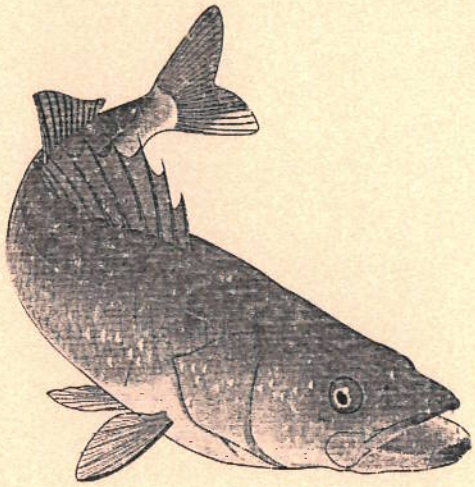
Standard Operating Procedures for Fish and Shellfish Tissue Collection



Technical Report No. 003-01

South Carolina Department of Health and Environmental Control
Bureau of Water, Division of Water Monitoring, Assessment and Protection, Aquatic Biology Section

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Fish and Shellfish Tissue Collection and Analysis

1.1 Introduction

The collection of fish and shellfish for the purpose of tissue analysis is necessary to detect the presence and levels of heavy metals, pesticides and toxic organic compounds in edible tissue which may concentrate through aquatic food chains and threaten the health of human consumers. Aquatic organisms may accumulate contaminants through gills and epithelial tissue directly from water and sediment (bioconcentration), a combination of bioconcentration and dietary sources (bioaccumulation), or a process by which the tissue concentrations increase as the contamination is passed up the food chain (biomagnification). Data collected is used to issue consumption advisories for the protection of public health when necessary and to access adverse biological effects caused by environmental contaminants. A collecting permit is required from South Carolina Department of Natural Resources to collect fish for scientific research.

1.1.1 Species Selection

In most cases a piscivorous species will be targeted for collection. In most fresh waters of the state the targeted species for collection is the largemouth bass, *Micropterus salmoides*. Five largemouth bass with a minimum weight of one pound each should be collected from each waterbody that is capable of being sampled with an electrofishing boat. A minimum weight of one pound is not always possible, especially in some of the smaller rivers and ponds. Bowfin, *Amia calva*, are available in most low country waterbodies (lakes, rivers, and swamps) and a few piedmont water bodies. When available five bowfin at least one pound each should be collected and analyzed for mercury only. In waterbodies where the targeted species are not available in sufficient numbers or sizes, substitutions are made based on the field crews judgement. All fish collected must be of legal size according to South Carolina Department of Natural Resources Rules and Regulations. Substitutions for targeted species may include:

Chain pickerel (*Esox niger*)
Blue catfish (*Ictalurus furcatus*)
Channel catfish (*Ictalurus punctatus*)
Flathead catfish (*Plyodictis olivaris*)
Smallmouth bass (*Micropterus dolomieui*)
Spotted bass (*Micropterus punctutus*)
Rainbow trout (Oncorhychus mykiss)
Brown trout (*Salmo truta*)

Incidentals are any nontarget species readily taken for human consumption of edible size and may be collected while sampling for target species. No more then five fish of each species should be collected from each site. Incidentals may include, but are not limited to the following species:

White catfish (*Ictalurus catus*)
White bass (*Morone crysops*)
Redbreast sunfish (*Lepomis auritis*)
Warmouth (*Lepomis gulosus*)
Bluegill sunfish (*Lepomis macrochirus*)

Redear sunfish (*Lepomis microlophus*)
White crappie (*Pomoxis annularis*)
Black crappie (*Pomoxis nigromaculatus*)
Yellow perch (*Perca flavecens*)

In estuaries, oysters, *Crassostrea virginica,* and blue crabs, *Callinictes sapidus,* are collected for tissue analysis. The South Carolina Department of Natural Resources provides fish from estuarine environments for tissue analysis. Targeted species collected from estuarine environments are red drum, *Sciaenops ocellatus,* spotted seatrout, *Cynoscion nebulosus,* and southern flounder, *Paralichthys lethostigma.* Incidental species may be collected from marine environments and include striped mullet, *Mugil cephalus,* and spot, *Leiostomus xanthurus.* No more than five of each species will be collected from each site per year. All fish collected must fall within size limits set by the South Carolina Department of Natural Resources.

