Section 9
Benthic Macroinvertebrates

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Benthic macroinvertebrates inhabit the sediment or live on the bottom substrates of lakes, streams, and rivers. The macroinvertebrate assemblages in rivers reflect the overall biological integrity of the benthic community such that monitoring these assemblages is useful in assessing the status of the water body and monitoring trends. Benthic communities respond differently to a wide array of stressors. As a result of this, it is often possible to determine the type of stress that has affected a benthic macroinvertebrate community (Plafkin et al., 1989; Klemm et al., 1990; Barbour et al., 1999). Because many macroinvertebrates have relatively long life cycles of a year or more and are relatively immobile, macroinvertebrate community structure is a function of past environmental conditions.

EMAP scientists are currently evaluating two different approaches to developing ecological indicators based on benthic invertebrate assemblages. The first is a multimetric approach, where different structural and functional attributes of the assemblage are characterized as "metrics". Individual metrics that respond to different types of stressors are scored against expectations under conditions of minimal human disturbance. The individual metric scores are then summed into an overall index value that is used to judge the overall level of impairment of an individual stream reach. Examples of multimetric indices based on benthic invertebrate assemblages include Kerans and Karr (1994), Fore et al. (1996), Barbour et al. (1995; 1996), and Karr and Chu (1999).

The second approach being investigated is to develop indicators of condition based on multivariate analysis of benthic assemblages and associated abiotic variables. Examples of this type of approach as applied to benthic invertebrate assemblages include RIVPACS (Wright 1995), and BEAST (Reynoldson et al., 1995). Rosenberg and Resh (1993) present various approaches to biological monitoring using benthic invertebrates, and Norris (1995) briefly summarizes and discusses approaches to ana-

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lyzing benthic macroinvertebrate community data.

Field procedures for collecting and processing benthic invertebrate samples from non-wadeable streams are presented in Section 9.1. These procedures are based upon draft procedures developed for the Mid-Atlantic Integrated Assessment (MAIA) study conducted in the eastern U.S. Section 9.2 contains an equipment and supply checklist for benthic invertebrate sampling.

9.1 Sampling Procedures for Non-wadeable Streams

The length of river reach established for larger non-wadeable streams and rivers is much larger than for wadeable streams, making a visual estimate of the number of riffle and pool macrohabitat units impossible. In addition, mid channel depths of larger streams and rivers will make it impractical to collect kick net samples from mid-river habitats. In non-wadeable streams and rivers, samples are collected at each of eleven transects established for physical habitat characterization. At each transect, two kick net samples are obtained from shallow area (< 1m) near the bank of the river. Kick net samples collected from each transect are composited into a single sample for the river; samples collected from different macrohabitat types are not composited separately. A kick net modified for use by one person is shown in Figure 9-1. In addition to the mesh size used in the two EMAP studies other mesh sizes such as 250 - 800 m can be used depending upon the objectives of

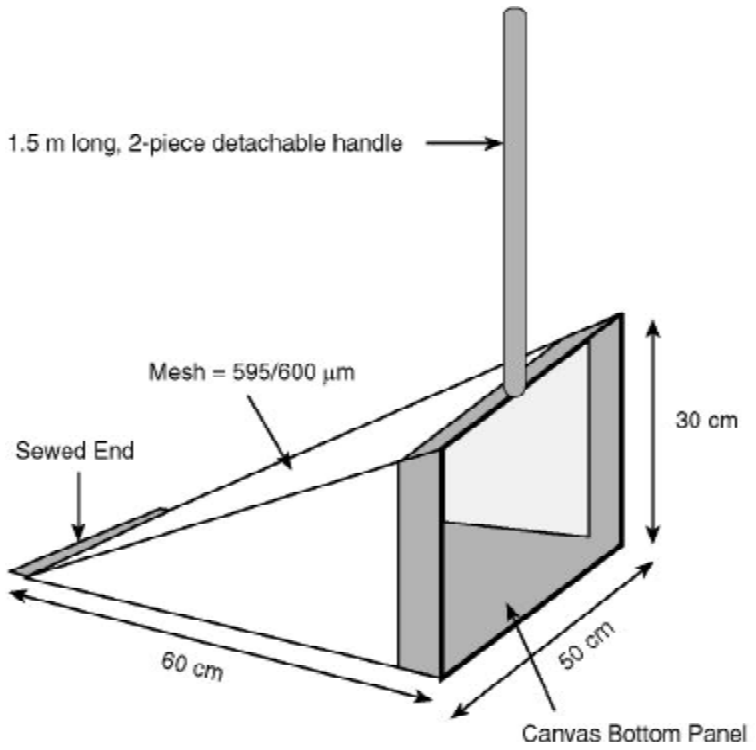


Figure 9-1. Modified kick net. (Not drawn to scale.).

the program and potential for clogging. For deep rivers that are extremely difficult or hazardous to obtain benthic samples with a kick net, a ponar or core grab sample could be used instead.

