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ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM-SURFACE WATERS:

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

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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) and adopted for use by many states. The two man kick net procedure of the Rapid Bioassessment Protocol (RBP) is replaced in the EMAP-SW protocol with a kick net modified for use by one person (Figure 11-1), as is used by the U.S. Geological Survey for their National Water-Quality Assessment Program (NAWQA; Cuffney et al., 1993). This protocol 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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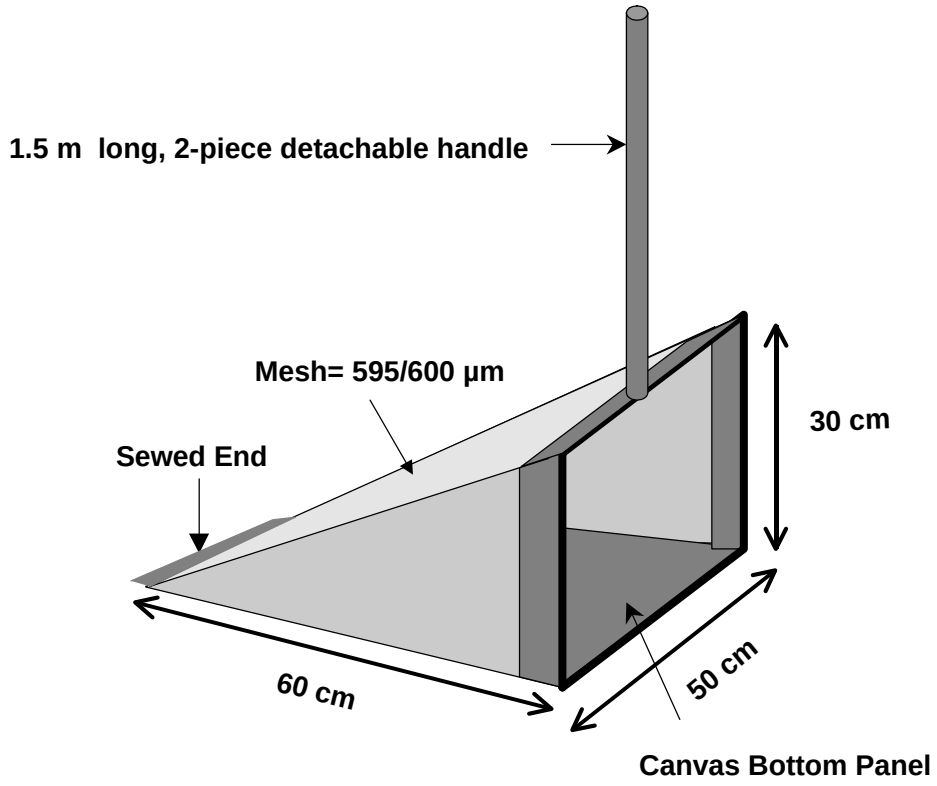

Figure 11-1. Modified kick net. (Not drawn to scale.)

The “biomorphs” (refer to Figure 2-1) collect kick net samples for benthic macro-invertebrate at sampling points located on each cross-section transect. Kick net samples are collected at the same time as periphyton samples (Section 8) and sediment samples (Section 9). Kick net samples collected from flowing water habitats (e.g., riffles, runs) are combined into a single composite sample for the stream reach. Kick net samples collected from pool habitats are combined into a separate composite sample.

11.1 SAMPLE COLLECTION

The index sample design for benthic macroinvertebrates is shown in Figure 11-2. This design is used in the EMAP and R-EMAP stream studies in the mid-Atlantic region (refer to Section 1 for project descriptions). A modified index sample design was developed and implemented in some studies conducted in the western U.S. In the modified design, an equal number of kick net samples are collected from available riffle and pool habitats located within the sampling reach. This modified index sampling design is described in more detail in Appendix E.

A kick net sample is collected from each of the nine interior cross-section transects (Transects "B" through "J") at an assigned sampling point (Left, Center, or Right). These 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 "B" 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".

