

US EPA ARCHIVE DOCUMENT

**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 B

QUICK REFERENCE GUIDES

The following pages are tabular summaries of different field activities and procedures described in this manual. These were developed by the principal investigators for each ecological indicator to provide a field team with a quick way to access information about each procedure. They are intended to be laminated and taken to the stream site after the crew has been formally trained in the detailed procedures as presented in the manual. They are arranged here in the general sequence of their use in the field.

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QUICK REFERENCE GUIDE FOR INITIAL SITE ACTIVITIES

1. Find the stream location in the field corresponding to the "X" on 7.5" topo map (X-site). Crews should use all available means to insure that they are at the correct site, as marked on the map, including: 1:24,000 USGS map orienteering, topographic landmarks, county road maps, and global positioning system (GPS) confirmation of site latitude and longitude.
2. Classify the site, **AT THE X-SITE**, as:

NON-TARGET	No Stream Channel Impounded Stream Marsh/Wetland Unwadeable Stream (> 50% of reach is unwadeable)
TARGET	Regular Stream Intermittent Stream Dry Channel Altered Channel (stream channel different from map representation)
INACCESSIBLE	Physical Barriers (Physically unable to reach the X-site) No Permission

Record class on Site Verification form, do not sample Non-target or inaccessible sites. Take samples from Target sites as discussed in field operations and methods manual.

3. Measure the stream width at five "typical" places within 10 m of the X-site. Average and round the width to the nearest meter. Record width on the stream site verification form. Lay out a sample reach with a length of 40 times the stream width. If the stream is less than 4 m wide, use 150 m as the sample reach length.
4. Do a reconnaissance of the sample reach.
5. Proceed downstream half the required reach length; measure the distance with a tape measure down the middle of the stream. Mark it as the reach start point (Transect "A").
6. Proceed upstream marking 10 more cross-section transects (Transects "B" through "K") at 1/10 intervals along the calculated reach length (every 4 channel widths or 1.5 meters in small streams). At Transect "B", assign a sampling point (Left, Center, or Right as you face downstream) for collecting periphyton and benthic macroinvertebrate samples by throwing a die. Once the initial point has been determined, assign sampling points for Transects "C" through "J" systematically using the order Left, Center, and Right.

NOTE: If there is a lake/pond or a stream order change (100,000 map-based) along the survey reach, end the sample reach at the barrier. Make up for the loss of stream length by adding length to the other end of the reach ("slide" the reach). Locations where the stream order changes will be noted on the topo maps provided to the field teams. Do not "slide" the reach to avoid bridges, riprap, small flow control structures, culverts and the like.

QUICK REFERENCE GUIDE FOR WATER CHEMISTRY

I. EQUIPMENT TO CARRY IN FIELD FOR WATER CHEMISTRY

Rinse/Test bottles of QCCS in self-sealing plastic bag

D.O./Temperature/Conductivity Meter

Field Forms

One 500-mL plastic beaker with handle, in clean self-sealing plastic bag

One cubitainer in clean self-sealing plastic bag (barcode label attached)

Two 60-mL syringes in a plastic container (each one with a bar code label attached)

Two syringe valves in the plastic container

Opaque garbage bag

II. EXTRA EQUIPMENT TO CARRY IN VEHICLE

Cooler with 4 to 6 one-gallon self-sealing plastic bags filled with ice

Back-up labels, forms, syringes, and syringe valves

III. DAILY ACTIVITIES AFTER SAMPLING

1. Check that cubitainer lid is on tight and has a flush seal.
2. Prepare the sample for shipping (label and seal cooler, replace ice as close as possible to shipping time).
3. Call Overnight shipping company to arrange pick-up of cooler.
4. Rinse the sampling beaker with deionized water three times.
5. Make sure field meters are clean and are stored with moist electrodes.
6. Label the next days sample containers (cubitainer and syringes), pack cubitainer and sample beakers in clean self-sealing plastic bag, and pack two syringes and syringe valves in a plastic container with a snap-on lid.

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QUICK REFERENCE GUIDE FOR WATER CHEMISTRY (Continued)

.SUMMARY OF SITE PROCEDURE FOR WATER CHEMISTRY

I. COLLECT WATER SAMPLE

- A. Make sure cubitainers and syringes are labeled and have the same barcode ID.
- B. Rinse the 500-mL sample beaker three times with streamwater from mainstream.
- C. Rinse cubitainer three times with 25-50 mL of streamwater, using the sample beaker. Rinse cubitainer lid with stream water.
- D. Fill cubitainer with streamwater using the 500 mL sample beaker. Expel any trapped air and cap the cubitainer. Make sure that the lid is seated correctly and that the seal is tight.

DO NOT EXPAND CUBITAINER BY BLOWING IN IT.

