The South Carolina Stream Assessment

Standard Operating Procedures

A document prepared by the

SCDNR Stream Assessment Task Group

of the Freshwater Fisheries Section.

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Introduction

A. Background

Freshwater species worldwide face accelerated extinction rates relative to most other wildlife taxa. The Southeastern U.S. in particular has been suffering long-term declines in native species of fish and aquatic invertebrates. As noted in the SCDNR Freshwater Fisheries Section's Priority Planning document (2003), the traditional role of the SCDNR in aquatic resource management has been sportfish management. Therefore, we currently have a critical lack of information on the distribution, population status, and most pervasive threats to their shared habitats associated with pollution (from point and nonpoint sources) and hydrological disruptions. South Carolina faces rapid growth and increasing demands for land development and water resources for municipal and industrial needs. We know that the consequences of certain human activities (e.g., runoff of silt and excessive nutrients, flow alteration) eventually wind up as degraded water quality and aquatic habitat in the streams, rivers, reservoirs, and coastal systems of the State. The quality of water and aquatic habitat reflects the condition of the uplands drained by the stream. As has been widely noted in conservation literature, successful aquatic conservation must focus on landscapes and watersheds (Allan 2004). A reversal of the decline of native aquatic species requires an understanding of factors that are critical for maintenance of suitable water quality and habitat capable of supporting sensitive forms. Extending this logic, we must also understand the threats that degrade the water quality and aquatic habitats to the point where they no longer support sensitive species. We do not currently possess this information in sufficient detail to recommend efficient and effective on-the-ground conservation actions. The foundation of such an approach should include a system-led (e.g., watershed) rather than species-led focus; biological integrity goals applied in the context of preventing degradation of high-quality systems and restoring poor-quality systems; recognition of land and water resources as integrated parts of the same system; and commitment to implementing effective land-water management practices (Angermeier 1995, Warren et al. 1997). The primary goal of this probabilistic assessment is to gather the appropriate data that will allow the SCDNR to design effective management strategies to protect, conserve, and restore the aquatic resources of the state.

B. Intent

The purpose of the South Carolina Stream Assessment (SCSA) and its standardized sampling program is multifold. First, we seek to document distribution of many poorly-known taxa, including fish, invertebrates, and herptiles. Second, we seek information on population status of aquatic fauna, as indicated by standardized catch rates. Third, we seek to quantify associations between biological populations and communities with the condition of their habitats in terms of water quality, channel geomorphology, and physical heterogeneity. Fourth, we seek to quantify the relationships among watershed and riparian condition and aquatic habitat. Finally, we intend to use this information to construct models to form the basis for decision-support networks that can

help SCDNR make decisions to achieve effective and efficient management and conservation of aquatic resources. In addition, model output will be available for public outreach to serve as scientific input to resource decision-makers interested in sustaining natural resources. These include but are not limited to managers of public and private lands, county and municipal zoning and planning commissions, state and regional development planners, and environmental regulators.

C. Project Design

The South Carolina Stream Assessment (SCSA) was initiated in 2006 to collect data with standardized procedures necessary to support decision-making with respect to aquatic resources in the state. The design of the SCSA is based on the drainage and ecoregional patterns that exist in South Carolina. Seven river basins (Savannah, ACE [Ashepoo-Combahee-Edisto], Saluda, Broad, Catawba/Wateree, Congaree/Lower Santee, and Pee Dee) were divided according to seven level IV ecoregions that they intersect on the state's landscape: Blue Ridge, Inner Piedmont, Outer Piedmont, Slate Belt, Sand Hills, Atlantic Southern Loam Plains, and Carolina Flatwoods. The resulting 30 landscape units, termed "ecobasins", are used in stratification of sampling effort and are intended to partition the variation in ecological structure and function of aquatic communities across the state. Watersheds selected are restricted to wadeable streams, approximately 4km^2 to 150km^2.

The sampling design consists of two parallel approaches that differ only in method of site selection and sample scheduling. One approach utilizes approximately 85 least-impacted fixed sites, selected to be highest quality "reference" sites that are sampled annually. This method is intended to provide expected resource conditions for comparative purposes as well as range due to temporal variability. The fixed sites are identified and sampled by biologists familiar with the region. The second method employs random selection of 450 watersheds allocated proportionally among ecobasin strata to allow statistically defensible estimates of statewide resource parameters from the sample data. Data collection is identical in both sampling designs, occurring at two spatial scales:

- *Watershed* – nonpoint sources as measured by appropriate land use/land cover classes in entire basin and within riparian buffer, point sources as measured by NPDES permits, hydrological disruption as measured by impounded area or occurrence of dams;
- *Stream Reach* – Selected measures of channel geomorphology and flow characteristics, water quality, vertebrate and invertebrate species composition and abundance (Table 1).

Random sites are selected with known probability using a multistage design from a list frame of all stream segments in the state, stratified by ecobasin and stream size. This "stream population" was constructed using the ArcGIS Spatial Analyst extension with Flow Direction and Flow Accumulation data, derived from existing Digital Elevation Models (30 m resolution). Each 100 m segment of stream length that drains watersheds between 4 km^2 and 150 km^2 in area was assigned a unique site identification number and stored in a database with flow information. A query was constructed using

VisualBasic that selects segments randomly from the ecobasin specified by the user. A novel component of the site selection routine allows users to avoid a common pitfall in stream sampling design and site selection: dependence among sample sites. That is, a stream located downstream of another and sharing much of its drainage area can be expected to be similar in terms of physical, chemical, and biological characteristics. Our site selection routine examines each potential site for upstream sites that share drainage. The user can specify how much dependence, defined as shared drainage, will be allowed in the site selection process; the default value is less than 50%. This translates into a set of sample sites that share no more than half of the drainage of any downstream site, which we believe ensures a reasonable level of independence among samples.

Table 1. Suite of measurements corresponding to stream sample locations, in conjunction with Clemson University collaborators.

Variables associated with each stream site (units)	
Stream reach ID Longitude (decimal degrees) Latitude (decimal degrees)	Drainage area of watershed (km^2) Elevation (m above mean sea level) Channel gradient (percent slope)
Water quality/chemistry	
Dissolved oxygen (mg/L) Conductivity (µS/cm) pH Hardness Turbidity (NTU) Total suspended solids (mg/L) Total dissolved solids (anions, cations; mg/L)	Nitrogen (mg/L): nitrate, nitrite, TN Phosphorus (mg/L): ORP, TP Metals (water & sediment concentrations): Al, Cd, Cr, Cu Fe, Pb, Mn, Ni, Ag, Zn Organic compounds: selected polycyclic aromatic hydrocarbons & nonylphenols
Physical/Geomorphological	
Water temperature (ºC) continuous hourly logging Channel dimensions: ratios of width to depth, bank height/angle, cross sectional area Channel substrate particle size distributions	Mean wetted width (m) Mean and standard deviation (STD) water depth (m) Mean and STD water velocity (m^3/sec) Percent occurrence of organic debris and wood in stream channel
Biological	
Biomarkers indicating exposure to pollutants in sunfish individuals: EROD activity, bile fluorescence, and induction of metallothionein and vitellogenin Indicators of fish health: hepatosomatic index, gonadosomatoc index and splenosomatic index	Biological Community Structure: aquatic insects crayfishes mussels fishes reptiles & amphibians (herps)

Field sampling follows standard operating procedures cited in this document for sampling wadeable streams. This protocol mainly proscribes fish, habitat and water quality data collection. Other taxa that have become the focus of interest include aquatic insects, crayfish, mussels, and herpetofauna. Aquatic macroinvertebrates are collected according to SC Department of Health and Environmental Control protocols. Crayfish, mussels, and herpetofauna collection protocols are cited in this document.

