FISH HANDLING AND PROCESSING

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1.0 SCOPE AND DESCRIPTION

This standard operating procedure (SOP) describes the basic procedures for field processing of fish collected at hazardous waste sites. Fish can be used to determine whether contaminants in aquatic habitats accumulate in fish tissue, cause histopathological damage, or affect fish condition or growth. Impacts on aquatic community structure can also be assessed.

2.0 METHOD SUMMARY

Specific procedures used to process fish will depend on the project objectives. Regardless of the objectives, data that should always be collected on fish in the field include length, weight, species, and information on parasites or other abnormalities. Scales, otoliths, or fin rays should be collected for aging fish. When possible, sex and stage of maturity should also be noted.

Fish that are collected for contaminant analysis should be measured, then filleted or frozen whole. If study objectives include histopathology, fish should be dissected so sections of target tissues can be collected.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

If tissues are being analyzed for contaminants, fish should be kept on dry ice after processing. Fish for heavy metal analysis may be placed directly into plastic bags. If fish are going to be analyzed for organic compounds, they should be wrapped in aluminum foil and then placed into plastic bags.

Fish collected for population studies can be preserved in ethanol or 10 percent formalin. If otoliths are going to be collected to age fish, fish should not be preserved in formalin until the otoliths have been collected ,as formalin will decalcify the bones. Specimens should be stored in glass jars or plastic buckets. Small fish can be preserved by simply placing them in ethanol or formalin. When preserving large fish, a slit should be made along the belly on the right side of the midline. Incisions should also be made in the dorsal muscle mass, on either side of the vertebral column. For proper preservation, the specimen volume should be no more than 50 percent of the total volume occupied by specimen and preservative.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

4.1 Length

Factors, which contribute to length measurement errors, are muscular tension in live fish, eroded fins, shrinkage of fish due to preservation, and failure to consistently squeeze the tail to get maximum total length.

4.2 Weight

When taking weights, an attempt should be made to have fish at a standard degree of wetness. Variation in stomach contents or amount of water swallowed at capture will also affect fish weights. Other sources of error include movement of the scale due to fish movements, wind or boat motion.

4.3 Aging

Because scales initially appear on different body parts at different times, scales collected to age fish must be removed from the same location. Scales should not be removed from areas likely to shed scales or where there are irregularly shaped scales.

Otoliths are brittle cna crack easily. Because there are only two large otoliths per fish, care should be taken not to damage them when removing them. Field crews should practice locating otoliths on target species before the fieldwork begins.

4.4 General

Extreme temperatures can alter tissue characteristics, making tissues unsuitable for analysis. Exposure of dead specimens to extreme cold can cause tissue to freeze, making histopathological analysis difficult. Extreme heat can cause rapid decomposition of tissue. An effort should be made to keep fish alive until they are processed. Dead animals should be processed as soon as possible.

All members of the processing staff should be trained in techniques used to make length and weight measurements. Inconsistencies in the way these measurements are taken can lead to errors. Aging fish using scales or otoliths should be done by one person if possible. Meaningful age estimates can only be obtained if hard parts are read in a consistent manner.

In some cases, fish collected may not have sufficient body mass for analysis of a containment to a given detection limit. If this occurs, then individuals of the same species from the same sampling location may be pooled for analysis. If multiple analyses of contaminants in tissues are being done, these may need to be prioritized if body mass of the specimens is insufficient to conduct all of the analysis. Analyses to be conducted on each specimen should be carefully documented.

5.0 EQUIPMENT/APPARATUS

Equipment needed for processing fish is listed below:

Processing Fish

Data Sheets	Measuring board
Balance or scale	Field guides or keys
Coin envelopes	Knife
Forceps	Saw
Probe	Pliers
Ziploc bags	Aluminum foil
Large scissors	Small scissors
Dissecting microscope	Glass scintillation vials with lids
Glass jars with lids	Preservative
Scalpel	Fillet knives
Knife sharpener	Dissecting trays

6.0 REAGENTS

No reagents are needed for fish processing if fish are being collected for residue analysis. Tissue sections

collected for histopathological analysis should be preserved in glass scintillation vials filled with 4 percent buffered paraformaldehyde. Buffered paraformaldehyde can be purchased through commercial chemical supply companies. Tissue sections for histopathology should be collected before fish are frozen. Fish being collected for population studies can be preserved in either 70 percent ethanol or 10 percent formalin.