- E. Rinse each of the two, 60-mL syringes three times with 10-20 mL of streamwater.
- F. Fill each of the syringes with streamwater from mid-stream by slowly pulling out the plunger. If any air gets into the syringe, discard the sample and draw another.
- G. Invert the syringe (tip up) and cap the syringe with a syringe valve. Open the valve, tap the syringe to move any air bubbles to the tip, and expel any air and a few mL of water. Make sure there is 50-60 mL of stream sample in the syringe. Close the valve and place the syringes in their transport container.
- H. Place the cubitainer and syringes in cooler/stream to keep cool (keep dark as well) while the rest of the sampling is taking place. When you return to the vehicle, put the samples in the cooler and surround with 4 to 6 one-gallon self-sealing plastic bags filled with ice.

II. IN SITU MEASUREMENTS

A. Conductivity

- 1. Turn on and check the zero and red line (if applicable) of the conductivity meter.
- 2. Measure and record the conductivity of the QCC solution. Rinse the probe in the "Rinse" bottle of QCC solution before immersing in the "Test" bottle of QCC solution.
- 3. Measure and record stream conductivity in mid-stream.

B. Dissolved Oxygen/Temperature

- 1. Calibrate the DO meter following meter instructions.
 - 2. Measure the DO and temperature in mid-stream. If water velocity is slow, jiggle the DO probe as you take the reading.
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QUICK REFERENCE GUIDE FOR PHYSICAL HABITAT CHARACTERIZATION

FIELD SUMMARY: P-HAB LAYOUT AND WORKFLOW

1. Habitat Sampling Layout:

Thalweg interval: 1.0 m for streams <2.5 m wide (from initial estimate).
1.5 m for streams 2.5 - 3.5 m wide
0.01 × (reach length) for streams >3.5 m wide

100 thalweg measurement intervals in each sample reach, except 150 in streams <2.5m wide

Channel/Riparian Cross Section Transect every 10th thalweg interval (every 15th for channels <2.5m wide). Eleven of them, marked "A" thru "K".

Wetted Width at every cross-section transect and halfway in between transects (total of 21 measurements).

2. Work Flow:

- At the downstream start point (Transect "A"), one person makes channel dimension, substrate, bank, and canopy densiometer measurements. The second person records those measurements while making visual estimates of riparian vegetation structure, fish cover, and human disturbance. No bearing or slope at first cross section.
- Proceed upstream between Transects "A" and "B", making measures at each thalweg measurement station. One person in channel measures width (when required), thalweg depth, and determines presence of soft/small sediment at thalweg. The other person records those measurements, classifies channel habitat, and makes large woody debris estimates.
- When you complete 10 thalweg intervals and reach one of 11 pre-marked cross section transect flags, stop and take out a new cross-section form for Transect "B". Repeat all the Channel/Riparian measurements at this new location. In addition, do the slope & bearing backsites together. Intermediate flagging (of a different color) may have to be used if the stream is extremely brushy, sinuous, or steep to the point that you cannot site for slope and bearing measures between the 11 points. (Note that you could tally woody debris while doing the backsite, rather than during the thalweg profile measurements.)
- Repeat the cycle of thalweg and cross section measurements until you reach transect 11 ("K") at the upstream end.
- Discharge measurements made any time after choosing suitable location nearest to the "X" site. Discharge measurements are done by the Chemistry/Macroinvert pair (rather than the Habitat/Fish pair) just after chemical samples are taken.

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QUICK REFERENCE GUIDE FOR PHYSICAL HABITAT CHARACTERIZATION (Continued)**FIELD SUMMARY: COMPONENTS OF P-HAB PROTOCOL****Width, Depth Profile, Hab Classes, Woody Debris:**

- At 10 (15) equally spaced intervals between each of 11 channel cross-sections (100 or 150 along entire reach):
 - Measure max. depth ("Thalweg") at each increment and wetted width at the required increments.
 - Classify habitat and pool-forming elements.
 - Determine presence of soft/small sediment at thalweg measurement points.
- Between each of the channel cross sections, tally all Large Woody debris within and above the bankfull channel according to size class. In the tally boxes provided on the form, make separate tally for LWD wholly or partially within the bankfull channel and then for LWD only bridging above the channel.

NOTE: If initial width estimate is <2.5 m, then 150 thalweg measurements are made at 1.0 m intervals over a 150 m reach. If width is 2.5 to 3.5 m, then make 100 thalweg measurements at 1.5 m intervals. In all other cases, 100 measurements are made at an interval 1/100th the length of the sample reach.

Channel and Riparian Cross-Sections:

- Measurements: Bankfull width, bankfull height, incision height, wetted width, bar width, undercut, bank angle (with rod and clinometer); gradient (clinometer), sinuosity (compass backsight), riparian canopy cover (densiometer).
- Visual Estimates: Substrate size class and embeddedness; areal cover class and type of riparian vegetation in Canopy, Mid-Layer and Ground Cover; areal cover class of fish cover features, aquatic macrophytes, and filamentous algae; presence and proximity of human disturbances

Discharge:

In medium and large streams measure water depth and velocity (at 0.6 depth from surface) at 15 to 20 equally spaced intervals across one carefully chosen channel cross-section. Let meter equilibrate to average velocity for 20 seconds. In very small streams, measure discharge by timing the passage of a neutrally-buoyant object 3 times or the filling of a bucket 5 times in succession.