In addition, two daytime drift net samples are collected from as near to the downstream end of the defined reach as is practical. The drift net assemblies are positioned when the crew drops off a vehicle at the take-out point, and are retrieved when the crew reaches the sampling point in the boat.

9.1.1 Sample Collection Using Kick Nets

9.1.1.1 Selection of Sampling Points

Samples are collected from non-wadeable streams during a downstream traverse of the sample reach. At each transect location, locate a suitable sampling point on the same side of the river as fish sampling is conducted (Section 10). Locate the sampling point in an area away from the river margin, but at a depth less than or equal to 1 m.

9.1.1.2 Sample Collection

At each sampling point, obtain TWO kick net samples using the procedures presented in Table 9-1 (if the sampling point is located in a riffle or glide macrohabitat) or Table 9-2 (if the sampling point is located in a pool macrohabitat). If there is insufficient flow to sample a transect with the modified kick net following this protocol, spend about 60 seconds hand picking a sample from approximately 0.25 m^2 of substrate at the station and combine it with samples from other transects in the bucket. If there is too little water to collect the sample with the kick net, randomly pick up 10 rocks, and pick and wash the organisms off of them into the bucket. Keep a note of this on the field sheets and in all databases generated from sites where more than one transect has to be sampled in this manner. Results may show a bias due to the larger organisms picked in this approach.

9.1.2 Sample Processing: Kick Net Samples

After all transects have been sampled, the composite sample is processed as described in Table 9-3. Sample labels to put on and in the jar are shown in Figures 9-2 and 9.5, respectively, and the sample collection form is shown in Figure 9-4. Ensure the sample is preserved and that the jar is completely sealed. Place the sealed sample jar upright in a cooler or plastic bucket for transport. Blank labels for use inside of sample jars are presented in Figure 9-5. These can be copied onto waterproof paper.

9.1.3 Description of Drift Nets and Habitat Sampled

Drift nets are stationary nets designed to sample organisms from flowing waters such as streams and rivers. The drift net sampler is designed to obtain qualitative and quantitative samples of macroinvertebrates which either actively or passively enter the water column from all types of substrates in flowing water with a velocity of not less than 0.05 m/s. They can be used to capture organisms at and below the surface of the water. Drift nets can be used individually or in groups with nets strung out side by side or arranged vertically.

Table 9-1. Collecting Kick Net Samples From Non-wadeable Streams: Riffle/Run Macrohabitats.

1. Attach the four foot handle to the kick net. Care should be exercised to be sure the handle is on tight or the net might become twisted in strong current or while dragging it through the water causing the loss of part of the sample.
2. Position the sampler quickly and securely on the river bottom with the net opening upstream so as to eliminate gaps under the frame. Reposition the sampling point to avoid large rocks that prevent the sampler from seating properly.
3. The sampling area (or quadrate) has a width and length equal to the width of the net frame (0.5 m) or a total area = 0.25 m 2.
4. Hold the sampler in position on the substrate and check the quadrat directly in front of the net for heavy organisms, such as mussels and snails. Place these organisms into the net.
5. Continue to hold the sampler securely while vigorously kicking the substrate within the quadrat for 20 seconds (use stopwatch).
6. After 20 seconds, hold the net in place with the knees. Pick up any loose rocks in the quadrat and scrub off organisms in front of the net. Place any additional mussels and snails found in the quadrat in the net.
7. Remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net. Immerse the net several times to remove fine sediments and to concentrate organisms at the end of the net. Avoid having any water or additional material enter the mouth of the net during this operation.
8. Transfer the contents of the net into a plastic bucket half filled with water by inverting the net into the bucket.
9. Inspect the net for clinging organisms. Use forceps and remove any organisms found and place them into the bucket.
10. Carefully inspect large objects (rocks, sticks, leaves, etc.) in the bucket. Wash off any organisms, then discard the objects. Remove as much detritus, sediment, and debris as possible without losing any organisms.
11. See Table 9.3 for processing kick net samples.