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. To collect a kick net sample from a sampling point classified as "riffle/run" habitat, follow the procedure presented in Table 11-1. To collect a kick net sample from a sampling point classified as a "pool/glide" habitat, follow the procedure presented in Table 11-2. Record the habitat type and sampling point 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 collected from "riffle/run" habitats into a bucket or similar container labeled "RIFFLE". Combine kick net samples collected from "pool/glide" habitats into a second bucket labeled "POOL". Fill in the checklist shown in Figure 11-4 as individual activities are completed.

If it is impossible to sample at the sampling point with the modified kick net following either procedure, spend about 60 seconds hand picking a sample from about 0.25 m^2 of

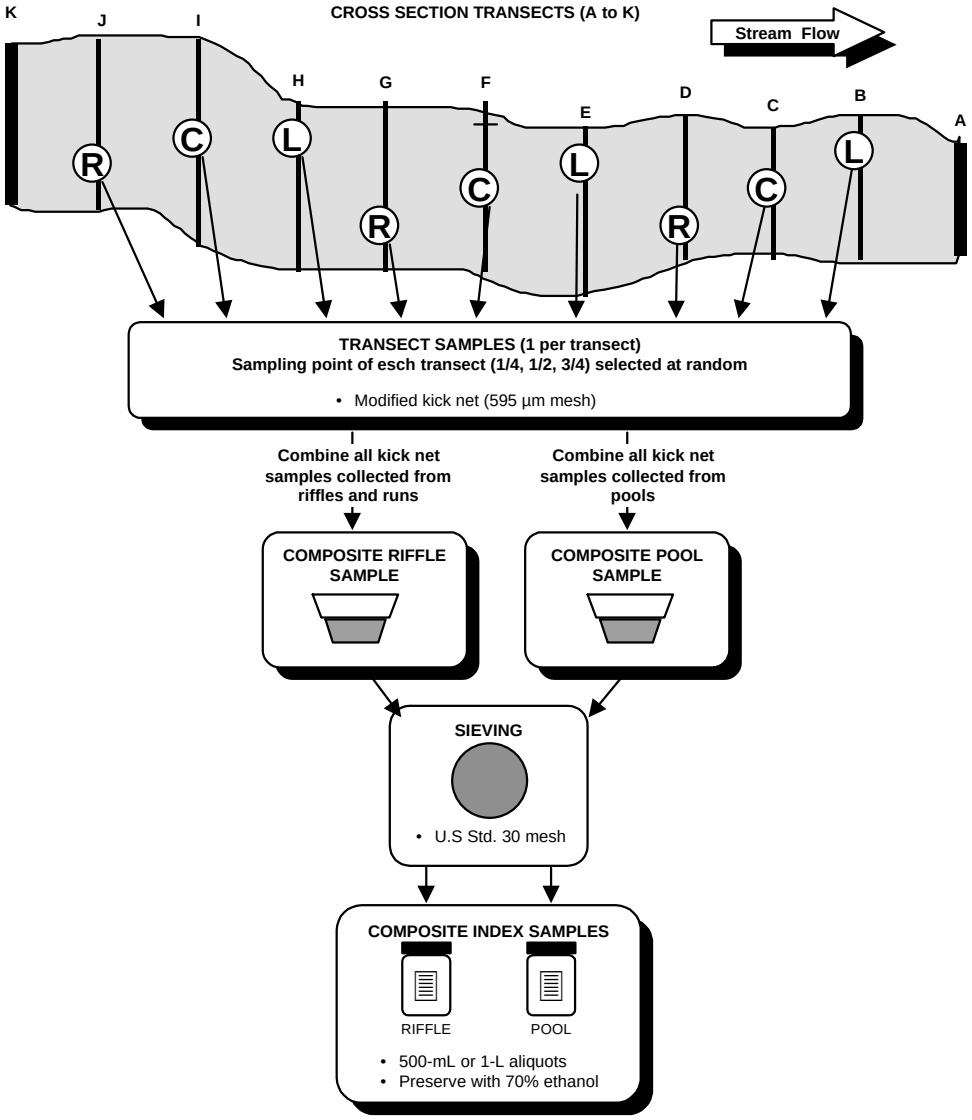

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Figure 11-2. Index sampling design for benthic macroinvertebrates.