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QUICK REFERENCE GUIDE FOR PHYSICAL HABITAT CHARACTERIZATION (Continued)

FIELD SUMMARY: RIP. VEG., HUMAN DISTURB., IN-CHANNEL COVER:

- Observations upstream 5 meters and downstream 5 meters from each of the 11 cross-section transects.
- For riparian vegetation and human disturbances, include the visible area from the stream back a distance of 10m (30 ft) shoreward from both the left and right banks. If the wetted channel is split by a mid-channel bar, the bank and riparian measurements shall be for each side of the channel, not the bar.
- Three vegetation layers:
 - CANOPY LAYER (>5 m high)
 - UNDERSTORY (0.5 to 5 m high)
 - GROUND COVER layer (<0.5 m high).
- Canopy and Understory Vegetation Types:

(Deciduous, Coniferous, Broadleaf Evergreen, Mixed, or None) in each of the two taller layers (Canopy and Understory). "Mixed" if more than 10% of the areal coverage made up of the alternate type.
- Areal Cover Classes for Vegetation and In-Channel Cover:
 - 0: (absent -- zero cover)
 - 1: (sparse -- cover <10%)
 - 2: (moderate -- cover 10-40%)
 - 3: (heavy -- cover 40-75%)
 - 4: (very heavy -- cover >75%).
- Tallying Human Disturbances:
 - B: The human activity or structure is ON THE STREAMBANK
 - C: CLOSE to the Bank (within 10m)
 - P: PRESENT, but farther than 10m from the bank
 - 0: NOT PRESENT.

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QUICK REFERENCE GUIDE FOR PHYSICAL HABITAT CHARACTERIZATION (Continued)**FIELD SUMMARIES: SUBSTRATE AND WOODY DEBRIS SIZE CLASSES**

Substrate size class and embeddedness are estimated, and depth is measured for 5 particles taken @ 5 equally-spaced points on each cross-section. The cross-section is defined by laying the surveyor's rod or tape to span the wetted channel.

SUBSTRATE SIZE CLASSES:

RS	Bedrock (Smooth)	>4000 mm	smooth surface rock or hardpan (bigger than a car)
RR	Bedrock (Rough)	>4000 mm	(bigger than a car)
BL	Boulders	>250 to 4000 mm	(basketball to car size)
CB	Cobbles	64 to 250 mm	(tennis ball to basketball size)
GC	Gravel(Coarse)	16 to 64 mm	(marble to tennis ball size)
GF	Gravel (Fine)	2 to 16 mm	(ladybug to marble size)
SA	Sand	0.06 to 2 mm	(smaller than ladybug size, but visible as particles - gritty between fingers).
FN	Fines	<0.06 mm	Silt-Clay-Muck (not gritty between fingers)
HP	Hardpan	>4000 mm	(consists of firm, consolidated fines)
WD	Wood	Regardless of Size	Wood or other organic material
OT	Other	Regardless of Size	Metal, Tires, Car bodies, asphalt, concrete, etc. (Describe in comments if you enter "OT").

LARGE WOODY DEBRIS SIZE CLASSES

LWD Definition: Diameter (small end) ≥ 0.1 m (≥ 4 in)
Length ≥ 1.5 m (≥ 5 ft) -- count only part with diam ≥ 0.1 m.

Two Tallys:

- (1) LWD at least partially within bankfull channel.
- (2) LWD not within bankfull channel, but at least partially bridging above bankfull stage (idea is that it will eventually fall into channel).

Size Categories for Tally (12 potential combinations):

Diameter (large end):		Length:	
0.1 to <0.3 m	(4 to 12 inches)	1.5 - <5 m	(5 - 16 ft)
0.3 to <0.6 m	(1 to 2 ft)	5 - 15 m	(16 - 49 ft)
0.6 to <0.8 m	(2 to 2.6 ft)	>15 m	(>49 ft)
>0.8 m	(>2.6 ft)		

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QUICK REFERENCE GUIDE FOR PHYSICAL HABITAT CHARACTERIZATION (Continued)**FIELD SUMMARY: HABITAT CLASSIFICATION AT CHANNEL UNIT SCALE****Channel Unit Habitat Classes^a**