7.0 PROCEDURES

When fish are collected for residue analysis, generally the largest fish captured are the ones, which should be analyzed. All animals captured should be held until a sufficient number and weight of fish are caught either at a station, or until the end of the day. If necessary, fish should be marked or tagged as they are captured so that individual fish can be identified later. Length, weight and species should be determined at the time a fish is tagged. Other data can be collected after fish that will be analyzed have been selected.

A data sheet should be completed for each specimen processed. Sampling location, tag number, date, species, and data on the specimen metrics described below should be recorded.

7.1 Length

Fish length is measured using a measuring board on which the anterior end of a fish is placed against a stop at the beginning of a measuring scale. The fish should be measured with one mouth closed, and the body positioned on its right side with the head to the measurers left. Any one of three measurements can be taken: total, fork or standard length (Figure 1, Appendix A). Total length is the greatest length of a fish from its anterior most extremity (usually the mouth) to the end of the tail fin. For fish with a forked tail, the two lobes should be pressed together, and the length of the longest lobe should be taken. Fork length is measured from the anterior end of the fish to the tip of the middle rays of the tail. Standard length is the length of a fish from the anterior end of the fish to the tip of the middle rays of the tail. Standard length is the length of a fish from the anterior end to where the base of the median tail fin rays joins the caudal peduncle. This spot can be located by bending the tail sharply. A crease should form where the tail fin rays end. Total length or fork length measurements are used most often. Determination of standard length is very difficult on some species.

7.2 Weight

Spring balances or electronic digital scales are generally used to weigh individual fish. Fish can be weighed by themselves, or by placing them in a container of water. Taking the weight in water reduces error due to fish movement, but may not be practicable for large fish. Large numbers of fish can be weighed in bulk if individual weights are not needed (e.g., for population studies).

Because most fish maintain near-neutral buoyancy in water, their specific gravity is close to 1.0 and body volume is proportional to weight. Therefore, the amount of water displaced in a container can also be used to determine weight.

7.3 Species Identification

Study objectives will dictate what level of identification is needed for a fish. Fish collected for residue analysis should be identified to species, as different genera may have different feeding habits.

Local authorities should be consulted before fieldwork begins to determine whether regional taxonomic references exist.

7.4 Aging

Hard parts collected for aging can be scales, otoliths, spines or fin rays. Prior to field work, a literature search should be conducted to determine the best method for aging a particular target species.

Scales are the easiest hard structure to collect, but only scales from a particular area on a fish can be used for aging (Figure 2, Appendix A). Lateral line scales should not be collected. Before collection, gently scrape the target area to remove mucus, dirt and epidermis. Remove scales by scraping towards the head, or by firmly pressing a knife point on a scale and pushing towards the tail. Place the scales in a small, labeled coin envelope.

Otoliths are a more accurate method of determining age, but collection and reading requires more skill and time than scales. To collect otoliths, grasp the head firmly and cut the top of the skull slightly behind the eyes back to the upper edge of the gill cover (Figure 3, Appendix A). If the cut is made correctly, the large sacculus otoliths should be exposed behind the brain. If not, carefully probe around the area until the otolith is located. Gently remove both large otoliths with forceps, clean them, and store dry in a coin envelope.

7.5 External Examination

While processing fish, note any external abnormalities or parasites on data sheets or in field logbooks. Information on sex and stage of maturity should also be noted. If fish are collected during spawning season, some fish can be sexed based on breeding colors. Mature fish may release eggs or milt when they are handled.