Grab samples of stream water from each site are returned to the SCDNR Analytical Lab for analysis of standard water quality, including nutrients (Table 1). In-situ water samples are taken at the time of sample (Table 1). Metals and selected organic components of water and sediment are collected for analysis at Clemson University. A subsample of as many as ten sunfish (genus *Lepomis*) are processed at each site for tissue biomarkers and individual health indicators (Table 1). Population data includes distribution and catch per effort of each species, while community data is given by species composition and abundance among sites. Stream geomorphological surveys will be conducted in all sites resulting in measures of channel dimensions listed in Table 1. Physical estimates of habitat conditions (depth, velocity, substrate) are taken at the time of sample in a standardized habitat survey (Table 1).

Considering the linkages between aquatic corridors and surrounding terrestrial habitats, it is best to view aquatic habitat quality as a combination of factors at three spatial scales: (1) aquatic (e.g., water quality), (2) local (e.g., stream buffer quality), and (3) landscape (e.g., extent and configuration of preferred habitats). To derive predictive models, then, we need data from each scale. The South Carolina Stream Assessment will provide an outstanding database on faunal communities and their distributions, as well as aquatic and local conditions (e.g., water quality, geophysical conditions, and riparian buffer characteristics). Remotely sensed land use/land cover data can be analyzed at multiple scales (e.g., 30m LANDSAT to LIDAR) to provide metrics of landscape integrity. Ultimately, predictive models will be built with explanatory variables drawn from all three habitat scales (aquatic-local-landscape) and response variables being the abundance and diversity of species as recorded in the Stream Assessment. These predictive models will be projected and validated using reserve data sets and field visits, with the resulting maps being made available through SCDNR to help local officials and conservation groups prioritize actions.

Literature Cited

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Site Selection and Reconnaissance

A. Random Site Selectability

Random sites will be assigned by a Geographic Information Systems (GIS)-based selection program and sampled by the Stream Team on a random ecobasin schedule. In some cases an assigned random site may not be suitable for sampling due to physical and/or methodological reasons. Reasons for rejecting a randomly selected sample site include:

1. Physical:

- Access/sampling hazardous to sampling crew
- Access denied by property owner
- Site inaccessible (all potential routes)
- Site too large (mean wetted width ≥12 m)
- Site too deep for backpack sampling (i.e., not wadeable)
- Channel highly unconstrained (e.g., flooded swamp)
- Site artificially impounded (within 1 km of a dam)
- Site naturally impounded (e.g., beaver)
    - Note: naturally impounded sites may be sampled at the discretion of the supervisor if degree of impoundment is minor and does not affect entire sample section
- Stream dry / no water at time of reconnaissance
- Channel extensively braided (cannot be blocked off or main channel cannot be discerned)
    - Note: channels with only minor braiding may be sampled at the discretion of the crew or regional supervisor
- Channel altered by road crossing(s)
    - Note: sample should be conducted at least 50 m from a road crossing which alters the stream channel
- Sample reach contains tributary confluence

2. Methodological:

- Computer-generated site coordinates erroneous / target stream channel cannot be determined
- Watersheds nested (watershed area comprises ≥50% of an existing sample site's watershed)
    - Note: the nested site with higher rank on the randomized sample generation list, if suitable, should be sampled. Only if the site of higher rank is not suitable should the site of lower rank be sampled.

B. Random Site Reconnaissance Procedures

1. General Procedures / Forms

All random sites should be reconnaissanced prior to sampling. Reconnaissance activities should generally take place from October to May. Site selection should be based on physical and methodological criteria listed above, as well as consistent with certain safety concerns listed in the Safety section of this document.

Prior to reconnaissance, crews should develop a generalized reconnaissance itinerary and report such information to their supervisor. In addition, crews should keep in consistent contact with supervisors and/or other reconnaissance crews while active in the field. Reconnaissance activities should always involve two persons, each equipped with a radio, and other pertinent tools / safety devices. A full list of equipment that should be brought on each reconnaissance trip is listed in Appendix 1.

If a site is deemed suitable for sample, a random site reconnaissance form should be completed (Appendix 2). In addition to the written material cited on the reconnaissance form, at least one photo (preferably 2 or more with at least one photo facing downstream, and another facing upstream) should be taken of the site. Any unusual conditions that exist at the site should also be documented, such as point-source inputs, unusual water coloration, excess sediment inputs, riparian deforestation or other disturbance or unique condition, etc.

2. Temperature Logger Deployment / Retrieval

Temperature loggers should be deployed at all SCSA sites at the time of reconnaissance. Temperature loggers should be programmed with the capacity to record temperature for a minimum of one full calendar year (12 months). Each logger should be set to record the highest temperature during an interval not to exceed 4 hours in duration. The serial number and coordinate location for each logger should be recorded on the random site reconnaissance form. Temperature loggers should be deployed within the limits of a sample reach, preferably in a stable location that will receive the least amount of sheer stress during high water events, but also remain submerged during low flow events. It is highly recommended that the general location of the logger be marked with flagging tape. When temperature loggers are retrieved, the date, time, and condition (e.g. submerged, not-submerged, etc.) should be recorded, along with any other pertinent descriptive information. Verifying that the serial number for the logger matches with the serial number entered on the random site reconnaissance form is recommended.

C. Reference Site Selectability

In addition to the random sites, approximately 85 reference sites have been selected statewide to represent the least disturbed watersheds in each ecobasin. Reference sites have been selected to minimize anthropogenic and natural features that may alter the

biological assemblages. The number of reference sites per ecobasin is distributed in proportion to ecobasin area.

In the event that existing reference sites become unrepresentative of least disturbed conditions due to changes in anthropogenic or natural conditions, alternate reference sites may be selected. However, natural hydrological fluctuations (e.g., drought or above-average flows), and changes in surrounding land use, general habitat conditions or assemblage attributes should not be considered criteria for elimination/replacement of a reference site. Criteria for selection of reference sites / alternate sites should follow physical and methodological criteria listed below. Reasons for rejecting a reference sample site include:

1. Physical:

- Access/sampling hazardous to sampling crew
- Access denied by property owner
- Site inaccessible (all potential routes)
- Site too large (mean wetted width ≥12 m)
- Site too deep for backpack sampling (i.e., not wadeable)
- Channel highly unconstrained (e.g., flooded swamp)
- Site artificially impounded (within 1 km of a dam)
- Site naturally impounded (e.g., beaver)
    - Note: naturally impounded sites may be sampled at the discretion of the supervisor if degree of impoundment is minor and does not affect entire sample section
- Channel extensively braided (cannot be blocked off or main channel cannot be discerned)
    - Note: channels with only minor braiding may be sampled at the discretion of the crew or regional supervisor
- Channel altered by road crossing(s)
    - Note: sample should be conducted at least 50 m from a road crossing which alters the stream channel
- Sample reach contains tributary confluence

2. Methodological:

- Watersheds nested (watershed area comprises ≥50% of an existing sample site's watershed)

Safety

There are a number of potential health and safety considerations specific to SCSA field activities. This section lists a number of safety precautions that should be followed to minimize health and safety risks.

A. Safety Training / Safety Equipment

All SCSA crew members must fill out an emergency medical information sheet prior to any field activity (Appendix 3). The crew leader must review the specific needs of all crew members, as stated in each individual's emergency medical information sheet, and facilitate each individual's specific medical needs. Copies of each participant's emergency medical information sheet should be on-hand during all field activities. A first-aid kit should be fully stocked and readily available during all SCSA field activities. All crew members must be informed as to the location of any medical / first-aid supplies. In the event of a medical or other emergency, the crew leader and/or other qualified crew member should take all appropriate immediate actions and should send for assistance using the fastest available means. A means of mobile communication (cellular phone, 2-way radio, etc.) must always be on hand in the field.

