EPA/XXX/X-XX/XXX
April 2001

ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM-SURFACE WATERS:

WESTERN PILOT STUDY FIELD OPERATIONS MANUAL FOR WADEABLE STREAMS

Edited by

David V. Peck[1], James M. Lazorchak[2], and Donald J. Klemm[2]

[1] U.S. Environmental Protection Agency
Regional Ecology Branch
Western Ecology Division
National Health and Environmental Effects Research Laboratory
Corvallis, OR 97333

[2] U.S. Environmental Protection Agency
Ecosystems Research Branch
Ecological Exposure Research Division
National Exposure Research Laboratory
Cincinnati, OH 45268

NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

NATIONAL EXPOSURE RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

SECTION 12
AQUATIC VERTEBRATES

by
Frank H. McCormick[1] and Robert M. Hughes[2]

Sampling amphibian, fish, and crayfish (no reptiles) species to determine their proportionate abundances and the presence of external anomalies is conducted after all other field sampling and measurement activities are completed. The objective is to collect a representative sample of all except very rare species in the assemblage. Backpack electrofishing equipment is used as the principal sampling gear (Section 12.1.1). Bank or towed electrofishers are recommended for wide but shallow streams (Section 12.1.2), and seining (Section 12.1.3) is used in habitats where high conductivity or turbidity preclude electrofishing. All team personnel are involved in collecting aquatic vertebrates. In addition to gathering data on the assemblage, fish specimens are retained for analysis of tissue contaminants and microbial pathogens (Section 13).

The procedures and activities presented here differ slightly from those previously published for EMAP-SW (McCormick and Hughes, 1998). These changes are summarized in Table 12-1. In 2000, aquatic vertebrates collected between transects were tallied and recorded separately to provide a means to evaluate sampling efficiency. Crayfish collected during aquatic vertebrate sampling are counted and included as part of the aquatic vertebrate sample. Identifying and tallying specific types of external anomalies, and measuring total lengths of 30 individuals of dominant species are no longer included. Beginning in 2001, aquatic vertebrates are tallied and recorded on a single data form for the entire reach. If a wadeable river is too shallow to sample by boat and too wide (> 20 m) to sample efficiently with backpack electrofishers or seines, the stream is probably more effectively sampled using methods similar to those presented in the field operations manual for nonwadeable rivers and streams, and may require more than 1 day to sample completely.

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[1] U.S. EPA, National Exposure Research Laboratory, Ecological Exposure Research Division, 26 W. Martin Luther King Dr., Cincinnati, OH 45268.

[2] Dynamac International Corp., 200 SW 35th St., Corvallis, OR 97333

TABLE 12-1. SUMMARY OF CHANGES IN AQUATIC VERTEBRATE PROCEDURES FOR THE WESTERN PILOT STUDY

Changes from McCormick and Hughes (1998)

1. Aquatic vertebrates collected between each pair of transects are tallied and recorded on separate field data forms.
2. Crayfish collected during aquatic vertebrate sampling are counted and included as part of the aquatic vertebrate sample.
3. Recording the occurrence of specific types of external anomalies is not required.
4. Determination of total lengths of 30 individual fish of each dominant species collected is not required.

Changes from Year 2000 Western Pilot Study Activities

1. Aquatic vertebrates and crayfish are tallied and recorded on a single data form for each stream; all transects where a species is collected are noted on the form
2. Procedures for dealing with wide (>20 m) yet wadeable streams have been clarified and/or included.

12.1 SAMPLE COLLECTION

The entire channel within the sampling reach is sampled through use of transects (see Section 4) so that effort is distributed along the entire reach. Collection time should be 45 minutes to 3 hours within the reach (Section 4) to obtain a representative sample. If a stream is very wide, however, it may take 2 days to effectively sample it. Sampling data and general comments (perceived fishing efficiency, missed fish, gear operation, suggestions) are recorded on the Vertebrate Collection Form (Figure 12-1).

12.1.1 Electrofishing

Primary responsibility for safety while electrofishing rests with the crew chief (Section 2). Electrofishing units may deliver a fatal electrical shock. While electrofishing, avoid contact with the water unless sufficiently insulated against electrical shock. Use chest waders with nonslip soles and linesman gloves (***NOTE: some types of "breathable" waders do not provide adequate insulation against electric current when wet***). If waders become wet inside, stop fishing until they are thoroughly dry or use a dry pair. **Avoid contact with the anode and cathode at all times due to the potential shock hazard.** If you perspire heavily, wear polypropylene or some other wicking and insulating clothing instead of cotton. If it is necessary for a team member to reach into the water to

