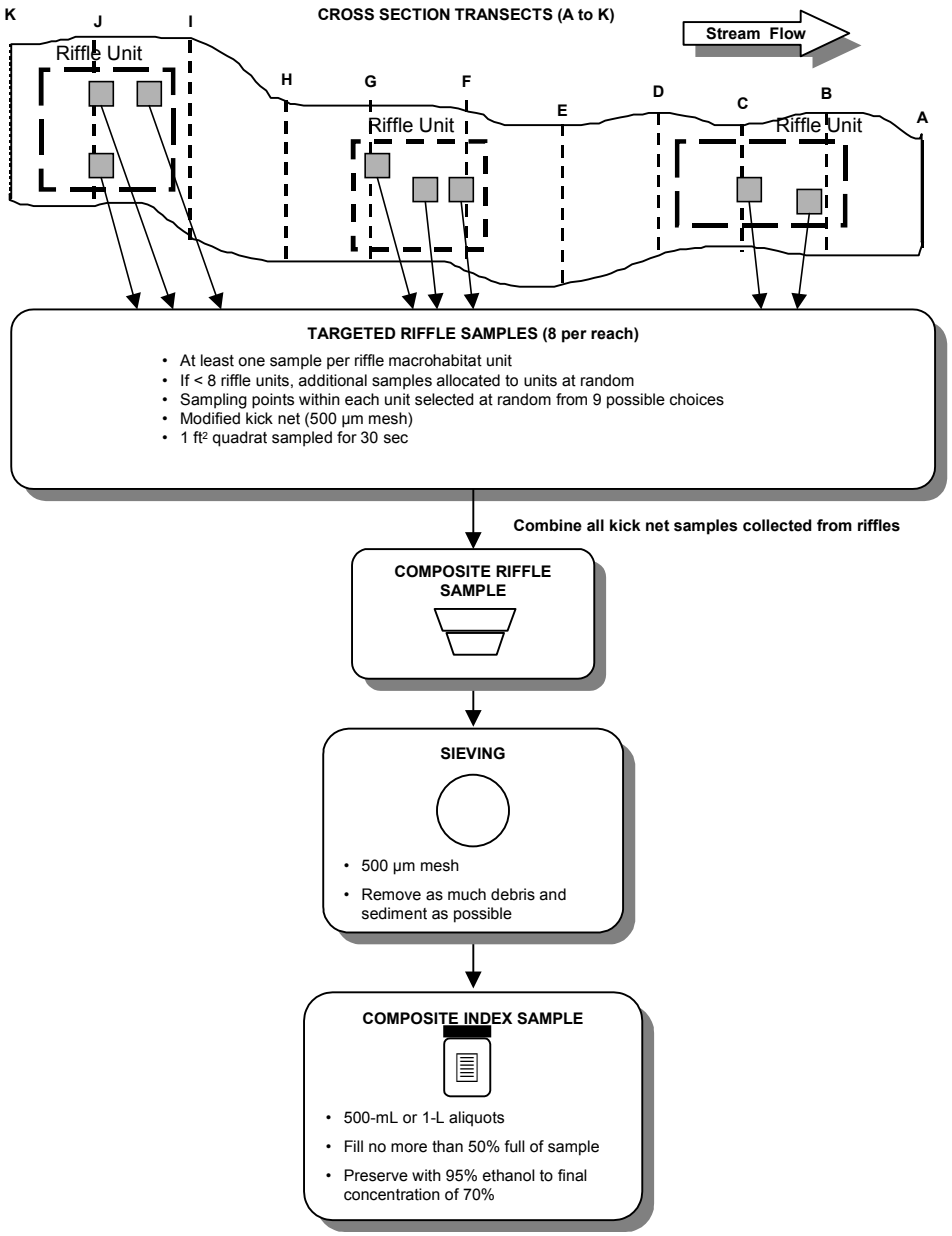

Figure 11-4. Index sampling design for benthic macroinvertebrate targeted riffle sample.

TABLE 11-3. LOCATING SAMPLING POINTS FOR KICK NET SAMPLES: TARGETED RIFFLE SAMPLE

1. Before sampling, survey the stream reach to estimate visually the total number (and area) of riffle macrohabitat "units" contained in the defined stream reach. To be considered as a unit, the area of the riffle must be greater than 1 ft^2.

   A. Do not sample poorly represented habitats. If the reach contains less than 8 ft^2 of riffle macrohabitat, then do not collect a targeted riffle sample.

   B. If the reach contains more than one distinct riffle macrohabitat units but less than eight, allocate the eight sampling points among the units so as to spread the effort throughout the reach as much as possible. You may need to collect more than one kick sample from a given riffle unit.

   C. If the number of riffle macrohabitat units is greater than eight, skip one or more habitat units at random as you work upstream, again attempting to spread the sampling points throughout the reach.

