Fish Collection Protocols for Streams

A. Overview

Backpack electrofishing is the primary method for sampling fish populations in wadeable streams in South Carolina. Electrofishing methods vary slightly depending on geographic location within the state. The sampling method cited in this protocol is not meant to provide an exhaustive survey of the fish fauna; it is meant to produce a representative sample of the fish population in the sampled portion of stream. Please see Appendix A for equipment specifications to be used during sampling efforts.

B. Sampling Season

Fish sampling should be conducted during periods of base flow and appropriate water temperatures. In South Carolina, the duration of appropriate flow and temperature conditions exist approximately from late March through October. Random fluctuations in weather conditions may shorten or lengthen this given period. Sampling should not be conducted during high flow events (e.g., during or after rain events). Sampling during a high flow event may result in reduced sampling efficiency and induce bias due to changing chemical and biological conditions. Winter sampling should be avoided due to cold water temperatures. When daytime water temperatures consistently remain below 10 °C (50 ^{o}F), fish tend to be inactive and not representative of primary habitats, moving into deep water or heavy cover. A minimum of two samples should be conducted during optimum sampling season for site certification when water temperatures are 50^{o}F and above.

C. Sampling Techniques

<u>1. Sample Reach Length/Number of Electrofishing Passes</u>
Sample reach length is dependent on both average wetted width (m) at the time of sample and geographic location within the state. Average width is determined by measuring the wetted width (m) at the downstream limit of the sample reach (0 m) and then every subsequent 25 m upstream for a total of five measurements. Average all observations and enter the average width on the data sheet. To determine sample length based on geographic location and average wetted width, follow the guidelines listed in Table 1. **The minimum sample reach length is 100 m for all sites statewide.** The number of electrofishing passes to be conducted is also dependent on geographic location within the state. Sites located above the fall line are sampled with one electrofishing pass and sites located below the fall line are sampled with three electrofishing passes. To determine the number of passes based on geographic location, follow the guidelines listed in Table 1.

Table 1: Sample reach length and number of electrofishing passes by Level III Ecoregion.

Level-III Ecoregion	**Sample Reach Length (m)**	**Number of Electrofishing Passes**
Blue Ridge Piedmont	= 30x average wetted width	1
Southeastern Plains Middle Atlantic Coastal Plain	= 20x average wetted width	3

2. Crew Size

The appropriate number of backpack electrofishing units is dependent on average wetted width at the time of sample (Table 2). The appropriate number of backpack units is one for every three meters of average stream wetted width. The sampling crew must also include netters and a bucket-carrier. The number of netters and bucket-carriers are dependent on the required number of backpack electrofishing units (Table 2).

Table 2: Appropriate crew size for stream electrofishing.

Average Wetted Width	Number of Backpack Units	Number of Netters	Number of Buckets
< 3 m	1	1	1
3-6 m	2	2	1
6-9 m	3	2	2
9-12 m	4	3	2-3

3. Backpack Electrofishing Procedures

General electrofishing procedures apply statewide (section 3a). However, research from South Carolina streams has shown that the efficiency of certain electrofishing procedures varies according to geographic location. Therefore, procedures have been developed specific to geographic location in order to maximize electrofishing efficiency (sections 3b and 3c). All stream sampling should follow general electrofishing procedures as well as those specific to the geographic location of the sample site.

3a. General Electrofishing Procedures

1. Before electrofishing, all members of the crew should be equipped with the proper gear to ensure personal safety. Important examples of proper equipment needs include:
    a. To reduce the risk of electrical shock, all persons participating in electrofishing must wear watertight waders and gloves.
    b. Polarized sunglasses should be considered essential equipment under certain light conditions. The use of polarized glasses reduces glare and improves capture efficiency.

2. Backpack electrofishing units should be set to an appropriate electrical output voltage to capture fishes effectively. Refer to manufacturer's guidelines for determining optimal settings of backpack electrofishing units. Adjustments of electrical output will vary depending on the conductivity of water in different streams. Pulsed direct current (DC) is preferred over alternating current when conductivity is high enough for DC to be effective, generally over 20 microsiemens/cm conductivity. Prior to sampling, each backpack electrofishing unit should be tested outside of the sample reach to determine appropriate electrical output settings. Generally, higher frequencies (i.e. >100) and shorter pulse-widths (i.e., duty cycles of a millisecond or less) are more effective for stream fishes.