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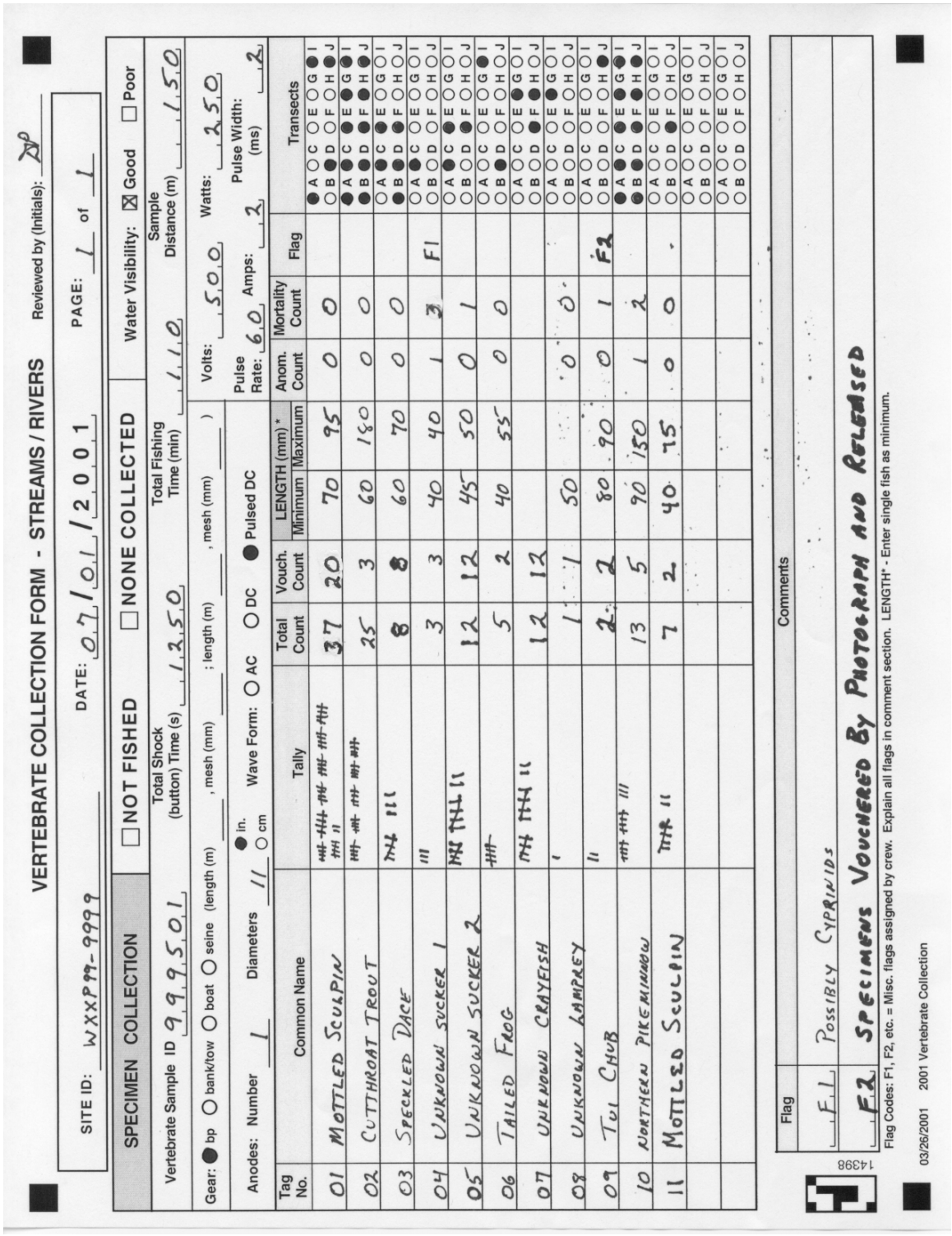

VERTEBRATE COLLECTION FORM - STREAMS / RIVERS

SITE ID: WXXP99-9999 DATE: 07/01/2001 PAGE: 1 of 1 Reviewed by (Initials): DP

SPECIMEN COLLECTION ☐ NOT FISHED ☐ NONE COLLECTED Water Visibility: ☒ Good ☐ Poor

Vertebrate Sample ID 99950 1 Total Shock (button) Time (s) 1250 Total Fishing Time (min) 110 Sample Distance (m) 150

Gear: ● bp ○ bank/tow ○ boat ○ seine (length (m) ____, mesh (mm) ____; length (m) ____, mesh (mm) ____) Volts: 500 Watts: 250

Anodes: Number 1 Diameters 11 ● in. ○ cm Wave Form: ○ AC ○ DC ● Pulsed DC Pulse Rate: 60 Amps: 2 Pulse Width: (ms) 2

Tag No.	Common Name	Tally	Total Count	Vouch. Count	LENGTH (mm)* Minimum	LENGTH (mm)* Maximum	Anom. Count	Mortality Count	Flag	Transects
01	MOTTLED SCULPIN	卌 卌 卌

pick up a fish or something that has been dropped, do so only after the electrical current is off and the anode is removed from the water. Do not resume electrofishing until all individuals are clear of the electroshock hazard. The electrofishing equipment is equipped with a 45° tilt switch that interrupts the current. Do not make any modifications to the electrofishing unit that would hinder turning off the electricity. Avoid electrofishing near unprotected people, pets, or livestock. Discontinue activity during thunderstorms or heavy rain. Team members should keep each other in constant view or communication while electrofishing. For each site, know the location of the nearest emergency care facility. Although the team leader has authority, each team member has the responsibility to question and modify an operation or decline participation if it is unsafe.

