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ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM-SURFACE WATERS:

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

Edited by

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SECTION 12
AQUATIC VERTEBRATES

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

Sampling amphibian and fish 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), supplemented by block netting (when necessary) and seining (Section 12.1.2) in habitats where flow, substrate and structure affect capture of benthic species. 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 (Section 13).

12.1 SAMPLE COLLECTION

The entire channel within the sampling reach is sampled. Complex, very large, or wide systems without clearly-defined habitat types are sampled through use of transects so that effort is distributed along the entire reach relative to the mean width of each transect, as illustrated in Figure 12-1. Fish and other aquatic vertebrates are collected according to time **and** distance criteria. Collection time should continue for not less than 45 minutes and not longer than 3 hours within the defined sampling reach (Section 4) to obtain a representative sample. Sampling information is recorded on the Vertebrate Collection Form (Figure 12-2). Record general comments (perceived fishing efficiency, missed fish, gear operation, suggestions) on the blank lines of the form.

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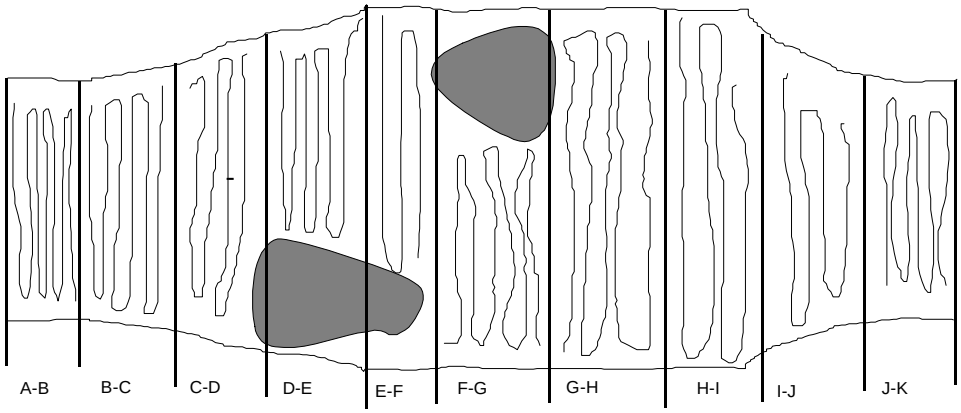
Stippled areas represent habitats too hazardous to sample.



Transects correspond to cross-section transects established every 10 channel widths or 15 m

Transect	Mean Transect Width (m)	Time Allotment (sec)	Shock Time (estimated)
A-B	8m	8*84= 672	350
B-C	9m	9*84= 756	400
C-D	12m	12*84=1008	600
D-E	12m	12*84=1008	600
E-F	15m	15*84=1260	800
F-G	15m	15*84=1260	800
G-H	15m	15*84=1260	800
H-I	15m	15*84=1260	800
I-J	10m	10*84= 840	500
J-K	8m	8*84= 672	350

Example Calculation
Sum of Mean Transect Widths
8 + 9 + 12 + 12 + 15 + 15 + 15 + 15 + 10 + 8 = 128m.
3 hrs = 10800s sampling time.
10800s / 128m = 84 s/m of mean width.
Multiply mean width by # units to calculate time in seconds to be spent in each transect.
Depending on complexity of habitat, actual shock time may vary from 50% - 75% of fishing time.

Figure 12-1. Index sample design for allocating aquatic vertebrate sampling effort in very complex or very large wadeable streams. Note distribution of effort in narrow and wide sections.

12.1.1 Electrofishing

Because fishes and amphibians are collected using portable electrofishing units, safety procedures must be followed meticulously at all times (refer to Section 2). Primary responsibility for safety while electrofishing rests with the team leader. Electrofishing units have a high voltage output and may deliver a dangerous electrical shock. While electrofishing, avoid contact with the water unless sufficiently insulated against electrical shock. Use chest waders with nonslip soles and watertight rubber (or electrician's) gloves that cover to the elbows. If they become wet inside, **stop fishing until they are thoroughly dry. 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. While electrofishing avoid reaching into the water. If it is necessary for a team member to reach into the water to pick up a fish or something that has been dropped, **do so only after the electrical current has been interrupted 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 operating electrofishing equipment 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. Use 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. Always wear gloves and safety glasses when handling gasoline. Keep gasoline only in approved plastic containers and store in a tightly closed container in safety cabinet or cooler lined with vermiculite.

The procedure to sample with the backpack electrofisher unit is presented in Table 12-1. Record information on the Vertebrate Collection Form as shown in Figure 12-2. If the stream cannot be sampled by either electrofishing or seining, complete the "NOT FISHED" field on the form. Select the initial voltage based on the measured conductivity of the stream (see Section 5). Select the initial frequency based on the expected size of fish. If fishing success is poor, increase the pulse width first and then the voltage. Increase the frequency last to minimize mortality or injury to large fish.

Determine that all team members are wearing waders and gloves and are clear of the anode. Wear polarized sunglasses to aid vision. Start the electrofisher, set the timer to zero, and depress the switch to begin fishing. Starting at the bottom of the reach, fish in an upstream direction. Adjust voltage and waveform output according to sampling effectiveness and incidental mortality to specimens. The backpack unit is equipped with an audio alarm that sounds when the output voltage exceeds 30 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 and adjust voltage and waveform and continue fishing.