B. Vehicle Safety

All field activities are inherently associated with the risk of a vehicular accident / incident. In order to minimize risk associated with vehicles, the following measures should be taken. Each driver is responsible for visually inspecting their vehicle for potential problems prior to field departure. Any crew member who perceives a vehicular risk must communicate that risk to their crew leader. Only crew members who have been authorized by the SCDNR may operate a SCDNR vehicle. All applicable SCDNR protocols associated with operating a SCDNR vehicle must be followed; refer to SCDNR policy manual directive D202 (equipment use and care) for details.

C. Dangerous Plants and Animals

All crew members should be aware of the risks associated with dangerous plants and/or animals. Field crew members should be able to identify and avoid dangerous plants such as but not limited to poison ivy, poison sumac, and poison oak. Stings or bites associated with the certain terrestrial invertebrates may cause allergic reaction, disease, or death. Field crew members should be able to identify and avoid dangerous insects such as, but not limited to, wasps, yellow jackets, hornets, bees, fire ants, saddleback caterpillars, puss caterpillars, slug caterpillars, black widows, brown recluses, centipedes, millipedes, scorpions, ticks, and chiggers. Appropriate courses of first-aid / medical treatment should be sought if bitten or stung by a dangerous insect. Additional risks are associated with bites/attacks associated with dogs, cats, snakes, catfish/madtoms, wild hogs, bears, and rabid animals. All crew members should stay alert and notify the crew leader of any potential risks associated with dangerous plants or animals.

D. Electric Shock

The failure to observe appropriate safety precautions when using backpack electrofishing equipment can result in electric shock, and worse case scenario, respiratory arrest and/or cardiac fibrillation and death. Therefore, certain precautions must be taken. Only persons specifically designated by field crew leaders may operate backpack electrofishing equipment. Appropriate gear should be worn to minimize the amount of body surface area exposed to electric shock. This includes non-leaking waders and gloves. Gloves should be rated to resist electricity above the maximum power generating capacity of the battery source (normally 12 Volt, 7.5 Ah). All backpack electrofishing equipment should be checked prior to use to verify that it is working properly. All backpack electrofishing units should have operational safety shut-off switches. Neither the anode or cathode, or any electrical conduit linked to either should be touched while a backpack electrofishing unit is in operation. Voltage should not be adjusted higher than what is necessary to conduct a proper sample of stream fishes at a given locality. 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. DC is safer for practitioners and easier on fish. Backpack electrofishing should be conducted with a minimum of three field crew; therefore, in the case of an emergency, one person can tend to the injured person and the other member can summon help. Backpack electrofishing should never be conducted if stream, terrain, and weather conditions are deemed unsafe.

E. Hazardous Stream and Terrain Conditions

Certain hazardous stream and terrain conditions are routinely associated with SCSA activities. In order to minimize risk, crew members should wear appropriate footwear, distribute equipment weight appropriately among crew members, work only during daylight hours, and avoid terrain deemed too dangerous to traverse. If possible, the stream sampling reach should be inspected prior to initiating electrofishing for obstacles, deep holes, fast water, slippery substrate, and other potential hazards. All crew members should be aware of such potential safety hazards and that other hidden hazards may be present. Sites deemed too dangerous to enter and/or sample should be avoided.

F. Hazardous Localities

Certain localities may pose risks to personal safety and should be avoided. The following are examples of locations that may pose inherent safety risks: hunt lands, private property, areas where illegal activity is conducted, areas contaminated by high bacterial levels, and areas contaminated by hazardous wastes. Localities determined to be too dangerous to sample safely should be avoided.

G. Hazardous Weather Conditions

Crew leaders are responsible for monitoring weather conditions and adjusting field activities accordingly. Electrofishing should be avoided during rain events. Field activities should be stopped in the event of an electrical storm.

H. Hyperthermia and Dehydration

Because most sampling activities occur during the summer, the risk of hyperthermia and dehydration is prevalent. Water should be available to all crew members at all times, and all crew members are encouraged to drink copious amounts of water. If a crew member shows signs of dehydration or any heat-related illness, the person should stop work, and be cooled and hydrated. Additional medical attention should be applied as necessary.

Sampling Procedures

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. The success of SCSA research is dependant upon consistency in the collection of biological data, including strict adherence to sampling and documentation protocols. A complete list of the equipment needed to conduct stream sampling for fishes is cited in Appendix 4. All data collected at the time of sample should be recorded on the Stream Assessment Data Sheet (Appendix 5).

B. Sampling Season

Fish sampling should be conducted during periods of base flow and appropriate water temperatures. In South Carolina, appropriate flow and temperature conditions exist approximately from May through October. Stochastic fluctuations in weather conditions may shorten or lengthen this given period. Sampling in the early spring, fall and winter is typically precluded due to increased precipitation, and resultant high flows. 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. In addition, spring, fall, and winter sampling is typically precluded due to cool and cold water temperatures. In cold water, fish tend to be inactive, moving into deep water or heavy cover.

C. Sampling Techniques

1. Sample Reach Length / Number of Electrofishing Passes

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 2. **The minimum sample reach length is 100 m for all sites statewide.**

The number of electrofishing passes to be conducted is also dependant 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 2.

Table 2: Sample reach length and number of electrofishing passes by level-IV ecoregion.

Level-IV Ecoregion	Sample Reach Length (m)	Number of Electrofishing Passes
Blue Ridge Inner Piedmont Outer Piedmont Slate Belt	= 30x average wetted width	1
Sand Hills Atlantic Southern Loam Plains Carolina Flatwoods	= 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 3). The appropriate number of backpack units is at least 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 3).

Table 3: Appropriate crew size for stream electrofishing.

Wetted width (m)	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
9-12m	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. **A complete review of the risks and appropriate precautions for backpack electrofishing are listed in the Safety section of this document**. 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 pulsewidths (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 out of 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 IV Slatebelt, Inner/Outer 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 (minimum length 100m) (Table 2).** 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 8-10ft seine of mesh size 1/4". 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 5-7m 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 moments 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 taut. 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 much 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 IV Coastal Plain ecoregions (Sandhills, Atlantic Southern Loam Plains, Carolina Flatwoods), **three electrofishing passes should be conducted over a reach length of 20 times the average stream width in meters (minimum length 100 m) (Table 2)**. Block nets should be placed at the upstream and downstream extents of the sample reach. Block nets should have ¼" 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 these 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 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 (Appendix 5).

1. On-site Fish Sample Processing Protocol

1. Identify all fish to species and enter the three-character species codes (Appendix 6) 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 OR fixed in either a 10% buffered formalin or 90% ethanol solution and returned to the lab for identification (see Quality Assurance and Quality Control section for protocols)**.** Containers returned to the lab should include a waterproof label in the jar which records the stream name, site number, ecoregion, drainage basin, collection date, latitude and longitude (decimal degrees), and supervising biologist(s).

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 either be omitted from the collection, or counted and recorded separately. YOY fish populations can be highly variable and their inclusion may introduce bias into sample analyses.

5. Recording of total length and weight is optional. Record this information in the Length/Weight/Anomalies column.

6. During enumeration, examine fish for anomalies, and record the codes of all anomalies observed (Table 4) for each individual in the Length/Weight/Anomalies column. Simply note presence or absence; do not enumerate anomalies.

Table 4: Codes for external fish anomalies (EPA 1994).

