FS 6000. GENERAL BIOLOGICAL TISSUE SAMPLING

1. INTRODUCTION

1.1. The assessment of contaminant bioaccumulation and biomagnification may require analysis of the tissues of various plant or animal organisms. Top predators may be at risk if their food bioaccumulates toxic contaminants. If these organisms are also part of the human food chain, it is very important to monitor them for human health reasons. This may be a concern as, for example, in the contamination of sport fish by methyl mercury. The presence of contaminants in biological tissues is also important when assessing pollution impact across environmental media (e.g., air, water, soil and sediment). Because of biomagnification and bioaccumulation, tissue analysis can be a useful surrogate for investigating the extent of contamination and bioavailability in a more dilute medium such as water or sediment.

1.2. "The bioavailability of contaminants in sediment is a function of the type of chemical and the chemical speciation, as well as the behavior and physiology of the organism. The two basic routes of exposure for organisms are transport of dissolved contaminants in pore water across biological membranes and ingestion of contaminated food or sediment particles with subsequent transport across the gut. For upper-trophic-level species, ingestion of contaminated prey is the predominant route of exposure, especially to hydrophobic chemicals. Uptake through ingestion of or direct exposure to water or sediment can also be important depending on the trophic level of the organism and the physical-chemical characteristics of the contaminant (EPA 2000)."

1.3. The most common target analytes in animal tissues are the toxic metals (e.g., arsenic, lead, mercury, tin, copper, cadmium, nickel, zinc, etc.), their organic compounds (e.g., methyl mercury, tributyltin, etc.), chlorinated phenols, polycyclic aromatic hydrocarbons (PAHs), pesticides, dioxins, furans and PCBs. The levels commonly found in these matrices are in the ppt to low-ppb range. A principal concern in sampling and processing tissues is avoiding sample contamination and degradation.

1.4. Tissues commonly used for analysis are those of algae, vascular plants, shellfish (e.g. oysters, clams and mussels), finfish, birds and mammals. In general, the kind of sample analyzed depends upon study or program objectives. Some of the variables are:

- The target species, e.g., largemouth bass, Florida gar, redear sunfish, bowfin, alligator, raccoon, etc.
- Size, age, sex or other characteristic of the target species, e.g., largemouth bass are often standardized to age 3
- Specific tissues, e.g., edible tissue, entire organism, blood, brain, liver or other organs
- Whole tissue homogenate, composites, or a discrete portion of a dissected organ

2. SELECTION OF APPROPRIATE PROTOCOLS

2.1. In general, obtain the tissue sample from a specimen that was captured alive and take the necessary precautions to avoid contamination and degradation of the sample. These precautions are discussed in each of the specific sampling SOPs in this compendium. These SOPs cover sampling of Shellfish Tissue (FS 6100) and Finfish Tissue (FS 6200). The SOPs for shellfish and finfish were adapted from EPA 1995 and DER 1984 references.

2.2. Study design and the choice of the other parameters mentioned above in section 1.4, although important, are not further discussed in this compendium, but are established as a part of the Data Quality Objectives (DQOs) by the program area that oversees the collection of tissue data. The choice of protocols is also dictated by the nature of the monitoring program, i.e., if for screening studies or for intensive studies (EPA 1995). The procedures - described in FS 6100 and FS 6200 - are comprehensive enough to satisfy the needs of the current monitoring programs requiring tissue analysis in Florida.

NOTE: In general, a 20 g sample is required for the analysis of metals (including Hg) and a 200 g sample is required for the analysis of organics. The sample size must also include enough tissue for QC analyses per laboratory methods. Always check with the laboratory on their specific requirements.

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FS 6100. SHELLFISH TISSUE SAMPLING

1. INTRODUCTION

1.1. Procedures are described here for the collection and processing of tissues from oysters, clams, and mussels. Although not required, it is recommended that the processing of the shellfish to extract the tissue from the shell be carried out at the laboratory or at a field office with appropriate conditions for this work. This step, however, is also described in this SOP. In general, only the edible tissue is submitted for analysis and compositing is employed because single organisms do not provide enough tissue mass to satisfy the analytical requirements (EPA 1995).

1.2. The shellfish collection methods can be divided into two major categories, active and passive. Each collection method has advantages and disadvantages. Various types of sampling equipment, their use, and their advantages and disadvantages, are summarized in Table FS 6100-1.

1.3. The procedures described in this SOP may also be adapted for collection of tissues from shrimp, scallops, crabs, crayfish, spiny or clawed lobsters, and turtles. Specific procedures for these animals are not provided in these SOPs.

1.4. Unless specifically exempted in research projects, the collection of shellfish for these purposes must always satisfy any legal requirements of harvest size or weight, or at least be of consumable size if no legal harvest requirements are in effect.