When fishing, **slowly** sweep the electrode wand from side to side in the water in riffles and pools. Sample available cut-bank and snag habitat areas as well as riffles and pools. Move the wand in and out of large snags or deep cuts or release the electrode switch, move the wand away slightly, depress the switch again and sweep the wand away from the cover to draw fish out into open. In fast, shallow water, it may be more effective to use a seine as a block net; sweep the anode and fish downstream into the net.

In extremely wide streams, it may be necessary to 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 into deep water. In larger or more complex streams, allocate the fishing time between transects based on differences in the mean wetted width of the stream (Figure 12-1).

One or two netters follow along beside or slightly behind the person operating the electrofisher (on the anode side). Each netter uses an insulated dip net to retrieve stunned individuals, which are then deposited into a bucket for later processing (Section 12.3).

TABLE 12-1. PROCEDURE TO COLLECT AQUATIC VERTEBRATES BY ELECTROFISHING

1. Survey the sampling reach and set block nets at each end (Transects "A" and "K") if necessary (e.g., the majority of the reach is one large, continuous pool). If necessary, allocate the total shocking time among transects based on mean stream widths.

2. Complete the header information on a copy of the Vertebrate Collection Form. Indicate that all transects are being sampled in the "TRANSECT" field on the form.

   - NOTE: Make an effort to search and sample for aquatic vertebrates at all streams, even if the stream is extremely small, and it appears that sampling may not collect any specimens. If no specimens are collected, complete the "NONE COLLECTED" field on the Vertebrate Collection Form. Provide an explanation in the comments section of the form.

3. If the conductivity measured during the water chemistry sampling is less than 10 uS/cm, or if the depth or velocity make electrofishing unsafe, sample by seining if possible, otherwise do not sample. If you do not sample, complete the "NOT FISHED" field on the Vertebrate Collection Form. Provide an explanation in the comments section of the form.

4. Set unit to 300 volt-amperes (VA) and 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 frequency of 30 Hz with a pulse width of 2 msec. If mostly small fish are expected, use a frequency of 60-70 Hz. Start the generator, set the timer, and depress the switch to begin fishing.

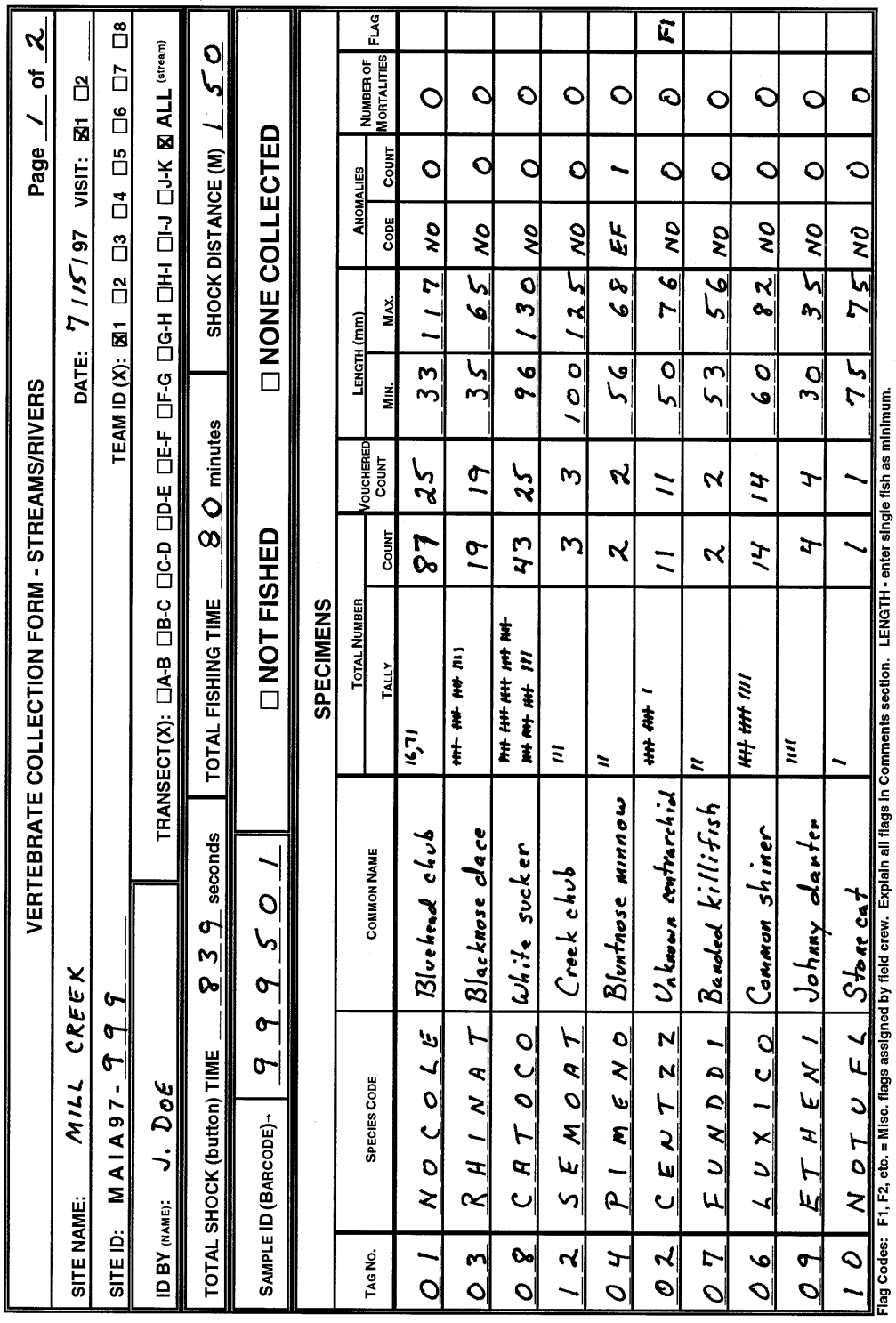
5 Beginning at the downstream end of the reach (Transect "A"), fish in an upstream direction, parallel to the current. Depress the switch and sweep the electrodes from side to side in the water. Sample available cut-bank and snag habitats as well as riffles and pools.