3. Re-zero electrofishing unit timers. Start electrofishing at the downstream block net/start location and electrofish in an upstream direction, sampling all habitats until you have reached the upstream extent of the sample reach. To reduce bias introduced by the selective placement of electric field, a continuous electric field should be applied to the entire sampling reach (i.e., do not switch power on and off). Netters should follow along slightly behind the person operating the backpack electrofisher, making an equal effort to collect each observed fish. Fish collected should be immediately transferred to a collection bucket. In order to reduce mortality, handling of fish should be minimized and bucket contents should periodically be emptied into mesh holding cages placed within the stream. Holding cages reduce mortality by exposing fish to fresh, flowing water.

4. When not actively netting fish, dipnetters should always have their nets deployed on the bottom downstream of the electrodes. It is particularly important that dipnetters keep their nets in the water, as much as possible, in turbid or fast-water areas.

5. Once each pass is completed, turn off each unit and record the timer reading for each backpack electrofishing unit on the Stream Assessment Data Sheet.

6. If block nets are required, check the upstream and downstream block nets for fishes. Ensure that the block nets are still effectively blocking the movement of fish in the sample reach.

7. If additional passes are required, repeat steps 3 through 6. Expend an adequate amount of effort on each pass, thoroughly covering all habitats and collecting all observed fish. The number of electrofishing units and netters should be the same for all electrofishing passes. If water clarity is reduced due to substrate disturbance by the first or any subsequent electrofishing passes, delay subsequent electrofishing passes until water clarity resembles undisturbed conditions.

3b. Specific Electrofishing Procedures for the Piedmont/Blue Ridge

For sample sites within the level III Piedmont and Blue Ridge ecoregions, sampling should consist of a **single electrofishing pass with a reach length equal to 30 times the average wetted width of the stream (or minimum length 100m) (Table 1).** Block nets are not required in these ecoregions. Most habitats (glides, pools, etc.) should be sampled in a downstream to upstream direction. However, riffle habitats are sampled by electrofishing in a downstream direction into an 8-10 foot seine of mesh size ¼ of an inch. Two persons should hold the seine perpendicular to the direction of flow, allowing the seine to form a bag which will allow for the capture of all descending fish. In order to avoid fish escaping beneath the seine, special attention should be given to keeping the seine's lead line flush with the bottom of the stream. It may be necessary for one person to stand on the lead line to hold it flush to the stream bottom. Dip nets should be used in conjunction with the seine to collect stray fish. The person(s) operating the electrofishing unit(s) should begin shocking no more than 5-7 meters upstream of the seine, aggressively kicking the substrate as they descend, dislodging fish that may be caught in the substrate. Once the electrofisher(s) reach the seine, they should shock the seine for several seconds to ensure that all fishes are stunned. Then, the two seine operators should lift the seine out of the water, each stepping backwards to stretch the seine taught.

One member then should scoop fish from the seine, and place all specimens in a bucket. Repeat this procedure until the entire riffle area has been covered, checking the seine and removing captured fish after each set. This method is more effective than dip netting alone at capturing benthic species from riffle habitats.

3c. Specific Electrofishing Procedures for the Coastal Plain

For sample sites within level III Coastal Plain ecoregions, **three electrofishing passes should be conducted over a reach length of 20 times the average stream width in meters (or minimum length 100 m) (Table 1)**. Analyses of South Carolina stream sampling data have shown that multiple passes are necessary to document the majority of species in coastal plain streams and extract fish from dense habitats such as undercut banks, root wads and heavy aquatic vegetation. Block nets should be placed at the upstream and downstream extents of the sample reach. Block nets should have ¼ inch or smaller mesh and be free of tears and holes. Block nets should be long enough to extend across the entire width of the stream and wide enough to vertically reach from the bottom to above the surface of the stream. Any minor braids or stream confluences that are present within the reach should also be blocked to avoid fish escaping or entering the sample reach. Where possible, no person should enter the stream until all block nets have been deployed. The downstream block net should be set first and care should be taken to ensure that the lead line is in contact with the stream bottom along the entire width of the stream. Although most block nets have a lead line, it is often useful to stake or anchor the net to the bottom of the stream. Once the downstream net is set, the sampling crew will then walk along the bank (not in the stream) to the upper extent of the reach and set the upstream block net. Upon completion of each electrofishing pass, the downstream block net should be checked for fish and checked to ensure that it still effectively blocks the entire stream. Any fish collected out of the block net should be added to the fish totals for that particular pass.