The drift net consists of a bag of nylon or nylon monofilament frame. The standard drift net is approximately 1 m (39.3") long and has a closed end. The drift net open end is 30.48 cm (12") x 45.72 cm (18"). The net frame is made of stainless steel rods or PVC pipe. The frame of the drift net is anchored into the river bed by a pair of steel rods, 15.46 cm (18") long or can be attached to a "floating drift assembly" device (Figure 9-3), Wildlife Supply Co., 1999-2000. Drift net frames can also be fitted anteriorly with a mouth reducing rectangular plexiglass enclosure (Rutter and Ettinger, 1977; Wefring, 1976) to increase filtration efficiency. For EMAP, MAIA sampling in Regions 2, 3, and 4 rivers, a drift net with 600 m mesh openings has been used in conjunction with the floating drift assembly device (other mesh sizes such as 250 - 800 m can be used depending upon the objectives of the program and potential for clogging).

The drift collection usually represents a wide spectrum of the habitats found in a river. Drift nets are effective for the collection of emigrating and dislodged benthic macroin-vertebrates drifting in the water column of flowing streams and rivers. Sampling efficiency of this gear is a function of current velocity and sampling period. Data collected can be used to estimate macroinvertebrate drift densities and rates (individuals per unit volume of water per unit time passing through the net). However, this requires an estimate of the volume of water passing through the sampling nets. This is accomplished by averaging repeated measures of the water velocity at the mouth of the drift net and recording the total time the drift net is set in the

Table 9-2. Collecting Kick Net Samples From Non-wadeable Stream: Pool\Glide Macrohabitats.

1. Attach the four foot handle to the kick net. Care should be exercised to be sure the handle is on tight or the net might become twisted in strong current or while dragging it through the water causing the loss of part of the sample.
2. The sampling area (or quadrate) has a width and length equal to the width of the frame (0.5 m) or a total area = 0.25 m 2.
3. Inspect the river bottom within the quadrat for any heavy organisms, such as mussels and snails. Remove and place these organisms into the net.
4. Disturb the substrate within the quadrat by kicking vigorously with the feet. Drag the net repeatedly and continuously through the disturbed area just above the bottom whole continuing to kick for 20 seconds (use a stopwatch). Keep moving the net so that the organisms trapped in the net will not escape.
5. Remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net. Immerse the net several times to remove fine sediments and to concentrate organisms at the end of the net. Avoid having any water or additional material enter the mouth of the net during this operation.
6. Hold the net so that the mouth is out of the water and the net is partially submerged. Pick up any loose rocks in the quadrat and rub or brush any organisms found on them into the net. Also recheck the quadrat for any additional snails or clams and place them in the net.
7. Transfer the contents of the net into a bucket half filled with water by inverting the net into the bucket.
8. Inspect the net for clinging organisms. Use forceps and remove any organisms found and place them in the bucket.
9. Carefully inspect large objects (rocks, sticks, leaves, etc.) in the bucket. Wash any organisms found into the bucket, then discard the objects. Remove as much detritus, sediment, and debris as possible without losing any organisms.
10. See Table 9.3 for processing kick net samples.

water column. Repeated measures of the water velocity are most representative of the sample period if they are taken when the nets are first set and just prior to removal of the net from the system.

Limitations and hazards of daytime drift net sampling include:

- Unknown where organisms come from; terrestrial species may make up part of sample in summer and periods of wind and rain. It is not the ideal time of day for sampling drifting benthic macroinvertebrates.
- Installing and retrieving drifts from areas of swift current can be hazardous. Placing nets in highly visible areas may result in tampering or theft. Boats and skiers may damage drift net assemblies.

9.1.3.1 Drift Net Sampling Procedures

For EMAP, MAIA pilot studies of Regions 2, 3, and 4 rivers, install two drift nets at transect K (See Table 9-4), one about 25 cm from the bottom substrate and one about 10 cm below the surface in water not exceeding 3 m in depth, using cable and anchor attached to a "floating drift assembly" device. The installation procedure for drift nets is presented in Table 9-4. Install the net in an area of river that is receiving part of the main channel flow, but that can be safely accessed by wading. A location that you would consider to provide a representative water chemistry sample is probably also suitable for positioning a drift net. Do not use drift nets if the current velocity at the sampling point is less than 0.05 m/s or more than same rate.

Table 9-3. Processing Kick Net Samples: Non-wadeable Streams.