Occasionally the South Carolina Department of Natural Resources will provide edible portions of alligator meat collected during their nuisance alligator trappings.

1.1.2 Fish Collection Equipment

Smith Root 16S Electrofishing Boat
Duracraft 16' Electrofishing Boat
Duracraft 14' Electrofishing Boat
Backpack Electrofisher
Gillnets
Jugs, Trotlines, Limblines

1.1.3 Electrofishing Introduction

A current is passed between submerged electrodes. The conductivity of the water and the conductivity of the fish's flesh affect electrofishing the most. The quantity of dissolved salts and minerals determine the conductivity of the water. Low conductivity water (0.5 to 5.0 microSiemens/cc) requires high voltage , up to 12,000 volts, to pass a current thru fish. High conductivity water (greater then 2,000 microSiemens/cc) requires low voltages and high currents, up to 60 amps. Brackish water and industrial waste water may have conductivities as high as 10,000 microSeimens/cc.

The current flowing through the water is directly proportional to the amount of voltage applied. The higher the voltage, the greater the current will be. There are two types of current available, alternating current (AC) and direct current (DC). Alternating current is an electrical current in which the direction of the electrical current reverses a number of times each second between the anode and the cathode. During electrofishing with alternating current the fish attempt to face the anode and the cathode successively. Alternating current results in strong contractions of the body muscles. At high voltages these muscle contractions may be so severe that vertebrae are fractured and brain damage occurs. Alternating current should not be used for the collection of fish for tissue analysis due to its ability to kill unwanted fish during collections.

Direct current is electrical current that flows in only one direction. The current passes

from the negative electrode (cathode) to the positive electrode (anode). The reaction of the fish is to turn and swim towards the anode until it reaches a field strong enough to stun it (galvanonarcosis). There are no severe muscle contractions and therefore less injury to the fish. Only direct current should be used for the collection of fish for tissue analysis.

1.1.4 Smith Root Boat Electrofishing Procedures

1. Allow the outboard motor to warm idle for 1- 2 minutes before leaving the landing.

2. Remove caps from end of booms. Have a crew member move the booms from the trailering position to the upper underway position and attach boom extensions, and folded umbrella arrays. Attach the folded arrays connecting the quick connector first and then the attached safety line. Connect the quick connector by sliding the female quick connect fitting from the array over the male quick connect fitting on the end of the boom extension. Release the sleeve and pull on the female end to determine the connection is secure.

3. Throttle up slowly and head for the work site.

4. Trim bow to suit boat load and water conditions.

5. Throttle down slowly when reaching the work site.

6. Unfold the umbrella arrays and adjust the boom extensions to the desired position.

7. With the electrofisher off, start the generator after the booms are extended and allow the generator to warm up for a few minutes. The generator must be on water to run. The generator has a "water cooled exhaust", and damage will occur if the generator is ran out of water.

8. Adjust the foot switch system to desired sequence. On the lower right-hand corner of the console control panel is a foot switch workdeck control switch. In the " both position" both workdeck foot switches and the boat operators foot switch must be engaged simultaneously to activate the electrofisher. In the "separate position" only one workdeck foot switch and the boat operators foot switch need to be engaged to activate the electrofisher. The boat operators foot-switch has a separate active/inactive switch in the patch panel on the front of the console. The boat operators foot switch can be disengaged by placing this switch in the active position. The electrofisher can perform with use of only one workdeck foot switch (either the port or starboard foot switch).

9. Smith Root Boat Electrofisher Controls:

* Mode selects the type of output pulses. Direct current pulse rates are selectable in pulses per second. Alternating current frequency is fixed at 60 pulses per second.
* Range selects the output voltage range, or switches the output off.
* Percent Of Range limits the peak voltage of the pulses to a percentage of whatever range is selected.
* High Voltage Indicator Lamp indicates when voltage is present.
* Enunciator Volume controls the audio alarm that indicates an output voltage.
* Output Current shows the current flowing between the anode and cathode in amps.

* Time In Seconds records the actual time high voltage is applied and can be reset to zero by pushing the small red button on front panel.
* Emergency Shutdown provides an override of remote switches. The electrofisher can be shut down by pushing this large red switch down. Switch is located on top of front panel.