D. On-site Sample Processing

All fishes captured are identified to species and enumerated. The numbers of fish by species are recorded separately for each independent electrofishing pass. Special care should be taken to minimize the mortality of all fish. All biological data should be entered on the Biological Parameters section of the Stream Assessment Data Sheet. Fish species identification can be tricky for certain species which could require a voucher specimen to be preserved for further confirmation by another fishery biologist. Also, a fish may be captured that has yet to be identified in a watershed or stream system that it has never been documented. In these incidences, it is also necessary to preserve a voucher specimen to confirm this undocumented occurrence by another fishery biologist. Fish voucher specimen protocols for preservation and photographs are outlined in Appendix B.

<u>1. On-site Fish Sample Processing Protocol</u>

1. Identify all fish to species and enter data on the Stream Assessment Data Sheet in the Species column of the Biological Parameters section. Fish that are difficult to identify due to their small size or indistinguishable characteristics should be photo documented. Photo documented species should be placed against a light background (gray, white or light blue) of foamboard, plastic or some other semi-waterproof material. The left side of the fish should be photographed near a small ruler for relative size reference as seen in Figure 1. Detailed Photo Voucher information is provided in Appendix B.

2. Enumerate the total number of each species from each pass and record the total in the Total Column of the Biological Parameters section.

3. Record the correct corresponding pass number in the Pass Number column of the Biological Parameters Section.

~~4.~~ Fish that are clearly young of year (YOY) should be counted and recorded separately. Abundance of YOY fish can be highly variable and their inclusion may introduce bias into sample analyses. However, it is important to document the presence of YOY, which may represent species of which adults are absent. For most spring-spawning families of South Carolina stream fishes including the Cyprinidae, YOY are generally large enough to be captured with 3/8-inch net mesh by July. YOY cyprinids may reach 20-30 mm TL by July and 30-40 mm by later in the summer and fall. Placement of fishes into general size/age classes can aid in distinguishing YOY; most cyprinids, for example, occur in three to four predominant size/age classes.

Habitat Assessment

A. Habitat Assessment Protocol

Habitat assessments should be conducted after fish sampling has been completed. A modified ‘zig-zag’ method is used to quantify habitat heterogeneity in current velocity, depth and substrate (Bevenger and King, 1995). This method requires traversing a random ‘zig-zag’ longitudinal transect in a downstream to upstream direction along the sample reach. A total of 50 individual measurements of depth, current velocity and substrate are taken along the random longitudinal transect. Habitat data should be recorded in the Habitat Characterization section of the Stream Assessment Data Sheet.

1. The ‘zig-zag’ method requires two people to conduct the procedure efficiently. One person will take habitat measurements and is outfitted with a flow meter, top-set wading rod and meter stick with millimeter increments. The second person records data on the habitat characterization section of the Stream Assessment Data Sheet.

2. Begin at the downstream end of the sampled stream reach. The measurer should enter the stream and stand in the deepest part of the channel (the thalweg). Begin a random walk upstream through the reach, zig-zagging across the wetted channel and stopping to record the depth, velocity and substrate measurements at 50 random points throughout the section. The measurement points should be distributed approximately in proportion to major habitat types (runs, riffles, pools), but the measurer should try to avoid bias in selecting particular locations. If the top of the reach is approached before 50 points have been measured, turn around and proceed in a downstream direction to complete the assessment.

Depth and Velocity Measurement

At each of the 50 points, use the top-set wading rod to take a depth measurement and record. Record depth in meters. Adjust the flow meter to take a water velocity reading at 6/10 depth (60% of the depth from the surface and 40% from the bottom). Record velocity in meters per second.