2. Begin sampling at the most downstream riffle unit, and sample units as they are encountered to minimize instream disturbance.

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 riffle macrohabitat unit. Define a core area for each riffle 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 at random from the following list of locations (right and left are determined as you look downstream):

   Lower right quadrat
   Lower center quadrat
   Lower left quadrat
   Right center quadrat
   Center quadrat
   Left center quadrat
   Upper right quadrat.
   Upper center quadrat.
   Upper left quadrat

5. Collect the kick sample in the center of the selected quadrat as described in Table 11-4.

6. If a second sample is required from a single macrohabitat unit, select additional quadrats at random from the list in Step 4.

TABLE 11-4. COLLECTING A KICK NET SAMPLE FROM WADEABLE STREAMS FOR THE TARGETED RIFFLE COMPOSITE SAMPLE

1. Beginning at the most downstream riffle unit within the sampling reach, locate the sampling point within the macrohabitat unit as described in Table 11-3.

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 (total area = 0.09 $m^{2)}$.

4 Hold the net in place with your knees and pick up any loose rocks or other larger substrate particles in the quadrat. Use your hands or a small scrub brush to dislodge organisms so that they are washed 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. After scrubbing, place the substrate particles outside of the quadrat.

~~5~~6. Keep holding the sampler securely in position. Start at the upstream end of the quadrat, vigorously kick the remaining finer substrate within quadrat for 30 seconds (use a stopwatch).

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 "TARGETED RIFFLE" and transfer the sample. Inspect the net for any residual organisms clinging to the net and deposit them into the "TARGETED RIFFLE" bucket. Use watchmakers' forceps if necessary to remove organisms from the net.

9. Record the nearest transect location in the box for the sample on the Sample Collection Form. Also note the dominant substrate type by checking the appropriate box on the Sample Collection Form.
   Fine/sand: not gritty (silt/clay/muck < 0.06 mm diam.) to gritty, up to ladybug sized (2 mm diam.)
   Gravel: fine to coarse gravel (ladybug to tennis ball sized; 92 mm to 64 mm diam.)
   Coarse: Cobble to boulder (tennis ball to car sized; 64 mm to 4000 mm)
   Other: bedrock (larger than car sized; > 4000 mm), hardpan (firm, consolidated fine substrate), wood of any size, aquatic vegetation, etc.). Note type of "other" substrate in comments on field form.

(continued)

TABLE 11-4. (Continued)

10. Thoroughly rinse the net before proceeding to the next sampling location (either the next riffle unit or a different quadrat location within the same riffle unit).

11. Repeat steps 1-10 at subsequent riffle sampling points until 8 kick samples have been collected and placed into the "TARGETED RIFFLE" bucket.

selecting sampling points within the available riffle macrohabitat units is to allocate points throughout the sampling reach as much as possible.

Procedures for collecting a point sample using the kick net from riffle macrohabitat units are presented in Table 11-4. At each sampling point, a quadrat having a total area of 0.09 m^2 (1 ft^2) is sampled. Because the reach-wide and targeted riffle samples are collected in the order they are encountered during a single pass through the reach, it is very important to rinse the kick net thoroughly between samples to avoid carryover and possible cross-contamination of the targeted riffle sample and the reach-wide sample.

11.2 SAMPLE PROCESSING

After collecting kick net samples for both the reach-wide and targeted riffle samples, prepare two composite index samples from the contents of the "REACH-WIDE" and "TARGETED RIFFLE" buckets as described in Table 11-5. Record tracking information for each composite sample on the Sample Collection Form as shown in Figure 11-3. A set of completed sample labels, including the label that is used if more than one jar is required for a single composite sample, is shown in Figure 11-5. Note that each composite sample has a different sample number (barcode). The ID number is also recorded on a waterproof label that is placed inside the jar (Figure 11-5, lower right). If more than one jar is used for a composite sample, a special label (Figure 11-5, lower left) is used to record the ID number assigned to the sample. DO NOT use two different barcode numbers on two jars containing one single sample. Blank labels for use inside of sample jars are presented in Figure 11-6. These can be copied onto waterproof paper.

Check to be sure that the prenumbered adhesive barcoded label is on the jar and covered with clear tape, and that the waterproof label is in the jar and filled in properly. Be sure the inside label and outside label describe the same sample. Replace the cap on each jar and seal them with plastic electrical tape. Check to make sure the cap is properly marked with site number, habitat type (reach-wide or targeted riffle). Place the samples in a cooler or other secure container for transporting and/or shipping the laboratory (see Section 3). The container and absorbent material should both be suitable for transporting ethanol. Check to see that all equipment is in the vehicle.