10. Set the electrofisher to the desired mode. Turn the direct current/alternating current switch from the off position to the direct current position. Turn the Mode selector to 120 pulses per second DC.

11. Set the Percent Of Range to the minimum.

12. Set the Range selector switch to low.

13. Set the Emergency Shutdown switch to on.

14. Set the Enunciator Volume to a range crew members can hear.

15. With anode and cathode in water activate the electrofisher by stepping on the foot switch. The enunciator and high voltage indicator lamp should both come on. Look at the ammeter to determine amount of amps generated.

16. Deactivate the electrofisher by stepping off the foot switch and adjust the Percent Of Range and the Range selector switch to achieve optimum amperage. Generally 4 amps is an optimum range for our fish collections. Most often the Range is set at 1000 volts DC and the Percent Of Range is adjusted until 4 amps is reached. On some of the upstate reservoirs 4 amps is not possible and collections are made using as little as 1.5 amps due to the low conductivity of the water. Do not adjust the Range selector switch while the electrofisher is activated. Damage may occur.

17. If erratic operation occurs in the high range, switch to low range. Do not operate the generator above power ranges indicated on the meter.

18. Electrofish the site at likely fish habitat. Place fish in live well.

19. After completing fish collections allow the generator to run for a few minutes to allow it to cool down. Switch the Mode and Range controls to off. Switch the Percent Of Range and Enunciator Volume controls to the lowest possible settings.

20. Fold and disassemble the umbrella arrays, disconnect the boom extensions, and place caps on end of booms. Boat is ready to be loaded on trailer.

21. Place fish in a labeled cooler with ice. Include station location and date on label.

1.1.5 Duracraft Boat Electrofishing Procedures

1. Generator and electrofisher should be placed in boat and connections made before launching boat. Connections are the same for the 14' and 16' Duracraft Boat.

2. Check generator engine oil level and replace with SAE 10-30 detergent oil classified for service SF, SE, SD, or SC. Do not overfill.

3. Refuel generator outdoors. Use gas with a minimum rating of 85 octane.

4. Place Smith Root Type VI-A Electrofisher in front of the generator.

5. Join the generator, anode booms, and electrofisher together by connecting the three pin male end black cable to the female three pin receiver on the left front of the electrofisher. Plug the male end of the adjoining cable into the 240V AC outlet on the front of the generator.

6. Join the netters foot switch, operators switch, and electrofisher together by connecting the four pin male end black cable to female four pin receiver on the front of the electrofisher.

7. Join the boat ground to the electrofisher by connecting the two pin male end black cable to the female two pin receiver on the right front of the electrofisher. Connect the opposite male end into the outlet located in front of the steering console. This outlet is connected to the boat hull to provide a ground for electrofishing.

8. Allow the outboard motor to warm idle for 1-2 minutes before leaving the landing.

9. After arriving at the electrofishing site deploy the anode booms in front of the boat by sliding them forward. Plug the boom ends into the outlet box connected to the electrofisher.

10. Turn the electrofisher Input Power Switch (located on the electrofisher console) to off.

11. Turn the generator Power Switch from "off" to "on".

12. Start the generator by pulling the pull cord.

13. Adjust the Mode Selector Switch to 120 PPS DC.

14. Turn on the power switch (labeled Input Power). The red light located to the left of the power switch should come on.

15. Adjust the Pulse Switch Control to approximately 3.5 ms.

16. Place the Voltage Selector Switch to the lowest setting.

17. Insert the key into the key switch labeled Ready on the front panel and, turn it to the right (on position).

18. Lift the cover (bright red) on the Emergency Shutdown switch and move the switch to the right (on position)

19. Boat operator should activate the control switch by flipping the operators switch to the on position.

20. The netter can now stand on the foot control switch and activate the electrofisher. The High Voltage indicator lamp located to the left of the Voltage Selector should come on. The ammeter should deflect and the timer (labeled Seconds on the front panel) should start recording seconds.

21. Adjust the Pulse Width Control and Voltage Selector Switch as necessary to obtain the desired amperage to stun fish (usually approximately 4 amps). Never adjust the Voltage Selector or the Mode Selector under load. Turn the Key Switch off or depress the Emergency Shutdown Switch before making adjustments. Damage to switches may occur while switching under a load.