7.6 Final Processing

To assess environmental risk through food chain concentration of contaminants, the whole body should be analyzed for tissue residue. Based on the objectives of the study, the stomach contents of the fish may be removed (using dissection technique) prior to analysis. Alternately, fish may be held in aerated chambers for 24 hours to depurate stomach contents. This will allow for a determination of the concentration of contaminants accumulated in the tissue versus contaminants entrained in the gut.

To assess risk to humans from fish consumption, the fish should be filleted and only muscle tissue sent to the laboratory for analysis. Fish should be dissected if tissues are being collected for histopathology or for residue analysis on specific organs.

Procedures for filleting or dissecting a fish are described below. Fish should be killed by a blow to the head immediately before processing.

7.6.1 Filleting

To fillet a fish, an initial cut should be made from the dorsal fin to the pelvic fin, just behind the opercular flap. Run the tip of the knife along the dorsal side of the fish, from the initial cut to the caudal fin. Continue making successively deeper cuts, running the knife blade as close to the neural spines and ribs as possible. After the fillet is obtained, remove the skin. Place the skin side of the fillet down on the dissecting tray, hold on to the tail portion of the fillet, and run the knife between the skin and the muscle tissue. Turn the fish over and repeat the process to obtain the other fillet.

7.6.2 Dissecting

Begin the dissection by laying the fish on its right side and making an incision from just above the vent to the top of the rib cage. Cut along the rib cage, forward through the pectoral girdle. Make a shallow incision to avoid damage to internal organs. Pull the flap downward to open the body cavity. Note any gross abnormalities or parasites observed in the body cavity. Also record sex and stage of maturity.

Liver, gill and kidney tissues are the fish tissues collected most often for histopathology or residue analysis. The liver should be located near the anterior end of the stomach. It is connected to the gut by the gall bladder and bile duct. The liver should be removed and weighed to the nearest 0.001 g. A hepatosomatic index, liver weight expressed as a percentage of body weight, can be used as an indicator of fish condition. For histopathology, two tissue sections should be obtained from the distal end of the medial lobe. The sections should be cut 1.0 centimeter (cm) towards the center of the lobe, and 0.5 cm thick. Cut the section using a scalpel, and handle carefully to avoid crushing the tissue. Place the tissue sections in a glass scintillation vial filled with 4 percent buffered paraformaldehyde.

The gills are located beneath the opercular flap. Pull back or remove the operculum to expose the gills. Carefully remove a section of gill tissue, taking care not to crush it. Place the gill tissue in the scintillation vial with the liver tissue.

The kidney is located along the backbone above the gas bladder. Kidney tissue is difficult to remove from fish because it adheres to the body wall and is soft. Thin slices can be taken through the vertebral columns that include the kidney. These tissue sections should be preserved with the liver and gill tissue sections. Again, for proper preservation, the specimen volume should be no more than 50 percent of the total volume occupied by specimen and preservative.

Unless specific organs are being analyzed for residues, place all tissues back in the body cavity and wrap the fish in plastic or aluminum foil. Samples should be labeled and shipped following ERTC/REAC SOP #2002. *Sample Documentation* and ERTC/REAC SOP #2004, *Sample Packaging and Shipping.*

8.0 CALCULATIONS

No calculations are needed for the above procedures.

9.0 QUALITY ASSURANCE/QUALITY CONTROL

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

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

2) Samples will be duplicated in an unimpacted reference area.

3) A quality assurance work plan (QAWP) will be prepared prior to field work, which specifies the methods, target species, and sample size.

4) All deliverables will be peer-reviewed prior to release.

10.0 DATA VALIDATION

Data generated will be reviewed according to the QA/QC considerations listed in Section 9.0

If possible, species identifications will be confirmed by a regional biologist familiar with the site's aquatic fauna.

11.0 HEALTH AND SAFETY

A site-specific Health and Safety plan will be prepared prior to any field activity, and must be approved by the REAC Health and Safety officer. All members of field crews should be trained in CPR.

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 falls occur. Boating safety guidelines should be followed for activities that require transportation by boat.