1. Fill out a sample label for the composite samples. Attach the label to a 500-mL (or 1-L) plastic jar. If the sample contains a large volume of material, complete a sample label for additional containers and attach them. Make sure the barcode numbers on each label are identical.
2. Hand-pick large organisms from the bucket containing the composite sample and place them into the appropriately labeled jar.
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 a sieve (600 m) mesh, and then transfer into the labeled jar. NOTE: Do not fill the jar more than ½ full of material. If necessary, use a larger jar and/or distribute the sample among two or more labeled jars. Rinse residue from the bucket into the jar using a wash bottle and a small volume of water.
5. Add 95% ethanol to each labeled 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.
6. Place the waterproof label with the following information inside each jar:
   - Stream Number
   - Type of sampler and mesh size used
   - Habitat type (riffle/run, pool/glide)
   - Name of stream
   - Date of collection
   - Collectors initials
   - Number of transects composited
7. Rinse the bucket well to eliminate any residue.
8. Complete the Sample Collection Form and on the jars (1 of 2, 2 of 2, etc). Record the barcode number of the composite sample, and the habitat type (shore). If more than one container was required for a sample, record the number of containers on the collection form. Replace the lid on the jar. Seal the container lid(s) with plastic or electrician's tape. Also note any peculiarities associated with a particular samples by using a flag code and/or a written comment on the collection form.

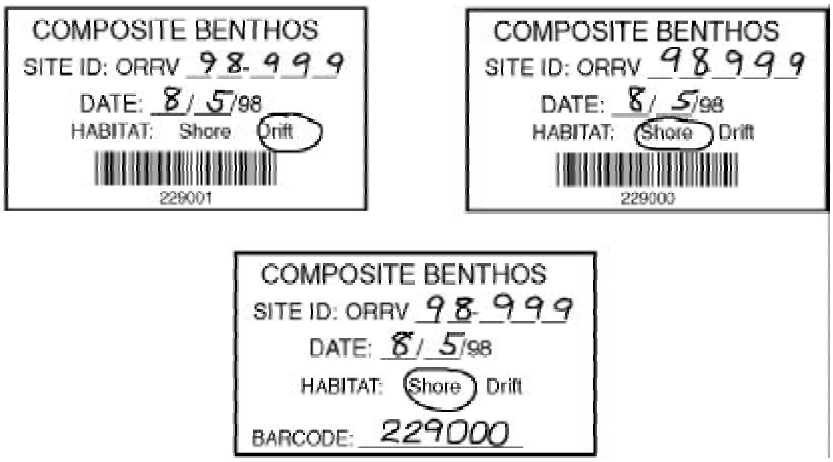


Figure 9-2. Completed labels for benthic macroinvertebrate samples. The bottom label is used if more than one jar is required for a composite sample.

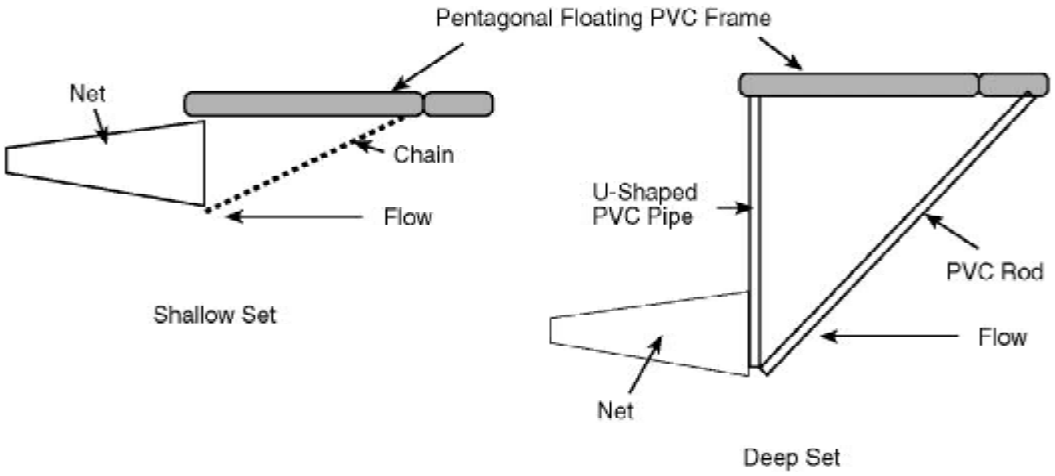


Figure 9-3. Shallow and deep set drift net assemblies. (Not drawn to scale).