Class (Code)	Description
Pools: Still water, low velocity, smooth, glassy surface, usually deep compared to other parts of the channel:	
Plunge Pool (PP)	Pool at base of plunging cascade or falls.
Trench Pool (PT)	Pool like trench in stream center
Lateral Scour Pool (PL)	Pool scoured along one bank.
Backwater Pool (PB)	Pool separated from main flow off side of channel.
Impoundment Pool (PD)	Pool formed by impoundment above dam or constriction.
Pool (P)	Pool (unspecified type).
Glide (GL)	Water moving slowly, with <u>smooth, unbroken surface</u> . Low turbulence.
Riffle (RI)	Water moving, with <u>small ripples, waves and eddies</u> -- <u>waves not breaking</u> , surface tension not broken. Sound: "babbling", "gurgling".
Rapid (RA)	Water movement <u>rapid and turbulent</u> , <u>surface with intermittent whitewater</u> with breaking waves. Sound: continuous rushing, but not as loud as cascade.
Cascade (CA)	Water movement rapid and very turbulent over steep channel bottom. <u>Most of water surface broken in short irregular plunges</u> , mostly whitewater. Sound: roaring.
Falls (FA)	<u>Free falling water</u> over vertical or near vertical drop into plunge, water turbulent and white over high falls. Sound: from splash to roar.
Dry Channel (DR)	No water in channel

Categories of Pool-forming Elements

Code	Category
N	Not Applicable, Habitat Unit is not a pool
W	Large Woody Debris.
R	Rootwad
B	Boulder or Bedrock
F	Unknown cause (unseen fluvial processes)
WR, RW, RBW	Combinations
OT	Other (describe in comments section of field form)

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QUICK REFERENCE GUIDE FOR PHYSICAL HABITAT CHARACTERIZATION (Continued)

FIELD SUMMARY: P-HAB PROBLEM AREAS

Mid-channel Bars: dry at baseflow, inundated at bankfull flow.

Measure wetted width across and over mid-channel bars, but record bar width in the column provided on thalweg profile and cross-section form.

Islands: as high as the surrounding flood plain; dry even at bankfull flow.

Measure only the width of the main channel between island and shore; then if required, measure the side channel width separately (record on another form). Handle the side channels created by islands as follows:

- * Visually estimate the percent of flow in the side channel.
- * If <15% -- Indicate presence of side channel on field data form.
- * If 16-49% -- Indicate presence of side channel, plus obtain and record detailed channel & riparian cross-section measurements on the side channel. Designate additional cross section transects as "XA", "XB", etc. corresponding to nearest main channel Transect location.

Note continuous presence of side channels on the Thalweg Profile Form until channels converge. In addition, note the points of side channel convergence and divergence in the comment section on the thalweg profile form.

Dry and Intermittent Streams, where no water is in the channel:

- Record zeros for depth and wetted width.
 - Record habitat type as dry channel ("DR").
 - Make all Channel Cross-section transect measures across the unvegetated portion of the channel. For substrate, DISTLB = the width of the unvegetated channel, and substrate measurements are made along that transect.
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QUICK REFERENCE GUIDE FOR PERIPHYTON

FIELD EQUIPMENT

1. Large funnel (15-20 cm diameter).
2. Scrape area delimiter (3.8 cm diameter pipe, 3 cm tall).
3. Stiff-bristle toothbrush with handle bent at 90° angle.
4. Wash bottle.
5. Collection bottle to catch removed periphyton.
6. 60 mL syringes with 3/8" hole bored into the end.
7. 50 mL centrifuge tubes or similar sample vials.
8. Formalin.
9. Glass-fiber filters (0.45 μ m average pore size) for chlorophyll *a*.
10. Pre-leached, pre-ashed, weighed glass-fiber filters (0.45 μ m average pore size) in numbered pans for ash free dry mass (AFDM).
11. Forceps for filter handling.
12. Millipore®-type filtration apparatus with plastic or stainless steel filter base, and Nalgene® funnel and suction flask.
13. Nalgene® hand-operated vacuum pump (need one additional pump as a backup).
14. Aluminum foil.
15. Ice chest.

FIELD PROTOCOLS

1. Periphyton samples will be collected using a random-systematic procedure. The location (left, middle, or right 1/3 of the channel) of the first sample (Transect B) will be chosen randomly. Subsequent samples (Transects C-J) will be collected sequentially from the left, middle, then right 1/3 of the channel, resulting in three samples from each side and middle.
2. Periphyton are collected, using the appropriate method, from flowing (riffles) and slack water (pools) habitats.
3. Rock and wood samples which are small enough (< 15 cm diameter) and can be easily removed from the stream are collected by placing the substrate in a funnel which drains into a sample bottle. A defined area of substrate surface (12 cm²) is enclosed, and attached periphyton is dislodged with 30 seconds of brushing with a stiff-bristled toothbrush. **Care must be taken to ensure that the upper surface of the rock is the surface that is being scraped.**
4. Loosened periphyton is then washed, using stream water from a wash bottle, from the substrate into the 500-mL sample bottle.
5. Soft-sediments are collected by vacuuming the upper 1 cm of sediments confined within the 12-cm² sampling ring into a 60-mL syringe.
6. All samples, regardless of substrate type, are composited by habitat (riffle or pool) and mixed thoroughly.
7. **Record total volume of composited sample before proceeding to the next step!**

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QUICK REFERENCE GUIDE FOR PERIPHYTON (Continued)

