

US EPA ARCHIVE DOCUMENT

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 river 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 river 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, local contacts, boat launches, 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 River
TARGET	Marsh/Wetland
	Regular Wadeable Stream
	Regular - Partial Boatable and Wadeable Combination
	Regular Boatable
	Intermittent Stream
INACCESSIBLE	Dry Channel
	Altered Channel (channel different from map representation)
	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. At the launch site, unload the rafts and all equipment, supplies, and sample containers. Shuttle the vehicles.
4. Using a laser rangefinder, measure the river width in several places, specifically the X-site and the two boat launches. Record the width on the site verification form. Lay out a sample reach with a length of 100 times the river width by rolling a map wheel on the topographic map and marking the reach boundaries.
5. Do a reconnaissance of the sample reach while shuttling vehicles, obtaining widths, and evaluating launch sites. Extensive shallows, large log jams, absence of launch sites or vehicle access, and hazardous whitewater may all preclude rafting.
6. Determine the float distance, if any, from the put-in to the first transect (Transect "A"), and from the last transect (Transect "K") to the take-out.
7. Using a laser rangefinder at the most upriver transect (Transect "A"), measure 10 channel widths downriver to the next transect (Transect "B"). This distance is a profile.
8. Sample odd numbered site ID's along the left shore (facing downriver); sample even numbered sites along the right shore.

NOTE: There are some conditions that may require adjusting the reach about the X-site (i.e., the X-site no longer is located at the midpoint of the reach) to accommodate river access or to avoid river hazards or obstacles. If the beginning or end of the reach cannot be sampled due to obstacles or hazards, make up for the loss of reach length by moving ("sliding") the other end of the reach an equivalent distance away from the X site. Similarly, access points may necessitate sliding the reach. Do not "slide" the reach so that the X-site falls outside of the reach boundaries. Also, do not "slide" a reach to avoid man-made obstacles such as bridges, rip rap, or channelization.

Quick Reference Guide for Water Chemistry and Microbiology

I. Equipment to Carry in Field for Water Chemistry and Microbiology

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)
One 200-mL sterile square glass microbial bottle (barcode label attached)
Two syringe valves in the plastic container
Plastic cooler and several bags of ice
Opaque garbage bag
Electricians tape

II. Extra Equipment to Carry in Vehicle

Back-up labels, forms, cubitainers, syringes, syringe valves, and microbial bottles

III. Daily Activities after Sampling

1. Check that cubitainer lid is on tight, has a flush seal, and is taped. Also tape the microbial cap.
2. Prepare the sample for shipping (label and seal cooler, replace ice as close as possible to shipping time)
OR direct delivery to the laboratory.
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, syringes, and microbial bottle), pack cubitainer and sample beakers in clean self-sealing plastic bag, and pack two syringes, syringe valves, and a microbial bottle in a plastic container with a snap-on lid.

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Quick Reference Guide for Water Chemistry and Microbiology (continued)

Summary of Site Procedure for Water Chemistry and Microbiology

I. Collect Water Sample

- A. Make sure cubitainers and syringes are labeled and have the same barcode ID.
- B. Make sure the microbial bottle is labeled with a barcode ID.
- C. Cubitainer, syringe, and microbial samples are taken only from the **middle of the flowing river at the last sample transect (Transect "K")**.
- D. Rinse the 500-mL sample beaker three times with river water from the mid-channel.
- E. Rinse cubitainer three times with 25-50 mL of river water, using the sample beaker. Rinse cubitainer lid with river water.
- F. Fill cubitainer with river water 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.
- G. Rinse each of the two, 60-mL syringes three times with 10-20 mL of river water .
- H. Fill each of the syringes with river water from mid-river by slowly pulling out the plunger. If any air gets into the syringe, discard the sample and draw another.
- I. 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 river sample in the syringe. Close the valve and place the syringes in their transport container.
- J. Keep the microbial bottle closed until filled. Do not contaminate inner surface of cap or bottle. **Fill the bottle without rinsing.**
- K. Take sample from upriver side of boat by holding bottle near base and plunge neck downward below water's surface. Turn bottle until neck points slightly upward and mouth is directed toward the current.
- L. After sample is collected, leave ample air in the microbial bottle (~ 2.5 cm) and tape the cap tight.
- M. Place the cubitainer, syringes, and microbial bottle on ice in a cooler to keep cool (keep dark as well) until shipment.

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 river conductivity in mid-river **AT EACH TRANSECT**.
- B. Dissolved Oxygen/Temperature
 1. Calibrate the DO meter following meter instructions.
 2. Measure the DO in mid-river at the **middle of the flowing river of the last sample transect (Transect "K")**. If water velocity is slow, jiggle the DO probe as you take the reading. Measure temperature mid-river **AT EACH TRANSECT**.