Substrate Measurement

Without looking at the streambed, reach down and pick up the piece of substrate nearest the big toe (pick a spot on the wader boot to consistently use). Substrate can be either inorganic or organic (see below for details on how to treat both).

If the substrate is **inorganic**, measure the intermediate axis of the bed particle to the nearest millimeter (Table 3). If the particle is clay or fine sand, record as 0.5 mm. For inorganic particles too large to pick up and measure, make the measurement on the streambed using the meter stick. If the substrate is bedrock, record as 999 mm.

Organic substrates (e.g., detritus, animal inputs, leaves, wood or aquatic vegetation) should be assigned to one of five categories according to size and composition: fine particulate organic matter (FPOM), coarse particulate organic matter (CPOM), fine woody debris (FWD), large woody debris (LWD) or aquatic vegetation (AV) (Table 3).

3. If a sample point falls on a dry section of the stream, record "DRY" for that point. In low water and drought situations in which a stream has been reduced to broken or isolated pools, habitat assessment should be conducted over the entire sample reach rather than only the pool portion. That is, the number of DRY points should be approximately proportional to the amount of normally wet channel that is dry at the time of the assessment. A completely dry stream would result in 50 DRY measurements. However, if the stream channel is reduced in width, but there is a continuously watered channel throughout the sample reach, there should be **NO** "DRY" measurements. **If there is a continuous watered channel, all measurements should be taken within the watered channel.**

Table 3: Habitat assessment and substrate categories and values.*	
Inorganic Substrate	
Code/Value	*Description*
0.5 mm CLAY	Clay
0.5 mm SAND	Silt, fine sand < 1 mm
Intermediate axis diameter (mm	Sand > 1 mm to large boulders (If particle embedded/too large to pick up, measure exposed portion.)
999 mm	Bedrock
Organic Substrate	
Code/Value	*Description*
FPOM	Fine Particulate Organic Matter: < 1 mm diameter, organic matter that has been broken down into fine pieces
CPOM	Coarse Particulate Organic Matter: > 1 mm diameter and <50 cm in length, leaves/fragments, small sticks, plant parts, animal inputs (feces/carcasses)

FWD	Fine Woody Debris: 3-10 cm diameter and > 50 cm in length, sticks/logs
LWD	Large Woody Debris: > 10 cm in diameter and > 50 cm in length, sticks/logs
AV	Aquatic Vegetation: all sizes/shapes, rooted aquatic vegetation and filamentous algae
Other	
Code/Value	*Description*
DRY	Dry point that is normally wet under base flow
*Modified from Bevenger, G.S., and R. M. King. 1995. A pebble count procedure for assessing watershed cumulative effects, USDA FS Research Paper RM-RP-319.	

Water Quality

Water quality parameters should be measured/collected on the date of biological sampling. Parameters should be measured prior to fish and habitat sampling or any other stream disturbance. If access to the sample section requires entering the stream channel, water quality measurements should be taken upstream of the disturbed section. Water quality is measured by on-site measurements.

A. On-Site Water Quality Measurement

1. Overview

On-site measurements should be made using well-calibrated portable meters and should include the following parameters:

- Water temperature (ºC)
- Dissolved oxygen (mg/L)
- Conductivity (μS/cm)
- pH
- Salinity (ppt)
- Turbidity (NTU)

All on-site measurements and a record of the instrument used to measure them are recorded on the Chemical Parameters section of the Stream Assessment Data Sheet.

2. On-Site Water Quality Measurement Protocol

Sensors should be placed in an area of moderate or representative flow and suspended in the middle of the water column if possible. Do not place sensors in fine bottom sediments or organic debris as this can affect water quality parameters (e.g. reduce dissolved oxygen), produce unrepresentative readings or potentially damage the equipment. If little or no flow is present, sensors should be slowly circulated according to manufacturer's guidelines to ensure adequate circulation. Allow sensors to remain in the water for the duration recommended by the manufacturer for all parameters to fully stabilize (dissolved oxygen and pH typically take longest to stabilize). Record measurements in the Chemical Parameters section of the Stream Assessment Data Sheet.