12.1.1.1 Backpack Electrofishing--

The backpack electrofishing procedure is presented in Table 12-1; record information on the Vertebrate Collection Form (Figure 12-1). If the stream cannot be sampled by either electrofishing or seining, mark the “NOT FISHED” field on the form. Determine that all team members are wearing waders and gloves and are clear of both electrodes. Wear polarized sunglasses and caps to aid vision. The backpack unit is equipped with an audio alarm that sounds when the output voltage exceeds 300 V. It also serves as an input current indicator for pulse cycles greater than 5Hz. It begins as a strong continuous tone and begins to beep slowly at currents of 1.25 amps. It beeps faster as input current increases. In case of an overload (in excess of 3 amps), the beep becomes very rapid and the overload indicator comes on. Release the anode switch, adjust voltage and waveform, and resume fishing.

The anode is fitted with a net and a second netter uses an insulated dip net to retrieve stunned individuals, which are immediately deposited into a bucket for later processing (Section 12.3). If individuals show signs of stress (loss of righting response, gaping, gulping air, excessive mucus), change water or stop fishing and process them. This should only be necessary on very warm days, in long transects, or if large numbers or biomasses of aquatic vertebrates are collected. Cease electrofishing to process and release listed threatened or endangered species or large game fish as they are netted (see Section 12.2). If periodic processing is required, be sure to release individuals downstream to reduce the likelihood of collecting them again.

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TABLE 12-2. BACKPACK ELECTROFISHING PROCEDURES

1. Allocate the total fishing time (45-180 min) among all transects based on stream size & complexity. It may be necessary to spend 2 days on extremely wide wadeable streams.

2. Review all collecting permits to determine if any sampling restrictions are in effect for the site. In some cases, you may have to cease sampling if you encounter certain listed species, or use alternate gear types.

3. Search for aquatic vertebrates and crayfish even if the stream is extremely small, and it appears that sampling may produce no specimens. If none are collected, check the "NONE COLLECTED" box on the Vertebrate Collection Form. Explain why in comments section.

4. If conductivity, turbidity, or depth preclude backpack electrofishing, sample by seining or bank/ towed electrofishing if possible, otherwise do not sample. If you do not sample, complete the "NOT FISHED" field on the Vertebrate Collection Form and comment why.

5. Set unit to pulsed DC. Select initial voltage setting (150-400 V for high conductivity [>300 S/cm]; 500-800 V for medium conductivity [100 to 300 S/cm]; 900-1100 V for low conductivity [< 100 S/cm] waters). In waters with strong-swimming fish (length >200 mm), use a pulse rate of 30 Hz with a pulse width of 2 msec. If mostly small fish are expected, use a pulse rate of 60-70 Hz. Start the electrofisher, set the timer, and depress the switch to begin fishing. If fishing success is poor, increase the pulse width first and then the voltage. Increase the pulse rate last to minimize mortality or injury to large fish. If mortalities occur, first decrease pulse rate, then voltage, then pulse width. Start cleared clocks. Note, some electrofishers do not meter all the requested header data; provide what you can.

6. Once the settings on the electrofisher are adjusted properly to sample effectively and minimize injury and mortality, begin sampling at the downstream end of the reach (Transect A), and fish in an upstream direction. Depress the switch and slowly sweep the electrode from side to side in the water in riffles and pools. Sample available cut-bank and snag habitat as well. Move the anode wand into cover with the current on, then remove the wand quickly to draw fish out. In fast, shallow water, sweep the anode and fish downstream into a net. In extremely wide streams, work from the midline of the stream channel to the banks. Be sure that deep, shallow, fast, slow, complex, and simple habitats are all sampled. In stretches with deep pools, fish the margins of the pool as much as possible, being extremely careful not to step or slide into deep water. Keep the cathode near the anode if fish catch is low.

7. The netter, with the net 1 to 2 ft from the anode, follows the operator, nets stunned individuals, and places them in a bucket.

8. Continue upstream until the next transect is reached. Process fish and/or change water after each transect to reduce mortality and track sampling effort.