TABLE 11-1. PROCEDURE TO COLLECT KICK NET SAMPLES FROM RIFFLE AND RUN HABITATS

1. At each cross-section transect, beginning with Transect "B", locate the assigned sampling point (Left, Center, or Right as you face downstream) as 25%, 50%, and 75% of the wetted width, respectively.

   If the sampling points were not assigned previously when laying out the sampling reach, proceed to Transect "B". Roll a die to determine if it is a left (L), center (C), or right (R) sampling point for collecting periphyton and benthic macroinvertebrate samples. A roll of 1 or 2 indicates L, 3 or 4 indicates C, and 5 or 6 indicates R (or use a digital wristwatch and glance at the last digit (1-3=L, 4-6=C, 7-9=R). Mark L, C, or R on the transect flagging. Assign sampling points at each successive transect in order as L, C, R after the first random selection.

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 3. If not, use the sampling procedure described for "pool/glide" habitats.

3. 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 "RIFFLE" which is half-full of water.

4. Holding the net in position on the substrate, visually define a rectangular quadrat that is one net width wide and two net widths long upstream of the net opening. The area within this quadrat is $\sim 0.5\ m^2$.

5. 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.

6. Hold the net securely in position while kicking the substrate within the quadrat vigorously for 20 seconds (use a stopwatch).

7. After 20 seconds, hold the net in place with your knees and pick up any loose rocks within the quadrat. Use your hands to rub any clinging organisms off the rocks (especially those covered with algae or other debris) in front of the net. Also, place any additional mussels and snails found into the net. Remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net.

(continued)

TABLE 11-1. (Continued)

8. Invert the net into a bucket labeled “RIFFLE”, which is about half full of water, to rinse organisms out of the net. Inspect the net for clinging organisms. Use watchmakers’ forceps to remove any organisms from the net and place them in the bucket. 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.

9. Place an “X” in the appropriate habitat type and sampling point boxes for the transect on the Sample Collection Form.

10. Proceed upstream to the next transect and repeat Steps 1 through 9. Combine all kick net samples from “riffle/run” habitats into the “RIFFLE” bucket.

TABLE 11-2. PROCEDURE TO COLLECT KICK NET SAMPLES FROM POOL AND GLIDE HABITATS

1. At each cross-section transect, beginning with Transect "B", locate the assigned sampling point (Left, Center, or Right as you face downstream) as 25%, 50%, and 75% of the wetted width, respectively.

   If the sampling points were not assigned previously when laying out the sampling reach, proceed to Transect "B". Roll a die to determine if it is a left (L), center (C), or right (R) sampling point for collecting periphyton and benthic macroinvertebrate samples. A roll of 1 or 2 indicates L, 3 or 4 indicates C, and 5 or 6 indicates R (or use a digital wristwatch and glance at the last digit (1-3=L, 4-6=C, 7-9=R). Mark L, C, or R on the transect flagging. Assign sampling points at each successive transect in order as L, C, R after the first random selection.

2. Attach the 4-ft handle to the kick net. Make sure that the handle is on tight or the net may become twisted during the collection process, 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, use the sampling procedure described for "Riffle/run" habitats. If not, classify the habitat as "Pool/glide" and proceed to Step 4. NOTE: If the pool is too deep (much more than 1 m) to sample safely at the designated spot, move downstream till a safe sampling spot is found.

4. Visually define a rectangular quadrat that is one net width wide and two net widths long at the sampling point. The area within this quadrat is ~0.5 m^2.