2. EQUIPMENT AND SUPPLIES

2.1. Boat and Supplies- A boat may be required, and the following supplies must be available:

- Fuel supply (primary and auxiliary)
- Spare parts kit
- Life preservers (USCG approved)
- First aid kit (including emergency phone numbers of local hospitals and family contacts for each member of the sampling team)
- Spare oars
- Nautical charts with sampling site locations
- GPS or Loran instrumentation

2.2. Shellfish Collection Equipment- See Table FS 6100-1. Examples of equipment include:

- Nets
- Grabs
- Rakes
- D-traps

2.3. Recordkeeping and Documentation Supplies:

- Field notebook or field forms (waterproof paper is recommended)

- Specimen identification labels
- Indelible pens
- Digital camera (optional)

2.4. Sample Processing Equipment and Supplies:

- Holding trays
- Shucking knife (SS)
- DI water for rinsing (in plastic or Teflon bottles)
- Non-powdered latex surgical gloves
- Calipers (metric units)
- Balance to weigh representative specimens and tissues (metric units)
- Aluminum foil (extra heavy duty)
- Freezer tape
- String (to tie plastic bags)
- Several sizes of plastic bags for holding discrete or composite samples
- Resealable watertight plastic bags for storage of field records, COC forms, sample request forms

2.5. Sample Preservation and Shipping Supplies:

- Ice (wet ice, blue ice packets, or dry ice)
- Ice chests
- Wide-mouth sample containers (100 mL, plastic) w/ plastic caps
- Wide-mouth sample containers (250 mL, glass) w/ Teflon-lined plastic caps
- Filament-reinforced tape to seal ice chests

3. COLLECTION OF SHELLFISH

3.1. Equipment Selection:

3.1.1. Select the appropriate sampling equipment from Table FS 6100-1 that is compatible with the plan of study, the measurement program and the site conditions.

3.1.2. Conduct any needed field measurements and collect sample(s) from other matrices (water, sediment) for laboratory analyses first.

3.2. Sampling:

3.2.1. Evaluate the catch immediately after the organisms are retrieved from the sampling device, and select those appropriate for analysis.

3.2.1.1. Keep only organisms of target species and discard the remainder.

3.2.1.2. Measure the dimension of each organism to a precision of 0.1 cm; the relevant dimension for bivalves is the height, which is the distance from the umbo to the anterior (ventral) shell margin.

3.2.1.3. Weigh each selected organism to a precision of 0.01 g. Record these measurements in the appropriate form. Collect a minimum of 200 g for the composite sample.

3.2.1.4. For the purpose of determining compliance with the methylmercury standard in Rule 62-302, at least twelve organisms of the species under consideration must be collected.

3.2.1.5. Regardless of the number, the shellfish should all be of similar size so that the height of the smallest individual is no less than 75% of the height of the largest individual in the composite sample.

3.2.1.6. Place all shellfish in plastic bags and into ice chests containing wet ice immediately after collection or measurement. The maximum holding time on ice is 24 hours.

3.3. Tissue Extraction: If the sampling program requires that only the tissues be shipped to the laboratory, follow these procedures in a suitable location, with a large stainless steel sink/counter and a source of tap water.

3.3.1. Place the organisms to be composited in the sink, rinse profusely with tap water and transfer them to the holding tray.

3.3.2. Use one tray for each sampling site.

3.3.3. Cover a portion of the counter with the heavy-duty aluminum foil and place the sample container(s) on it.

3.3.4. Wear the latex gloves at this point.

3.3.5. Using the shucking knife, carefully open each shellfish, extract the tissue and transfer it directly to the wide-mouth container.

3.3.6. If the tissue happens to fall in the counter, rinse it with analyte-free water before placing it in the container.

3.3.7. Rinse the shucking knife with tap and analyte-free water after each organism is processed.

3.3.8. Cap each sample container and write the field ID number on the label attached to it.

3.3.9. Glass containers may be used for all analytes (metals and extractable organics); do not use plastic containers for extractable organics.

3.3.10. After you have completed all extractions, place the labeled containers in the ice chest and prepare for shipping (see section 5.2, below).

4. FIELD MEASUREMENTS AND RECORDS: All information associated with the sampling event must be recorded in the appropriate field sheets or field notebook using indelible pens. These records are the required minimum:

4.1. Names of field personnel, date, time, waterbody name and type (river, lake, estuary), tide cycle (if applicable), site location (latitude/longitude), and weather conditions.

4.2. Field measurements commonly associated with shellfish sampling, e.g., depth, salinity, temperature, turbidity and pH of the water at the sampling site.

