
Appendix M1

COLLECTION, HANDLING, AND PROCESSING OF TISSUE SAMPLES FOR CONTAMINANT ANALYSIS

This appendix defines the specific procedures that should be applied to the collection, handling, and shipment of tissues samples that will be analyzed to quantify the bioaccumulation of contaminants of concern.

I. Procedures for Bioaccumulation Studies

Analyses of animal tissues (finfish and macroinvertebrates) provide information on the bioavailability of contaminants in the sediment and water column, and also on the bioaccumulation of these contaminants in top predators. Since the target level for analytes of interest (i.e., mercury, methylmercury, pesticides, and other toxicants) are in the parts per billion (ppb) range, an important concern in sampling and processing tissues is avoiding sample contamination and degradation. This text defines standard operating procedures for the collection, handling, and processing of representative tissue samples for contaminant analysis.

Specific topics include:

- General tissue sampling and processing considerations;
- Guidelines for the collection and processing of aquatic organisms (i.e. prey fish, sunfish, largemouth bass, grass shrimp, and crayfish);
- Post-field procedures for aquatic organisms (i.e. prey fish, sunfish, largemouth bass, grass shrimp, and crayfish);
- Procedures to be used when transporting and shipping aquatic organism samples to the laboratory for analysis.

I.I General Tissue Sampling and Processing Considerations

Unless specifically exempted in research projects, the collection of fish or macroinvertebrates (shrimps, crabs, or crayfish) for tissue sampling purposes must satisfy legal requirements of harvest size or weight. If no legal harvest requirements are in effect, the animal should be of consumable size, at the minimum.

The processing of fish or macroinvertebrates for the analysis of mercury or other toxicants must be performed under conditions that prevent contamination or degradation of the analytes in question. Although not required, processing the animals to extract tissue for analysis should be carried out at the laboratory or at a field office with appropriate conditions for this work. In general, only the edible tissue should be submitted for analysis, except in cases when whole organism analysis is required. The tissue targeted for analysis is driven by the intended use of the data and is defined in the project DQOs.

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

1 for some programs. The use of composites, however, depends upon the DQOs of the program.
2 The FDEP (FS 6200) recommends compositing samples of fish fillets (edible portion) for
3 analysis of target analytes in screening studies.

4 For health assessments, a composite sample must represent the portion of the individual
5 organism that is commonly consumed by the population at risk. Florida fish consumption health
6 advisories for mercury are based upon 3-year old largemouth bass, because that is the most
7 common fish in creel census. In this case, the edible portions of individual fish must be analyzed.
8 “Skin-on fillets (with the belly flap included) are recommended for most scaled finfish. Skinless
9 fillets are recommended for scaleless finfish, such as catfish” (USEPA 2000).

10 To assess risks to other predators, analyze whole fish of the species within the size range
11 common to the predators’ diet. Depending upon the study design and DQOs, compositing may or
12 may not be appropriate in this case. Note that it may be difficult to obtain a representative aliquot
13 of homogenized whole fish in the laboratory.

14 **I.2 Guidelines for the Collection and Processing of Aquatic Organisms**

15 Guidelines for the collection and processing of fish samples can be found in the EPA document
16 *Guidance For Assessing Chemical Contaminant Data For Use In Fish Advisories*, EPA 823-B-
17 00-007, and in the FDEP SOP, DEP-SOP-001/01, FS 6000 and FS 6200. Instructions specific to
18 the mercury compliance monitoring are given below. Guidelines for the collection and
19 processing of macroinvertebrates can also be found in the DEP SOP, FS 6100. The procedures in
20 FS6100 for shellfish tissue sampling may be used for the collection of tissues from shrimp,
21 crabs, and crayfish.

22 Sample collection equipment construction materials must be consistent with the analytes of
23 interest (see QASR **Chapter 3, Tables 3.3 and 3.4**). The site locations will be identified and
24 characterized during the initial trip and recorded by differential Global Positioning Satellite
25 (GPS) using NAD83 datum for mapping, and by digital camera. A written site description should
26 accompany the photographs and site officially registered in the database.

27 Collect other matrices for laboratory analysis first, as well as any field measurements. The
28 equipment used for sample collection must be compatible with the plan of study, the
29 measurement program, and the site conditions.

30 Measure the dimensions of each organism to a precision of 0.5 cm; the relevant dimension for
31 finfish is the maximum body length, which is the distance from the anterior-most part of the fish
32 to the tip of the longest caudal fin. Weigh each selected organism to a precision of 0.01 g. For
33 small fish (length <10 cm), the precision of these measurements must be increased to 0.2 cm and
34 0.001 g, respectively. These measurements must be documented.