9. Complete header information on the Vertebrate Collection Form.

10. Repeat Steps 6 through 8 until Transect "J-K" is finished.

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12.1.1.2 Bank/towed Electrofishing--

Bank/towed electrofishing sampling procedures are presented in Table 12-3. The primary electrofishing gear is a 9 ft. inflatable kayak modified to carry all fishing equipment. Kayak configuration consists of a frame mounted generator and electrofishing control box, port and starboard cathodes, and one or two hand-held anodes fitted with netting. Alternatively, the generator and control box may remain on the riverbank connected to the electrodes by a 100 m long heavily insulated wire. The kayak is maneuvered by one or two persons, and the vertebrates are collected by the others wading in the water. Wear chest waders and linesman gloves to avoid electric shock, and polarized sunglasses and caps to minimize glare. Starting at the bottom of the reach and along the designated shoreline, fish in an upriver direction. Adjust voltage and output according to sampling effectiveness and incidental mortality to specimens.

The netters use a dip net and an insulated anode with a net ring to retrieve stunned individuals, which are then deposited into a livewell in the kayak for later processing (Section 12.2). **Note, to avoid shocking crew members, insure that the thumb switch is off anytime the anode leaves the water.** Change the water in the live well at each transect to minimize mortality. If individuals show signs of stress (loss of righting response, gaping, gulping air, excessive mucus), stop fishing and process them. This should only be necessary on very warm days, in long transects, or if very large biomasses of aquatic vertebrates are collected. Cease electrofishing to immediately process and release specimens (e.g., listed species or large game fish) as they are netted (Section 12.2). If periodic processing is required, be sure to release individuals downriver and away from the shoreline to reduce the likelihood of collecting them again. At the completion of electrofishing each transect, record information on the Vertebrate Collection Form (Figure 12-1). Use ear protection and hand signals to communicate direction and power on or off when using generators.

Gasoline is extremely volatile and flammable. Its vapors readily ignite on contact with heat, spark or flame. Never attempt to refill the generator while it is running. Always allow the generator to cool before refilling. Keep gasoline out of direct sunlight to reduce volatilization and vapor release. Keep gasoline only in approved, tightly closed plastic containers.

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TABLE 12-3. BANK/TOWED ELECTROFISHING PROCEDURES

1. Select river bank for initial fishing (left for odd numbered sites [e.g., WXXP99-0503], right for even) unless immediate hazards or obstructions preclude this. Stay along the selected bank for two transects to the degree it is safely wadeable. Switch to the opposite bank for the next two transects, alternating in this manner until the entire site is fished or hazards prevent it. Using a rangefinder, determine a downstream point that is 4 mean channel widths distant (this is the transect length). Record this distance on the Vertebrate Collection Form.

2. Fill tank with gas, check all electrical connections and potential conductors, and place the anodes and cathodes in the water. Fill livewell and put on linesman gloves. Verify that all electrical switches are off, that cathodes are submerged, that all non-target organisms are clear of the water or 20' away, and that barge surfaces are dry.

3. Review all collecting permits to determine if any sampling restrictions are in effect for the site. In some cases, you may have to cease sampling if you encounter certain listed species, or use alternate gear types.

4. Start generator, switch to pulsed DC, a pulse rate of 30 pps, low range (duty cycle) and 40%. Increase % (voltage) as needed to roll fish. If success is poor, reduce %, switch to high range, and again increase % as needed. If effectiveness is still low, switch to 60 pps and repeat the process. If the current (amperage needle) is reduced, switch back to low range to avoid over-loading the generator. **Switching should occur when power to the control box is off.** If the conductivity of the river is > 1700 &S/cm, use a larger generator or seine. Netters activate thumb switches and insure that when they are off current ceases. Crew members towing the barge activate the generator and pulsator switches. Verify that fish are rolled and relaxed but not rigid before beginning transect. Record settings on the Vertebrate Collection Form and clear clocks.

5. Zero the timer, and depress the thumb switch to begin fishing. With system activated and safety switches on, fish upstream near shore. Maneuver the anode(s) to cover a swath 3-4 meters wide, near cover, and at depths less than 1 meter wherever possible. Do not place yourself or the gear in danger in order to fish particular habitats; cut the generator and stow the gear before negotiating hazards.

6. Place fish directly in livewell; do not hold them in the electrical field. Pay special attention to netting small and benthic fishes as well as fishes that respond differently to the current--not just the big fish that move to the surface. Try to net all fish seen, but in productive areas this is impossible. Do not chase individual fish or place yourselves in unbalanced positions to net them. If benthic fish are not being collected, occasionally hold a net in the current and along the bottom, then sweep the anode downstream into the net. Draw fish from cover by thrusting the anode into the cover with the power on, then quickly removing it.

7. Cease sampling at the end of the transect. Process the fish quickly and carefully, returning them to the water unless they are vouchered or saved for tissue.

8. Return to step 1 for each of the subsequent 9 transects, but begin upstream from where fish were released and alternate banks on every other transect.