6. The netters follow the operator and net stunned aquatic vertebrates. Deposit individuals in buckets for processing. If necessary, use seines to block riffles, pools and snags. The operator should adjust voltage and waveform output according to sampling effectiveness and the mortality of fish specimens.

7. Continue upstream until the next transect is reached. In large or complex streams, allocate the fishing time between the two transects as calculated based on the mean transect widths (Step 1). Process fish after each transect to reduce mortality.

8. Repeat Steps 5 through 7 until Transect "K" is reached. Record the following on the Vertebrate Collection Form:
   - The reading from the electrofisher timer in the "TOTAL SHOCK TIME" field on the Vertebrate Collection Form.
   - The total distance sampled by electrofishing
   - The total fishing time, if no additional sampling is conducted (e.g., by seining) once electrofishing is completed. Total sampling time should be between 45 minutes and 3 hours.

9. If no aquatic vertebrates were collected, complete the "NONE COLLECTED" field on the Vertebrate Collection Form.



VERTEBRATE COLLECTION FORM - STREAMS/RIVERS — Page 1 of 2

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

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

ID BY (NAME): J. Doe — TRANSECT(X): ☐A-B ☐B-C ☐C-D ☐D-E ☐E-F ☐F-G ☐G-H ☐H-I ☐I-J ☐J-K ☒ ALL (stream)

TOTAL SHOCK (button) TIME 839 seconds — TOTAL FISHING TIME 80 minutes — SHOCK DISTANCE (M) 150

SAMPLE ID (BARCODE)→ 99950 1 — ☐ NOT FISHED — ☐ NONE COLLECTED

SPECIMENS

Tag No.	Species Code	Common Name	Total Number: Tally	Total Number: Count	Vouchered Count	Length (mm): Min.	Length (mm): Max.	Anomalies: Code	Anomalies: Count	Number of Mortalities	Flag
01	NOCOLE	Bluehead chub	16,71	87	25	33	117	NO	0	0	
03	RHINAT	Blacknose dace	++++ ++++ ++++ ++++ 111	19	19	35	65	NO	0	0	
08	CATOCO	White sucker	++++ ++++ ++++ ++++ ++++ ++++ ++++ 111	43	25	96	130	NO	0	0	
12	SEMOAT	Creek chub	111	3	3	100	125	NO	0	0	
04	PIMENO	Bluntnose minnow	11	2	2	56	68	EF	1	0	
02	CENTZZ	Unknown centrarchid	++++ ++++ 1	11	11	50	76	NO	0	0	F1
07	FUNDDI	Banded killifish	11	2	2	53	56	NO	0	0	
06	LUXICO	Common shiner	++++ ++++ 1111	14	14	60	82	NO	0	0	
09	ETHENI	Johnny darter	1111	4	4	30	35	NO	0	0	
10	NOTUFL	Stoat cat	1	1	1	75	75	NO	0	0	

Flag Codes: F1, F2, etc. = Misc. flags assigned by field crew. Explain all flags in Comments section. LENGTH - enter single fish as minimum.

Reviewed by (initial): DP

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VERTEBRATE COLLECTION FORM - STREAMS/RIVERS - 1

Figure 12-2. Vertebrate Collection Form (page1).

Change the water in the bucket periodically to minimize mortality prior to processing. If individuals show signs of stress (loss of righting response, gaping, gulping air, excessive mucus), stop and process them. This should only be necessary on very warm days, in long reaches, or if very large numbers of aquatic vertebrates are collected. Electrofishing may also need to cease at times to immediately process and release specimens (e.g., listed 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.

At the completion of electrofishing, record the total operating time (shock time) shown on the electrofisher timer and the distance sampled by electrofishing on the Vertebrate Collection Form (Figure 12-2). If sampling activities (electrofishing and seining) are completed, also record the total fishing time on the Vertebrate Collection Form. If no aquatic vertebrates were collected, indicate this on the form as shown in Figure 12-2.

12.1.2 Seining

Seining may be used in conjunction with electrofishing to ensure sampling of those species which may otherwise be underrepresented by an electrofishing survey alone (e.g., darters, sculpins, madtoms, and benthic cyprinids). Seining may also be used in sites where the stream is too deep for electrofishing to be conducted safely or in turbid, simple, soft-bottomed streams where it is more effective.