35 The following precautions should be taken: Make sure the collecting area is free of grease,
36 spilled engine fuel, engine exhaust, and dust. If the weight and dimensions will be measured in
37 the laboratory or field office, wrap each fish in aluminum foil, and place all fish in plastic bags
38 and into ice chests containing wet ice, soon after collection. With small fish, such as
39 *Gambusia sp.*, water loss or gain may affect the apparent weight. Do not allow the fish to freeze
40 prior to processing.

41 The following sections describe the methods to be used to collect prey fish, predator fish, grass
42 shrimp, and crayfish.

1 Collection of Prey Fish (*Gambusia* spp.)

2 Prey fish are collected from the dock or airboat using a long-handled dip net and/or seine net
3 with $\leq 3/16$ in. diameter mesh. Mosquitofish (*Gambusia* spp.) are the target species. Non-
4 mosquitofish are released in the field. The collector should visually identify captured fish to
5 ensure the collection of a minimum of 100 - 250 mosquitofish or the required volume; if after 90
6 minutes, 100 mosquitofish have not been collected, schedule a return trip to this location and
7 move on to the next station. The collected specimens, which should be free of mud or any other
8 clinging particulates, are placed in a labeled resealable bags containing in-situ water. Samples
9 are then placed on double-bagged ice and/or “Blue Ice” in a cooler for transport to the processing
10 lab. To reduce the chances of contamination, do not mix “Ultra trace Blue Ice” with “fish tissue
11 Blue Ice” to preserve samples. Upon returning to the lab, samples are placed in the refrigerator or
12 freezer until ready for processing (DO NOT FREEZE INDIVIDUAL SMALL FISH). Schedule
13 sampling and access to processing equipment well in advance so that samples are processed no
14 later than the day after collection. Prompt processing is required to avoid possible deterioration
15 or desiccation of samples. If samples are not processed within a day after collection, samples
16 should be processed as soon as possible. Fish should be in their in-situ water if frozen.

**17 Collection of Predator Fish (Largemouth Bass [*Micropterus salmoides*] and Sunfish [*Lepomis*
18 spp.]**

19 Predator fish, bass, and sunfish, are collected either by electroshocking or by hook and line. Fish
20 should be identified by species immediately after removal from the collection device, and fish
21 should be inspected and sorted, so that length and size requirements are met. Sex determination
22 and notations of morphological abnormalities are optional, unless specified as a DQO. Target
23 weight for bass is 0.5 – 1.0 kg (first to second year-class). The specimens are collected and then
24 placed in labeled resealable bags with in-situ water and put on ice. The bass are then individually
25 tagged. In the lab, the fish samples are weighed and measured for total length (TL) in
26 millimeters, and total weight (TW) in grams. Fish are weighed and measured prior to freezing to
27 eliminate bias due to shrinkage. Required number of replicates (typically 20 individuals of each
28 species) will depend on the DQOs for the project and may be found in the Monitoring Plan or the
29 specific SOP for the project. All relevant collection information should be recorded. Upon
30 returning to the processing lab, the samples are placed in the freezer until ready for processing.
31 Holding time should not exceed six months in a standard freezer to avoid desiccation.

32 Collection of Grass Shrimp (*Palaemonetes* spp.)

33 Grass shrimp (*Palaemonetes* spp.) should be put into labeled resealable plastic bags, placed on
34 ice, and transported to the laboratory for processing. At the lab, samples are immediately placed
35 in the refrigerator until ready for processing (DO NOT FREEZE INDIVIDUAL SHRIMP).
36 Schedule sampling and access to processing equipment well in advance so that samples are
37 processed no later than the day after collection. Prompt processing is required to avoid possible
38 deterioration or desiccation of samples. If samples are not processed within a day after
39 collection, samples should be processed as soon as possible. All actions that deviate from this
40 SOP should be documented in detail in the field and laboratory notebook. Deviations in SOP
41 must be approved by the CERP QAOT before implementation.

42 Collection of Crayfish (*Procambarus* spp.)

1 Crayfish (*Procambarus spp.*) are collected using crayfish traps. The traps can be used unbaited
2 or baited. Unbaited traps have been found to be as effective in catching crayfish as baited traps.
3 If baiting is desired, the traps can be baited with dry dog food or any cheap, readily available,
4 frozen baitfish. The traps should be settled on the bottom of the canal, in shallow water close to
5 shore, and in torpedo grass or other suitable cover. The traps should be checked at least twice per
6 week to avoid accidental death of the specimens. Only live crayfish are to be collected and
7 placed in labeled, resealable bags. The crayfish samples should then be placed in a cooler on ice.
8 Other animals found in the traps should be released unharmed. On the day of collection, place
9 the specimens in the freezer at the processing laboratory until ready for processing.