TABLE 11-5. PROCEDURE FOR PREPARING COMPOSITE SAMPLES FOR BENTHIC MACROINVERTEBRATES

1. Pour the entire contents of the "REACH-WIDE" bucket through a sieve with 500 µm mesh size). Remove any large objects and wash off any clinging organisms back into the sieve before discarding.

2. Using a wash bottle filled with stream water, rinse all the organisms from the bucket into the sieve. This is the composite reach-wide sample for the site.

3. Estimate the total volume of the sample in the sieve and determine how large a jar will be needed for the sample (500-mL or 1-L). Avoid using more than one jar for each of the composite samples.

4. Fill in a "REACH-WIDE" (or "TARGETED RIFFLE") sample label with the stream ID and date of collection. Attach the completed label to the jar and cover it with a strip of clear tape.

5. Wash the contents of the sieve to one side by gently agitating the sieve in the water. Wash the sample into a jar using as little water from the wash bottle as possible. Use a large-bore funnel if necessary. If the jar is too full pour off some water through the sieve until the jar is not more than ¼ full, or use a second jar if a larger one is not available. Carefully examine the sieve for any remaining organisms and use watchmakers' forceps to place them into the sample jar.

   - If a second jar is needed, fill in a sample label that does not have a pre-printed ID number on it. Record the ID number from the pre-printed label prepared in Step 4 in the "SAMPLE ID" field of the label. Attach the label to the second jar and cover it with a strip of clear tape.

6. Place a waterproof label with the following information inside each jar:

   - Stream Number
   - Type of sampler and mesh size used
   - Habitat type (riffle or pool)
   - Name of stream
   - Date of collection
   - Collectors initials
   - Number of transect samples composited

7. Completely fill the jar with 95% ethanol (no headspace) so that the final concentration of ethanol is between 75 and 90%. It is very important that sufficient ethanol be used, or the organisms will not be properly preserved.

   - NOTE: Prepared composite samples can be transported back to the vehicle before adding ethanol if necessary.

8. Replace the cap on each jar. Slowly tip the jar to a horizontal position, then gently rotate the jar to mix the preservative. Do not invert or shake the jar. After mixing, seal each jar with plastic tape.

9. Repeat Steps 1 through 8 for the "TARGETED RIFFLE" bucket.

10. Store labeled composite samples in a container with absorbent material that is suitable for use with 95% ethanol until transport or shipment to the laboratory.

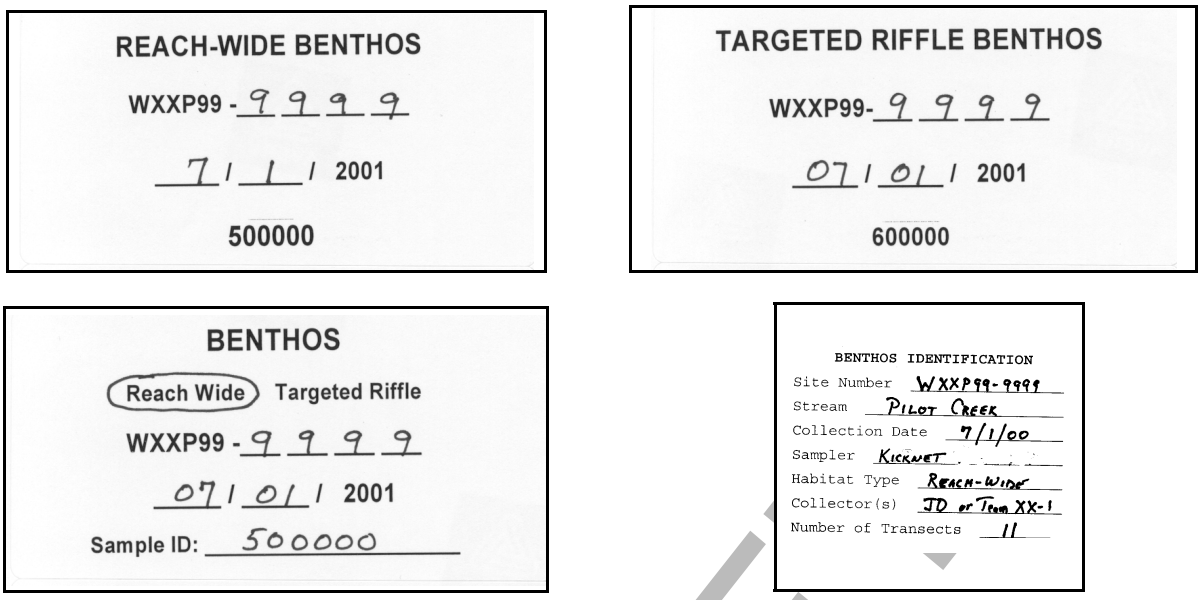

Figure 11-5. Completed labels for benthic macroinvertebrate samples. The label at lower left is used if more than one jar is required for a composite sample. The label at lower right is placed inside the sample container.

