DEPARTMENT OF ECOLOGY
State of Washington

Standard Operating Procedure EAP007, Version 1.3

Resecting Finfish Whole Body, Body Parts, or Tissue Samples

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Purpose of this Document

The Washington State Department of Ecology develops Standard Operating Procedures (SOPs) to document agency practices related to sampling, field and laboratory analysis, and other aspects of the agency's technical operations.

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SIGNATURES AVAILABLE UPON REQUEST

Please note that the Washington State Department of Ecology's Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.

Although Ecology follows the SOP in most instances, there may be instances in which the Ecology uses an alternative methodology, procedure, or process.

SOP Revision History

Revision Date	Revision History	Summary of changes	Sections	Reviser(s)
08/30/06	V1.0	SOP Publication		
10/19/10	V1.0	Three year review		
10/21/10	V1.0	QA approval, recertified		
03/21/14	V1.0	Three year review		
04/21/14	V1.1	QA approval, recertified		
04/20/17	V1.2	Three year review, minor changes	all	Sandvik, Sargeant
5/3/2017	V1.2	Recertification, Cover page and footer	all	Kammin
4/29/2019	V1.2	updated cover page and recert. page	cover, p. 1	Ponzetti
10/15/21	1.3	Revised in new template. Updated and added accessibility.	all	Sandvik, Kaza

1.0 Purpose and Scope

1.1 This document is the Environmental Assessment Program (EAP) Toxics Study Unit (TSU) Standard Operating Procedure (SOP) for resecting finfish tissue samples.

1.2 Washington Department of Ecology (Ecology) investigates the occurrence and concentrations of toxic contaminants in fish. This SOP is intended to provide consistent techniques that ensure the quality of tissue preparation (including whole finfish or other body parts) for the purpose of homogenizing samples for chemical analysis by an accredited analytical laboratory. Procedures for this SOP were adapted from the Environmental Protection Agency's (EPA) Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1 Fish Sampling and Analysis. Third Edition (2000).

2.0 Applicability

2.1 This procedure is for Ecology person(s) conducting finfish processing in part or as a whole.

3.0 Definitions

3.1 Analyte – The substance or chemical constituent that is undergoing analysis. It is the substance being measured in an analytical procedure.

3.2 Caudal fin – The tail of fishes.

3.3 Composite – Composite samples are homogeneous mixtures of equal amounts and types of tissue from two or more individual organisms of the same species collected at a particular site and analyzed as a single sample.

3.4 Ecology – Washington State Department of Ecology.

3.5 DOH – Department of Health.

3.6 Dorsal fin – The main fin located on the back of fishes.

3.7 EAP – Environmental Assessment Program.

3.8 Lab Analysis & Tracking Plan – A table, usually created in Excel®, used to plan and document lab analyses of samples for single or multiple projects (Attachment 1).

3.9 LAR – Laboratory Analysis Required form.

3.10 MEL – Manchester Environmental Laboratories.

3.11 Operculum – Any one of the bony plates, which support the gill covers of fishes.

3.12 OSWER – U.S. Environmental Protection Agency Office of Solid Waste and Emergency Response.

3.13 Otolith – One of many minute calcareous particles found in the inner ear of vertebrates. Fish species have three pairs of otoliths. The largest pair is used for aging.

3.14 PBDEs – Polybrominated diphenyl ethers.

3.15 PCBs – Polychlorinated biphenyls.

3.16 PCDDs – Polychlorinated dibenzo-p-dioxins.

3.17 PCDFs – Polychlorinated dibenzofurans.

3.18 Pectoral fin – Either of the anterior pair of fins attached to the pectoral (mid) girdle of fishes.

3.19 Processing Benchsheet – A table, usually created in Excel®, used to plan and document sample processing data for each fish collected (Attachment 2).

3.20 QAPP – Quality Assurance Project Plan.

3.21 QC – Quality control.

3.22 SDS – Safety Data Sheets provide both workers and emergency personnel with the proper procedures for handling or working with a particular substance. SDS include information such as physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment and spill/leak procedures.