10 **II.I Post-Field Procedures for Aquatic Organisms**

11 **Post-Field Procedures for Prey Fish (*Gambusia spp.*) Samples**

- 12 1. Work only on clean glass or PTFE cutting boards, rinsing 3 times with DI water after
13 each use.
- 14 2. Remove specimens from refrigerator.
- 15 3. Put on disposable powder-free PVC gloves.
- 16 4. For each sampling location, separate prey fish by species (generally done in the field).
- 17 5. Record the number of individuals of each species collected, site name, collection date,
18 collector, and sample set I.D.
- 19 6. Rinse samples with DI water.
- 20 7. For each station, pool/composite all individual mosquitofish (*Gambusia spp.*) and
21 homogenize using a tissue homogenizer (e.g., Polytron or equivalent). Mercury
22 concentrations in these samples are expected to exceed ultra-trace levels. As a result,
23 processing does not have to be carried out in a mercury-clean lab. However, to control
24 cross-contamination, rinse all utensils and glass / PTFE cutting boards 3 times with DI
25 water after each use.
- 26 8. Split homogenized sample using stainless steel or Teflon coated spatula and transfer the
27 sample into a 20ml vial for shipment to the lab. Ensure that the weight of the vial
28 containing the sample is a minimum of 5 g (6 g is optimal) of composite that is required
29 for each of the five aliquots or subsamples. Short-term archive the remainder in a small
30 (2x3 in (5x8 cm)) resealable bag, then double bag. Place the short-term archive samples
31 in a rigid plastic container for freezing and include a permanent label.
- 32 9. Samples for analyses can now be frozen until shipment.

33 **Post-Field Procedures for Sunfish (*Lepomis spp.*) Samples**

- 34 1. Work only on clean glass/PTFE cutting boards, rinsing 3 times with DI water after each
35 use.
- 36 2. Remove specimen from the freezer and allow the specimen to thaw.
- 37 3. Put on disposable powder-free PVC gloves. Record (in notebook and on Field data log
38 sheet) the sample number of each specimen, along with, sex, site name, collection date,
39 and collector.

- 1 4. Rinse samples with DI water.
- 2 5. Homogenize whole sunfish (i.e., with stomach contents) in a two step process. First,
3 whole fish are ground using a commercial meat grinder or food processor with stainless
4 steel blades. Ground fish tissue is collected in a clean vessel (e.g., plastic bag, glass,
5 plastic vial bottle, and/or stainless steel) as it exits grinder. If the homogenization
6 process is incomplete (i.e., organs, skin or bone still visible), homogenize a second time
7 using a tissue Homogenizer (e.g., Polytron or equivalent) or food processor. (If grinder
8 or Polytron is not available, homogenize in food processor, manually cut up large
9 tissues and bones, re-homogenize in food processor).
- 10 6. To control cross-contamination, rinse all utensils, glass/PTFE cutting boards,
11 disassembled parts of grinders or food processors 3 times with DI water after each use.
- 12 7. Scoop homogenate out of container using a stainless steel or Teflon coated spatula and
13 transfer into a 2 x 3 in (5 x 8 cm) resealable bag for archiving, and a 20 mL vial for the
14 laboratory. Ensure equivalent consistency between splits. Each bag is then placed into a
15 second bag (i.e., double bagged) with a permanent label placed between bags.
- 16 8. The 20mL vial (weight should exceed 6 grams) is placed into a cardboard container and
17 frozen pending shipment to the analytical laboratory.
- 18 9. When shipping to a contract laboratory, samples are frozen overnight, packed in a
19 cooler with “Blue Ice” for shipment.
- 20 10. The larger portion of the sample is placed into another rigid plastic container
21 (i.e. Tupperware or equivalent) and archived on a short-term basis. For long-term
22 archiving, 10 – 20% of samples will be transferred to glass bottles with Teflon lined
23 closure.

24 **Post-Field Procedures for Largemouth Bass (*Micropterus salmoides*) Samples**

- 25 1. Work only on clean glass/PTFE cutting boards, rinsing 3 times with DI water after each
26 use.
- 27 2. Remove samples from the freezer and allow to thaw. Fish should be thawed only to the
28 point where it becomes possible to make an incision into the flesh.
- 29 3. Put on disposable powder-free PVC gloves.
- 30 4. Record (in notebook and on field data log sheet) the sample number of each specimen,
31 sex, site name, collection date, and collector.
- 32 5. Rinse samples with DI water.
- 33 6. Remove otoliths and place in labeled sealed bag, if aging will be performed.
- 34 7. Cut a fillet from each specimen, remove skin, and place fillet in a labeled resealable
35 freezer bag. A sub-sample of the fillet is collected by removing a strip of tissue from
36 the center of the fillet for analysis. Then place the remaining bagged fillet into a second
37 bag (i.e., double-bagged) with a permanent label placed between bags for archiving.
38 Place at least 6 grams of sample into the vial for laboratory analysis. Mercury
39 concentrations in these samples are expected to exceed ultra-trace levels; therefore,
40 processing does not have to be done in a mercury-clean laboratory. However, to control