11.3 EQUIPMENT AND SUPPLY CHECKLIST

Figure 11-7 shows the checklist of equipment and supplies required to complete the collection of benthic macroinvertebrates from streams. This checklist is similar to the checklist presented in Appendix A, which is used at the base location (Section 3) to ensure that all of the required equipment is brought to the stream. Use this checklist to ensure that equipment and supplies are organized and available at the stream site in order to conduct the activities efficiently.

11.4 LITERATURE CITED

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish*. Second Edition. EPA/841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Assessment and Watershed Protection Division, Washington, D.C.

BENTHOS IDENTIFICATION

Site Number ________________

Stream ______________________

Collection Date _____________

Sampler _____________________

Habitat Type ________________

Collector(s) ________________

Number of Transects _________

BENTHOS IDENTIFICATION

Site Number ________________

Stream ______________________

Collection Date _____________

Sampler _____________________

Habitat Type ________________

Collector(s) ________________

Number of Transects _________

BENTHOS IDENTIFICATION

Site Number ________________

Stream ______________________

Collection Date _____________

Sampler _____________________

Habitat Type ________________

Collector(s) ________________

Number of Transects _________

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Site Number ________________

Stream ______________________

Collection Date _____________

Sampler _____________________

Habitat Type ________________

Collector(s) ________________

Number of Transects _________

BENTHOS IDENTIFICATION

Site Number ________________

Stream ______________________

Collection Date _____________

Sampler _____________________

Habitat Type ________________

Collector(s) ________________

Number of Transects _________

BENTHOS IDENTIFICATION

Site Number ________________

Stream ______________________

Collection Date _____________

Sampler _____________________

Habitat Type ________________

Collector(s) ________________

Number of Transects _________

Figure 11-6. Blank labels for benthic invertebrate samples.

EQUIPMENT AND SUPPLIES FOR BENTHIC MACROINVERTEBRATES

QTY.	ITEM	
1	Modified kick net (D-frame with 500 µm mesh) and 4-ft handle (Wildco #425-C50)	
	Spare net(s) and/or spare bucket assembly for end of net	
1	Watch with timer or a stopwatch	
2	Buckets, plastic, 8- to 10-qt capacity, labeled “REACH-WIDE” and “TARGETED RIFFLE”	
1	Sieve with 500 µm mesh openings	
1	Sieve-bottomed bucket, 500 µm mesh openings	
2 pr.	Watchmakers’ forceps	
1	Wash bottle, 1-L capacity labeled “STREAM WATER”	
1	Small spatula, spoon, or scoop to transfer sample	
1	Funnel, with large bore spout	
4 to 6 each	Sample jars, HDPE plastic with screw caps, 500-mL and 1-L capacity, suitable for use with ethanol	
2 gal	95% ethanol, in a proper container	
2 pr.	Rubber gloves, heavy rubber	
1	Cooler (with suitable absorbent material) for transporting ethanol and samples	
2	Composite Benthic sample labels, with preprinted ID numbers (barcodes)	
4	Composite Benthic sample labels without preprinted ID numbers	
6	Blank labels on waterproof paper for inside of jars	
1	Sample Collection Form for site	
	Soft (#2) lead pencils	
	Fine-tip indelible markers	
1 pkg.	Clear tape strips	
4 rolls	Plastic electrical tape	
1	Knife, pocket, with at least two blades	
1	Scissors	
1	Pocket-sized field notebook (optional)	
1 pkg.	Kim wipes in small self-sealing plastic bag	
1 copy	Field operations and methods manual	
1 set	Laminated sheets of procedure tables and/or quick reference guides for benthic macroinvertebrates	

Figure 11-7. Equipment and supply checklist for benthic macroinvertebrates.

Cuffney, T.F, M.E. Gurtz, and M.R. Meador. 1993. *Methods for Collecting Benthic Invertebrate Samples as Part of the National Water-Quality Assessment Program.* U.S. Geological Survey Open-File Report 93-406, Raleigh, North Carolina.

Klemm, D.J., P.A. Lewis, F. Fulk, and J.M. Lazorchak. 1990. *Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters.* EPA/600/4-90/030. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.

Klemm, D.J., J.M. Lazorchak, and P.A. Lewis. 1998. Benthic Macroinvertebrates. pp. 147-182 IN: J.M. Lazorchak, D.J. Klemm, and D.V. Peck (Eds.). *Environmental Monitoring and Assessment Program-Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams*. EPA/620/R-94/004F. U.S. Environmental Protection Agency, Washington, D.C.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish.* EPA/440/4-89/001. U.S. Environmental Protection Agency, Assessment and Watershed Protection Division, Washington, D.C.

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