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12.1.2 Seining

Seining is used when the conductivity of the stream is too high and/or in streams with extremely high turbidity where electrofishing is ineffective. Avoid high mortality rates by employing multiple short seine hauls at each of the 11 transects by using either the "riffle" or "pool" method (Table 12-4). Allocate the total sampling time (240 minutes) among the 11 transects (i.e., from 16 to 22 minutes per transect). If no aquatic vertebrates were collected, indicate this on the form (Figure 12-1). Record the seine length, mesh size, the time spent seining ("SAMPLING TIME") and the length of the seine haul ("SAMPLING DISTANCE") on the Vertebrate Collection Form (Figure 12-1). If more than one size or type of seine is required, record the information for the primary seine used on the collection form, and note the alternative types used in the comments section of the form.

12.2 SAMPLE PROCESSING

Sample processing involves tallying and identifying fish, crayfish and amphibians, examining individual specimens for external anomalies, obtaining length measurements from selected specimens, preparing voucher specimens for taxonomic confirmation and archival at a museum, and selecting specimens to prepare samples for fish tissue contaminants (Section 13). Process collections as quickly as possible to minimize stress to live specimens. One person can process fish from one bucket while the other team members continue to collect fish and deposit them into a second bucket. One person can identify, measure, and examine individuals while another person records information on the field data forms.

12.2.1 Taxonomic Identification and Tally

Table 12-5 presents the procedure for identifying and tallying aquatic vertebrates. Record identification, tally data, and comments for each species on the Vertebrate Collection Form (Figure 12-1). It is important to note all transects where a species is collected, as this is information is needed to develop estimates of sampling efficiency. Use common names from Page and Burr (1991) or similar keys. Taxonomic identification should be performed only by trained ichthyologists familiar with the fish species and other aquatic vertebrate taxa of the region. Use taxonomic reference books and other materials that contain species descriptions, ranges, and identification keys to make species identifications in the field. Where there are many individuals of easily identified species, processing is facilitated by keeping a tally count of the number of individuals of each species and totaling

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TABLE 12-4. SEINING PROCEDURES

12. Allocate the sampling effort throughout the reach so that the total fishing time will be between 45 minutes (small stream) and 3 hours (large stream). It may be necessary to spend 2 days on extremely wide wadeable streams.

13. Review all collecting permits to determine if any sampling restrictions are in effect for the site. In some cases, you may have to cease sampling if you encounter certain listed species.

14. Search for aquatic vertebrates & crayfish even if the stream is extremely small and it appears that sampling will produce no specimens. If none are collected, complete the "NONE COLLECTED" field on the Vertebrate Collection Form. Explain why in the comments section.

15. Begin at the downstream end of the sampling reach (Transect A). Proceed along the reach, sampling available habitats using the appropriate methods below:

    4A. Riffle habitats: Use a seine 2 m long (1.25 m high with 0.6 cm mesh.

        1. Place the seine perpendicular to the current across the downstream end of the riffle. Ensure that the lead line is on the bottom. Tilt the net slightly downstream to form a pocket to trap aquatic vertebrates.
        2. Starting about 2 m upstream, kick the substrate and overturn rocks, proceeding downstream toward the net.
        3. Raise the net quickly and examine it carefully for aquatic vertebrates (and crayfish).

    3B. Pool habitats: Use a seine 3-9 m long × 2 m high with 0.6 cm mesh size.

        1. Two people pull the seine across the pool, using the shore or riffles as barriers.
        2. In areas with current, pull the net downstream and then sweep toward the shore with one or both poles, or keep one end of the seine near shore and sweep the other end in a wide arc from midstream to the same shore.
        3. Pull the net onto the shore and examine it carefully for aquatic vertebrates (and crayfish).

    3C. Snags & undercut banks: Use a seine 2 m long × 1.25 m high; 0.6 cm mesh size.

        1. Jab the seine under the cover and near the stream bottom, then quickly lift it above the stream surface.

16. Place individuals in buckets for processing, and continue upstream to the next habitat area.

17. Complete the header information on the Vertebrate Collection Form.

18. Repeat Steps 4 through 6 for successive habitat areas until Transect "I-K" is finished.

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TABLE 12-5. PROCEDURE TO IDENTIFY, TALLY, AND EXAMINE AQUATIC VERTEBRATES

1. Complete all header information accurately and completely. If no vertebrates or crayfish were collected, complete the "NONE COLLECTED" field on the Vertebrate Collection Form.

2. Identify and process each individual completely, ideally handling it only once. Record the common name (PRINT USING CAPITAL LETTERS) on the first blank line in the "SPECIMENS" section of the Vertebrate Collection Form. If a species cannot be positively identified, assign it as "unknown" followed by its common family name (e.g., UNKNOWN SCULPIN A). Note every transect where a species is collected (letters represent the downstream transect).

3. Process species listed as threatened and endangered first and return individuals immediately to the stream. Photograph specimens for voucher purposes if conditions permit and stress to individuals will be minimal. Indicate if photographed on Vertebrate Collection Form. If individuals have died, prepare them as voucher specimens and preserve in formalin.

4. Keep voucher specimens (up to 20) of smaller individuals of each species. If no smaller individuals are collected, photograph each species and indicate so on the data form. Large, questionable species may be placed on ice and then frozen.