8. **Four** subsamples will be taken from each composite sample. These are:

a. Identification/Enumeration

- 1) Withdraw 50 mL of mixed sample and place in a labeled sample vial (50-mL centrifuge tubes work well). Cover label with clear tape.
- 2) Preserve sample with 2 mL of 10% formalin. Gloves should be worn.
- 3) Tightly cap tube and tape with electrical tape.

b. Chlorophyll a

- 1) Withdraw 25 mL of mixed sample and filter onto a glass-fiber filter (0.45 um pore size) using a hand-operated vacuum pump. (Note: for soft-sediment samples, allow grit to settle before withdrawing sample).
- 2) Fold filter so that the sample on the filter surface is folded together, wrap in aluminum foil, and affix the tracking label to the outside, and seal with clear tape.
- 3) Freeze filter as soon as possible by placing it in a freezer.
- 4) Store frozen for laboratory analysis.

c. Ash Free Dry Mass (AFDM)

- 1) Withdraw 25 mL of mixed sample and filter onto a pre-leached, pre-weighed glass-fiber filter. (Note: for soft-sediment samples, allow grit to settle before withdrawing sample).
- 2) **Do not fold this filter.** Return filter to it's numbered container, wrap in aluminum foil, affix tracking label to outside, and seal with clear tape.
- 3) Freeze filter as soon as possible by placing it in a freezer.
- 4) Store frozen for laboratory for analysis.

d. Alkaline/Acid Phosphatase

- 1) Withdraw 50 mL of mixed sample and place in a labeled sample vial (50-mL centrifuge tubes work well). Cover label with clear tape.
 - 2) Tightly cap tube and tape with electrical tape.
 - 3) Freeze sample as soon as possible by placing it on dry ice.
 - 4) Store frozen for laboratory analysis.
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QUICK REFERENCE GUIDE FOR SEDIMENT METABOLISM

FIELD EQUIPMENT

1. Ice chest for floating centrifuge tubes during incubation
2. 1000 mL Nalgene® beaker for holding centrifuge tubes during incubation.
3. Small scoop sampler for sediments.
4. 50-mL, screw-top, centrifuge tubes.
5. Digital dissolved oxygen meter (e.g. YSI 58) with a stirring probe (e.g., YSI 5730).
6. Spare batteries for D.O. meter.
7. Permanent markers for labeling tubes.
8. Sample labels and field data sheets.
9. Ice chest with dry ice for sample freezing.

FIELD PROTOCOLS

Dissolved Oxygen Meter Calibration (for YSI model 58, with YSI model 5730 stirring BOD probe)

1. Zero meter according to manufacturer's directions, and
2. Calibrate meter using the water-saturated atmosphere method described in the meter's operations manual.

QUICK REFERENCE GUIDE FOR SEDIMENT METABOLISM (Continued)

Sediment Collection and Experimental Set-up

1. Collect and combine fine-grained, surface sediments (top 2 cm) from all depositional areas along the stream reach (Transects B-J) established for the Physical Habitat Characterization.
 2. Fill ice chest 2/3 full with stream water and record temperature and dissolved oxygen (D.O.).
 3. Thoroughly mix composite sediment sample.
 4. Place 10 mL of sediment in each of 5 labeled, 50 mL screw-top centrifuge tubes.
 5. Fill each tube to the top (no head space) with stream water from the ice chest and seal.
 6. Fill one additional tube with stream water only to serve as a blank.
 7. Incubate tubes in closed ice chest for 2 hours.
 8. Measure D.O. in each tube, including the blank.
 9. Decant overlying water and save sediment.
 10. Tightly seal tubes and freeze as soon as possible.
 11. Store frozen for laboratory analysis.
 12. If you are collecting samples for sediment toxicity tests, save 1 to 2 L of the remaining sediment sample by placing it in a labeled plastic bag.
 13. Store sediment toxicity sample chilled (but not frozen!) for laboratory analysis.
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QUICK REFERENCE GUIDE FOR SEDIMENT TOXICITY

SAMPLE COLLECTION AND SHIPMENT FOR SEDIMENT TOXICITY SAMPLES

1. Use the sediment left over from the benthic (sediment) metabolism indicator in Section 9, Benthic (Sediment) Metabolism: Field Methods.
 2. Mix sediment well with a stainless steel or plastic mixing spoon or gloved hand.
 3. Fill a 2-gallon polyethylene (4 mil) bag with at least 1 L of sediment.
 4. Close bag, squeeze the air out and tie a knot in the remaining portion of the bag to seal.
 5. Fill out ID label; place label on the outside of the bag. Place this bag inside a second 2-gallon polyethylene bag and tie off the top to seal.
 6. Place these bags inside a cooler with only sediment samples in them.
 7. Hold sediment samples on ice (do not freeze!) for laboratory analysis.
 8. Ship samples to the designated contact person or laboratory.
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QUICK REFERENCE GUIDE FOR BENTHIC MACROINVERTEBRATES

TABLE I. BASE PROTOCOLS FOR COLLECTING MACROINVERTEBRATES

1. Do the water chemistry.
2. Locate first sampling station (second flag) from downstream end of the study segment and roll die to pick left (1), middle (2), or right side (3) of transect to sample. If stream is narrower than three nets, pick left or right. If wide enough for only one net, then sample entire stream width. After first transect, systematically sample remaining transects left, middle, or right so that three samples are collected on left, middle, and right at the site.
3. If riffle or run use protocol in Table II. If pool use protocol in Table III or hand pick for 60 seconds if kick net cannot be used.
4. Go to next upstream station and repeat. Combine all riffle samples in one bucket and pool samples in another. Check net after each sample for clinging organisms and transfer to bucket.
5. After a sample is collected from each of nine interior transects and all samples are combined in the proper bucket, obtain a composite sample as described in Table IV.
6. Assist with the fish collection.
7. Preserve and label each sample as described in Table V.