22. Adjust the Pulse Width to achieve approximately 4 amperes. Often 4 amperes is not possible and electrofishing is done with less amperes. The Output Mode and Voltage Selector may have to be adjusted downward if to many amperes are generated.). Generally the Voltage Selector Switch is set at 1061 VDC and the Output Mode at 120 PPS, and the Pulse Width is adjusted to obtain needed amperes.

23. Electrofish the site at likely fish habitat and place collected fish in a labeled cooler with ice. Label should include the station location and date.

24. After collections are completed turn the Pulse Width to the minimum setting, Voltage Selector to off, Output Mode to off, and Input Mode to off .

25. Allow the generator to run for a few minutes to allow it to cool off.

26. Retract anode booms. Boat is ready to be loaded on trailer.

1.1.6 Backpack Electrofishing Procedures

Backpack electrofishing is performed in wadable streams in pools and around snags, boulders, and other likely fish habitat. Waders must be worn at all times, and rubber gloves should be worn. Backpack electrofishing is performed with a Smith Root Model 12-B POW Electrofisher.

1. Make sure power switch is in the off position, and secure battery in battery box. Connect input power plug to the battery.

2. Connect the cathode (rat tail) to the electrofisher by connecting the four pin male end of the cathode to the four pin female connection on the electrofisher labeled "Cathode".

3. Connect the anode (aluminum ring and fiberglass pole) to the electrofisher by connecting the four pin male end of the anode to the four pin female connection on the electrofisher labeled "Anode".

4. Select voltage and frequency ranges. Set voltage ranges to 100V, and select mode settings of D and 4 when water conductivity is unknown.

5. Place power switch in the "on" position.

6. Place anode and cathode in water, and press pole switch to generate electricity. Audio tone and self test indicator should come on.

7. Observe reaction of fish. Voltage can be increased after releasing the pole switch. If electrofisher is not holding fish, increase pulse width or frequency. If fish are being stunned before reaching anode, decrease the voltage, pulse width or frequency. While the person wearing the electrofisher activates the electrofisher, other field crew can adjust the voltage, frequency, and pulse width until the needed voltage and amperes is obtained. Do not make adjustments with the pole switch pressed.

8. Electrofishing is performed by holding the anode pole button down and holding the anode ring in likely fish habitat.

9. The person dipping should stay in close proximity to the person wearing the backpack to assist with any problems that may arise.

10. After completing collections the fish are placed in a labeled cooler. Station name and date are on the label.

11. Turn the Power Switch to off, Frequency Switch, Pulse Width Switch, and Voltage Switch to minimum settings before removing the battery.

12. Recharge battery before next sampling event. Batteries should be recharged as soon as possible. Connect charger to battery, and connect the charger to the AC power supply, and switch on. Charging time will depend on size and depth of discharge of battery. A minimum of one hour is needed and possibly twelve hours may be needed to recharge a battery. Allow charger to complete its full cycle, indicated by green "Ready" LED. The charger will not overcharge the battery.

13. Surface of anode must be conductive to operate properly. It may become anodized and nonconductive during normal operation. To restore conductivity to anode clean with a Scotch-Brite pad until it shines. Wire brushes and cleaning solutions may also be used.

14. Model 12-B POW Electrofisher Controls and Features:

* Voltage Range Switch is located on bottom left side of electrofisher and has ten ranges. The range can be adjusted according to the conductivity of the water. Use 100 to 300 volt ranges for high conductivity waters (400 to 1600 microSiemens/cc), 400 to 700 volt ranges for medium conductivity waters (200 to 400 microSiemens/cc), and 800 to 1000 volt ranges for low conductivity waters (10 to 200 microSiemens/cc).
* Mode Switches are located on the middle of the left side of electrofisher and are able to produce 256 different waveforms. One switch is labeled A-P and the other 1-16.
* Output Voltage Indicator is an audio indicator that produces a tone warning field crew that voltage greater than 30 volts is being generated between the anode and cathode. The indicator beeps slowly when an input of 4 Amps is generated. The indicator beeps faster as the input increases.
* Timer is a six digit timer located on top of the left side of the electrofisher. The timer records actual shocking time in seconds and can be reset by placing a magnet over the