3.23 Supplemental Benchsheet for processing fillet and carcass tissue – A table, usually created in Excel®, for documentation of fish and parts of fish at different stages of processing. Data are used in mass balance equation to estimate contaminant concentrations in tissue that cannot be analyzed (Attachment 3).

3.24 Resecting – Surgical removal of all or part of an organ, tissue or structure.

3.25 U.S. EPA – United States Environmental Protection Agency.

3.26 WDFW – Washington Department of Fish and Wildlife.

4.0 Personnel Qualifications/Responsibilities

4.1 Because this procedure requires use of hazardous materials, training is required as per the Ecology Chemical Hygiene Plan and Hazardous Materials Management Plan (Section 1) (Ecology, 2019), which includes a Laboratory Safety Orientation, Job-Specific Orientation and must know Chemical Safety Procedures and follow the Standard Operating Procedures (Section 16).

5.0 Equipment, Reagents, and Supplies

5.1 Deionized (DI) water and Nalgene wash squeeze bottles.

5.2 Forceps – Fine point, flat and rounded.

5.3 Stainless steel fillet knives with 6-8 inch stainless steel blade.

5.4 Cleaver, hacksaw and blades.

5.5 Pliers (needle nose and regular) for pulling skin off. Wire cutters for cutting through bone and cartilage.

5.6 Surgical scalpels and box of new blades (stainless steel).

5.7 Fume hood.

5.8 Personal protection gear.

5.8.1 Talc-free nitrile exam gloves.

5.8.2 Gloves for solvents (see *Chemical Hygiene Plan and Hazardous Materials Management Plan* (Section 6) (Ecology, 2019).

5.8.3 Eye protection.

5.8.4 Full body apron.

5.8.5 Facial shield.

5.9 Sample jars – Short jars with Teflon lids (ICHEM certified 300 series, references for container cleaning procedures can be found in U.S. EPA OSWER directive 9240.0-05, *Specifications and Guidance for Obtaining Contaminant-Free Containers*, April 1992) (Figure 1). Determine the sizes of jars needed, based on the amount of tissue and analyses being performed, by using EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories* (U.S. EPA, 2000), Manchester Environmental Laboratory (MEL) contacts and MEL's *Manchester Environmental Laboratory Lab Users Manual* (MEL, 2016). The 4 oz (item#320-0125) and 2 oz (item#220-0060) jars are currently the predominant choices. See full instructions for requesting sample containers from MEL's *Manchester Environmental Laboratory Lab Users Manual*, and order based on the analytical plan prior to initial field sampling.

5.10 Sample jars for PFAS -- CapSure Oblong HDPE WM (Figure 2). Do not use sample containers with Teflon due to possible contamination of PFAS.

5.11 Create and print custom labels, or use labels provided with sample jars.

5.12 Heavy-duty aluminum foil (Reynolds Foodservice Foil 45.7cm x 152.4mm (624) and 38.1cm x 152.4mm (622)).

5.13 Sponges and scrub brushes.

5.14 Decontaminating fluids.

5.14.1 10 % nitric acid. See the TCT SharePoint Site (Toxics Documents) for SDS and concentration grade before dilution. Dilute to 10% with DI water. Note – use 5% nitric acid dilution on the Hobart grinder's tin plated finish grinder parts. If sampling for metals and plan to use Hobart grinder for tissue sample preparation, consider including equipment blank analysis to account for potential metals introduced from tin plated grinder parts.

5.14.2 Acetone (solvent) ACS, HPLC Grade, 99.9% min. See the TCT SharePoint Site (Toxics Documents) for SDS. See Section 4.1 of this document for safety requirements.

5.14.3 Hexane (solvent) ACS HPLC Grade, 99.9% min. See the TCT SharePoint Site (Toxics Documents) for SDS. See Section 4.1 of this document for safety requirements.