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QUICK REFERENCE GUIDE FOR BENTHIC MACROINVERTEBRATES (Continued)

TABLE II. PROCEDURES FOR RIFFLES AND RUNS USING KICK NET SAMPLER

1. Attach four foot pole to the sampler.
2. Position sampler quickly and securely on stream bottom with net opening upstream.
3. Hold the sampler in position on the substrate while checking for snails and clams in an area of about 0.5 m² in front of the net; kick the substrate vigorously for about 20 seconds in front of the net.
4. Inspect and rub off with the hands any organisms clinging to the rocks, especially those covered with algae or other debris.
5. Remove the net from the water with a quick upstream motion to wash the organisms to the bottom of net.
6. Rinse net contents into the "riffle" bucket containing one or two gallons of water by inverting the net in the water.
7. Inspect the net for clinging organisms. With forceps remove any organisms found and place them into the bucket.
8. Large objects (rocks, sticks, leaves, etc.) in the bucket should be carefully inspected for organisms before discarding.
9. Combine all riffle samples in the "riffle" bucket.
10. After all stations are sampled and all riffle samples combined in the "riffle" bucket, obtain a composite sample as described in Table IV.

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QUICK REFERENCE GUIDE FOR BENTHIC MACROINVERTEBRATES (Continued)

TABLE III. PROCEDURES FOR POOLS USING THE MODIFIED KICK NET SAMPLER

1. Attach four-foot pole to the sampler.
2. Inspect about 1/2 square meter of bottom for any heavy organisms, such as mussels and snails, which have to be hand picked and placed in the net.
3. While disturbing about 0.5 m² of substrate by kicking, collect a 20-second sample by dragging the net repeatedly through the area being disturbed. Keep moving the net all the time so that the organisms trapped in the net will not escape.
4. After 20 seconds remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net.
5. Rinse net contents into a small bucket of water (about one or two gallons) by inverting the net in the water.
6. Inspect the net for clinging organisms. With forceps remove any organisms found and place them in the bucket.
7. Large objects in the bucket should be carefully inspected for organisms which are washed into the bucket before discarding.
8. Combine this sample with the other pool samples in the "pool" bucket.
9. After all stations are sampled and all pool samples are combined together in the "pool" bucket, obtain a composite sample as described in Table IV.

(continued)

QUICK REFERENCE GUIDE FOR BENTHIC MACROINVERTEBRATES (Continued)**TABLE IV. PROCEDURES FOR OBTAINING THE COMPOSITE SAMPLE**

1. Pour the contents of the riffle bucket through a U.S. Standard 30 sieve. Examine the bucket while rinsing it well to be sure all organisms are washed from the bucket onto the sieve.
2. Wash contents of the sieve to one side by gently agitating in water and wash into jar using as little water from the squirt bottle as possible. Carefully examine the sieve for any remaining organisms and place them in the jar.
3. Place properly filled out waterproof label in the jar and replace the cap.

TABLE V. SAMPLE PRESERVING AND LABELING

1. Fill in special pre-numbered barcoded label and place on jar. All additional jars used for a sample must be labeled with same number. Enter this number which will be used for tracking purposes in the computer.
2. Preserve samples in ethanol as follows:
 - a. If jar is more than 1/4 full of water, pour off enough to bring it to less than 1/4 full using proper sieve to retain organisms.
 - b. Fill jar nearly full with 95% ethanol so that the concentration of ethanol is 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.
 - c. Transfer any organisms on the sieve back into the jar with forceps.
3. Check to be sure waterproof label is in jar with the required information on it.
4. Check to be sure that the pre-numbered stick-on barcoded label is the on jar and agrees with the inside label. Cover the entire label with clear, waterproof tape.
5. With a grease pencil write the site number, sample type (Riffle or Pool), and the number of transects sampled for either Riffle or Pool on the cap.
6. Seal the caps with electrical tape.
7. Complete the check off sheet and place samples in cooler or other secure container for transport.
8. Secure all equipment in the vehicle.