Measure the current velocity at the entrance to each net at the time the net is installed and again when it is retrieved. Velocity is determined by timing a neutrally buoyant object over a known distance or using a flow meter.

9.1.3.2 Processing and Preservation of Drift Samples

After retrieving the drift nets from the stream or river, process the sample as described in Table 9-5. Sample labels are shown in Figure 9-2, and the sample collection form is shown in Figure 9-4. Note that the material from the two nets is combined to yield a single composite sample of drift for the stream or river. Blank labels for use inside of sample jars are presented in Figure 9-5. These can be copied onto water-proof paper.

9.1.3.3 Maintenance of the Drift Nets

After the drift sample has been processed and preserved, thoroughly wash the drift nets with water from the stream or river to remove all debris, etc.

9.2 Equipment Checklist

A list of all equipment and supplies required to conduct benthic invertebrate sampling is presented in Figure 9-6.

Reviewed by (initial) DKA

SAMPLE COLLECTION FORM - RIVERS

SITE NAME: BEAVER RIVER DATE: 8 / 5 / 98 VISIT ☐0 ☒1 ☐2 ☐3

SITE ID: ORRV 98-999 TEAM ID (X): ☒1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8

COMPOSITE BENTHOS SAMPLES

BANK SAMPLED (LOOKING DOWNSTREAM): ☒LEFT ☐RIGHT

S = SHORE M = MID-CHANNEL D = DRIFT

SAMPLED (BARCODE)	HABITAT (CIRCLE ONE)	NO. OF JARS	FLAG	COMMENTS
243612	S M (D)	1		RIGHT BANK - SAMPLED AT LAUNCH
243611	(S) M D	1		NO SHORE KICK AT TRANSECTS
	S M D			A,G,K => TOO DEEP

STATION (CIRCLE IF DONE)	A	B	C	D	E	F	G	H	I	J
SHORE KICK	S	(S)	(S)	(S)	(S)	(S)	S	(S)	(S)	(S)
MID-CHANNEL	M	M	M	M	M	M	M	M	M	M

DRIFT NET	TRANSECT	TIME (24-HR CLOCK) START	TIME (24-HR CLOCK) END	VELOCITY (FT/SEC) XX.XX
	☒A ☐B ☐C ☐D ☐E ☐F ☐G ☐H ☐I ☐J ☐	09 : 40	19 : 45	3.0

COMPOSITE PERIPHYTON SAMPLES

SAMPLE ID (BARCODE) ➤ 244013 COMPOSITE VOLUME xxxx mL ➤ 325

ASSEMBLAGE ID (50-mL TUBE)	CHLOROPHYLL (GF/F FILTER	BIOMASS (TARED FILTER)		APA SAMPLE (50-mL TUBE)
SUB. SAMPLE VOL. xx mL	VOL. FILTERED xxxx mL	FILTER NO.	VOL. FILTERED xxxx mL	SUB. SAMPLE VOL. xxxx mL
50	25		25	50

COMMENTS:

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

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SAMPLE COLLECTION FORM - RIVERS - 1

Figure 9-4. Sample Collection Form (page 1), showing information for benthic macroinvertebrate samples.

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 _____	**BENTHOS IDENTIFICATION** Site Number _____ Stream _____ Collection Date _____ Sampler _____ Habitat Type _____ Collector(s) _____ Number of Transects _____

Figure 9-5. Blank labels for benthic invertebrate samples.

Table 9-4. Collection Procedures for Drift Net Samples: Non-wadeable Streams.

NOTE: Do not use drift nets for large rivers with currents less than 0.05 m/s.

Installation and Retrieval of Drift Nets:

1. Ideally, the net should be installed at the downriver end of the designated sampling reach (transect K in non-wadeable streams). In practice, the net is installed at either the takeout point (1st choice) or put-in point, whichever is located closer to the designated sampling reach. Mark the nearest transect on the Sample Collection Form and note if the drift net location is outside of the designated sampling reach in the Comments section of the collection form.
2. Locate the drift net assembly in an area receiving the main flow of the river (i.e., avoid backwaters, river margins, eddies, etc.)
3. Anchor the net assembly using anchors and cables.
4. Record the START TIME of sampling on the Sample Collection Form.
5. Measure the current velocity at the entrance of the net, using the neutrally buoyant object technique (or a flow meter) as follows:
    A. Measure out a straight segment of the river reach just upstream of the drift net location in which an object can float relatively freely and passes through within about 10 to 30 seconds.
    B. Select an object that is neutrally buoyant, like a small rubber ball or an orange; it must float, but very low in the water. The object should be small enough that it does not "run aground" or drag bottom.
    C. Time the passage of the object through the defined river segment 3 times. Record the length of the segment and each transit time in the Comments section of the Sample Collection Form.
6. The net assembly should be left in the river for at least 3 hours or as long as possible at the site. Upon return to the net location after floating the designated sampling reach, retrieve the net assembly from the water, taking care not to disturb the bottom upstream of the net.
7. Record the END TIME on the Sample Collection Form.
8. Determine the current velocity again as described in Step 5 above. Record the three "final" velocity estimates in the Comments section of the collection form. Calculate the average velocity from the initial and final values (6 measurements). Record the average velocity in the "Velocity" field of the Sample Collection form. Exclude any gross outlier values from the computation of the average velocity.
9. Note in the comments section if the net is badly clogged, which may occur at locations with high discharge and/or where the float time of the sampling reach is long.

Table 9-5. Procedures for Processing Drift Net Samples: Non-wadeable Streams.

1. Fill out a sample label for the composite drift sample. Attach the label to a 500-mL or 1-L plastic jar. If the sample contains a large volume of material, complete a sample label for additional containers and attach it to a second jar. Make sure the barcode numbers on each label agree.
2. Concentrate the material in each net in one corner by swishing up and down in the stream or river. Wash the material into a bucket half-filled with water. Use a wash bottle and/or forceps to remove as much material as possible from the net.
3. Repeat Step 1 for the second drift net. The contents of both nets are combined into a single bucket.
4. After the two net samples are combined into a single bucket, pour the composite sample into a sieving bucket (595 micron mesh).
5. Hand-pick large organisms from the sieve bucket containing the composite sample and place them into the appropriately labeled jar.
6. 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.
7. Lightly "tapping" the bottom of the sieve bucket on the surface of the stream or river helps to remove fine material. Remove as much material as possible using the sieve bucket.
8. Empty the contents of the bucket into the labeled jar. If necessary, distribute the sample among two or more labeled jars. Rinse residue from the bucket into the jar using a wash bottle and a small volume of water.
9. Add 95% ethanol to each labeled 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.**
10. Place a waterproof label with the following information inside each jar:
    - Stream Number
    - Type of sampler and mesh size used
    - Habitat type (drift net)
    - Name of stream
    - Date of collection
    - Collectors initials
    - Number of transects composited
11. Rinse the bucket well to eliminate any residue.
12. Complete the Sample Collection Form. Record the barcode number of the composite sample, and the habitat type (drift). If more than one container was required for a sample, record the number of containers on the collection form and on the jars. Replace the lid on the jar, and seal the container lid(s) with plastic or electrician's tape. Also note any peculiari ties associated with a particular samples by using a flag code and/or a written comment on the collection form.

Equipment and Supplies for Benthic Macroinvertebrates

Qty.	Item	
1	Modified kick net with 595 m mesh openings and closed bag (Wildco #425-J50-595)	
1	Handle for Kick Net Sampler, four foot length	
1	Floating drift net assembly (PVC frame, chains, snap-clips, and carabineers)	
2	Drift nets, 595 m mesh, closed end	
1	Sieve-bottomed bucket, 595- m mesh openings (optional)	
2 pr.	Watchmakers' forceps	
1	Wash bottle, 1-L capacity	
1	Small spatula, spoon, or scoop to transfer sample	
1	Funnel, with large bore spout	
12	Sample jars, plastic with screw caps, 500 mL and 1 L capacity, suitable for use with ethanol	
2	Buckets, plastic, eight to ten quart capacity	
1 gal	95% ethanol, in a proper container	
2 pr.	Rubber gloves, heavy rubber	
1	Cooler (with suitable absorbent material) for transporting ethanol and samples	
6	Sample labels, pre-numbered barcoded, stick-on type	
6	Sample labels, blank, stick-on type (for additional containers)	
2	Sample Collection Form for site	
1	Field check list sheet	
	Soft (#2) lead pencils	
1 pkg.	Clear waterproof tape strips	
4 rolls	Plastic electrical tape	
1	Knife, pocket, with at least two blades	
1	Stopwatch	
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 9-6. Equipment and supply checklist for benthic macroinvertebrates.

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