- 1 cross-contamination, rinse all utensils and glass/PTFE cutting boards 3 times with DI
2 water after each sample.
- 3 8. Place processed samples in a freezer until ready for shipment. When using a contract
4 laboratory, samples are placed in a cardboard container and packed in a cooler with
5 “Blue Ice” for shipment to analytical lab.
- 6 9. Follow the same procedure for the other fillet for the purposes of archiving (i.e.,
7 double-bag). The archive sample is to be placed into a rigid plastic container (i.e.
8 Tupperware or equivalent) and archived on a short-term basis. For long-term archiving,
9 10 – 20% of samples will be transferred to glass bottles with Teflon lined closure.

10 **Post-Field Procedures for Grass Shrimp (*Palaemonetes* spp.)**

- 11 1. Work only on clean glass/PTFE cutting boards, 2 times rinsing with tap water and once
12 with Milli-Q water before and after each use.
- 13 2. Remove specimens from refrigerator.
- 14 3. Put on disposable powder-free PVC gloves.
- 15 4. Record the number of individuals collected, the total weight of pooled individuals, site
16 name, collection date, collector, and sample set I.D.
- 17 5. Rinse samples with Milli-Q water.
- 18 6. Composite (pool) all individuals from a given site and homogenize. Note, because
19 mercury concentrations in these samples are expected to exceed ultra-trace levels,
20 processing does not have to be done in a mercury-clean laboratory. However, to control
21 cross-contamination, rinse all utensils and glass /PTFE cutting boards 2 times with tap
22 water and once with Milli-Q water before and after each use.
- 23 7. Split homogenate and place one aliquot into a small-labeled Ziploc® freezer bag, the
24 other into a clean jar with Teflon closure. Sample in jar is to be archived.
- 25 8. Record weight of bag containing sample.
- 26 9. Samples for analyses can now be frozen; when ready, pack in a cooler and prepare for
27 shipment to analytical laboratory.

28 **Post-Field Procedures for Crayfish (*Procambarus* spp.)**

- 29 1. Work only on clean glass/PTFE cutting boards, rinsing 2 times with tap water and once
30 with Milli-Q water before and after each use.
- 31 2. Remove specimens from freezer and allow them to thaw.
- 32 3. Put on disposable powder-free PVC gloves.
- 33 4. Measure each specimen, distinguishing between carapace length and total length in
34 field notebook. Also record the weight of each specimen, along with collection site,
35 date, collector, and sample I.D.
- 36 5. Rinse each sample with Milli-Q Water.
- 37 6. Individually bag each specimen, with sample I.D. recorded on each bag. Specimens
38 collected at a particular site may occasionally be homogenized as a composite. In this

1 case, record the number of crayfish in each composite and total weight, as well as
2 individual sample information.

- 3 **7.** Samples for analyses can now be frozen; when ready, pack in a cooler and prepare for
4 shipment to analytical laboratory.

5 **III.I Transport and Shipping of Aquatic Organism Samples to the Analytical Laboratory**

6 The following procedure should be used when shipping aquatic organism samples to the
7 laboratory for analysis:

- 8 **1.** Freeze samples overnight (if permitted for the species).
9 **2.** Place samples in a sturdy cooler in good condition.
10 **3.** Tape the drain plug closed with fiber or duct tape.
11 **4.** Place "Blue Ice" (or ice that has been "double bagged" in heavy duty polyethylene bags
12 and properly sealed) on top of and/or between the samples.
13 **5.** Fill all remaining space between the bottles with bubble wrap or other packing material.
14 Place the COC record into a resealable plastic bag, and tape the bag to the inner side of
15 the cooler lid. Note: Field data log sheet for mosquitofish must indicate that the
16 analytical laboratory is to sub-sample homogenized composite five times and analyze
17 for THg.
18 **6.** Close the cooler and securely tape (preferably with fiber tape) the lid of the cooler shut.
19 **7.** Place a label containing the name and address of the shipper on the outside of the
20 container.
21 **8.** Attach a completed airbill (ship Priority Overnight, Weekday Delivery). The airbill
22 must be filled out, signed and kept on file by the sample collector to maintain chain-
23 of-custody.
24 **9.** If the samples are stored temporarily in the freezer prior to shipping, document this in
25 the logbook.
26 **10.** Notify analytical laboratory (contact by phone or email) of impending arrival of
27 samples.
28 **11.** The recipient of the samples at the analytical laboratory signs the COC, verifying
29 receipt in good condition, and faxes this back to the originator.
30