(continued)

QUICK REFERENCE GUIDE FOR BENTHIC MACROINVERTEBRATES (Continued)

TABLE VI. 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 label inside jar.
3.	Composite pool/glide sample collected with label inside 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 sealed with tape.
8.	Photos of site.
9.	Sample jars in cooler or otherwise secured.
10.	All equipment accounted for and secured in vehicle.
Signature:	
Time sampling completed:	

QUICK REFERENCE GUIDE FOR AQUATIC VERTEBRATES

FIELD PROTOCOLS FOR FISH COLLECTION

1. Site Selection

- a. Determine channel width.
- b. Survey sample reach.
- c. Determine if reach requires block nets.
- d. If conductivity is below 10 :S/cm or if flow, depth or turbidity make it unsafe to electrofish, crew may elect to use seine only or not sample. THIS IS A SAFETY DECISION.
- e. **In case of emergency, determine location of means of easy egress from stream.**

2. Electrofishing

- a. Set unit to 300VA and pulsed DC. Select initial voltage setting. Start generator, set timer, and depress switch to begin fishing.
- b. Fish in an upstream direction, parallel to the current. Adjust voltage and waveform output according to sampling effectiveness and mortality fish specimens.
- c. With switch depressed, sweep electrodes from side to side in the water. Sample available cut-bank and snag habitat as well as riffles and pools.
- d. Netters follow operator and net fish. Deposit fish in buckets. Block with seines in riffles, pools and snags.
- e. Continue for 40 channel widths. Record total time spent collecting and shocking time on data sheets.
- f. Identify and release any threatened and endangered species.
- g. Identify and measure (SL, TL) sport fish and very large specimens, record external anomalies, and release unharmed.
- h. Identify other specimens. Determine number of individuals in species, measure largest and smallest individuals, and voucher as described in Voucher Protocol.
- i. Retain a subsample of target species for Fish Tissue Contaminants analysis.

3. Seining will be used in conjunction with electrofishing and in sites where stream is too deep for electrofishing to be conducted safely.

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QUICK REFERENCE GUIDE FOR AQUATIC VERTEBRATES (Continued)**ANOMALY CATEGORIES AND CODES**

Categories	Code	Definition
Absent	AB	Absent eye, fin, tail.
Blisters	BL	In mouth, just under skin.
Blackening*	BK	Tail or whole body with darkened pigmentation.
Extensive Black spot disease	BS	Small black cysts (dots) all over the fins and body.
Cysts	CY	Fluid-filled swellings; maybe small dots or large.
Copepod	CO	A parasitic infection characterized by a worm like copepod embedded in the flesh of the fish; body extends out and leaves a sore/discoloration at base, may be in mouth gills, fins, or anywhere on body.
Deformities	DE	Skeletal anomalies of the head, spine, and body shape; amphibians may have extra tails, limbs, toes.
Eroded fins	EF	Appear as reductions or substantial fraying of fin surface area.
Eroded gills	EG	Gill filaments eroded from tip.
Fungus	FU	May appear as filamentous or "fuzzy" growth on the fins, eyes, or body.
Fin anomalies	FA	Abnormal thickenings or irregularities of rays
Grubs	GR	White or yellow worms embedded in muscle or fins.
Hemorrhaging	HM	Red spots on mouth, body, fins, fin bases, eyes, and gills.
Ich	IC	White spots on the fins, skin or gills.
Lesions	LE	Open sores or exposed tissue; raised, granular or warty outgrowths.
Lice	LI	Scale-like, mobile arthropod.
Mucus	MU	Thick and excessive on skin or gill, as long cast from vent.
None	NO	No anomalies present.
Other	OT	Anomalies or parasites not specified.
Scale anomalies	SA	Missing patches, abnormal thickenings, granular skin
Shortened operculum	SO	Leaves a portion of the gill chamber uncovered
Tumors	TU	Areas of irregular cell growth which are firm and cannot be easily broken open when pinched. (Masses caused by parasites can usually be opened easily.)
Leeches	WR	Annelid worms which have anterior and posterior suckers. They may attach anywhere on the body.
Exophthalmia	EX	Bulging of the eye.

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QUICK REFERENCE GUIDE FOR AQUATIC VERTEBRATES (Continued)

GUIDELINES AND PROCEDURES FOR PREPARING FISH VOUCHER SPECIMENS

Category 1. Large easily identified species OR adults may be difficult to identify **OR** the species is uncommon in that region. Preserve 1-2 small (<150 mm total length) adult individuals per site plus 2-5 juveniles. If only large adults are collected, reserve smallest individual until voucher procedure is complete and preserve **ONLY** if space is available. Photograph if considered too large for the jar.

Category 2. Small to moderate-sized fish **OR** difficult to identify species. Preserve 25 adults and juveniles. If less than 25 individuals are collected, voucher all of them.

Category 3. Species of "special concern." These are state or federally listed species. **Photograph and release.** If specimens have died, include in voucher collection, note on data sheet and notify appropriate state official as soon as possible.