4.3. Number and size of organisms in each composite sample, and the collection method.

4.4. Any other relevant information if water chemistry samples are also collected.

5. SAMPLE PACKAGING AND SHIPPING

Prior to shipping samples, notify the analytical laboratory and confirm the amount of sample required for sample analysis. The maximum holding time on ice is 24 hours. Freeze the samples if they will be held longer than 24 hours. To freeze, place the samples in air tight glass or plastic containers (wide-mouth bottles or vials) and freeze at -20°C. Ship the samples using an overnight courier service. Use dry ice or wet ice for shipping. Frozen samples must arrive at the laboratory in the frozen state.

5.1. Whole Organisms:

5.1.1. Place all shellfish, presorted or not, into plastic bags, tied with string, and inside the shipping cooler with plenty of wet ice (see 5.1.4 below).

5.1.2. Attach an ID label to the string of each plastic bag.

5.1.3. The volume of ice should be at least as large as the volume of the bagged samples.

5.1.4. Place all documentation (field sheets; COC, sample request, and shipping forms) in a resealable plastic bag and attach it to the inside of the cooler's cover using the freezer tape.

5.1.5. Seal the cooler with reinforced tape and proceed with shipment.

5.2. Dissected Tissues:

5.2.1. Place each sample container inside a plastic bag and seal it.

5.2.2. Place all containers inside the shipping cooler.

5.2.3. Use enough wet ice so that samples arrive at the analyzing laboratory at 4°C or if shipped frozen maintain a frozen state.

5.2.4. Dry ice is optional if allowed by the shipping company.

5.2.5. Place all documentation (field sheets; COC, sample request, and shipping forms) in a resealable plastic bag and attach it to the inside of the cooler's cover using the freezer tape.

5.2.6. Seal the cooler with reinforced tape and proceed with shipment.

Appendix FS 6100
Tables, Figures and Forms

TABLE FS 6100-1. SUMMARY OF SHELLFISH SAMPLING EQUIPMENT

Device	Use	Advantages	Disadvantages
ACTIVE METHODS			
Seines	Shallow shoreline areas in estuaries.	Relatively inexpensive and easy to operate. Mesh size selection available for crustaceans.	Cannot be used in deep water or over substrates with an irregular contour. Not completely efficient for crustaceans.
Trawls	Various sizes can be used from boats in 10 to >70 m depths	Efficient in deep waters not accessible by other methods. Allows collection of large number of samples.	Requires boat and trained operators.
MECHANICAL GRABS:			
Bucket Type/ Double-Pole	Used from boat or pier. Most useful in shallow waters (lakes, rivers and estuaries)	Very efficient for bivalves that are located or buried in bottom sediments.	Difficult to operate at depths greater than 6 m.
Tongs/Double-Handed Grab Sampler	Most useful in shallow waters (lakes, rivers and estuaries). Used generally from a boat.	Very efficient for oysters, clams, and scallops. Allows washing of collected specimens to remove sediments.	Difficult to operate at depths greater than 6 m.
LINE OR CABLE-OPERATED GRAB BUCKETS:			
Ekman Grab	Used from boat or pier to sample soft to semi- soft substrates.	Can be used in lakes, rivers and estuaries of varying depths.	Possible incomplete closure can result in sample loss. Grab is small and not effective for large bivalves.
Petersen Grab	Deep lakes, rivers and estuaries; for most substrates.	Large sample can be obtained; grab can penetrate most substrates.	Grab is heavy, may require winch for deployment. Possible incomplete closure can result in sample loss. Must be repeatedly deployed.
Ponar Grab	Deep lakes, rivers and estuaries; for sand, silt, or clay substrates.	Most universal grab sampler. Adequate for most substrates. Large sample is obtained intact.	Possible incomplete closure can result in sample loss. Must be repeatedly retrieved and deployed.
Orange Peel Grab	Deep lakes, rivers and estuaries; for most substrates.	Designed for hard substrates.	Grab is heavy, small, and not effective for large bivalves.
Biological or Hydraulic Dredges	Dragged along bottom of deep-water bodies to collect large stationary invertebrates.	Qualitative sampling of large area of bottom substrate. Length of tows can be relatively short if high density of shellfish exists in the collection area.	If the length of tow is long, it is difficult to know the exact sample location. Because of scouring, some bivalve shells may be damaged during collection.
Scoops /Shovels	Used in shallow areas for soft or hard-shell clams, and bay scallops.	Does not require a boat; sampling can be done from shore.	Care must be taken not to damage the shells while digging in the substrate.
Scrapers	Used in shallow areas for oysters or mussels.	Does not require a boat; sampling can be done from shore.	Care must be taken not to damage the shells while digging in the hard substrate.
Rakes	Used in shallow areas by wading or can be used from a boat.	Does not require a boat; sampling can be done from shore. Can be used in soft sediments for clams or scallops. Can be used for oysters or mussels attached to rocks or pier pilings.	Care must be taken not to damage the shells while raking or dislodging them from the substrate.
PASSIVE METHODS			
D-traps	Used to capture slow- moving crustaceans (crabs and lobsters).	Can be used in a variety of environments. Useful to capture bottom dwelling organisms in deep water or inaccessible areas. Rather inexpensive to make.	Catch efficiency is highly variable. Not a good choice for a primary sampling device, but valuable as a backup method.