Anomaly	Description	Code
None	No anomalies are present	N
Deformities[1]	Can affect the head, spinal vertebrae, fins, stomach shape, scales, operculum, eyes, pugheadedness, barbel and jaw deformaties and clubtail.	D
Eroded or frayed fins[1, 2]	Includes necrosis at the base of the caudal fish (peduncle disease) and erosions of the preopercle and operculum.	E
Lesions or Ulcers[1]	Appear as open sores or exposed tissue. Prominent bloody areas on fish should also be included. Small, characteristic sores left by anchor worms and leeches should not be included, unless they are enlarged by secondary infection.	L
Tumors	Result from proliferative cellular growth with tissue that is firm and not easily broken.	T
Fungus	Appears on the body or eyes as a white cottony growth and usually attacks an injured or open area of the fish.	F
Black Spot Disease	Is caused by a parasite and appears as small black cysts on the fins and body.	B
Ich	Is caused by a protozoan infestation and appears as white spots on the fins and body.	I
Anchor Worm	Is a parasitic infection characterized by a worm imbedded in the flesh of the fish.	A
Leeches	Are oligochaete worms which have anterior and posterior suckers; may attach anywhere on the body.	W
Exopthalmia	'Popeye disease' is an anomaly seen as the bulging of the eye.	X
Missing	Eyes, blindness or deterioration of the lens should be noted.	M
Other	Anomalies or parasites not specified.	O

[1] Fish can be damaged during capture and handling; do not note anomalies of this origin.
[2] Do not include damage to fins associated with spawning.

Habitat Assessment

A. Habitat Assessment Protocol

Habitat assessments should be conducted after fish sampling has been completed. The 'zig-zag' method is used to quantify habitat heterogeneity in current velocity, depth, and substrate. 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 (Appendix 5).

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 (m). 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 (m/s).

   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 5). 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), substrates 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 5).

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 5: Habitat assessment substrate categories and values. 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.

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 is embedded/ too large to pick up, measure exposed portion
999 mm	Bedrock	

ORGANIC SUBSTRATE

Code/Value	Description
FPOM	Fine Particulate Organic Matter (<1 mm dia.) organic matter that has been broken down into fine pieces)
CPOM	Coarse Particulate Organic Matter (>1 mm diameter, <50 cm in length) leaves/fragments, small sticks, plant parts, animal inputs (feces/carcasses)
FWD	Fine Woody Debris (3-10 cm diameter >50 cm in length) sticks/wood
LWD	Large Woody Debris (>50 cm in length and >10 cm in diameter) 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

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 two methods: on-site measurement, and grab sampling for laboratory analysis.

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 (see the Quality Assurance / Quality Control section for equipment calibration procedures):

- 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. Save the datafile in the water quality meter's internal memory, if applicable.

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.

B. Grab Water Sampling for Laboratory Analysis

1. Overview

In addition to on-site measurement of basic water quality parameters, two grab samples should be obtained for laboratory analysis of chemical and nutrient characteristics. Procedures for obtaining and processing grab samples are described in the section below. Parameters measured from grab samples include:

- Nitrogen: Nitrite (ppm), Nitrate (ppm), Total Nitrogen (ppm)
- Phosphorus: Total Phosphorus (ppm), ORP, Phosphate (ppm)
- Fluoride (ppm)
- Chloride (ppm)
- Bromide (ppm)
- Sulfate (ppm)
- Total Dissolved Solids (mg/L)
- Total Suspended Solids (mg/L)
  - Volatile (organic) Solids (mg/L)
  - Fixed (inorganic) Solids (mg/L)
- Conductivity (µS/cm)
- Resistivity (KΩ)
- Salinity (ppt)
- Hardness (mg CaCO3/L)
- pH
- Turbidity (NTU)
- Dissolved Oxygen (mg/L)

2. Grab Water Sampling Protocol

Two water samples should be taken at each site, one that is preserved with 2mL of concentrated sulfuric acid, and one that is not preserved. Sample bottles for use with the preserved samples will be prepared and distributed by the state analytical lab. Distributed bottles containing sulfuric acid will be labeled as 'corrosive' and should be handled with caution.

1. For each site, label one pre-prepared 1L sample bottle containing sulfuric acid and one sterile 1L sample bottle with the stream name, site number, date

sampled, and sampler name. The pre-prepared bottle should have already been labeled as 'corrosive'.

2. Rinse the sterile 1L bottle and cap that do not contain the sulfuric acid preservative 3 times prior to filling with sample water. Rinse bottles and caps by filling bottle (and caps) ½ full, rinse, and discard.

3. Collect the sample prior to or upstream of any stream or sampling disturbances. Collect water in the sample bottle that does not contain the sulfuric acid preservative, and carefully transfer the water sample into the bottle that does contain the preservative. Fill the sample bottle (with the preservative) approximately 75% full and cap.

4. Re-fill the unpreserved sample bottle with a final water sample. Fill the bottle to approximately 75% full.

5. Return both bottles to the vehicle immediately after water samples are taken.

6. Ensure that the caps on both bottles are sealed tightly and are dry, then tightly wrap the necks of each bottle with electrical tape to prevent the cap from loosening during transport.

7. Place the samples into a cooler of ice, and maintain at 4°C while in the field. Transfer the samples to a freezer set at -80°C as soon as possible.

8. Frozen samples should be transferred to the analytical lab as soon as possible. Sample analysis is time dependant.

9. Complete a chain of custody sheet (Appendix 7), and keep this sheet with the samples at all times.

Specific Precautions: Any contact with sulfuric acid should be immediately washed off the skin and out of the eyes. In the event of eye contact, the person should be referred to a health care professional for evaluation. Sulfuric acid should be stored in the provided transport cases and transported in a manner so as to prevent damage. When not transporting sulfuric acid for use in sample preservation, the sulfuric acid should be stored in an area approved for storing acids.
Do not allow sulfuric acid to enter the water system. Any spills of sulfuric acid, not in the water system, should immediately be cleaned up and neutralized with sodium bicarbonate.

Quality Assurance and Quality Control

Key to the integrity of a statewide stream assessment program is the strict adherence to quality assurance/quality control standards. The establishment of a quality control framework represents a commitment to maintaining credibility, with the specific objectives of maximizing the precision/accuracy, consistency, and representativeness of program methods and data.

A. Voucher Specimens

Note: the following are the minimum required procedures for obtaining voucher specimens. The crew leader may collect additional specimens as necessary if a situation warrants.

1. Fixed Reference Sites

For species positively identifiable on site, a minimum of **one adult and one juvenile per species per ecobasin per year** should be preserved as voucher specimens. High-quality photographs of live specimens are recommended to supplement preserved specimens, as well as for large specimens in which preservation is not practical (see Photographic Vouchers section). Special procedures apply to species that cannot be distinguished and/or identified, unusual occurrences, and protected species, and are described below.

2. Randomly Selected Sites

For each identifiable species, a minimum of **one adult and one juvenile per species per ecobasin per year** should be preserved as voucher specimens. High-quality photographs of live specimens are recommended to supplement preserved specimens, as well as for large specimens in which preservation is not practical (see Photographic Vouchers section). Special procedures apply to species that cannot be distinguished and/or identified, unusual occurrences, and protected species, and are described below.

3. Special Cases

3a. Questionable Identification

If a species can be distinguished but not identified, it should be recorded with a preliminary descriptive title including the greatest possible taxonomic identification plus a unique identifier (e.g., *Lepomis* sp. A), such that it can be distinguished from all other species on the data sheet. Record corresponding count. Keep a minimum subsample of 5 individuals (or all individuals if <5 present) as voucher specimens in a separate container from other unidentifiable specimens. A plastic bag placed within the larger voucher jug works well in separating specimens. Label the separate bag or container with the temporary species designation and site information (see Labeling and Storage section). Return specimens to the laboratory or send them to the appropriate taxonomic authority for identification. Upon confirmation, update the original data sheets by marking out the

preliminary label with a single line, recording the confirmed species name or code, and initialing beside the correction.