word "reset" next to the timer.
* Input Power Connector is a quick-twist positive locking connector with index tabs for proper polarization of the connector halves.
* Input Power Switch is a 25A toggle circuit breaker switch that protects electrofisher from excessive input currents.
* Self Test Indicator is a SelfTest LED indicating that the control circuit wiring and pole switch are operating correctly under normal conditions. Problems exist with the battery or control circuit if the indicator doesn't come on when the pole switch is pressed.
* Batt/Gen is a LED that comes on only when the battery is discharged. It can be cleared by turning the electrofisher off and placing a charged battery in the unit.
* Average Current Overload is an LED indicator that turns on if the electrofisher draws to much current from the battery. Turn down the voltage range, select a narrower pulse width, select a lower frequency, or a combination of all three to correct the problem.
* Peak Current Overload is indicated by the Overload LED flashing, and SelfTest Led will also be on. Release pole switch and reduce voltage setting to correct the overload. Anode and cathode touching will also cause a peak current overload.
* A Tilt Switch will trip at approximately 15 degrees backward tilt, and 30 degrees sideways or forward tilt. Correct the problem by standing straight and releasing the pole switch.
* Operator Error is caused by changing the mode switches with output on or by having the pole switch pressed while the on/off circuit breaker is turned on. Release the pole switch to correct this problem.
* Over Temperature begins once the internal temperature of the unit reaches 182 degrees Fahrenheit (83 degrees Celsius). The unit will shutdown automatically. Allow the unit to cool for at least 15 minutes with the on/off circuit breaker turned off to correct the overheating problem.
* Startup Failure indicates an internal problem, and Smith-Root should be contacted.

1.1.7 Electrofishing Safety

1. Members of the electrofishing crew should be trained in cardiopulmonary resuscitation and artificial respiration.

2. Rubber gloves and boots should be worn.

3. Never touch an electrode while the circuit is energized.

4. Do not work on the system while the generator is running.

5. Do not enter the water while the system is running.
6. Never electrofish alone.

7. Inspect all equipment before each sampling event.

8. Use only nonconductive dip nets.

9. Wear personnel flotation devices.

10. Do not operate an electrofisher if you have had prior heart aliments.

11. Ground the generator to the boat hull.

12. Do not electrofish during rain or hazardous weather.

1.1.8 Gill-netting procedure

Gill-netting is used only when the target species is not readily available by electrofishing (e.g. striped bass). Gill-netting is usually performed in reservoirs. If gill-netting is to be performed in rivers, the net should be set parallel to the current.

1. The net is rigged with weights and floats before setting.

2. Place a weight (anchor) on the bottom of the net and a float with a section of rope on the top of the net.

3. Before setting the net, drop the anchor over the bow and back the boat as the net is played out. Remove tangles while keeping the net relatively taut.

4. When the end of the net is reached placed an anchor on the bottom of the net and a float on the top of the net and release the net while making sure it is relatively taut.

5. The nets are set near nightfall and collected at daylight the next morning.

6. Start retrieving the net at the downwind end of the net.

7. Remove anchor and float from downwind end.

8. Remove fish as they come out of the water and place on ice in a labeled cooler. The label will include the station location and date.

9. The net is stacked in a basket as it is retrieved.

10. Remove remaining anchor and float.

1.1.9 Jugs, Trotlines, and Limbline Procedure

Jugs, trotlines, and limblines are used for the collection of catfish when necessary. They are fished overnight and collected as soon as possible the next morning. Trotlines should be marked with clearly labeled floats. Cut bait (shad) is the preferred bait. The number of hooks, jugs, and limblines fished depends on the study requirements.

1.1.10 Sample Collection And Preservation

When the collection of fish or shellfish samples are complete, care should be taken to

insure the freshness and integrity of each sample. Fish or shellfish samples collected from the same site should be immediately placed in a cooler on wet ice for transport to the lab. Each cooler should be labeled with the station information including the site description, station number and date of collection (Ex. Congaree River @ Hwy 601, C-007, 8/11/98). When samples are left unattended, coolers should be placed inside the vehicle and locked to avoid theft and tampering.