FS 6200. FINFISH TISSUE SAMPLING

1. INTRODUCTION: These procedures describe the collection and processing of fish tissues.

   1.1. Perform fish processing activities under conditions that prevent contamination by or degradation of the analytes in question.

   1.2. Although not required, DEP recommends that the processing of the fish to extract the tissue for analysis be carried out at the laboratory or at a field office with appropriate conditions for this work. Follow these procedures regardless of the location.

   1.3. In general, submit only the edible tissue for analysis. There are, however, occasions where whole fish analysis is required and this procedure is also addressed in this SOP.

   1.4. Samples may consist of tissue dissected from a single organism, the entire organism, composite of dissected tissues, or composite of many organisms.

      1.4.1. Composite samples are homogeneous mixtures of samples from two or more individual organisms of the same target species collected at a particular site and analyzed as a single sample. Because the analytical costs are usually higher than those of collection and preparation of samples, composite samples are cost-effective for some programs. The use of composites, however, depends upon the Data Quality Objectives (DQOs) of the program.

      1.4.2. DEP recommends compositing samples of fish fillets (edible portion) for analysis of target analytes in screening studies.

      1.4.3. For health risk assessments, a composite sample must represent the portion of the individual organism that is commonly consumed by the population at risk. Florida fish consumption health advisories for mercury are based upon 3-year old largemouth bass, because that is the most common fish in creel census. In this case, the edible portions of individual fish must be analyzed. “Skin-on fillets (with the belly flap included) are recommended for most scaled finfish. Skinless fillets are recommended for scaleless finfish, such as catfish” (EPA 1995).

      1.4.4. For the purpose of determining compliance with the methylmercury standard in Rule 62-302, at least twelve fish of the species under consideration must be collected. Game fish must be of legal size and within a size or weight range that represents fish most likely to be caught and eaten as determined by the Florida Fish and Wildlife Conservation Commission (FWCC).

      1.4.5. To assess risks to other predators, analyze whole fish of the species and size range common to their diet. Depending upon the study design and DQOs, compositing may not be appropriate. Note that it may be difficult to homogenize the whole fish.

   1.5. Fish collection methods can be divided into two major categories, active and passive. Each collection method has advantages and disadvantages. Various types of sampling equipment, their use, and their advantages and disadvantages are summarized in Table FS 6200-1.

   1.6. Unless specifically exempted in research projects, the collection of fish for these purposes must always satisfy any legal requirements of harvest size or weight, or at least be of consumable size if no legal harvest requirements are in effect.

2. EQUIPMENT AND SUPPLIES

2.1. Boat and Supplies- A boat may be required, and the following supplies must be available:

- Fuel supply (primary and auxiliary)
- Spare parts kit
- Life preservers (USCG approved)
- First aid kit (including emergency phone numbers of local hospitals and family contacts for each member of the sampling team)
- Spare oars
- Nautical charts with sampling site locations
- GPS or Loran instrumentation (recommended)

2.2. Fish Collection Equipment- e.g., nets, traps, electroshocking device (see Table FS 6200-1).

2.3. Recordkeeping and Documentation Supplies:

- Field notebook or forms (waterproof paper is recommended)
- Specimen identification labels
- Indelible pens
- Digital camera (optional)

2.4. Sample Processing Equipment and Supplies:

- Holding trays
- Fish measuring board (metric units)
- Small Teflon cutting board (about 5 x 10 inches)
- Fish de-scaler or blunt knife (stainless steel)
- Non-powdered latex surgical gloves
- DI water for rinsing (in plastic or Teflon bottles)
- Dissection kit w/ disposable sterile surgical blades of stainless steel or carbon steel (recommended is rib-back No. 21 by Bard-Parker or equivalent) and a steel holder (such as the Bard-Parker No. 4)
- Balance to weigh representative specimens and tissues (metric units)
- Aluminum foil (extra heavy duty)
- Freezer tape
- String (to tie plastic bags)
- Several sizes of plastic bags for holding discrete or composite samples
- Resealable watertight plastic bags for storage of field records, COC forms, sample request forms

2.5. Sample Preservation and Shipping Supplies:

- Ice (wet ice or dry ice)
- Ice chests
- Wide-mouth sample containers (100 mL, plastic) w/ plastic caps
- Wide-mouth sample containers (250 mL, glass) w/ Teflon-lined plastic caps
- Pyrex culture tubes (25 x 200 mm) w/ Teflon-lined plastic caps **[pre-weighed]**
- Filament-reinforced tape to seal ice chests

3. COLLECTION OF FISH

3.1. Equipment Selection: Select the appropriate sampling equipment from Table FS 6200-1 that is compatible with the plan of study, the measurement program and the site conditions. Conduct field measurements and/or collection of other matrices (water, sediment) for laboratory analyses first.