5. Tally the number of individuals of each species collected in the "TALLY" box on the Vertebrate Collection Form and record the total number in the "COUNT" field on the form.

6. Measure the total length (body length for amphibians, no lengths for crayfish) of the largest and smallest individual to provide a size range for the species. Record these values in the "LENGTH" area of the Vertebrate Collection Form.

7. Examine each individual for external anomalies and tally those observed. Readily identified external anomalies include missing organs (eye, fin), skeletal deformities, shortened operculum, eroded fins, irregular fin rays or scales, tumors, lesions, ulcerous sores, blisters, cysts, blackening, white spots, bleeding or reddening, excessive mucus, and fungus. After all of the individuals of a species have been processed, record the total number of individuals affected in the "ANOMALIES" area of the Vertebrate Collection Form. Photograph specimens with especially extreme anomalies and save them for fish tissue microbial samples.

8. Record the total number of mortalities due to electrofishing or handling on the Vertebrate Collection Form.

9. Follow the appropriate procedure to prepare voucher specimens and/or to select specimens for tissue samples. Release all remaining individuals so as to avoid their recapture.

10. For any line with a fish name, ensure that all spaces on that line are filled in with a number, even if it is zero.

11. Repeat Steps 1 through 10 for all other species.

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TABLE 12-5 (continued)

12. After processing the fish from all 10 transects, calculate a Jaccard Coefficient (JC) to assess sample adequacy. To help randomize the calculation, list the species collected from transects A-B, C-D, E-F, G-H, and I-J in group "A" and those from B-C, D-E, F-G, H-I, and J-K in group "B". Calculate the Jaccard coefficient as:

$$\mathbf{JC} = \frac{\mathbf{S}}{\mathbf{S} + \mathbf{A} + \mathbf{B}}$$

where **S** is the number of species shared by both groups, **A** is the number of species unique to group A, and **B** is the number of species unique to group B. Record JC = "n" in the comments section; if JC < 0.7, sample two additional transects. List the species in the appropriate group and recalculate JC. Continue until JC $\geq$ 0.7 or there is insufficient time or space to sample.

EXAMPLE: You have collected 6 different species from the group "A" transects, and 4 different species from the group "B" transects. Of these, three species were shared by both groups.

JC = (3) / (3) + (6-3) + (4-3)
JC = (3) / (3 + 3 + 1)
JC = (3) / (7)
JC = 0.4 In this case, sample two additional transects and recalculate.

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the tally once processing is complete. If protected fish have died, voucher them. Notify the appropriate state officials as soon as possible.

After the entire reach has been sampled , sampling adequacy is estimated based on the Jaccard coefficient of similarity, using the presence/absence of species between the downstream and upstream halves of the reach (i.e., the top and bottom rows of transects on the form. If the calculated Jaccard value is < 0.7, sample an additional 2 transects (8 channel widths) upstream of the reach. Continue with additional transects until the calculated Jaccard value is $ 0.7, or until there is insufficient time or space to sample. For the data presented in Figure 12-1, a total of 9 vertebrate species were collected. Six species are shared between the two groups of transects, 2 species were only collected in the "A" group of transects, and 1 species was only collected in the "B" group of transects. The Jaccard coefficient is calculated as:

$$JC = \frac{6}{6+2+1} = 0.67 \approx 0.7$$

For this site, the sampling effort is adequate, and no additional transects are sampled.

12.2.2 External Examination and Length Measurements

During the tallying procedure for each species (Table 12-5), examine each individual for the presence of external anomalies. Record the number of individuals affected on the Vertebrate Collection Form (Figure 12-1). Blackening and exopthalmia (popeye) may occasionally result from electrofishing. Injuries due to sampling are not included in the tally of external anomalies, but should be noted in the comments section of the Vertebrate Collection Form (Figure 12-1). Blackening from electrofishing usually follows the myomeres or looks like a bruise. If fish die due to the effects of sampling or processing, record the number for each species on the Vertebrate Collection Form (Figure 12-1).

For each species, use a measuring board or ruler to determine the length of the largest and smallest individuals collected at a site. Measure total length for fish (nose to distal end of caudal fin) and body length for amphibians (tip of snout to vent) on the Vertebrate Collection Form (Figure 12-1). No length measurements are taken for crayfish.

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12.2.3 Preparing Voucher Specimens

With the exception of very large individuals or easily identified species, up to 20 individuals of each species are vouchered from each stream site to provide a permanent, archived, historical record of fish collections. Prepare the voucher sample for a site according to the procedure presented in Table 12-6. Retain additional specimens of the appropriate species for the fish tissue contaminants samples (Section 13). **For each species, voucher specimens take priority over specimens for the tissue contaminants samples.**

The number of voucher specimens and the method of vouchering varies with species. Large, easily identified species, larger species that are difficult to identify in the field, or species that are uncommon in the region require a few specimens of both adults and juveniles, if both were collected. Very large specimens, especially of easily identified game fish, are “vouchered” by photographing them and then releasing them alive. More voucher specimens are required for smaller species, which are typically more difficult to identify in the field. Species of "special concern" (state and federally protected species), are processed first, vouchered by photographs, and released alive. Include any individuals of protected species that die before they can be released as part of the preserved voucher sample for the stream.