Seining procedures are presented in Table 12-2. Depending on the particular use (block netting vs. active seining) and the habitat, different sizes of seines are used. In riffle habitats, the seine is held stationary while team members disturb the substrate immediately upstream of the net. In pools, the seine is pulled back and forth across the pool, using the shore and other natural habitat breaks as barriers, or pulled rapidly downstream through the pool and then swept toward the shore. Block nets may be used in very large pools to limit escape or as seines. Large nets are typically deployed parallel to the current and swept to shore.

Proceed upstream through the reach, allocating the seining effort among habitat areas (riffles and pools) so that the entire reach is sampled within the required sampling time (45 minutes to 3 hours). Deposit aquatic vertebrates collected by seining into a bucket for later processing as described in Section 12.1. At the completion of sampling activities (electrofishing and/or seining), record the total fishing time on the Vertebrate Collection

TABLE 12-2. PROCEDURES TO COLLECT AQUATIC VERTEBRATES BY SEINING

NOTE: Seining is used in place of electrofishing where a stream is too deep for electrofishing to be conducted safely, or in turbid, simple, soft-bottomed streams where it is more effective.

1. Survey the sampling reach and set block nets at each end (Transects "A" and "K") if necessary (e.g., the majority of the reach is one large, continuous pool). Allocate the sampling effort throughout the sampling reach so that the total fishing time will be between 45 minutes (small stream) and 3 hours (large stream).

2. Complete the header information on a copy of the Vertebrate Collection Form. Indicate that all transects are being sampled in the "TRANSECT" field on the form.

   - NOTE: Make an effort to search and sample for aquatic vertebrates at all streams, even if the stream is extremely small, and it appears that sampling may not collect any specimens. If no specimens are collected, complete the "NONE COLLECTED" field on the Vertebrate Collection Form. Provide an explanation in the comments section of the form.

3. Begin at the downstream end of the sampling reach (Transect "A"). Proceed upstream, sampling available riffle and pool habitats using the appropriate method below:

   3A. Riffle habitats-- Use a small minnow seine (2 m long × 1.25 m wide; 0.6 cm mesh size).

      1. One or two persons 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 backward to form a pocket to trap aquatic vertebrates.
      2. Starting about 2 m upstream, the other two team members disturb the substrate in front of the net by kicking through the substrate and overturning rocks, and proceed downstream toward the nets.
      3. Raise the net and examine it carefully for aquatic vertebrates.

   3B. Pool habitats: Use a larger seine (3 m long × 2 m wide; 0.6 cm mesh size).

      1. Two people pull the seine back and forth across the pool, using the shore and other natural habitat breaks as barriers.
      2. Alternatively (in areas with some current), pull the net along in a downstream direction and then sweep toward the shore.
      3. Pull the net onto the shore and examine it carefully for aquatic vertebrates.

4. Deposit individuals in buckets for processing, and continue upstream to the next habitat area.

5. Repeat Steps 3 and 4 for successive habitat areas until Transect "K" is reached. If no aquatic vertebrates were collected by either seining or electrofishing, complete the "NONE COLLECTED" box on the Vertebrate Collection Form.

6. Record the total fishing time on the Vertebrate Collection Form. Include any time spent electrofishing in the total fishing time. Total fishing time should be between 45 minutes and 3 hours.

Form (Figure 12-2). If no aquatic vertebrates were collected, indicate this on the form as shown in Figure 12-2.

12.2 SAMPLE PROCESSING

Sample processing involves tallying and identifying fish 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 (see Section 13). Process collections as quickly as possible to minimize stress to live specimens. All team members can work to separate aquatic vertebrates into families or obvious "morphotypes". Alternatively, 1 or 2 persons can process fish from one bucket while the other team members continue to collect fish and deposit them into a second bucket. Once the rough sort has been completed, one person can identify, measure, and examine individuals while another person may record information on the field data forms.

12.2.1 Taxonomic Identification and Tally

Table 12-3 presents the procedure for identifying and tallying aquatic vertebrates. Record identification and tally data for each species on the Vertebrate Collection Form as shown in Figure 12-2. Record comments and data for additional species on page 2 of the Vertebrate Collection Form (Figure 12-3). Each team needs to be provided with a list of standardized names (required) and species codes (optional) for aquatic vertebrate species that are expected to be collected (see Appendix D for an example).

Sort aquatic vertebrates by species into small buckets and containers. 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. Try to process one species completely before going on to the next. However, where there are many individuals of easily identified species, processing may be facilitated by keeping a tally count of the number of individuals of each species and totaling the tally once processing is complete.

To minimize handling, process threatened and endangered species first, and immediately return all individuals to the stream. If conditions permit and stress to individuals will be minimal, photograph such fish for voucher purposes (Section 12.2.3).

TABLE 12-3. PROCEDURE TO IDENTIFY, TALLY, AND EXAMINE AQUATIC VERTEBRATES

1. Separate aquatic vertebrates retained in collecting buckets or live wells into families or obvious "morphotypes" (e.g., two dorsal fins vs. one, a sucker mouth, catfish, trout, etc.). Place each group into a separate bucket or similar container. All team members can participate in this "rough" sort. Alternatively, identify and process each individual completely, thus handling it only once.