5.14.4 Liqui-Nox (biodegradable, phosphate-free, interfering-residue free, concentrated soap from Valconox, Inc.).

5.15 Small and large stainless mixing bowls (approximately 3 quarts and 13 quarts).

5.16 Medium and large stainless mixing spoons (approximately 8 inch and 15 inch).

5.17 Paper towels.

5.18 Garbage bags.

5.19 Zip seal bags – Pint, quart and gallon sizes.

5.20 Blue painter's masking tape.

5.21 Pens, pencils, pencil sharpener, permanent markers.

5.22 Kitchen Aid mixer.

5.23 Kitchen Aid grinding units (Ecology currently using model #K45SS) (Figure 3).

5.24 Hobart grinders (Ecology currently uses model #4812 and #4732A) (Figure 4).

5.25 Bench scale including spare 9-volt battery, standard weights and standards logbook for pre and post accuracy checks (Figure 5).

5.26 Fish Measuring Board (Figure 6).

5.27 Magnifier (Ecology currently using an arm-mounted magnifier).

5.28 Polypropylene cutting board.

5.29 Sink and drain board.

Figure 1. Illustration of 8 oz, 4 oz and 2 oz jars (MEL, 2016).

Figure 2. PFAS sample container; CapSure oblong HDPE WM.

Figure 3. Kitchen Aid mixer with grinding unit

Figure 4. Hobart grinding units (small unit #4812, large unit #4732A)..


Figure 5. Scale log book, standard weights, spare 9-volt battery, and bench scale.

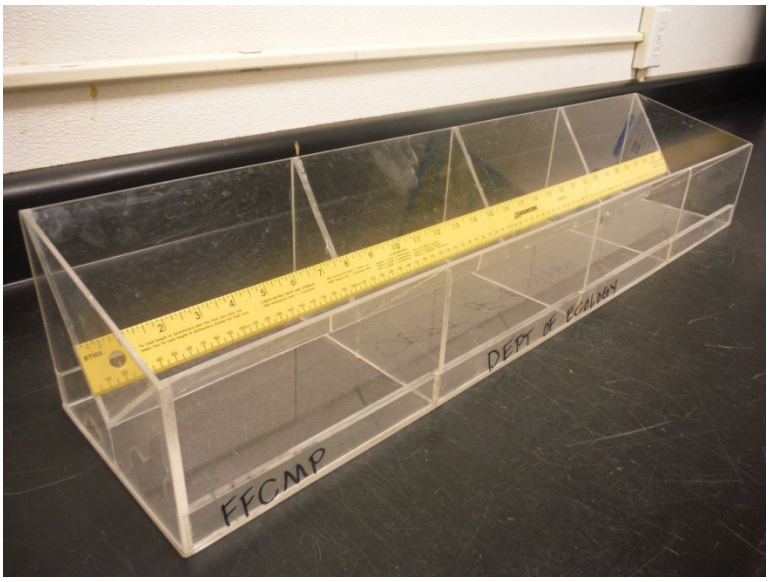


Figure 6. Fish measuring board

6.0 Summary of Procedure

6.1 Project officers make decisions about how to process each fish based on study objectives and the fish samples collected. Such decisions include whether to analyze individual fish or combine individuals to form composite samples, the number of fish used per composite sample, the size range of fish to be processed – either as individuals or as composite samples, whether the fish will be processed with or without the skin, and other related decisions. The Lab Analysis & Tracking Plan and Processing Benchsheet reflects such decisions.

6.2 Fish Processing Preparation.

6.2.1 Formulate processing plan: timing, location, staff resources, and equipment.

6.2.2 Decide which jar sizes to order from Manchester Laboratory before scheduling processing. See more comments for jar size and ordering under "Equipment, Reagents, and Supplies" section of this document. The general rule of thumb is to use the small 2 oz jar for mercury samples only, the 4 oz jar for the remaining tissue analysis, or special containers for PFAS analyses.