Quick Reference Guide for Physical Habitat Characterization

Field Summary: P-hab Layout And Workflow

1. Habitat Sampling Layout:
 - A. Thalweg Profile: At 10 equally spaced intervals between each of 11 channel cross-sections (100 along entire reach):
 - * Classify habitat type, record presence of backwater and off-channel habitats. (10 between cross-sections, 100 total)
 - * Determine dominant substrate visually or using sounding rod. (10 between cross-sections, 100 total)
 At 20 equally spaced intervals between each of 11 channel cross-sections (200 along entire reach):
 - * Tally mid-channel snags (20 between cross-sections, 200 total).
 - * Measure thalweg (maximum) depth using Sonar or rod (20 between cross-sections, 200 total)
 - B. Littoral/Riparian Cross-Sections: @ 11 stops ("transects") at equal intervals along reach length
2. Work Flow: In a single mid-river float down a 100 channel-width reach
 - At the upriver start point (Transect "A") and along the designated shoreline: Move boat in a "loop" within a 10 x 20 m littoral plot, measuring 5 littoral depths and probing substrate. Also estimate dominant and subdominant littoral substrate within the "loop." After the "loop," estimate areal fish cover within and tally LWD within or partially within the 10 x 20 m plot. Record densiometer measurements at the bank (up, down, left, right), and choose bank angle class, and estimate bankfull height, width and channel incision (for BOTH banks). Estimate and record distance to riparian vegetation on the chosen bank. Estimate visually riparian vegetation cover for the 10 x 20 m plot on BOTH sides of channel (plot starts at bankfull, continues back 10m from bankfull). For the largest riparian tree, estimate Dbh, height, species, distance from river edge. Visually tally human disturbances in the same plot as riparian vegetation. No bearing or slope at first cross section.
 - Proceed downriver between Transects "A" and "B", making 20 thalweg depth measurements and substrate snag probes; also classify habitat types. Estimate thalweg distance intervals by tracking boat lengths or channel-widths. One person measures thalweg depths and the other records those measurements. At the 20th thalweg measurement location (close to Transect "B"), backsit a compass bearing in mid-channel, then distance and % slope back to your visual "mark" on the bank at the previous transect ("A").
 - When you complete 20 thalweg intervals and reach one of 11 cross sections, stop at the chosen shore and take out a new Channel/Riparian Transect Form for Transect "B". Repeat all the Channel/Riparian measurements at this new location.
 - Repeat the cycle of thalweg and cross section measurements until you reach transect 11 ("K") at the downriver end.

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Quick Reference Guide for Physical Habitat Characterization

Field Summary: Components of P-Hab Protocol

Thalweg Depth Profile, Mid-Channel Snags, Hab. Type, Off-channel, Substrate:

- At 20 approximately equal spaced intervals between each of 11 channel cross-sections (200 along entire reach) while floating mid-channel:
 - Measure max. depth ("Thalweg") at each increment
 - Tally mid-channel snags
- At 10 approximately equal spaced intervals between each of 11 channel cross-sections (100 along entire reach) while floating mid-channel:
 - Classify habitat type and off-channel habitats
 - Determine dominant substrate

Channel and Riparian Cross-Sections:

- Measurements: Wetted width, mid-channel bar width, gradient (clinometer or Abney level), sinuosity (compass backsight), riparian canopy cover (densiometer).
- Visual Estimates: Bankfull width, bankfull height, incision height, bank angle, shoreline substrate, large woody debris, 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.

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Quick Reference Guide for Physical Habitat Characterization (continued)

Field Summary: Rip. Veg., Human Disturb., In-Channel Cover:

- Observations upriver 10 meters and downriver 10 meters from each of the 11 cross-section transects.
- For riparian vegetation and human disturbances, include the visible area from the river 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:
 - (**D**eciduous, **C**oniferous, **B**roadleaf **E**vergreen, **M**ixed, or **N**one) 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: **P**RESENT within the defined 20 m river segment and located in the river or on the wetted or bankfull river
 - C: **C**LOSE - Present within the 10 x 20 m riparian plot area, but above bankfull level
 - P: **P**RESENT, but observed outside the riparian plot area
 - 0: **N**OT **P**RESENT within or adjacent to the 20 m river segment or riparian plot

Quick Reference Guide for Physical Habitat Characterization (continued)

Field Summaries: Substrate And Woody Debris Size Classes

Observe bottom substrates within a 10m swath along the 20m of channel margin that is centered on each transect location. Determine and record the dominant and subdominant substrate size class at 5 systematically spaced locations estimated by eye within this 10m x 20m plot and 1m back from the waterline.