2. Sort each group created in Step 1 by species into separate containers. This should be done only by team members who are trained ichthyologists familiar with the fish species and other aquatic vertebrate taxa of the region.

3. Select a container and record the common name (from a standardized list) and species code (if required) on the first blank line in the "SPECIMENS" section of the Vertebrate Collection Form. If a species cannot be positively identified, assign it an "unknown" species code from the list provided.

    - NOTE: 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. Notify the appropriate state officials as soon as possible.

4. Tally the number of individuals collected (use the "TALLY" box on the Vertebrate Collection Form if necessary) and record the total number in the "COUNT" field on the form.

5. Measure the total length 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.

6. If the container has sport fish and other very large specimens, or if 3 or fewer species are captured at the stream, prepare a Vertebrate Length Recording Form.

    A. Complete the header information on the form, then enter the common name (from a standardized list) and the species code (if required) in the first blank line.

    B. Measure the total length of each individual (up to 30) and record the lengths in the boxes on the form (2 lines of boxes per species). For smaller species, measure and record lengths of a random set (up to 30) of the individuals collected.

7. Examine each individual for external anomalies and note the types of anomalies observed. After all of the individuals of a species have been processed, record the anomaly code and the total number of individuals affected in the "ANOMALIES" area of the Vertebrate Collection Form.

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 into the stream. If there is still a portion of the sampling reach that has not been sampled, release fish downstream to avoid their recapture.

10. Repeat Steps 3 through 9 for all other species.

Reviewed by (initial): DJP

VERTEBRATE COLLECTION FORM - STREAMS/RIVERS (continued) Page 2 of 2

SITE ID: MAIA97-999 DATE: 7/15/97 VISIT: ☒1 ☐2

Sample ID (Barcode) → 999501 TEAM ID (X): ☒1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8

ID BY (NAME): J. Doe TRANSECT(X): ☐A-B ☐B-C ☐C-D ☐D-E ☐E-F ☐F-G ☐G-H ☐H-I ☐I-J ☐J-K ☒ALL (stream)

SPECIMENS (continued)

TAG NO.	SPECIES CODE	COMMON NAME	TOTAL NUMBER		VOUCHERED COUNT	LENGTH (mm)		ANOMALIES		NUMBER OF MORTALITIES	FLAG
			TALLY	COUNT		MIN.	MAX.	CODE	COUNT		
11	COTTZZ	Unknown cottid	III	3	3	60	80	NO	0	0	

FLAG	COMMENTS
F1	Unknown centrarchid = Lepomis sp.

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Figure 12-3. Vertebrate Collection Form (page 2).

Indicate if photographed with an “F” series flag for the species on page 1 of the Vertebrate Collection Form (Figure 12-2) and record a notation in the comments section on page 2 of the form (Figure 12-3). If protected fish have died, they should be prepared as voucher specimens and preserved in formalin. Notify the appropriate state officials as soon as possible.

If a species cannot be confidently identified in the field (e.g., small individuals or suspected hybrids), record it as an “unknown” species on the Vertebrate Collection Form, using one of the names (and code, if required) provided for unknowns from the standardized list (see Figure 12-2 for an example). If possible, flag unknown species with an “F” series flag and provide your best guess at an identification in the comments section of the Vertebrate Collection Form (Figure 12-3).

12.2.2 External Examination and Length Measurements

During the tallying procedure for each species (Table 12-3), examine each individual for the presence of external anomalies. External anomalies may result from sublethal environmental or behavioral stress, diseases, and toxic chemicals. Readily identified external anomalies include deformities, eroded fins, lesions, tumors, diseases and parasites. Codes for different types of anomalies are presented in Table 12-4. Record the types of anomalies observed and the number of individuals affected on the Vertebrate Collection Form as shown in Figure 12-2.

Blackening and exopthalmia 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 12-3). Care should be taken in the early stages of electrofishing to use the most effective combination of voltage and pulse width while minimizing injury to fish. 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 12-2).

For each species, use a measuring board or ruler to determine the total length (Figure 12-4) of the largest and smallest individuals. Measure individuals on right side, and slide fish to touch the "Bump Board" on the measuring board. Measure total length to the nearest millimeter (mm) and record these values on the Vertebrate Collection Form as shown in Figure 12-2. For sport fish and other larger species, measure the total lengths of

TABLE 12-4. EXTERNAL 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; may be either small or large dots.
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 out-growths.
Lice	LI	Scale-like, mobile arthropods.
Mucus	MU	Thick and excessive on skin or gill, or 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.