A turbidity sample should be obtained from the thalweg and at mid-to-upper-water column depth. The vial or bottle should be rinsed at least twice with stream water, then filled and capped underwater (avoid bubbles), being careful not to disturb bottom sediments in the collection area. Turbidity should be measured as soon as possible following sample collection in order to prevent algal growth within the sample vial, which can artificially increase turbidity readings. Shake the sample immediately prior to measurement in order to re-suspend any particles that may have settled following sample collection. Use a lint-free non-abrasive tissue to clean the exterior of the vial. Insert the vial into the turbidimeter, pushing the vial down to ensure that it is completely inserted into the chamber. Index or rotate the sample following manufacturer's instructions. Record turbidity in NTU on the Chemical Parameters section of the Stream Assessment Data Sheet.

Literature Cited

Bevenger, G.S., and R. M. King. 1995. A pebble count procedure for assessing watershed cumulative effects, USDA FS Research Paper RM-RP-319.

Appendix A Equipment Specifications

Fish collection nets should conform to the specifications employed in South Carolina Stream Assessment (or equivalent):

Backpack Electrofishing Nets:

- Net head size: 11.5” (W) x 14” (L) (Loki Nets TN-1 or equivalent)
- Mesh size: 3/16” (Loki Nets Ace nylon or equivalent)
- Net depth: 6-8”
- Handle length: 48”

Seines:

- Length: 10’
- Height: 4’
- Mesh size: 3/16” (Loki Nets Ace nylon or equivalent)
- Bottom line with lead weights
- Top line with floats

Block Nets:

- Length: 30’
- Height: 6’
- Mesh size: 1/4" (Loki Nets square nylon #105 or equivalent)
- Bottom line with lead weights
- Top line with floats

Appendix B Fish Voucher Specimen Protocol

Specimen fixation/preservation:

Fish should be anesthetized in ice water and placed into 10% formalin solution for fixation. Specimens should remain in 10% formalin solution for a minimum of 7 days then transferred to water and soaked/rinsed regularly over a period of 1-2 days. Once rinsed, specimens should be transferred to 70% ethanol for long-term storage.

Detailed labels containing all relevant date, locality and identification information should accompany specimens (inside the jars) throughout the fixation/preservation process.

General voucher criteria:

Voucher specimens and/or documentation photographs should be obtained for every species collected at each site.

Small specimens should be preserved following the methods above; specimens too large to be retained should be photographed with a high-quality digital camera, obtaining at a minimum a lateral (left side) view of the entire fish with fins extended to greatest degree possible (see also specific protocols by family, below).

For relatively small species (to be preserved), **at least two specimens** (if present) of each species should be preserved. Effort should be made to retain at least one specimen of the smallest size class represented by each species, along with a representative larger individual.

To the greatest degree possible in the field, specimens should be housed and labeled separately by species, in order to avoid potential discrepancies.

General photography procedures:

For all voucher photographs, a minimum of a lateral view should be obtained of the left side of the fish (or right side if left side damaged), with fins extended to greatest degree possible. Photographs of specimens submerged in specialized viewing containers or similar 100% transparent enclosures are recommended. In general, effort should be made to photograph any diagnostic features for a given species and especially those distinguishing a species from similar species.

Photography procedures by family:

In addition to a full lateral view as described above, the following features should be photographed:

- Catostomidae (Suckers):
  - Dorsal fin (upright, enabling ray count);
  - Mouth (ventral view, “closed” position, enabling zoom examination of lower and upper lips)
- Ictaluridae (Bullhead Catfishes):
  - Maxillary barbel showing anterior edge
  - Anal fin, extended
- Clupeidae (Shads & Herrings):
  - Mouth (lateral view, held open, showing angle of upper edge of lower jaw)

Appendix C Helpful Supporting Documents

Freshwater Fishes of South Carolina (including taxonomic key to species). Rohde et al. 2009.

SC Mussel Field Identification Guide

http://www.dnr.sc.gov/aquaticed/pdf/MussellFieldGuide.pdf

Workbook and Key to the Freshwater Bivalves of South Carolina

http://www.dnr.sc.gov/aquaticed/pdf/WorkbookSCclams.pdf

SC Wildlife Action Plan

www.dnr.sc.gov/swap

Herpetology at Savannah River Ecology Lab

http://srelherp.uga.edu/