.1.1.11 Fish Work-up Procedure

Fish should be worked-up as soon as possible after collection.

1. Record station number in log book.

2. Record date station was sampled in log book.

3. Record sample collectors in log book.

4. Record gear used for collection in log book.

5. Cover table used for working up fish with clean aluminum foil.

6. Place fish on table to be worked up. Only fish from one station can be on fish work-up table at a time.

7. Identify each fish to species and record in fish log book.

8. Measure total length of each fish to the nearest millimeter and record in log book.

9. Weigh each fish 800 grams or smaller to the nearest gram and record in log book. Weigh each fish over 800 grams to the nearest 10 grams and record in log book. Use platform scale (800g x 1g) or electronic scale for fish 800 grams or smaller. Use hanging scale (15kg x 20g) for fish greater then 800 grams.

10. Assign a collection number to each fish, and record collection number in log book.. The first two numbers of the collection number will be the year the fish was collected. The next three numbers of the collection number will be the order in which the fish are worked-up. The 200th fish worked-up in 1998 would be assigned a collection number of 98-200. After fish number 98-999, the 9 is dropped from the year and 1000 will be the last four digits. The 1200th fish worked-up in 1998 would be assigned a collection number of 8-1200.

11. The right side of each fish is scaled. Catfish and other scaleless fish are skinned on the right side.

12. Standard fillets are taken from the right side of each fish for contaminant analysis. Standard fillets are skin on and scales off with the belly flap included. When filleting, care must be taken to ensure fish entrails are not punctured and visible bones are removed. Fish are filleted on clean aluminum foil or on a plastic fillet board that has been cleaned and rinsed first with deionized water and then isopropyl alcohol. Using an electric fillet knife with stainless steel

blades, fillet the right side of the fish. The electric knife blades are cleaned and rinsed first with deionized water and then isopropyl alcohol when the species being filleted changes or the station changes. The fillet board is also cleaned and rinsed with deionized water and isopropyl alcohol whenever the species or station changes.

13. The sex of each fish is determined during filleting and recorded in the log book.

14. Fat deposits, visible bones, and viscera are removed from the fillet with a stainless steel knife and deionized water. This stainless steel knife is cleaned and rinsed first with deionized water and then isopropyl alcohol when the species or the station changes.

15. The fillets from each fish are weighed and the weights recorded in the log book. The stainless steel platform scale pan is cleaned and rinsed first with deionized water and then with isopropyl alcohol when the species or station changes. Fillets are weighed to the nearest gram with the platform scales.

16. After weighing, the fillets are wrapped in clean aluminum foil(dull side to fillet), labeled with the assigned lab number, and frozen until processed for the SCDHEC Columbia Lab.

1.1.12 Fish Processing Procedure

After freezing, the fillets are ground and homogenized for analysis at the Aquatic Biology Section Lab.

1. Assign a lab sample number to each fish. The lab number is a 10 digit number. The first six numbers of the lab sample number will be the date processing of those fish began. The last four numbers will be the order the sample was processed beginning with the number 1000. The 20th sample processed on a start date of March 3, 1998 would be assigned lab sample number 0303981019.

2. Remove tissue samples (fillets) from the freezer as needed to prevent thawing.

3. Place the frozen fillet on a clean chopping board and cut into approximately 10 mm cubes using a stainless steel knife and hammer. The chopping board, knife, and hammer are cleaned and rinsed first with deionized water and then isopropyl alcohol after each fillet.

4. Place approximately 200 cc of dry ice in a clean stainless steel blender canister, then fill the canister approximately ½ with fish tissue. A new **clean** (see section **9.5.13)** canister is used for each fish.

5. The tissue and dry ice are ground into a fine powder.

6. The ground tissue is placed on clean aluminum foil.

7. If all of the fillet cubes cannot be ground at the same time , the ground tissue is placed in a clean stainless steel bowl and mixed throughly after the entire fillet is ground. The stainless steel bowl is cleaned following procedures outlined in section **9.5.13.** first with tap water, then

deionized water followed with isopropyl alcohol.

8. After mixing, tissue to be analyzed for metals is placed in a 50 ml conical tube. The lab sample number and the letters "mets" are placed on the tube. Place the letters "WPC" on the tube. Caps are loosely placed on the tubes to allow the dry ice to sublimate.