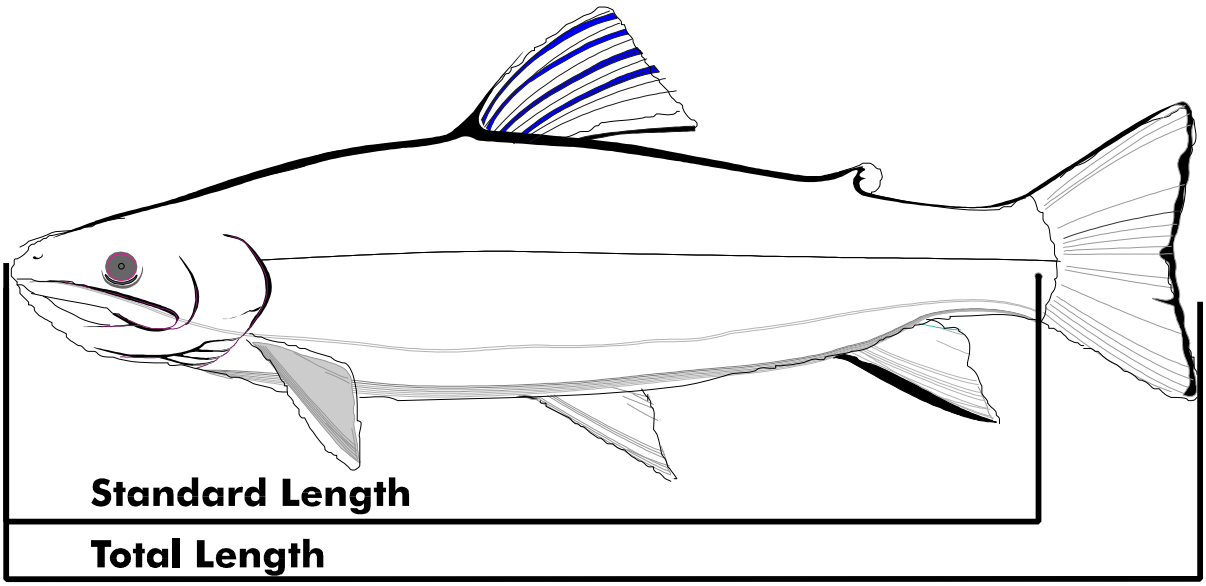


Figure 12-4. Fish length measurements.(modified from Lagler, 1956).

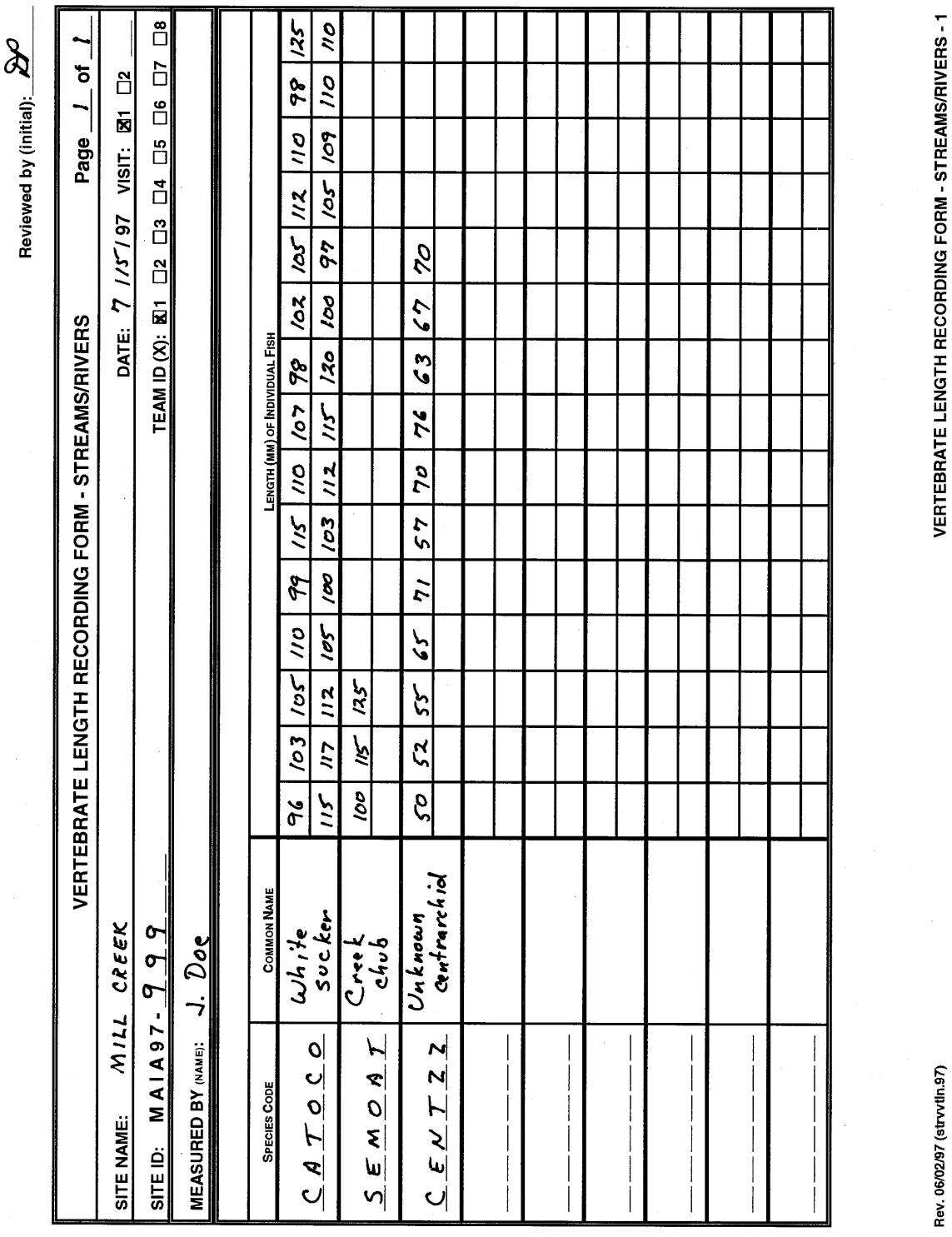
up to 30 individuals and record these values on the Vertebrate Length Recording Form as shown in Figure 12-5. If less than four species (large or small) are collected, randomly select up to 30 individuals of each species and determine the total length of each individual. Record these length measurements on the length recording form (Figure 12-5).