- a. After all individuals of a species have been processed, place the voucher sub sample in a kill jar containing a strong (approximately 20%) formalin solution. Individuals > 160 mm should be slit on the lower abdomen of the **RIGHT** side.
 - b. When specimens are dead, transfer to a small nylon bag containing a waterproof label with tag #. Place in "Voucher" jar in 10% formalin. **BE SURE THAT JAR IS LABELED INSIDE AND OUT WITH A **VOUCHER** LABEL** (site ID, barcode, and date).
 - c. Continue until all species are processed. Seal voucher jar with electrical or clear tape. Check that the jar is correctly labeled. Enter **BARCODE ID** in appropriate place on field data sheet.
 - d. Transport to storage depot at end of week. Store in a cool, dark, ventilated space.
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QUICK REFERENCE GUIDE FOR FISH TISSUE CONTAMINANTS

SELECTING FISH TISSUE SPECIMENS

If possible, obtain one sample each, containing the desired **weight** or **number** (see below) of **similarly sized individuals***, from the **primary** and **secondary** target species lists (**2 composite samples** total):

I. PRIMARY TARGET SPECIES

Small adult fish (in priority order)	DESIRED WEIGHT
1) Blacknose Dace	50** - 400 g
2) Another Dace species	50** - 400 g
3) Creek Chub or Fallfish	50** - 400 g
4) Slimy Sculpin/Mottled Sculpin	50** - 400 g
5) Stoneroller	50** - 400 g
6) A Darter species	50** - 400 g
7) A Shiner species	50** - 400 g

- A) Choose the **highest priority target species** from the above list, that has at least enough individuals to attain the minimum weight (50 g). Get as much weight of fish as possible within the desired weight range (50-400 g). Use scale provided to determine weight. With clean hands, place the fish in fresh aluminum foil (dull side towards fish) before placing fish in weighing container.
- (B) If **fewer than the desired number** of individuals of **any primary target species** are collected, send individuals of a small non-target species if 50 g or more are available.

* - Getting a sufficient sample amount is a higher priority than getting similar-sized individuals.

** - This weight represents the **minimum amount** needed for laboratory analysis. Crews should not settle for the minimum weight if more fish are present. They should **send as many fish as possible** up to 400 g weight goal.

QUICK REFERENCE GUIDE FOR FISH TISSUE CONTAMINANTS (Continued)

SELECTING FISH TISSUE SPECIMENS

II. SECONDARY TARGET SPECIES

Collect and save a sample of secondary target species if such a sample of desired number of individuals of desired size is available. Collect **similar sized individuals** if enough are present.

Larger adult fish (in priority order)	DESIRED SIZE	DESIRED NUMBER
1) White sucker	>120 mm	5
2) Hogsucker	>120 mm	5
3) A Bass species	>120 mm	5
4) A Trout species	>120 mm	5
5) A Sunfish species	>120 mm	5
6) Carp	>120 mm	5

- A) If **fewer than the desired number** of secondary target species individuals of desired size are collected, add smaller individuals of the same species, if available, to achieve the desired number (5).
- B) If **fewer than 5 fish** of **any size** are available, you may send **as few as 3 fish** that are at or at least near the minimum desired size (120 mm).
- C) If an acceptable secondary target species sample (by the above criteria) is not available send only the primary target species sample. If **neither a primary nor secondary species** sample that **meets these criteria** is available, use your best judgement in sending some type of fish sample (may be mixed species).

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QUICK REFERENCE GUIDE FOR FISH TISSUE CONTAMINANTS (Continued)

PROCESSING TISSUE SPECIMENS

1. Keep hands, work surfaces, and wrapping materials clean and free of potential contaminants (mud, fuel, formalin, sun screen, insect repellent, etc.)
2. Measure **total weight** of individuals for **primary target** species and count the total number of individuals. Measure the **total length** (TL) of each secondary target species individual. **Record** all of this information in the fish tissue section of the Sample Collection Form.
3. Write the bar-code number(s)*** on the collection form. Make sure that the form is filled out completely.
4. Wrap fish in aluminum foil. Place the dull side of the aluminum foil in contact with the fish. The **primary target fish** sample may be wrapped as a **group**. **Secondary target fish** should be wrapped individually. Once wrapped, place each sample in a self-sealing plastic bag or a garbage bag.
5. Expel excess air and seal the bag(s). Wrap clear tape around the bag(s) to seal and make a surface for each sample label.
6. Complete bar-coded fish tissue label(s). Make sure the number(s) is/are the same one(s) on the collection form. Apply it/them to the tape surface(s). Cover the label(s) with clear, waterproof tape. As labels will sometimes fall off, there should **always** be a **label on the inner bag**.
7. Place labeled bag(s) into a second plastic bag(s) and seal and label second bag(s). Repeat previous two steps.
8. Place double-bagged sample(s) in cooler with dry ice until shipment.
9. Ship weekly on dry ice by Federal Express next day service. **KEEP FROZEN UNTIL SHIPMENT**. If ice is used, double bag ice in self-sealing plastic bags and tape shut to avoid contamination of samples if ice should melt.

*** - If both primary and secondary target species are collected, the two samples should be wrapped and bagged separately, with separate bar codes and labels, but only one Sample Tracking Form.