9. After mixing, tissue to be analyzed for mercury is placed in a 50 ml conical tube. The lab sample number and the letters "Hg" are placed on the tube. Place the letters "WPC" on the tube. Caps are loosely placed on the tubes to allow the dry ice to sublimate.

10. If organic analysis is being performed, wrap all remaining tissue in clean aluminum foil. Wrap the aluminum foil with lab tape and write the lab sample number and the word "pesticides" on the tape. Write the letters "WPC" on the tape.

11. The samples are placed in a freezer until transport to the lab for analysis.

12. Tighten the caps on the conical tubes before delivery to the lab.

1.1.13 Cleaning and Sterilization Procedures

After each fish or shellfish sample is processed, the canister and other utensils need to be thoroughly cleaned and sterilized. Each sample should be processed with clean, dry equipment. The procedure for cleaning processing equipment following the grinding procedure is:

1. Each canister should be placed under **hot** running tapwater to allow the remaining powder to break free from the blade assembly and canister walls.

2. The canister should be scrubbed thoroughly with a brush inside and out.

3. Then the canister should be rinsed with deionized water and followed by a rinse with isopropyl alcohol and allowed to dry before use.

4. All knives, lids, bowls, spoons, etc ; should be cleaned following the same procedure. Scrub with a brush under **hot** running tapwater, rinse with deionized water and follow with isopropyl alcohol. Allow drying before use on the next sample.

1.2 Shellfish Collection

1.2.1 Oyster Sampling Procedure

Oysters are collected from the mid-intertidal portion of endemic reefs. Oysters are collected by hand using screwdrivers and hammers where necessary to break them free from clumps

1. In general, collect a minimum of 20 -30 legally harvestable (75 mm or greater) specimens from each station in order to produce 200 grams of shell liquor and meat.

2. Clean oysters in ambient water and place on wet ice in labeled coolers. Label should include station location and date.

1.2.2 Crab Sampling Procedure

Crabs are collected with baited commercial-style crab pots.

1. Bait traps with whole gut-slit shad or other fish.

2. Attach a float to each crab pot.

3. Deploy traps overnight at each station.

4. Remove legally harvestable (127 mm carapace width) blue crabs from trap as soon as possible the next morning. Approximately 20 crabs are collected from each station.

5. Place crabs on wet ice in a labeled cooler for transport to the lab. Label should include station location and date.

1.2.3 Oyster Work-up Procedure

1. Assign a collection number to each station of oysters. The first two numbers of the collection number will be the year the oysters were collected. The last three numbers of the collection number will be the order in which the oysters are worked-up. If the oysters are the 500th sample worked up in 1998 the lab number will be 98500. Record collection number in log book.

2. Record station name and number, collectors, and date of collection in log book.

3. Discard any gaping oysters

4. Shuck oysters at the Aquatic Biology Lab and weigh composite tissue on platform scales. Record composite weight of tissue in log book. Transfer the tissue with forceps that have been cleaned and rinsed first with deionized water and then isopropyl alcohol.

5. Transfer the tissue to clean aluminum foil.

6. Wrap lab tape around aluminum foil package of oysters and place sample number on tape.

7. Place oyster tissue in freezer until ready for processing.

1.2.4 Crab Work-up Procedure

Approximately twenty crabs are included in the composite sample to obtain the 100 g of somatic tissue needed from each station.

1. Assign a collection number to each station of crabs and record sample number in log book. The first two numbers of the collection number will be the year the oysters were collected. The last three numbers are the order in which the crabs were worked-up. If the crabs are the 500th sample worked-up in 1998 the collection number will be 98500. Record collection number in log book.

2. Record station number, date of collection, and collectors name in log book.

3. Obtain tissue by removing the claws, carapace, and hepatopancreatic material using stainless steel scissors and forceps rinsed in deionized water and isopropyl alcohol each time the station changes.

4. The body is broken in half and the exposed tissue is extracted from the shell with cleaned stainless steel scissors and forceps. Caution is taken to avoid contamination of tissue and instruments with any residual hepatopancreatic material.