If species cannot be identified and/or may represent multiple species indistinguishable in the field (e.g., where two or more closely related species overlap in range), keep all of the unknown specimens if possible (photograph representative larger specimens) in a separate container or bag if possible. Record the total count and designate a preliminary species title as above. Return specimens to the laboratory or send them to the appropriate taxonomic authority for identification. Upon confirmation, update the original data sheets by marking out the preliminary label with a single line, recording the confirmed species name or code, and initialing beside the correction.

3b. Unusual Occurrences

Voucher specimens are particularly important for species occurring outside of their documented range. In this case, a minimum of five individuals (or all individuals if <5 present) should be preserved (photo document larger specimens). Specimens should be sent to the appropriate taxonomic authority for confirmation. Notable occurrences of nonindigenous species should be reported to the United States Geological Survey Nonindigenous Aquatic Species program at http://nas.er.usgs.gov/SightingReport.asp or 877-STOP-ANS.

3c. SC Species of Concern / Protected Species

Prior to sampling, biologists should familiarize themselves with the state or federally listed taxa potentially encountered in a given area. A useful resource is the South Carolina Comprehensive Wildlife Conservation Strategy (http://www.dnr.sc.gov/cwcs/index.html), which contains descriptions of priority species of conservation concern. Under most circumstances, protected species should not be preserved and returned to the lab. Protected species should be photo vouchered (see Photographic Vouchers section). The process of photo vouchering should not jeopardize the survival of protected species.

4. Labeling and Storage

Voucher specimens should be preserved either in 10% buffered formalin or 90% ethanol within approved containers. Fish returned to the lab in 10% formalin solution should remain so for approximately 5 days, or until the fish are no longer floating. The formalin is then poured off in a well ventilated area and disposed of in an appropriate manner and replaced with fresh water. The water should be replaced every day with fresh water for a minimum of 3 days or until the formaldehyde odor is gone. Once the formaldehyde odor is gone, the water is replaced with a 70% ethanol solution for long term storage.

In the field, all jars with voucher specimens should be carefully labeled. The label should be placed inside the jar. Label field voucher jugs at a minimum with the following information in pencil on waterproof paper:

Date (mm-dd-yyyy)
Stream Name
Site Number
Level IV Ecoregion (e.g., Sand Hills)
River Basin (e.g., Pee Dee)
Latitude (decimal degrees)
Longitude (decimal degrees)
Collected By: (biologist name(s))

Once processed, specimens should be transferred to individual jars by species and labeled with the following information in pencil or laser printed on waterproof paper in addition to that above:

Species Common Name
Species Scientific Name
Confirming Biologist(s):

5. Photographic Vouchers

Photo vouchers can be taken of any fish for any reason. Photo vouchers are highly recommended for documenting SC species of concern, and large specimens that are difficult to preserve. Photos should show the anatomical features necessary for taxonomic identification. Prior to taking any photographs, a header photograph should be taken of the datasheet showing the stream name, site number, and date. When possible, the camera should be set to display the date and time on all photographs. Once photographs have been offloaded or scanned into the computer, they should be organized by site and the file names changed to the following format:

Example File Format:

[SiteID]_[Date]_[TypeCode]_[SpeciesCode]_[PhotoNumber][.jpg]

Where:
[SiteID] = assigned site identifer
[Date] = sample/collection date in this format: yyyymmdd
[TypeCode] = code for type of photo
s = site/sample photo
c = collection/fish photo
o = other
[SpeciesCode] = three letter fish ID code of specimen
[PhotoNumber] = a unique number to identify a photo.

Example Photo Voucher Files:

367389_20060615_s_001.jpg
367389_20060615_s_002.jpg
367389_20060615_c_BLG_003.jpg
R3ACELP3_20060608_s_004.jpg
R3ACELP3_20060608_o_005.jpg

This format will allow for easy sorting and retrieval using Windows Explorer. Photos will sort first by SiteID, then by Date and next by type. This will result in photos being grouped in a logical order for retrieval.

B. Equipment Maintenance

1. Backpack Electrofishing Units

Backpack electrofishing equipment should be maintained according to manufacturer's instructions. For battery-powered units, charge batteries at a rate consistent with manufacturer's guidelines. In addition, battery performance should be continually evaluated in the field, and batteries replaced at first sign of permanent decrease in capacity. Electrode poles and cables should be inspected and cleaned or replaced as necessary. All members of sampling crews are expected to be familiar with electrofishing unit troubleshooting procedures, and spare fuses should be considered standard equipment for all outings.

2. Habitat Measurement / Water Quality Equipment

A separate Calibration Documentation Notebook should be maintained for each water quality and habitat measurement instrument employed in stream sample procedures. Label each notebook with the instrument model number and serial number. Notebooks should also contain a copy of the manufacturer's operation manual.

Each instrument should be cleaned and/or calibrated according to manufacturer's instructions at a minimum frequency of once per week during the sampling season but not exceeding the maximum frequency recommended by the manufacturer. Additionally, calibration should be conducted following extended periods of storage without use.

Calibration activity should be documented on the Equipment Calibration Form contained within the Calibration Documentation Notebook for each instrument. Enter a separate row for each date and parameter calibrated.

All equipment should be stored according to manufacturer's instructions for periods of extended storage without use.

C. Data Management

1. Data Entry

Data should be transferred from field data sheets to the centralized electronic data entry file accessible at \\scdnradmin\data\fisheries, within the folder "StreamProject → Procedures and Forms → Data Entry → AssessmentData.xls." Access to the centralized data entry spreadsheet is limited to one user at a time. Furthermore, an entry status identifier is implemented for each sample in which entry forms must be designated as complete (i.e., all available data has been entered) or incomplete (i.e., pending further data) prior to exiting the data entry interface. A similar but separate identifier must be selected for species data designating whether all species identifications for a given sample have been confirmed or are pending confirmation.

1. Open 'AssessmentData.xls' on the shared network directory.
    a. Enter your stream assessment data.
    b. Save the file and close it.
2. If you have any photos from your sampling open the /photos folder and follow the instructions in the 'ReadMe.txt' file.

Note. These are shared files that reside on a server in Columbia. These files are backed up regularly. Only one person can use a file at a time so close the files when you are not using them to let others have access.

2. Data Verification

At least two persons should independently enter the data for a given sample. Following data entry by at least two persons, electronic cross-verification will be employed to detect potential data entry errors. Disagreeing entries detected by the verification program should be inspected against field data and corrected.

3. Data Validation

Habitat and water quality data will be electronically validated against acceptable ranges of parameter values pre-programmed into each data entry field. Values falling outside the programmed ranges will be automatically flagged by the data entry program. Flagged entries should be traced and all accountable sources inspected for accuracy and adherence to related quality assurance procedures. Entries linked to or strongly suspected of procedural and/or mechanical error should be deleted from the database.

4. Auditing

Periodic field and laboratory/database audits will be conducted to ensure that all personnel adhere to applicable protocol.

Snails, Crayfish and Mussel Collections

A. Snails, Crayfish, and Mussel Collection Protocols

1. Snails

No special efforts will be made to collect snails. If you happen to see any freshwater snails, collect them and preserve them in 100% ethanol.

2. Mussels

Do not collect live mussels, but photos of them including side views as well as views from the top and other angles may be helpful. It is difficult to identify freshwater mussels from a photo, but they may be somewhat helpful. Please note on your data sheet whether or not you have seen any live mussels. If you see shells from dead mussels, collect them, and keep them dried in a zip lock bag. Mussel shells may be easily crushed during transportation. It is helpful to place them in a hard plastic box or some other container to protect them.