3.2. Sampling:

3.2.1. Make sure the collecting area is free of grease spilled engine fuel, engine exhaust and dust.

3.2.2. Immediately after the organisms are retrieved from the sampling device, evaluate the catch and select those appropriate for analysis (section 3.3, below). Keep only organisms of target species and discard the remainder.

3.2.3. Measure the dimension of each organism to a precision of 0.5 cm; the relevant dimension for finfish is the maximum body length, which is the distance from the anterior-most part of the fish to the tip of the longest caudal fin.

3.2.4. Weigh each selected organism to a precision of 0.01 g. For small fish (length < 10 cm), the precision of these measurements must be increased to 0.2 cm and 0.001 g, respectively.

3.2.5. Record these measurements in the appropriate form.

3.2.6. Place all fish in plastic bags and into ice chests containing wet ice immediately after collection or measurement. The maximum holding time on ice is 24 hours. With small fish such as *Gambusia,* water loss or gain may affect the apparent weight. **Do not allow the fish to freeze prior to the next processing step**.

3.3. Initial Inspection and Sorting:

3.3.1. Select fish of the target species that do not have lacerated skin.

3.3.2. Rinse each individual fish of the selected target species in ambient water to remove any foreign material from the external surface.

3.3.3. If you are familiar with the physiology of the target species, you may evaluate the sex of the collected individuals and record it in the field sheet along with the other measurements (length and weight).

3.3.4. Place fish immediately in the ice chest if the sorting will be done later.

3.4. Tissue Extraction – Resections: If the sampling program requires that only the tissues be shipped to the laboratory, follow these procedures in a suitable location with a large stainless steel sink/counter and a source of tap water.

3.4.1. Place the organisms to be composited in the sink, rinse profusely with tap water and transfer to the holding tray.

3.4.2. Use one tray for each sampling site.

3.4.3. Cover a portion of the counter with the heavy-duty aluminum foil and place the sample container(s) on it.

3.4.4. Wear the latex gloves at this point. **The dissection is better performed when the sample is still cool; do not allow the fish to reach room temperature.**

3.4.5. Using the surgical knife, carefully open each fish, extract the target tissue (see below) and transfer it directly to the wide-mouth container.

3.4.6. If the tissue happens to fall in the counter, rinse it with analyte-free water before placing it in the container.

3.4.7. Rinse the surgical knife with tap and analyte-free water after each organism in a composite sample is processed.

3.4.8. Cap each sample container and write the field ID number on the label attached to it.

3.4.9. Use glass containers for all analytes (metals and extractable organics); use plastic containers if only metals will be analyzed.

3.4.10. After completing all dissections, place the labeled containers in the ice chest and prepare for shipping (see section 5.2, below).

3.4.11. Always use a new knife when dissecting a new fish of a discrete sample or the first fish of a composite sample.

3.4.12. Determine the sex and age of each specimen during this step and record on the field sheet, if it is necessary to confirm the presumed sex that was determined during the initial inspection and sorting.

3.5. Muscle Tissue Resections (Fillets): Depending on the study design, obtain only a portion of the muscle tissue, or obtain entire fillets from both sides of the fish, with or without the skin attached to the muscle tissue.

3.5.1. If the skin is to be included, place the fish in the lined counter top, wear the latex gloves, and carefully remove the scales from both sides using a blunt stainless steel knife-edge or a commercial de-scaler.

3.5.1.1. Be very careful not to puncture or rupture the skin of the fish.

3.5.1.2. Rinse the fish well with tap and analyte-free water and replace the aluminum foil liner of the counter top with a clean sheet.

3.5.1.3. Return the fish to the counter and proceed with filleting using the surgical knife.

3.5.1.4. For scaleless fish, remove the skin from each fillet after you have dissected the entire edible muscle tissue still attached to the skin.

3.5.1.5. Rinse each fillet with analyte-free water, shake the excess water, wrap each one tightly in aluminum foil and place inside a resealable plastic bag.