For taking photographs, use a film or digital camera with sufficient macro capability to take clear, close-up photographs of small fish or other aquatic vertebrates. The photographs must be of sufficient clarity or resolution that important external identifying characteristics can be distinguished. For each photograph, include a card with the site ID printed on it, and a measuring board, ruler, or some other object to provide a length reference.

Individuals selected as voucher specimens are first anaesthetized in a concentrated solution of carbon dioxide. Voucher specimens for each species are counted and placed in nylon mesh bags, stockings or plastic jars (1 or more bags per species). Each bag contains a numbered tag (Figure 12-2). Single specimens of easily identified and distinct species (e.g., sandroller, smallmouth bass) and crayfish may be placed directly in the jar with the tag. Record the tag number and the number of individuals vouchered for each species on the Vertebrate Collection Form (Figure 12-1). Preserve vouchers of sculpins, minnows, lampreys and other difficult species from throughout the reach. Use multiple bags and tags to do so. **This bagging, tagging, and recording is crucial, as it enables us to estimate**

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TABLE 12-6. GUIDELINES AND PROCEDURES FOR PREPARING AQUATIC VERTEBRATE VOUCHER SPECIMENS

1. Determine the voucher class of a species and the number of specimens to include in the voucher sample based on the following guidelines. Process **Class 1 species first.** Voucher samples take priority over tissue contaminant samples.

   Class 1--State or federally listed species. Photograph and **release immediately**. Photographs should include (1) a card with the stream ID and (2) an object of known length with the specimen. If specimens have died, proceed to Step 2 and include them in the voucher sample. Flag the species with an "Fn" on the Vertebrate Collection Form and note it is a listed species in the comments section of the form. Notify the appropriate state officials as soon as possible.

   Class 2--Large easily identified species **OR** adults that are difficult to identify **OR** species that are uncommon in that region (e.g., sunfish, suckers, bullheads, trout, crayfish). Preserve 1-2 small (<150 mm total length) adult individuals per site plus 2-5 juveniles. If only large adults are collected, reserve smallest individuals until voucher procedure is complete and preserve ONLY if space is available. Individuals with a total length > 160 mm should be slit on the lower abdomen of the RIGHT side before placing them into the container. Photograph if considered too large for the jar or place in a bag on ice for freezing **(Do not voucher large gamefish)**. Retain additional individuals for the tissue contaminant sample.

   Class 3--Small to moderate-sized fish **OR** difficult to identify species (e.g., lampreys, juvenile salmonids, minnows, sculpins). Preserve up to 20 adults and juveniles (several per transect). If fewer than 20 individuals are collected, voucher them all. Retain additional individuals for the tissue contaminant sample.

2. Anesthesize voucher specimens in a bucket with two carbon dioxide tablets and a small volume of water, then transfer them to a nylon mesh bag. Tally, then record the number of individuals included in the voucher sample in the "VOUCHERED COUNT" field for the species on the Vertebrate Collection Form.

3. Select a "FISH-BAG" tag with the same ID number as the voucher sample jar (Step 6). Record the tag number in the "TAG NO." field on the corresponding line for the species on the Vertebrate Collection Form. Place the tag into the mesh bag and seal. **This bagging, tagging, and recording is crucial, as it enables us to estimate species proportionate abundances in the assemblage even when 1 suspected species turns out to be multiple species.**

4. Immediately place the bag into a container large enough to hold all voucher specimens loosely and half-filled with 10% formalin. **Use additional jars if necessary to avoid close packing and bending of voucher specimens**.

5. Repeat Steps 1 through 4 for all species collected.

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TABLE 12-6 (continued)

6. Prepare two "FISH-JAR" labels (each having the same sample ID number) by filling in the stream ID and the date of collection. Place one label into the sample jar. Cap tightly and seal with plastic electrical tape. Attach the second label to the outside of the sample container by covering it with a strip of clear tape. Record the voucher sample ID number on page 1 of the Vertebrate Collection Form. Record general comments (perceived fishing efficiency, missed fish, gear operation, suggestions) in blank lines of form. NOTE: If more than one jar is required, use labels that have the same ID number printed on them and flag.

7. Place the preserved sample in a suitable container with absorbent material. Store the container in a well-ventilated area during transport. Follow all rules and regulations pertaining to the transport and shipment of samples containing 10% formalin.