3. Crayfish

Electrofishing is a very effective method for collecting freshwater crayfish in shallow streams. Collect all crayfish that are stunned during electrofishing for fish. Although crayfish are affected by the current, they are not stunned as completely as the fish. Usually you have to act quickly to scoop them up or they will swim away. Please do the best you can. Since crayfish regain consciousness and activity levels quickly after being shocked, they occasionally eat the stunned fish. Because of this, you may want to place them in a separate bucket while shocking.

Crayfish may either be preserved in 100% ethanol and placed in a jar with a well-sealed lid (see labeling instructions below), or delivered live to Eastover. Live delivery is recommended only if an Eastover staff member is present and will be making the trip back promptly.

To keep crayfish alive, place them in a zip lock bag with a small amount of fresh water (preferably not too muddy) and place them in a cooler with ice while you are sampling. When you return to the lab, refrigerate the bags of crayfish below 40^{o}F. If you collect very many crayfish from a site, please change the water (with cool aged tap water) in the bag when you return to the lab, and split the crayfish up so that no more than 5 are housed in the same bags. Crayfish can live for a long time refrigerated in small amounts of water, but they often die if they get too warm, or if too many crayfish are kept together and there isn't enough oxygen.

B. Labeling and Vouchering Snails, Crayfish, and Mussels

For all invertebrates, please label the containers with all of the location information:

- **Stream name & site ID number**
- **County**
- **Watershed name & 8 digit HUC**
- **Approximate distance from road crossing (note if the site is upstream or**
- **downstream from the road) and name of road at crossing**
- **GPS coordinates (decimal degrees)**
- **Name of collector/regional office**
- **Date collected**

It is critical that all location information stay with the specimens, which may be passed around for identification purposes.

Herpetofauna Collections

A. Overview and Protocols for Herpetofauna Collections

A field guide to the identification of herpetofauna should be carried into the field each sampling activity. All herpetofauna that are captured and/or sighted should be identified to species, enumerated, and recorded as a side-note on the Stream Assessment Data Sheet. Herpetofauna can be vouchered by preservation or photo; both protocols are identical to those used for vouchering fish.

Appendix 1

Stream Reconnaissance Equipment List

First aid kit(s)
Location(s) of nearest hospitals
Stream team personnel medical history sheets
Cell phone
Two-way radios
Water for drinking and water containers
SCDNR stream sampling SOP
GPS unit and associated cables
Computer and associated cables
Garmin MapSource and nRoute software
Delorme atlas
Stream List
Reconnaissance data sheets
Landowner letters
SCSA information brochures
Waterproof notebook
Temperature loggers (pre-launched)
PVC chambers for loggers
1½-2oz lead bullet weights
Copper couplers
Vice-grips
Zip-ties
Flagging tape
Waders (hip/chest)
Mud boots
Clipboard(s)
Backpack(s)
Camera
Laser range finder
Machete
Pruners/loppers
Calculator
50m measuring tape
Pencils
Pencil sharpener
Blaze orange hats and vests
Sun block
Insect repellant
Batteries (“AA”, “AAA”,”C”,”D”)
Polarized sunglasses
Hand sanitizer
Paper towels
Toolbox with assorted tools
Electric tape
Duct tape
Bungee Cords
Trash Bags

Appendix 2

South Carolina Stream Assessment Reconnaissance Form

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South Carolina Stream Assessment
Random Site Reconnaissance Form

Date:	**Investigator(s):**
Site Number:	**Stream Name:**
River Basin:	**Ecoregion:**
Watershed Size Class:	**Degree of Difficulty (1-10):**
Latitude:	**Longitude:**

Map

Property Contact Information:

Sample Section	
Estimated Average Width (m):	**Canopy (10 = most shaded):**
Estimated Number of Shockers:	**Access (10 = most difficult):**
Notes:	

Temperature Logger	
Serial Number:	**GPS Waypoint Name:**
Logger Type:	**Latitude:**
Logging Initiation Date:	**Longitude:**
Logging Initiation Time:	**Flagging:**
Location:	

Appendix 3
Emergency Medical Information Sheet

South Carolina Stream Assessment
Emergency Medical Information

Name: _____

Blood Type: _____

Allergies: _____

_____
_____
_____
_____
_____

Do you carry any emergency medications? If so, where are they and how do we use them?

_____
_____
_____
_____
_____
_____

In Case of Emergency, Call:

	Name	Relation	Work/Cell Phone	Home Phone
Primary Contact				
Second Contact				
Third Contact				

Insurance Information:

Company : _____
Phone Number: _____

Other:

Medi - Call: (800-925-9724) or (803-699-3337)

Appendix 4

Stream Sampling Equipment List

Safety equipment
- Rubber gloves (1 pair per person)
- Water-tight waders
- Hearing protection
- Insect repellent
- Sunscreen
- Polarized sunglasses

Location(s) of nearest hospitals
Stream team personnel medical history sheets
First aid kit(s)
Cell phone and/or two-way radios
Water for drinking and water containers
SC Stream Assessment Data Sheets
SCDNR stream sampling S.O.P.
Fish key
Landowner letters
SCSA informational brochures
Backpack electrofishers
- anode, cathode
- gas/batteries
- replacement fuses for batteries

Dip nets (4 or more) with ¼" mesh
Flow-through holding cages (2 or more)
Block nets
- 2 – 30' x 8' with ¼" mesh

Seine (8-10ft, ¼" mesh)
GPS unit and associated cables
Computer and associated cables
Camera
Formalin 10% 1 gal
Ethanol 95% 500 mL
Waterproof labels for voucher specimens
Wide-mouth jars (quart and gallon) for specimen preservation
Sterile 1L grab water sample jars (2 per site, plus extra)
Pre-apportioned sulfuric acid vials
Safety goggles
Nitrile gloves
Cooler stocked with fresh ice for grab sample preservation
Measuring board (mm increments)
Clipboard(s)
5-gallon buckets
Small aquarium nets
Small buckets for sorting fish
Water quality instrument (YSI, etc.)
Turbidimeter
Batteries ("AA", "AAA", "C", "D")
Laser range finder
100m measuring tape
30m measuring tape
Waterproof notebook
Blaze orange hats and vests
Electric tape
Duct tape
Wax-coated braided string for net repair
Bungee cords
Tire repair kit
Steel wool for cleaning electrodes
Temperature logger data offloading device
Portable aerators
Backpack(s)
Pencils
Pencil sharpener
Toolbox with assorted tools
Machete
Pruners/loppers
Delorme atlas
Garmin MapSource and nRoute Software
Flagging tape
Paper towels
Trash bags
Battery chargers
Calculator

Appendix 5

South Carolina Stream Assessment Data Sheets

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SOUTH CAROLINA DEPARTMENT OF NATURAL RESOURCES
STREAM ASSESSMENT DATA SHEET

DATE:	**SITE NUMBER**:

SAMPLE INFORMATION

STREAM NAME:			
LATITUDE:	(DD.DDDDDº N)	**ELEVATION:**	Ft ☐ m ☐
LONGITUDE:	(DD.DDDDDº W)		
RIVER BASIN:		**ECOREGION:**	
SUPERVISING BIOLOGIST:			
# of PARTICIPANTS:	(Include supervisor)	**RANDOM SITE** ☐	**FIXED SITE** ☐
NOTES/WEATHER:			

PHYSICAL PARAMETERS

WETTED WIDTH:	_____ (0m)	**AVERAGE WETTED WIDTH:**	(m)
	_____ (25m)		
	_____ (50m)	**SAMPLE LENGTH:**	(m)
	_____ (75m)		
	_____ (100m)	**NUMBER OF PASSES:**	
SUM:			

SAMPLE PARAMETERS

ELECTROSHOCKER TYPE/MODEL:						
VOLTS:	**AC / DC**	**ELECTROFISHING EFFORT** (s)				
FREQUENCY:	(Hz)		**UNIT 1**	**UNIT 2**	**UNIT 3**	**UNIT 4**
PULSE WIDTH:		**PASS 1**				
TOTAL SAMPLING TIME:		**PASS 2**				
		PASS 3				
_____ (s)						