4. 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 "POOL".

5. Vigorously kick the 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 20 seconds. NOTE: If there is too little water to use the kick net, stir up the substrate with your gloved hands and use the U.S. Standard #30 sieve to collect the organisms from the water in the same way the net is used in larger pools.

6. After 20 seconds, hold the net between your legs and partially submerged. Pick up any loose rocks within the quadrat. Rub or brush any organisms found on them into the net. Also recheck the area for any additional snails or clams and place them in the net.

7. Invert the net into a bucket labeled "POOL", which is about half full of water, to rinse organisms out of the net. Inspect the net for clinging organisms. Use watchmakers' forceps to remove any organisms from the net and place them in the bucket. 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.

8. Place an "X" in the appropriate habitat type and sampling point boxes for the transect on the Sample Collection Form.

9. Proceed upstream to the next transect and repeat Steps 1 through 8. Combine all kick net samples from "pool/glide" habitats into the "POOL" bucket.

Reviewed by (initial): DP

SAMPLE COLLECTION FORM - STREAMS

SITE NAME: MILL CREEK DATE: 7/15/97 VISIT: ☒1 ☐2 ___

SITE ID: MAIA97-999 TEAM ID (X): ☒1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8

COMPOSITE BENTHOS SAMPLES

SAMPLE ID (BARCODE)	HABITAT (X ONE) R	HABITAT (X ONE) P	NO. OF JARS	FLAG	COMMENTS
229001	X		2		
229002		X	1		

STATION	A	B	C	D	E	F	G	H	I	J	K
RIFFLE 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 ☐C ☒R	☒L ☐C ☐R	☐L ☒C ☐R	☐L ☐C ☒R	☒L ☐C ☐R	☐L ☒C ☐R	☐L ☐C ☒R	☒L ☐C ☐R	☐L ☒C ☐R	

COMPOSITE PERIPHYTON SAMPLES

HABITAT TYPE (X) → ☒ RIFFLE ☐ POOL ☐ OTHER

SAMPLE ID (BARCODE) → 229004 COMPOSITE VOLUME → 200 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.
50 mL	25 mL	999	25 mL	50 mL

COMPOSITE PERIPHYTON SAMPLES

HABITAT TYPE (X) → ☐ RIFFLE ☒ POOL ☐ OTHER

SAMPLE ID (BARCODE) → 229003 COMPOSITE VOLUME → 300 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.
50 mL	25 mL	1001	25 mL	50 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.

Rev. 06/02/97 (st_saco.97) SAMPLE COLLECTION FORM - STREAMS - 1

Figure 11-3. Sample Collection Form (page 1), showing information for benthic macroinvertebrate samples.

MACROINVERTEBRATE SAMPLING ACTIVITIES CHECKLIST		
Date:	**Time:**	**Site No.:**
Stream Name and Location:		
Crew ID: 1 2 3 4 5 6	**Collector:**	
1.	Initial observations, if any, on the Sample Collection Form - Streams.	
2.	Composite riffle/run sample collected with a label inside the jar.	
3.	Composite pool/glide sample collected with a label inside the jar.	
4.	Correct barcode and label on all jars and sealed with clear, waterproof tape.	
5.	All samples preserved.	
6.	With a grease pencil write site number, sample type (Riffle or Pool), and number of transects sampled for sample type on the cap. If two jars are used be sure to mark them as such.	
7.	Caps are sealed with plastic electrical tape.	
8.	Photos of the site.	
9.	Sample jars in cooler or otherwise secured.	
10.	All equipment accounted for and secured in the vehicle.	
Signature:		**Time sampling completed:**

Figure 11-4. Checklist for benthic macroinvertebrate sampling activities.

substrate at the sampling point. Place the contents of this hand-picked sample into either the "RIFFLE" or "POOL" bucket.

11.2 SAMPLE PROCESSING

After collecting kick net samples from all transects, prepare two composite index samples from the contents of the “RIFFLE” and “POOL” buckets as described in Table 11-3. 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.