12.2.3 Preparing Voucher Specimens

With the exception of very large individuals of easily identified species, voucher collections of up to 25 individuals (where allowed by collecting permits) of all species are made to provide a permanent, archived, historical record of fish collections. Prepare the voucher sample for a site according to the procedure presented in Table 12-5. Retain additional specimens of the appropriate species for the fish tissue contaminants sample (Section 13). **For each species, voucher specimens take priority over specimens for the tissue contaminants sample.**

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. A larger number of voucher specimens are required for smaller species, which are typically more difficult to identify in the field. As stated previously, species of "special concern" (state and federally protected species), are processed first, vouchered by photography, and released alive. Include any individuals of protected species that die before they can be processed and released as part of the preserved voucher sample for the stream. In some cases, special restrictions may apply to protected species (e.g., sampling may have to cease upon collecting an individual). These restrictions will be stipulated on the scientific collecting permits issued by state and federal agencies.

Individuals selected as voucher specimens are first anaesthetized in a concentrated solution of carbon dioxide. Voucher specimens for each species are counted and placed into individual nylon mesh bags (1 bag per species). Nylon stockings or panty hose may be substituted in place of nylon bags. Each bag contains a numbered tag (Figure 12-6). Record the tag number and the number of individuals vouchered for each species on the Vertebrate Collection Form as shown in Figure 12-2.



VERTEBRATE LENGTH RECORDING FORM - STREAMS/RIVERS

Reviewed by (initial): DO

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

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

MEASURED BY (NAME): J. Doe

Page 1 of 1

SPECIES CODE	COMMON NAME	LENGTH (MM) OF INDIVIDUAL FISH																	
CATOCO	White sucker	96	103	105	110	99	115	110	107	98	102	105	112	110	98	125			
		115	117	112	105	100	103	112	115	120	100	97	105	109	110	110			
SEMOAT	Creek chub	100	115	135															
CENTRZZ	Unknown centrarchid	50	52	55	65	71	57	70	76	63	67	70							

Rev. 06/02/97 (strvvtln.97)

VERTEBRATE LENGTH RECORDING FORM - STREAMS/RIVERS - 1

Figure 12-5. Vertebrate Length Recording Form (page 1).

TABLE 12-5. GUIDELINES AND PROCEDURES FOR PREPARING AQUATIC VERTEBRATE VOUCHER SPECIMENS

1. Determine the voucher category of a species and the number of specimens to include in the voucher sample based on the following guidelines. **NOTE: Category 3 species should be processed first.**

   A Category 1 — Large easily identified species **OR** adults may be difficult to identify **OR** the species is uncommon in that region. Examples include:

American Eel	White Sucker	Buffalo fishes	Drum
Sturgeon	Longnose Sucker	Bullhead catfish	Carp
Paddlefish	Hogsucker	Channel catfish	Salmonids
Gars	Quillback	Esocids	Crappies
Bowfin	Carpsuckers	*Morone spp*	*Micropterus spp.*
Mooneye and Goldeye	*Moxostoma spp.*	Shads	Walleye and Sauger

   1. 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.
      - NOTE: Individuals with a total length > 160 mm should be slit on the lower abdomen of the RIGHT side before placing them into the container.

   2. Photograph if considered too large for the jar. All photographs should include (1) a card with the stream ID, date, species code, and common name, and (2) a ruler or some other object of known length to provide some indication of the size of the specimen.

   3. Retain additional individuals of primary and secondary target species for the tissue contaminant sample.

   B. Category 2 — Small to moderate-sized fish **OR** difficult to identify species. Examples include:

Lampreys	Troutperch	Sculpins	Madtoms
Cyprinids	Chubsucker	Sunfish	Sticklebacks
Darters	Topminnows	Silversides	Mudminnows

   1. Preserve 25 adults and juveniles. If fewer than 25 individuals are collected, voucher all of them. **Voucher samples take priority over tissue contaminant sample**.

   2. Retain additional individuals of primary and secondary target species for tissue contaminants sample.

(continued)

TABLE 12-5 (Continued)

C. Category 3 — Species of "special concern." These are state or federally listed species.

1. Photograph as in Step 1.A.2 and then **release immediately**.

2. If specimens have died, proceed to Step 2 and include them as part of the voucher sample. Flag the species with an "F" series flag 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.

2. Place the voucher specimens in a bucket with two carbon dioxide tablets (e.g., Alka Seltzer®) and a small volume of water. When specimens are anaesthetized, transfer them to a nylon mesh bag. 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 that has the same ID number (barcode) as the voucher sample jar (Step 3). 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.