5. Weigh composite tissue on platform scales and record weight in log book.

6. Place tissue in clean aluminum foil. Place lab tape around aluminum foil, and write collection number on tape.

7. Place tissue in freezer until ready for processing.

1.2.5 Oyster And Crab Processing Procedure

Processing should be performed as soon as possible after the oysters and crabs have been worked-up.

1. Assign a lab sample number to each container of oysters or crab. The lab sample number is a 10 digit number. The first six numbers of the lab sample number will be the date processing of that tissue began. The last four numbers will be the order the sample was processed beginning with the number 1000. The 10th sample processed on April 01, 1998 would be assigned lab number 0401981009.

2. Remove samples from refrigerator as needed.

3. Place frozen tissue on a clean chopping board and cut into approximately 10 mm cubes using a stainless steel knife and hammer.

4. Place approximately 200cc of dry ice in a clean(see section **9.5.13)** stainless steel blender canister, then fill the canister approximately ½ with tissue.

5. The tissue and dry ice are ground into a fine powder.

6. The ground tissue is placed on clean aluminum foil.

7. If all of the tissue cannot be ground at the same time, the ground tissue is placed in a clean stainless steel bowl and mixed throughly after all tissue is ground.

8. After mixing, tissue to be analyzed for mercury is placed in a 50 conical tube. The lab sample number and the letters "Hg" and "WPC" are placed on the tube. Caps are loosely placed on tubes to allow the dry ice to sublimate.

9. After mixing, tissue to be analyzed for metals is placed in a 50 ml conical tube. The lab sample number and the letters "mets" and "WPC" are placed on the tube. Caps are loosely placed on the tubes to allow the dry ice to sublimate.

10. All remaining tissue is placed in clean aluminum foil. Wrap the aluminum foil with lab tape and write the lab sample number, the word "pesticides", and letters "WPC" on the tape.

11. The samples are placed in a freezer until transport to the lab for analysis.

12. Tighten the caps on the conical tubes before delivery to the lab.

1.3 Alligator processing

All Alligator samples will be processed for mercury , metals (cadmium, chromium, copper, lead, nickel, and zinc), and pesticides. Alligator meat is provided by SCDNR and all log book information may not be provided for each sample.

1. Enter all available information in the log book. This information is provided by the alligator trappers and may include sex, length, weight, date taken, and location. Record the SCDNR tag number also.

2. Assign a lab number to each portion of alligator meat. The lab number is a 10 digit number. The first six numbers of the lab sample number will be the date processing of that meat began. The last four numbers will be the order the sample was processed beginning with the number 1000. The 10th samples processed on a start date of May 15, 98 would be assigned a lab sample number 0515981009.

3.Remove tissue samples from freezer as needed to prevent thawing of samples.

4. Place meat on a clean chopping board and cut into approximately 10mm cubes with a stainless steel knife and hammer..

5. Place approximately 200cc of dry ice in a clean (see section **9.5.13)** stainless steel blender canister, then fill the canister approximately ½ with fish tissue.

6. The tissue and dry ice are ground into a fine powder.

7. The ground tissue is placed on clean aluminum foil.

8. If all of the meat cannot be ground at the same time, the ground tissue is placed in a clean stainless steel bowl and mixed throughly after the entire sample is ground.

9. After mixing, tissue to be analyzed for metals is placed in a 50 ml conical tube. The

lab sample number and the letters "mets" and "WPC" are placed on the tube. Caps are loosely placed on the tubes to allow the dry ice to sublimate.

10. After mixing, tissue to be analyzed for mercury is placed in a 50 ml conical tube. The lab sample number and the letters "Hg" and "WPC" are placed on the tube. Caps are loosely placed on the tubes to allow dry ice to sublimate.

11. All remaining tissue is wrapped in clean aluminum foil. Wrap the aluminum foil with lab tape and write the lab sample number and the word "pesticide" and letters "WPC" on the tape.

12. The samples are placed in a freezer until transport to the SCDHEC Lab for analysis.

13. Tighten the caps on the conical tubes before delivery to the lab.

APPENDIX 1

Fish Tissue Log Sheets

APPENDIX 2

Inorganic Analysis Fish Tissue Data Sheet

APPENDIX 3

Organic Analysis Fish Tissue Data Sheet