Substrate Size Classes:

RS	Bedrock (Smooth)	>4000 mm	smooth surface rock or hardpan (bigger than a car)
RR	Bedrock (Rough)	>4000 mm	Rough surface rock (bigger than a car)
HP	Hardpan	>4000 mm	(consists of firm, consolidated fines)
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)
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) > 30 cm (>1 ft.)
Length > 5 m (> 15 ft) -- count only part with diam > 30 cm.

Two Tallys:

- (1) LWD at least partially in the baseflow channel (wetted).
- (2) LWD presently dry but contained within the bankfull (active) channel, and LWD spanning above the active channel.

Size Categories for Tally (12 potential combinations):

Diameter (large end):		Length:	
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 - <30 m	(49 - 98 ft)
0.8 to <1.0 m	(2.6 to 3.3ft)	>30 m	(>98 ft)
>1.0 m	(> 3.3ft)		

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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 (PO):	Still water, low velocity, smooth, glassy surface, usually deep compared to other parts of the channel:
Plunge Pool	Pool at base of plunging cascade or falls.
Trench Pool	Pool-like trench in the center of the stream
Lateral Scour Pool	Pool scoured along a bank.
Backwater Pool	Pool separated from main flow off the side of the channel.
Dam Pool	Pool formed by impoundment above dam or constriction.
Glide (GL)	Water moving slowly, with a smooth, unbroken surface. Low turbulence.
Riffle (RI)	Water moving, with small ripples, waves and eddies -- waves not breaking, surface tension not broken. Sound: "babbling", "gurgling".
Rapid (RA)	Water movement rapid and turbulent, surface with intermittent whitewater with breaking waves. Sound: continuous rushing, but not as loud as cascade.
Cascade (CA)	Water movement rapid and very turbulent over steep channel bottom. Most of the water surface is broken in short, irregular plunges, mostly whitewater. Sound: roaring.
Falls (FA)	Free falling water over a 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 the channel
Off-Channel Areas	Side-channels, sloughs, backwaters, and alcoves that are separated from the main channel.

^aNote that in order for a channel habitat unit to be distinguished, it must be at least as wide or long as the channel is wide.

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 the Channel/Riparian Transect 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

Both bars and islands cause the river to split into side channels. When a bar or island is encountered along the thalweg profile, choose to navigate and survey the channel that carries the most flow.

Side channels (off-channel):

When present, check the "Off-channel" column on the Thalweg Profile Form. Begin checking at the point of divergence continuing until convergence. In the case of a slough or alcove, "off-channel" checkmarks should continue from the point of divergence downriver to where it is no longer evident.

Dry and Intermittent rivers:

Record zeros for depth and wetted width in places where no water is in the channel. Record habitat type as dry channel (DR).

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* and biomass (AFDM).
10. Forceps for filter handling.
11. Millipore®-type filtration apparatus with plastic or stainless steel filter base, and Nalgene® funnel and suction flask.
12. Nalgene® hand-operated vacuum pump (need one additional pump as a backup).
13. Aluminum foil.
14. Ice chest.

Field Protocols

1. Periphyton samples are collected from the designated shoreline at each transect location.
2. Collect a sample of substrate (rock or wood) that is small enough (< 15 cm diameter) and can be easily removed from the river. Place the substrate in a plastic funnel which drains into a 500-mL plastic bottle with volume graduations marked on it.
3. Use the area delimiter to define a 12-cm² area on the upper surface of the substrate. Dislodge attached periphyton from the substrate within the delimiter into the funnel by brushing with a stiff-bristled toothbrush for 30 seconds. Take care to ensure that the upper surface of the substrate is the surface that is being scrubbed, and that the entire surface within the delimiter is scrubbed.
4. Fill a wash bottle with river water. Using a minimal volume of water from this bottle, wash the dislodged periphyton from the funnel into the 500-mL bottle.
5. If no coarse sediment (cobbles or larger) are present, collect soft sediments by vacuuming the upper 1 cm of sediments confined within the 12-cm² sampling ring into a 60-mL syringe.
6. Place the sample collected at each sampling site into the single 500-mL bottle to produce the composite index sample.
7. After samples have been collected from all 11 transects, thoroughly mix the 500-mL bottle regardless of substrate type.
8. Record total volume of composited sample before proceeding to the next step!
9. 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 μ m 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.

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Quick Reference Guide For Periphyton (continued)

- c. Ash Free Dry Mass (AFDM)
 - 1) Withdraw 25 mL of mixed sample and filter onto a glass-fiber filter (0.45 μ m 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 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.