3.5.1.6. If the sample is a composite, place all fillets into one single bag. Prepare for shipping.

3.6. Muscle Tissue Resections (Sub-sample): If only a portion of the muscle tissue is to be used for metals analysis, follow these procedures.

3.6.1. Using the surgical knife make a 1½-inch incision along the dorsal edge of the fish (see diagrams in Figures FS 6200-1 and FS 6200-2).

3.6.2. Make two other incisions, perpendicular to the dorsal incision and forming a U-shaped cut, reaching over about 2/3 of the lateral ventral distance.

3.6.3. Extract only the scales necessary for the blade cuts.

3.6.4. Carefully separate the fish skin from the muscle tissue in the incised section.

3.6.5. Lift the skin, remove the portion of the underlying tissue, and rinse slightly with analyte-free water. This procedure removes any foreign material that may have contaminated the tissue from the skin surface.

3.6.6. Gently shake the excess analyte-free water from the tissue sample and place it on the Teflon cutting board.

3.6.7. Rinse the surgical knife with analyte-free water and cut the tissue into 0.5-inch strips in the cross-wise direction (across the muscle fibers).

3.6.8. Finally, insert the strips into the pre-weighed culture tube and cap it.

3.6.9. Prepare for shipping.

4. FIELD MEASUREMENTS AND RECORDS: All information associated with the sampling event must be recorded in the appropriate field sheets or field notebook using indelible pens. These records are the required minimum:

4.1. Names of field personnel, date, time, waterbody name and type (river, lake, estuary), tide cycle (if applicable), site location (latitude/longitude), and weather conditions.

4.2. Field measurements commonly associated with finfish sampling, e.g., depth, salinity, temperature, turbidity and pH of the water at the sampling site.

4.3. Size, sex and age of each individual organism in discrete or composite samples.

4.4. Number and size of organisms in each composite sample, and the collection method.

4.5. Any other relevant information if water chemistry samples are also collected.

5. SAMPLE PACKAGING AND SHIPPING

Prior to shipping samples, notify the analytical laboratory and confirm the amount of sample required for sample analysis. The maximum holding time on ice is 24 hours. Freeze the samples at -20°C if they will be held longer than 24 hours. Ship the samples using an overnight courier service. Use dry ice or wet ice for shipping. Frozen samples must arrive at the laboratory in the frozen state.

5.1. Whole Organisms:

5.1.1. Place all fish, pre-sorted or not, into plastic bags, tied with string, and inside the shipping cooler with plenty of wet ice.

5.1.2. Attach an ID label to the string of each plastic bag.

5.1.3. The volume of ice must be at least as large as the volume of the bagged samples.

5.1.4. Place all documentation (field sheets; COC, sample request and shipping forms) in a resealable plastic bag and attach it to the inside of the cooler's cover using the freezer tape.

5.1.5. Seal the cooler with reinforced tape and proceed with shipment.

5.2. Dissected Tissues:

5.2.1. Place each sample container inside a plastic bag and seal it.

5.2.2. Place all containers inside the shipping cooler.

5.2.3. Use enough wet ice so that samples arrive at the analyzing laboratory at less than 4°C or if shipped frozen maintain the frozen state.

5.2.4. The use of dry ice is optional if allowed by the shipping company.

5.2.5. Place all documentation (field sheets; COC, sample request and shipping forms) in a resealable plastic bag and attach it to the inside of the cooler's cover using the freezer tape.