Complete the check-off sheet (Figure 11-4). 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 (pool or riffle), and number of transects sampled. Record any additional pertinent information in the “Field Notes” section of the checklist. 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.

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.

TABLE 11-3. PROCEDURE FOR PREPARING COMPOSITE SAMPLES FOR BENTHIC MACROINVERTEBRATES

1. Pour the entire contents of the "RIFFLE" bucket through a U.S. Standard #30 sieve (or sieve-bottomed bucket with 595 :m 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 sample for that habitat (riffle or pool) 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 (half gallon or gallon). Do not use more than one jar for each of the samples unless it cannot be avoided.

4. Fill in a "Composite Benthos" sample label with the stream ID and date of collection. Circle the habitat type (Riffle or Pool). Attach the completed label to the jar and cover it with a strip of clear tape.

4. 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 barcode number on it. Record the barcode number from the pre-printed label prepared in Step 4 in the "BARCODE" field of the label. Attach the label to the second jar and cover it with a strip of clear tape.

5. Add 95% ethanol to each jar so that the final concentration of ethanol is at least 70%. If there is a small amount of water in the sample, it may not be necessary to fill the jar entirely full to reach a 70% concentration. It is very important that sufficient ethanol be used to reach a 70% concentration. Otherwise, the organisms will not be properly preserved.

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

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. Replace the cap on each jar. Seal each jar with plastic tape. Use a grease pencil to write the site number, sample type (Riffle or Pool), and number of transects on the cap of each jar.

8. Repeat Steps 1 through 7 for the "POOL" bucket.

9. Store labeled composite samples in a container with absorbent material that is suitable for use with 70% ethanol until transport or shipment to the laboratory.

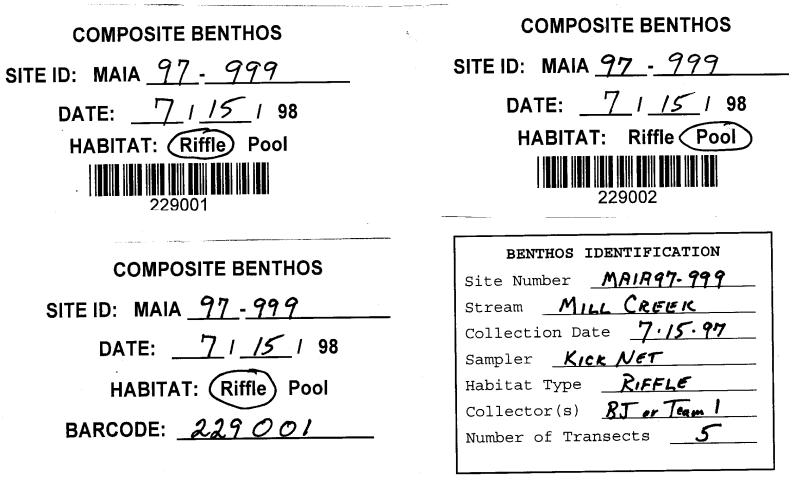

Figure 11-5. Completed labels for benthic macroinvertebrate samples. The label at lower left is used if more than one jar is required for a composite sample. The label at lower right is placed inside the sample container.

11.4 LITERATURE CITED

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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.

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.

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 (closed bag with 595/600 :m mesh) and 4-ft handle (Wildco #425-C50)	
	Spare net(s) for the kick net sampler or extra sampler	
1	Watch with timer or a stopwatch	
2	Buckets, plastic, 8- to 10-qt capacity, labeled “RIFFLE” and “POOL”	
1	Sieve, U.S. Standard 30	
1	Sieve-bottomed bucket, 595- :m 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, plastic with screw caps, ½ and 1 gallon 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	
1	Field check list sheet	
	Soft (#2) lead pencils	
	Fine-tip indelible markers	
	Grease pencils	
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.