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 95).
6. Spare batteries for D.O. meter.
7. Permanent markers for labeling tubes.
8. Sample labels and field data sheets.
9. Ice chest with ice for sample freezing.

Field Protocols

Dissolved Oxygen Meter Calibration (for YSI model 95)

1. Calibrate meter using the water-saturated atmosphere chamber described in the meter's operations manual. Allow at least 15 minutes for the probe to equilibrate before attempting to calibrate.

Sediment Collection and Experimental Set-up

1. Collect and combine fine-grained, surface sediments (top 2 cm) from all depositional areas at each transect (Transects A-K) along the designated shoreline of the river reach.
2. Fill ice chest 2/3 full with river 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.

Quick Reference Guide For Benthic Macroinvertebrates

Table I. Base Protocols for Collecting Macroinvertebrates

1. Set drift net assembly(s) near the put-in or take-out location.
2. Shore kick net samples are collected at each of the transect locations along the designated shoreline. Drift nets collect samples during the sampling day while the crew floats the river.
3. If riffle or run, use the kick net protocol in Table II. If pool, use the kick net protocol in Table III or hand pick for 60 seconds if kick net cannot be used.
4. Go to next downriver transect and repeat. Combine all riffle and pool samples into one bucket. Check net after each sample for clinging organisms and transfer to bucket.
5. After a sample is collected from each of the transects and all kick net samples are combined into one bucket, obtain a composite sample as described in Table V.
6. Drift net(s) procedures are described in Table IV. Processing is described in Table V.
7. Preserve and label each sample as described in Table VI.

Table II. Procedures for Riffles and Glides using Kick Net Sampler

1. Attach four foot pole to the sampler.
2. Position sampler quickly and securely on river bottom with net opening upriver.
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 upriver motion to wash the organisms to the bottom of net.
6. Rinse net contents into a 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. After all transects are sampled and all samples are combined in ONE bucket (riffle/glide + pool), obtain a composite sample as described in Table V.

Table III. Procedures for Pools using the Modified Kick Net Sampler

1. Attach four-foot pole to the sampler.
2. Inspect about ½ 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 upriver 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. After all transects are sampled and all samples are combined in ONE bucket (pool + riffle/glide), obtain a composite sample as described in Table V.

Table IV. Collection Procedures for Drift Nets

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

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Quick Reference Guide For Benthic Macroinvertebrates (continued)

2. Install the net at the downriver end of the reach (Transect K). The take-out location is 1st choice, otherwise the put-in location - whichever is closer to the reach.
3. Set the nets in the main flow of the river (avoid backwaters, eddies, river margins) at depths of about 25 cm from the bottom substrate and 10 cm below the water's surface.
4. Anchor the net assembly using anchors and cables. Record START TIME.
5. Measure the current velocity at the entrance of the net, using a neutrally buoyant object 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. After floating the river, retrieve the net assembly from the water, taking care not to disturb the bottom upriver of the net. Record the END TIME.
7. Determine the current velocity again as described above, calculate the average from the 6 measurements, and record on the form.
8. Concentrate the material in each net in one corner by swishing up and down in the river. Wash the material into a bucket half filled with water (NOT the shore sample bucket). Remove as much as possible from the nets.
9. The contents from both nets are combined into a single bucket. After this, pour the sample over a sieving bucket (same bucket used in the kick net samples).
10. Large objects in the bucket should be carefully inspected for organisms which are washed into the bucket before discarding.
11. After both nets are combined into one bucket, obtain a composite sample as described in Table V.

Table V. Procedures for Obtaining the Composite Sample

1. Pour the contents of the composite 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 appropriate jar labeled as either "shore" or "drift" sample.

Table VI. 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 that the pre-numbered stick-on barcoded label is the on jar. Cover the entire label with clear, waterproof tape.
4. Seal the caps with electrical tape.
5. Place samples in cooler or other secure container for transport.
6. Secure all equipment in the vehicle.