5.2.6. Seal the cooler with reinforced tape and proceed with shipment.

Appendix FS 6200
Tables, Figures and Forms

TABLE FS 6200-1. SUMMARY OF FISH SAMPLING EQUIPMENT

Device	Use	Advantages	Disadvantages
ACTIVE METHODS			
Electrofishing	Shallow rivers, lakes and streams.	Most efficient nonselective method. Minimal damage to fish. Adaptable to a number of sampling conditions. Useful when other active methods cannot be used.	Nonselective (stuns or kills most fish). Cannot be used in brackish, salt, or extremely soft water. Requires extensive operator training. Can be DANGEROUS.
Seines	Shallow rivers, lakes and streams; or shore line of estuaries.	Relatively inexpensive and easily operated. Mesh size selection is available.	Cannot be used in deep water or over substrates with an irregular contour. Not completely efficient (fish can evade the net while seining).
Trawls	Used from boats in moderate to deep open bodies of water (10 to >70 m depths).	Various sizes available. Effective in deep waters not accessible by other methods. Collects large number of fish.	Requires boat and trained operators.
Angling	Generally species selective using hook and line.	Most selective method. Does not require large number of personnel. Inexpensive.	Inefficient and not always dependable.
PASSIVE METHODS			
Gill Nets	Used in lakes, rivers and estuaries; where fish movement can be expected or anticipated.	Effective for pelagic fish. Selectivity by mesh size. Relatively easy to operate.	Not effective for bottom dwellers or populations that do not exhibit movement patterns. Nets are prone to tangling or damage by fish spines. Will kill captured specimens that may undergo physiological changes if left unattended for extended periods of time.
Trammel Nets	Used in lakes, rivers and estuaries; where fish movement can be expected or anticipated. Used where fish may be scared into the net.	Slightly more efficient than a straight gill net.	(Same as gill nets.) Tangling problems may be more severe. Method may require more personnel to scare the fish, and possibly boats in deep water.
Hoop, Fyke and Pound Nets	Used in shallow rivers, lakes and estuaries, where currents are present and predictable.	Unattended operation. Very efficient in regard to long-term return and expended effort. Useful when active methods are impractical.	Inefficient for short term. Difficult to set up and maintain.
D-traps	Used for long-term capture of slow-moving fish, particularly bottom species. Can be used in all environments.	Easy to operate and set up. Particularly useful for bottom dwelling fish in deep waters. Relatively inexpensive.	Efficiency is highly variable. Not effective for pelagic fish or fish that are visually oriented. Not a good choice as a primary sampling device but valuable as a backup method.