CHEMICAL PARAMETERS

PARAMETERS		**INSTRUMENT MODEL**
WATER TEMPERATURE:	(ºC)	
DISSOLVED OXYGEN:	(mg/L)	
CONDUCTIVITY:	(µS/cm)	
pH:	(pH)	
TURBIDITY:	(NTU)	
SALINITY:	(ppt)	
OTHER:		

SOUTH CAROLINA DEPARTMENT OF NATURAL RESOURCES
STREAM ASSESSMENT DATA SHEET

DATE:	**SITE NUMBER**:

HABITAT CHARACTERIZATION

	Depth (m)	Velocity (m/s)	Substrate: inorganic(mm) or organic category		Depth (m)	Velocity (m/s)	Substrate: inorganic(mm) or organic category
1				26			
2				27			
3				28			
4				29			
5				30			
6				31			
7				32			
8				33			
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HABITAT NOTES

SOUTH CAROLINA DEPARTMENT OF NATURAL RESOURCES
STREAM ASSESSMENT DATA SHEET

DATE:	**SITE NUMBER**:

BIOLOGICAL PARAMETERS

SPECIES (Use three letter code)	**TOTAL** (Count)	**LENGTH/WEIGHT/ANOMALIES** (OPTIONAL) (mm)/(g)/(code)			*PASS* #

Appendix 6

Codes for South Carolina Fish Species Occurring in Freshwater

Revisions to SCDNR List of Fish Species Occurring in Freshwater (last updated 01/2006)
Proposed Revisions for January 2009

ADDITIONS

- **Add to list/database**

Code (Proposed)	Common Name	Scientific Name
CRH	"Carolina" redhorse	*Moxostoma* sp.
FLG	Florida gar	*Lepisosteus platyrhincus*
TNC	"Thinlip" chub	*Cyprinella* sp. cf. *zanema*

SYNONYMIES

- **Database query for either code/name returns records for both codes/names**
- **Remove former codes from list?**

Former Code/Name	Currently Accepted Code/Name	Basis
SFR (Smallfin redhorse, *Scartomyzon* sp.)	BJR (Brassy jumprock, *Scartomyzon* sp.)	
SKR (Suckermouth redhorse, *Moxostoma pappillosum*)	VLR (V-lip redhorse, *Moxostoma pappillosum*)	Common name changed to V-lip redhorse (American Fisheries Society 1991)
SLD (Saluda darter, *Etheostoma saludae*)	CAD (Carolina darter, *Etheostoma collis*)	Currently considered the same species by regional experts (F. C. Rohde pers. comm.)
SRH (Silver redhorse, *Moxostoma anisurum*)	NLR (Notchlip redhorse, *Moxostoma collapsum*)	Southern Atlantic slope populations of *M. anisurum* elevated to new species (*collapsum*) (Warren et al. 2000). Since all of SC drains to Atlantic, former name SRH (*anisurum*) no longer applies.

UPDATES (database should still allow searches by former names and return records for both names when either is queried)

- Change genus of STC (Santee chub) and TLC (Thicklip chub) from *Hybopsis* to *Cyprinella*
- Change genus of STJ (Striped jumprock) from *Moxostoma* to *Scartomyzon*

Code	Common Name	Scientific Name
ALW	Alewife	*Alosa pseudoharengus*
AEL	American eel	*Anguilla rostrata*
AMS	American shad	*Alosa sapidissima*
ANF	Atlantic needlefish	*Strongylura marina*
ASS	Atlantic silverside	*Menidia menidia (9-26-01)*
AST	Atlantic sturgeon	*Acipenser oxyrhynchus*
BDD	Banded darter	*Etheostoma zonale (9-26-01)*
BDK	Banded killifish	*Fundulus diaphanus*
BPS	Banded pygmy sunfish	*Elassoma zonatum*
BDS	Banded sunfish	*Enneacanthus obesus*
BFS	Bannerfin shiner	*Cyprinella leedsi*
BYK	Bayou killifish	*Fundulus pulvereus*
BMF	Bigmouth buffalo	*Ictiobus cyprinellus (9-26-01)*
BLB	Black bullhead	*Ameiurus melas*
BLC	Black crappie	*Pomoxis nigromaculatus*
BBD	Blackbanded darter	*Percina nigrofasciata*
BBS	Blackbanded sunfish	*Enneacanthus chaetodon*
BND	Blacknose dace	*Rhinichthys atratulus*
BCF	Blue catfish*	*Ictalurus furcatus*
BLH	Blueback herring	*Alosa aestivalis*
BBP	Bluebarred pygmy sunfish	*Elassoma okatie*
BFK	Bluefin killifish*	*Lucania goodei*
BLG	Bluegill	*Lepomis macrochirus*
BHC	Bluehead chub	*Nocomis leptocephalus*
BLS	Bluespotted sunfish	*Enneacanthus gloriosus*
BNM	Bluntnose minnow*	*Pimephales notatus*
BFN	Bowfin	*Amia calva*
BJR	Brassy jumprock	*Scartomyzon sp. (1-27-06)*
BRS	Bridle shiner	*Notropis bifrenatus (9-26-01)*
BTM	Broadtail madtom	*Noturus* sp. n.
BSS	Brook silverside	*Labidesthes sicculus*
BRT	Brook trout	*Salvelinus fontinalis*
BBH	Brown bullhead	*Ameiurus nebulosus*
BNT	Brown trout*	*Salmo trutta*
CAD	Carolina darter	*Etheostoma collis*
CPS	Carolina pygmy sunfish	*Elassoma boehlkei*
CRP	Carp*	

CHP	Chain pickerel	*Esox niger*
CCF	Channel catfish*	*Ictalurus punctatus*
CMD	Christmas darter	*Etheostoma hopkinsi*
CSH	Coastal shiner	*Notropis petersoni*
CMS	Comely shiner	Notropis amoenus
CRC	Creek chub	*Semotilus atromaculatus*
CCS	Creek chubsucker	*Erimyzon oblongus*
DTG	Darter goby	*Gobionellus boleosoma*
DSF	Dollar sunfish	*Lepomis marginatus*
DKS	Dusky shiner	*Notropis cummingsae*
EMM	Eastern mudminnow	*Umbra pygmaea*
ESM	Eastern silvery minnow	*Hybognathus regius*
EPS	Everglades pygmy sunfish	*Elassoma evergladei*
FTD	Fantail darter	*Etheostoma flabellare*
FAS	Fat Sleeper	*Dormitator maculatus*
FHM	Fathead minnow*	*Pimephales promelas*
FBS	Fieryblack shiner	*Cyprinella pyrrhomelas*
FBH	Flat bullhead	*Ameiurus platycephalus*
FCF	Flathead catfish*	*Pylodictis olivaris*
FLR	Flier	
FWG	Freshwater gobie	*Gobionellus shufeldti*
GZS	Gizzard shad	*Dorosoma cepedianum*
GLS	Golden shiner	*Notemigonus crysoleucas*
GLT	Golden topminnow	*Fundulus chrysotus*
GLF	Goldfish*	*Carassius auratus*
GCP	Grass carp*	*Ctenopharyngodon idella*
GSF	Green sunfish*	*Lepomis cyanellus*
GFS	Greenfin shiner	*Cyprinella chloristia*
GHS	Greenhead shiner	*Notropis chlorocephalus*
HKS	Hickory shad	*Alosa mediocris*
HBC	Highback chub	*Hybopsis hypsinotus*
HFC	Highfin carpsucker	*Carpiodes velifer*
HFS	Highfin shiner	*Notropis altipinnis*
HCK	Hogchoker	*Trinectes maculatus*
ILS	Inland silverside	*Menidia beryllina*
ICS	Ironcolor shiner	*Notropis chalybaeus*
LKC	Lake chubsucker	*Erymyzon sucetta*
LMB	Largemouth bass	*Micropterus salmoides*
LSK	Least killifish	*Heterandria formosa*