Quick Reference Guide for Aquatic Vertebrates

Field Protocols For Fish Collection

1. Site Selection
 - a. Determine river bank to be sampled. Stay along this shore the entire day, unless river aspect is unchanging and the selected side is not representative of both.
 - b. Float downriver along the designated shoreline, stopping at each transect (A to K).
 - c. In case of emergency, determine location of means of easy egress from river.
2. Electrofishing
 - a. Check all electrical connections and potential conductors. Place cathodes and anodes in the water. Fill livewell with river water.
 - b. Start generator, switch to pulsed DC, a frequency of 30pps, low range and 40%. These are the initial settings. Set timer and depress pedal switch to begin fishing.
 - c. With switch depressed and floating downriver near shore, maneuver the raft or anode to cover a swath 3-4 meters wide, at an oar's length from shore, near cover, and at depths less than 3 meters wherever possible.
 - d. Deposit fish in the livewell as soon as possible; do not hold them in the electrical field.
 - e. Continue fishing until the next transect.
 - f. Process fish when stopped at each transect. Record total time spent collecting and shocking time on data sheets.
 - g. Identify and release any threatened and endangered species.
 - h. Identify and measure (TL) sport fish and very large specimens, record external anomalies, and release unharmed.
 - i. Identify other specimens. Determine number of individuals in species, measure largest and smallest individuals, and voucher as described in Voucher Protocol.
 - j. Large, questionable species should be placed on ice and then frozen.
 - k. Retain a subsample of target species for Fish Tissue Contaminants analysis.

(continued)

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	WG	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 (Please comment).
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	WL	Annelid worms which have anterior and posterior suckers. They may attach anywhere on the body.
Exophthalmia	EX	Bulging of the eye.

(continued)

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 up to 20 adults and juveniles. If less than 20 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.
- After all individuals of a species have been processed, place the voucher sub sample in a bucket with carbon dioxide tablets and a small amount of water. Individuals > 160 mm should be slit on the lower abdomen of the RIGHT side.
 - 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).
 - DO NOT over pack the sample jars with specimens OR use less formalin than is needed. If a fish will not fit in a jar, freeze the specimen.
 - 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.
 - Transport to storage depot at end of week. Store in a cool, dark, ventilated space.

Quick Reference Guide For Fish Tissue Contaminants

Selecting And Processing Fish Tissue Specimens

NOTE: If neither a primary nor secondary species sample is available, use your best judgement in sending some type of composite fish tissue sample.

Primary Sample (P)

After all voucher specimens have been prepared, choose a cottid, cyprinid, or salmonid that has enough similarly sized individuals to weigh to 400 g.

Secondary Sample (S)

After all voucher specimens have been prepared, select a large piscivore or omnivore species that has at least 5 individuals 120 mm. Include similar sized individuals if available.

1. Place the fish into a bucket with two carbon dioxide tablets (e.g., "Alka Seltzer®") and a small volume of water. After they have been anaesthetized, use clean hands to transfer them to aluminum foil.
2. Prepare a clean work surface to prepare the primary composite sample. Keep hands, work surfaces, and wrapping materials clean and free of potential contaminants (mud, fuel, formalin, sun screen, insect repellent, etc.)
- 3-P. For primary samples, record the common name (from a standardized list) of the species, its species code (if required), and the number of individuals in the sample in the appropriate fields on line "P1" of the Sample Collection Form (Figure 11-1).
- 3-S. Measure the total length (TL) of each secondary individual. Record the common name (from a standardized list) of the secondary target species, its species code (if required), and the total length for each individual on lines S1 through S5 in the secondary sample section of the Sample Collection Form.
4. If the individuals included in composite samples were collected from throughout the sampling reach, place an "X" in the "Yes" box in the sample section of the Sample Collection Form. If the individuals were only collected from a limited segment of the sampling reach, place an "X" in the "No" box and explain in the "Explain" field on the form.
- 5-P. Wrap all primary fish together in a single piece of aluminum foil, making sure the dull side of the aluminum foil is in contact with the fish. Place the sample in a self-sealing plastic bag.
- 5-S. Wrap each fish of the secondary sample separately in aluminum foil, with the dull side of the foil in contact with the fish. Place all the wrapped individuals into a single self-sealing plastic bag.
6. Expel excess air and seal the bag. Wrap clear tape around the bag to seal and make a surface for each sample label.
- 7-P. Prepare two Fish Tissue sample labels (each having the same sample ID number [Figure 11-2]) by filling in the stream ID and the date of collection. Circle "PRIMARY" on each label. Record the sample ID number (barcode) in the primary sample section of the Sample Collection Form.
- 7-S. Prepare two Fish Tissue sample labels (each having the same sample ID number [Figure 11-2]) by filling in the stream ID and the date of collection. Circle "SECONDARY" on each label. Record the sample ID number (barcode) in the secondary sample section of the Sample Collection Form.
8. Attach the appropriate label to the tape surface of the bag. Cover the label with a strip of clear tape. Place the labeled bag into a second self-sealing plastic bag. Seal the bag and attach the second label to the outside of the appropriate bag. Cover the label with a strip of clear tape.
- J. Place the double-bagged sample into a cooler containing bags of ice until shipment. Keep the sample frozen until shipment.