Figure FS 6200-1
Dissection Guide for Largemouth Bass, Redear Sunfish and Bowfin

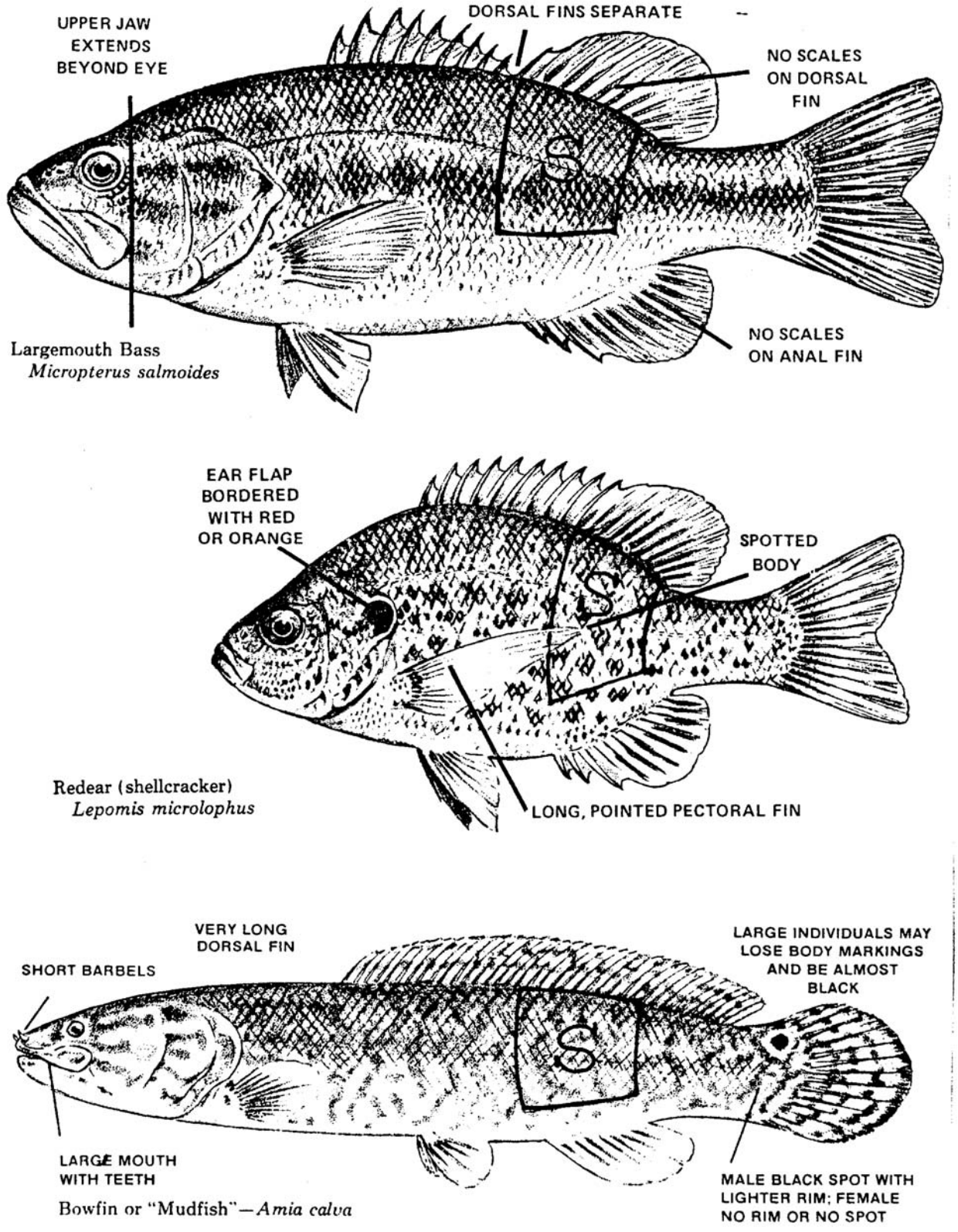
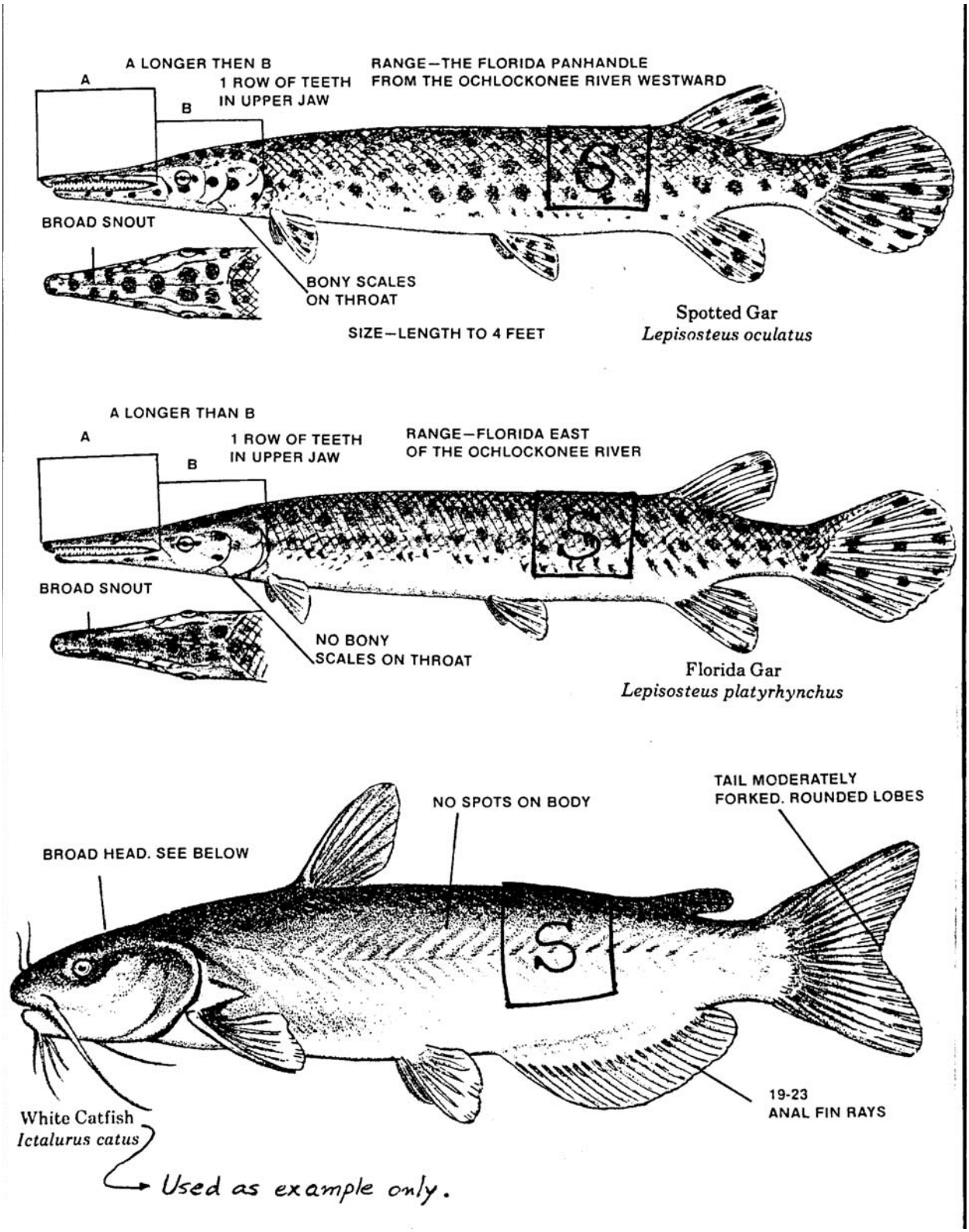


Figure FS 6200-1
Dissection Guide for Spotted Gar, Florida Gar and White Catfish



FS 6300. MISCELLANEOUS ANIMAL TISSUE SAMPLING (Reserved)

FS 6400. PLANT TISSUE SAMPLING (Reserved)