12.0 REFERENCES

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APPENDIX A
Figures
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FIGURE 1. Measurements of Fish Length - Standard, Fork, and Total
(From Anderson and Gutreuter 1983)

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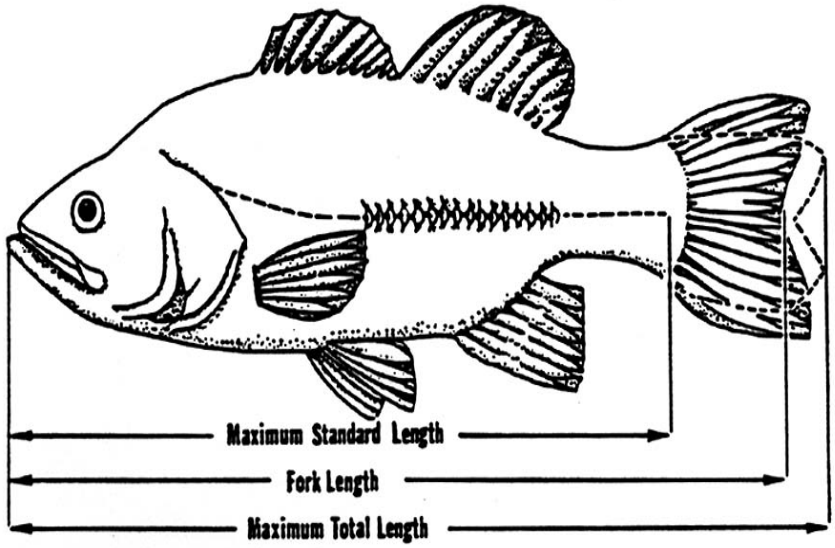

FIGURE 2. Location of Scales for Aging

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(From Jearld 1983)

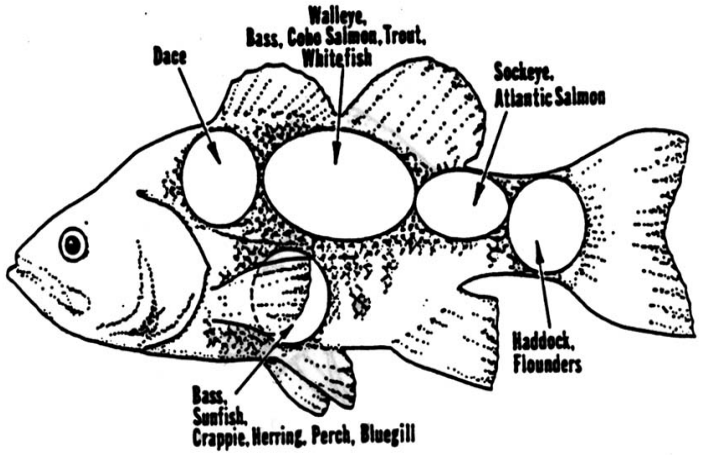

FIGURE 3. Method for Removing Otoliths
(From Jearld 1983)

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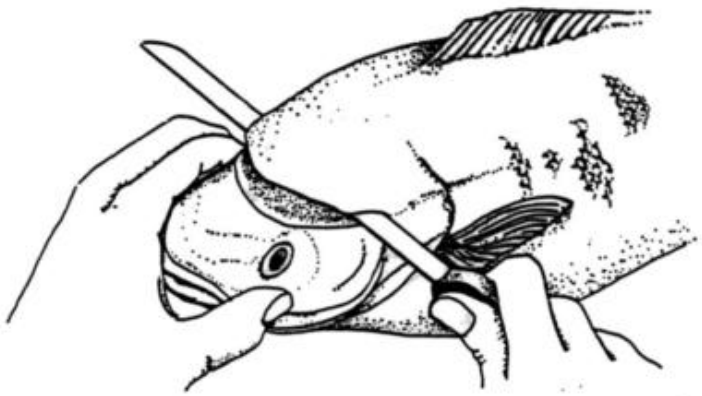

FIGURE 4. Location of Cuts for Filleting a Fish

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FIGURE 4. Location of Cuts for Filleting a Fish