4. Immediately place the bag into a container (½ or 1 gal plastic jar) large enough to hold all voucher specimens. Add a volume of 10% formalin solution equal to the volume of fish.

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

- Add additional 10% formalin solution as bags are added so that the final volume of formalin solution is equal to the total volume of fish specimens. **Use additional jars if necessary to avoid tight packing and bending of voucher specimens**.

6. Prepare two "FISH-JAR" labels (each having the same ID number [barcode]) 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.

7. 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 (barcode) on page 1 of the Vertebrate Collection Form. NOTE: If more than one jar is required, use labels that have the same ID number printed on them.

8. 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.

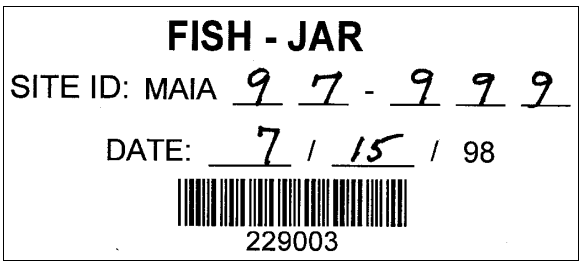


Figure 12-6. Completed voucher sample label and specimen bag tag for aquatic vertebrates.

Place specimen bags together into a large sample container. Preserve voucher specimens with a 10 percent formalin solution. See Section 3 for instructions for preparing a buffered formalin solution. Larger voucher specimens (total length > 160 mm) should be slit on the lower abdomen of the RIGHT side to allow for complete fixation of internal tissues and organs. Start with a concentrated solution of formaldehyde and dilute to the final volume with water. The final volume of 10% formalin in the sample container should equal the total volume of specimens. Use additional containers if necessary and avoid tight packing of specimen bags. Delays in carrying out the anaesthetization and preservation procedures, overpacking a sample container, or an inadequate volume of preservative will result in unidentifiable specimens.

Formaldehyde (37%) and formalin (10% formaldehyde by volume) are extremely caustic agents and may cause severe irritation on contact of vapors or solution with skin, eyes or mucus membranes. It is a potential carcinogen. Contact with vapors or solution 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 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 12-6. Place one label inside each sample container, and attach the second label to the outside of the jar with clear tape. Record the sample ID number on the Vertebrate Collection Form as shown in Figure 12-2. Some museums may also require that a separate collection card be completed and inserted into each jar of voucher specimens.

12.3 EQUIPMENT AND SUPPLIES

Figure 12-7 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 ensure that equipment and supplies are organized and available to conduct the protocols efficiently.

12.4 LITERATURE CITED

Lagler, K.R. 1956. *Freshwater Fishery Biology*. 2nd. Edition. William C. Brown Co., Dubuque, Iowa.

McCormick, F.H. 1993. Fish. pp. 29-36 IN: R.M. Hughes (ed.). *Stream Indicator Workshop.* EPA/600/R-93/138. U.S. Environmental Protection Agency, Corvallis, Oregon.

EQUIPMENT AND SUPPLIES FOR AQUATIC VERTEBRATES

QTY.	Item	
1	Gasoline or battery-powered backpack electrofishing unit with netted anode (electrode wand)	
	Extra battery (charged) or gasoline	
3 pr	Heavy-duty rubber gloves	
3 pr	Chest waders with non-slip soles	
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	
	Collapsible buckets for holding and processing aquatic vertebrates	
1	Minnow seine (2m × 1.25 m, 0.6 cm mesh) with brailles	
2	Large seines (3 m × 2 m, 0.6 cm mesh) with brailles	
2	Larger sized seines for block nets (if necessary)	
1 set	Taxonomic reference books and keys for fishes and amphibians of the region	
1	Fish measuring board or ruler	
1	List of vertebrate species common names (and species codes, if required)	
1	List of external anomaly codes	
15-20	Small nylon mesh bags for holding voucher specimens (bags can also be constructed from sections of nylon stockings or panty hose)	
1	Small fillet knife or scalpel for preparing larger voucher specimens for preservation	
2 ea.	½- or 1-gallon screw-top plastic jars for voucher sample	
2 gal	10% (buffered) formalin solution OR 0.2 gal buffered formaldehyde solution. Alternatively, fill each voucher sample jar one-half full of 10% formalin	
1	Container to hold formalin solution and preserved voucher sample jars	
1 pr	Safety glasses	
1 pr	Chemical-resistant gloves	

(continued)

Figure 12-7. Equipment and supplies checklist for aquatic vertebrates.

EQUIPMENT AND SUPPLIES FOR AQUATIC VERTEBRATES (Continued)

QTY.	Item	
1	Plastic bucket for anesthetization	
4	Carbon dioxide tablets (Alka-Seltzer® or equivalent)	
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 labels	
1 roll	Plastic electrical tape	
1 pkg.	Clear tape strips	
	Soft lead pencils for recording data and completing tags	
	Fine-tipped indelible markers for completing sample labels	
1 + extras	Vertebrate Collection Form	
1 + extras	Vertebrate Length Recording Form	
1	Field operations manual	
1 set	Laminated sheets of aquatic vertebrate procedure tables and/or quick reference guides	

Figure 12-7. (Continued).