LTM	Lined topminnow	*Fundulus lineolatus*
LES	Longear sunfish*	*Lepomis megalotis*
LND	Longnose dace	*Rhinichthys cataractae*
LNG	Longnose gar	*Lepisosteus osseus*
MGM	Margined madtom	*Noturus insignis*
MKF	Marsh Killifish	*Fundulus confluentus*
MRS	Mirror shiner	*Notropis spectrunculus*
MSQ	Mosquitofish	*Gambusia affinis*
MTS	Mottled sculpin	*Cottus bairdi*
MTM	Mountain mullet	*Agonostomus monticola*
MDS	Mud sunfish	*Acantharchus pomotis*
MMC	Mummichog	*Fundulus heteroclitus*
MSK	Muskellunge	*Esox masquinongy (9-26-01)*
NHS	Northern hogsucker	*Hypentelium nigricans*
NLR	Notchlip redhorse	*Moxostoma collapsum* ** (1/27/06)
OSS	Orangespotted sunfish*	*Lepomis humilis*
PDD	Piedmont darter	*Percina crassa*
PWD	Pinewoods darter	*Etheostoma mariae*
PIP	Pirate perch	*Aphredoderus sayanus*
PNM	Pugnose minnow	*Opsopoeodus emiliae*
PPS	Pumpkinseed	*Lepomis gibbosus*
QLB	Quillback	*Carpiodes cyprinus*
RBT	Rainbow trout*	*Oncorhynchus mykiss*
RWK	Rainwater killifish	*Lucania parva*
RDT	Redbelly tilapia*	*Tilapia zilli*
RBS	Redbreast sunfish	*Lepomis auritus*
RES	Redear sunfish	*Lepomis microlophus*
REB	Redeye bass	*Micropterus coosae*
RFP	Redfin pickerel	*Esox americanus*
RLS	Redlip shiner	*Notropis chiliticus*
RVC	River chub*	*Nocomis micropogon*
RBR	Robust Redhorse	*Moxostoma robustum ##*
RCB	Rock bass	*Ambloplites rupestris*
RFC	Rosyface chub	*Hybopsis rubrifrons*
RSD	Rosyside dace	*Clinostomus funduloides*
RSS	Rough silverside	*Membras martinica (9-26-01)*
SFM	Sailfin molly	*Poecilia latipinna*
SFS	Sailfin shiner	*Pteronotropis hypselopterus*
SLD	Saluda darter	*Etheostoma saludae*

SBS	Sandbar shiner	*Notropis scepticus*
SHC	Sandhills chub	*Semotilus lumbee*
STC	Santee chub	*Hybopsis zanema*
SNS	Satinfin shiner	*Cyprinella analostana*
SAU	Sauger	
SVD	Savannah darter	*Etheostoma fricksium*
SCD	Sawcheek darter	*Etheostoma serriferum*
SEL	Sea lamprey	*Petromyzon marinus*
SGD	Seagreen darter	*Etheostoma thalassinum*
SHM	Sheepshead minnow	*Cyprinodon variegatus*
SHR	Shorthead redhorse	*Moxostoma macrolepidotum*
SRS	Shortnose sturgeon	*Acipenser brevirostrum*
SRH	Silver redhorse	*Moxostoma anisurum* ** (1/27/06)
SFR	Smallfin redhorse	*Scartomyzon* sp. ##
SMB	Smallmouth bass*	*Micropterus dolomieu*
SLB	Smallmouth buffalo*	*Ictiobus bubalus*
SBH	Snail bullhead	*Ameiurus brunneus*
SFL	Southern flounder	*Paralichthys lethostigma*
SPM	Speckled madtom	*Noturus leptacanthus*
SWE	Speckled worm eel	*Myrophis punctatus*
SCS	Spinycheek sleeper	*Eleotris pisonis*
SFK	Spotfin killifish	*Fundulus majalis (9-26-01)*
SMO	Spotfin mojarra	*Eucinostomus argenteus*
STS	Spottail shiner	*Notropis hudsonius*
SPB	Spotted bass*	*Micropterus punctulatus*
SPG	Spotted gar	*Lepisosteus oculatus*
SPS	Spotted sucker	*Minytrema melanops*
SOS	Spotted sunfish	*Lepomis punctatus*
STR	Stoneroller	*Campostoma anomalum*
STB	Striped bass	*Morone saxatilis*
SWH	Striped bass X white bass hybrid*	
STJ	Striped jumprock	*Moxostoma rupiscartes*
STK	Striped killifish	*Fundulus majalis (9-26-01)*
STM	Striped mojarra	*Diapterus plumieri*
SRM	Striped mullet	*Mugil cephalus*
SKR	Suckermouth redhorse	*Moxostoma pappillosum*
SUF	Summer flounder	*Paralichthys dentatus*
SWS	Swallowtail shiner	*Notropis procne*
SWD	Swamp darter	*Etheostoma fusiforme*

SWF	Swampfish	*Chologaster cornuta*
TPM	Tadpole madtom	*Noturus gyrinus*
TLS	Taillight shiner	*Notropis maculatus*
TNS	Tennessee shiner	*Notropis leuciodus*
TSD	Tessellated darter	*Etheostoma olmstedi*
TLC	Thicklip chub	*Hybopsis labrosa*
TFS	Threadfin shad*	*Dorosoma petenense*
TQD	Turquoise darter	*Etheostoma inscriptum*
VLR	V-lip redhorse	*Moxostoma pappillosum (1/27/06)*
WEY	Walleye*	*Stizostedion vitreum*
WAR	Warmouth	*Lepomis gulosus*
WPS	Warpaint shiner	*Luxilus coccogenis*
WTB	White bass*	*Morone chrysops*
WCF	White catfish	*Ameiurus catus*
WTC	White crappie	*Pomoxis annularis*
WTP	White perch	*Morone americana*
WHS	White sucker	*Catostomus commersoni*
WFS	Whitefin shiner	*Cyprinella nivea*
WMS	Whitemouth shiner	*Notropis alborus*
WTS	Whitetail shiner	*Cyprinella galactura*
YBH	Yellow bullhead	*Ameiurus natalis*
YLP	Yellow perch	*Perca flavescens*
YFS	Yellowfin shiner	*Notropis lutipinnis*

*Denotes species known to be introduced to South Carolina waters.

**Currently the species on the Atlantic Slope from Georgia to Virginia referred to as silver redhorse is being elevated to species status with the name notchlip redhorse

M. robustum is currently being used as the scientific name for the robust redhorse, causing much confusion. It has been proposed to change the name of the smallfin redhorse to brassy jumprock and use the scientific name *Scartomyzon* n.sp.

Appendix 7

SC Stream Assessment Grab Water Sample Chain of Custody Sheet

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Sampler's Name _____	Page ___ of ____
Address:_____	
City_____State_____	
Zip Code_____Phone_____	
Project Name_____	

Sample ID / Description	Date Sampled	Time Sampled	North/South GPS Coord.	East/West GPS Coord.	Grab/ Composite	Matrix: Water	Matrix: Soil	Matrix: Other (List)	Preservation: None	Preservation: H2SO4	Preservation: HCl
Samples Relinquished by (Signature)_____ _____ _____	Samples Received by (Signature)_____ _____ _____										
Date _____ ___	Date_____ _____										

Time_____	Time_____
	Samples Received by (Signature)_____
	Date_____
	Time_____