SOP #EH-07

Non-Lethal Fish Tissue Plug Collection

(*Adapted from ERT/REAC SOP*)

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TECHNICAL STANDARD OPERATING PROCEDURE
NON-LETHAL FISH TISSUE PLUG COLLECTION

1.0 SCOPE AND APPLICATION

Because fish spend their entire life in a particular waterbody they can be important indicators of water quality, especially toxic pollutants (e.g., pesticides and trace elements). Toxic pollutants which may be present in the water column or the sediments at concentrations below our analytical detection limits may be exhibited in fish tissue analysis due to bioaccumulation.

Typical whole fish or skin on fillet fish tissue collection methods require the fish to be sacrificed. This can be problematic when there is a need to collect large trophy sized fish for contaminant analysis or when a large sample size is necessary for statistical analysis. The following describes an alternative method for the collection of fish tissue samples which uses a tissue plug instead of a skin on fillet. This method is advantageous in that it eliminates the need to kill the fish to obtain a fish tissue sample for mercury analysis. Secondly, skin on fillet sampling required homogenizing of samples through a grinder. Although the grinder is cleaned between samples, the risk of sample contamination is a concern. The plug method uses clean equipment and supplies each time a sample is collected, thus reducing the risk of sample contamination.

2.0 METHOD SUMMARY

In general, a plug tissue sample is collected by inserting a biopsy punch into a de-scaled meaty section of a live fish. After plug collection, an antibiotic salve is placed over the wound and the fish is released.

3.0 SAMPLE PRESERVATION, HANDLING, AND STORAGE

Fish for heavy metal analysis that includes mercury should be placed in glass sample jars or double packaged in plastic bags. If the fish specimens are double packaged in plastic bags, they should first be wrapped in aluminum foil. Each sample should be correctly labeled and the label should be visible on the outside of the package.

Fish tissues that are being analyzed for contaminants should be kept on ice (32^{o}F or 0^{o}C) immediately after field processing and should be frozen as soon as possible. Once frozen, the samples should not be allowed to thaw and should be delivered to the analytical laboratory as soon as possible to meet analytical holding times. The laboratory should be contacted to verify sample holding times for parameters to be analyzed. A laboratory sample chain-of-custody form should be properly completed at the time of collection and kept with the sample cooler until delivery to the laboratory. Standard information includes contact information, sample identification, sample date and time, number of packages per sample, analyses required, and custody signatures.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

There are two primary interferences or potential problems with fish tissue sampling. These include cross-contamination of samples and improper sample collection.

- To avoid cross-contamination, equipment used for the removal of fish tissues should be properly decontaminated between samples, such as knives used to filet fish. Other equipment, such as a biopsy punch, should be disposed of after each use. Once processed, fish specimens will either be stored in a glass jar when small enough or wrapped in aluminum foil and placed within double ziploc bags.

- Improper sample collection can involve direct contact of a captured fish with stream sediment, shoreline soil, boat floor, or other potential sources of chemical contamination (e.g. as may happen when a live fish is dropped during handling). Fish with foreign debris on the body should be adequately rinsed with site water before plug or filet samples are collected.

One or more of the sampling team members should have experience with the following aspects of fish tissue plug sampling:

- ✓ Safety issues associated with working near water at the study area stations.
- ✓ Fish species identification.
- ✓ Prior experience with fish sampling techniques described above.

5.0 EQUIPMENT/APPARATUS

- Glass cutting board.
- Fish measuring board.
- Fish weigh scale.
- Plastic bags.
- Sterile 20mL glass scintillation vials, ultra clean.
- Coolers with ice or frozen gel packs.
- Field Data and Sample Log forms.
- Sample labels.
- Latex gloves.
- 8 millimeter disposable biopsy punch (Acuderm brand Acu-Punch or equivalent).
- Decontaminated scalpel for scale removal
- Laboratory pipette bulb.
- Antibiotic salve.
- Pen.

6.0 REAGENTS

- 10 percent nitric acid rinse made from ultra-pure certified trace-metal grade concentrated nitric acid.
- 70 percent ethanol
- Laboratory-grade deionized water (for rinsing cutting equipment and board between samples)

7.0 PROCEDURES

1. Fish should be collected, held, and processed for physical measurements following guidelines provided in SOP #EH-07. Fish selected for tissue plug sampling should include up to five fish per species of similar size ranges. As a general guideline, the largest and smallest fish within each group should not exceed the average length of the group by more than 25%.

2. On left side dorsal area of fish, clear a small area of scales with a decontaminated scalpel.

3. Wearing clean double latex gloves, insert the 8 millimeter biopsy punch into the fish through the scale free area. The punch is inserted with a slight twisting motion cutting the skin and muscle tissue. Once full depth of punch is achieved a slight bending or tilting of the punch is needed to break off the end of the sample. Remove the biopsy punch taking care to ensure the sample remains in the punch. ***Note: The sample should result in a minimum of a 0.5 to 0.7 grams of fish tissue for mercury analysis.***

4. Apply a generous amount of antibiotic salve to the plug area and gently return the fish to the water.

5. Using a laboratory pipette bulb placed on the end of the biopsy punch, give a quick squeeze, blowing the tissue sample into a sterile 20 milliliter scintillation vial.

6. Dispose of gloves and biopsy punch.

7. Label the vial with the appropriate sample ID.

8. Immediately place vial in a plastic ziploc bag and put the bag and its contents in a cooler on ice or gel packs.

9. Fill out the appropriate Sample Identification, Custody, and Record Forms.

10. Place samples in a freezer within 48 hours to await analysis.

8.0 CALCULATIONS

This Section is not applicable to this SOP.

9.0 QUALITY ASSURANCE/QUALITY CONTROL

The following QA/QC procedures apply to fish collection and field processing:

a. All data will be documented on field data sheets or in logbooks. Photo documentation will be done when possible.

b. A sample plan, including numbers, target species, and sample size will be prepared before field work begins.

c. A field form will be developed which details the steps in the equipment decontamination process so that steps can be checked on the form as completed.

d. A low-level field check sample (designed to detect sample handling contamination) will be opened in the field, with the handling procedures mirroring the field sample collection method (e.g., place on cutting board, cut, put in new sample container, and submit to in-house lab for analysis). The field check sample matrix will be muscle tissue with a known mercury concentration, supplied by the National Institute of Standards and Technology.

10.0 DATA VALIDATION

Data generated will be reviewed according to the Quality Assurance Project Plan (QAPP).

11.0 HEALTH AND SAFETY

All sampling teams will perform sample collection in accordance with the health and safety requirements of their parent organization.

Any time fish are collected, water and boat safety precautions must be taken. Wading can be dangerous, especially in swift currents or if the bottom is uneven or algae-covered. Samplers should always work in pairs, and wader belts should be worn to prevent waders filling with water if fall occurs.

Many fish species have sharp fin rays, spines, and teeth, and may quickly cause lacerations or puncture wounds. Handle all fish with appropriate caution, and wear gloves or use pliers when necessary. A first aid kit should be kept at the job site.