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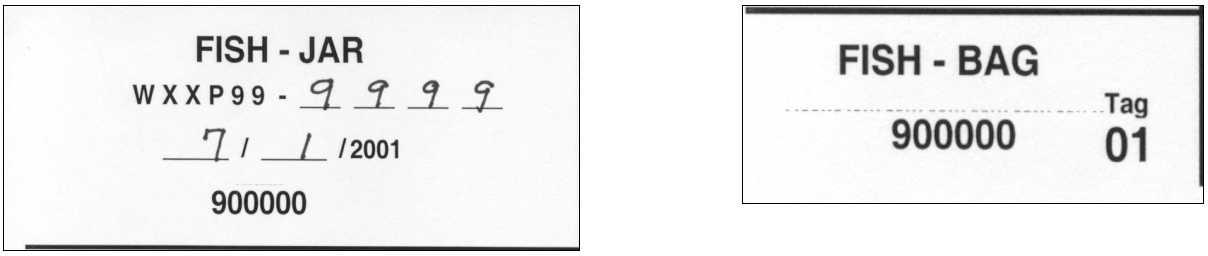

Figure 12-2. Completed voucher sample label and specimen bag tag for aquatic vertebrates. Note that two voucher sample labels (left) are filled out– one is placed inside the jar, the second is taped to the outside.

species proportionate abundances in the assemblage even when 1 suspected species turns out to be multiple species.

Place specimen bags into a large sample jar containing 10 % buffered formalin (Section 3). The final volume of 10% formalin in the sample container should equal to or exceed the total volume of specimens. Use additional containers if necessary and avoid tight packing of specimen bags or bending of specimens. Delays in carrying out the anaesthetization and preservation procedures, overpacking a bag or sample container, or an inadequate volume of preservative will result in unidentifiable (worthless) specimens. Formalin vapors and solution are extremely caustic and may cause severe irritation on contact with skin, eyes or mucus membranes, and it is a potential carcinogen. Contact with it should be avoided. Wear gloves and safety glasses and always work in a well-ventilated area. In case of contact with skin or eyes, rinse immediately with large quantities of water. Store stock solution in sealed containers in a safety cabinet or cooler lined with vermiculite. If possible, transport outside of the passenger compartment of a vehicle. A set of two sample labels is completed for each sample container as shown in Figure 12-2. Place one label inside each sample container, and tape the second label to the outside of the jar. Record the sample ID number on the Vertebrate Collection Form (Figure 12-1).

12.3 EQUIPMENT AND SUPPLIES

Figure 12-3 is a checklist of equipment and supplies required to conduct protocols described in this section. This checklist may differ from the checklists presented in Appendix A, which are used at a base site to ensure that all equipment and supplies are brought to the stream site. Field teams are required to use the checklist presented in this section to

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EQUIPMENT AND SUPPLIES FOR AQUATIC VERTEBRATE SAMPLING

QTY.	Item	
1	Gasoline or battery-powered backpack electrofishing unit with netted anode (electrode wand)	
	Extra battery (charged) or gasoline	
4 pr	Heavy-duty rubber gloves	
3 pr	Chest waders with non-slip soles & patch kit	
3 pr	Polarized sunglasses	
2	Long-handled dip nets (0.6 cm mesh) with insulated handles	
1	Watch or stopwatch to track elapsed fishing time	
4	Collapsible buckets for holding and processing aquatic vertebrates	
1	Minnow seine (2m X 1.25 m, 0.6 cm mesh) with poles	
1	Large seine (9 m X 2 m, 0.6 cm mesh) with poles	
1	Aquarium net	
1 set	Taxonomic reference books and keys for fishes and amphibians of the region	
1	Camera and film (or digital camera) with macro capability for photographing vouchers	
1-2	Fish measuring board & small plastic rulers (2)	
5-20	Small nylon mesh bags or stockings for holding voucher specimens	
1	Jackknife for preparing larger voucher specimens for preservation	
1 ea.	1, 2, and/or 4-L screw-top plastic jars (leakproof) for voucher samples	
2 L	10% (buffered) formalin or voucher sample jar half full of 10% formalin	
1	Container with absorbent material to hold formalin solution and preserved voucher sample jars	
1 pr	Safety glasses	
1 pr	Chemical-resistant gloves	
1	Covered clipboard	
1 box	Carbon dioxide tablets	
1	Sheet of pre-printed jar labels (4) and voucher bag tags (36), all with same preprinted sample ID number (barcode)	
1 pr	Scissors for cutting jar labels and tags	
1 roll	Plastic electrical tape	
1 pkg.	Clear tape strips	
2	Soft lead pencils for recording data and completing tags	
2	Extra fine-tipped indelible markers for completing sample labels	
12	Vertebrate Collection Forms	
3	Plastic safety whistles & ear protection if generators are used	
1	Field operations manual	
1 set	Laminated sheets of aquatic vertebrate procedure tables	
1 ea.	Vertebrate collection permits (State, Federal, Tribal)	

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Figure 12-3. Equipment and supplies checklist for aquatic vertebrates.

ensure that equipment and supplies are organized and available to conduct the protocols efficiently.

12.4 LITERATURE CITED

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