6.2.3 Determine how number of jars needed per process by consulting the Lab Analysis & Tracking Plan and counting one jar per type of analysis taken, plus one extra jar for archive samples. For example, if testing for pesticides (Pest), polychlorinated biphenyls (PCB), polybrominated diphenyl ether (PBDE), lipids and mercury (Hg), then two 4 oz jars and one 2 oz jar would be needed; a 4 oz jar each for "Pest PCB PBDE lipids" and "Archive," and a 2 oz jar for "Hg." Other combinations are possible.

6.2.4 Prepare and print out a Lab Analysis & Tracking Plan spreadsheet for samples to be processed. See Section 7.1 and Attachment 1 of this document for instructions and an example of the Lab Analysis & Tracking Plan.

6.2.5 Prepare and print out a Processing Benchsheet to record fish tissue processing data. See Section 7.2 and Attachment 2 of this document for instructions and an example of the Processing Benchsheet.

6.2.6 Prepare and set up lab.

6.2.6.1 Make sure plenty of DI water is available and that the solvent/acid squeeze bottles are full. Check the main supply of acid and solvent solutions to make sure there are adequate amounts stocked for the processing season. There should also be a rinsate container (stainless steel bowl) and a funnel in the fume hood for solvent rinsing. Keep solvent and acids stored in proper lab cabinets under the counter until ready for use. See Ecology's Chemical Hygiene Plan and Hazardous Materials Management Plan Section 16 Standard Operating Procedures (Ecology, 2019) for acid dilution and solvent handling procedures.

6.2.6.2 Put on protective equipment (apron, gloves, eye protection). Adhere to the safety procedures and training outlined in Section 4.1 of this document.

6.2.6.3 Clean lab space including dissecting areas, fume hood, equipment shelves, and floor (dust, sweep and wipe surfaces with damp cloth or sponge).

6.2.6.4 Decontaminate utensils for each individual fish and/or composite group before you begin to avoid sample-to-sample contamination. Refer to Ecology's Chemical Hygiene Plan and Hazardous Materials Management Plan Section 16 Standard Operating Procedures for decontamination procedures (Ecology, 2019). Decontaminated equipment needed for individual and composite samples include a fillet knife, scalpel, 3-quart SS bowl and large (approximately 8 inch) mixing spoon, and all the components of the Kitchen Aid grinding unit. Decontaminated equipment needed for individual and composite whole and carcass samples include knives, all the components of the Hobart grinding unit (5% nitric acid dilution should be used on Hobart grinder's tin plated finish grinder parts), a 13 quart SS bowl and an extra-large (approximately 15 inch) mixing spoon. Include any equipment that will come in contact with the sample.

6.2.6.5 Pull out the fish samples for processing per composite or group, to allow for thawing. Judge how long to thaw by the sizes of the specimens, by their field weight and length, or by visual inspection. Ideally, fillet fish while ice crystals are still present in the muscle tissue, just thawing them enough to insert a knife for filleting. Record the process date on the Lab Analysis & Tracking Plan spreadsheet and the Processing Benchsheet. Include names or initials of processing crew on the Processing Benchsheet.

6.2.6.6 Set up counter work area for ample space between resection processes. Five general stations are recommended: 1) desliming, descaling and rinsing in sink, 2) DNA, aging structures, and sexing, 3) filleting or chopping, 4) grinding and mixing, and 5) record keeping, labeling, and other documentation. Multiple lab workers can then utilize each station in an assembly line sequence. Take care not to cross-contaminate areas or samples. Thus, it is important to: frequently wash instruments, replace gloves, and reline each station with new foil dull-side-up after each process. The sink area, station 1 and 2, are considered the "dirty" areas, which are not acid and solvent cleaned as well as the equipment used in the other areas. Counter and equipment at station 3 and 4 are considered "clean" areas, and need to be covered, with instruments acid and solvent cleaned.

6.2.6.7 For station 1, clean and rinse thoroughly before starting on a new sample, to reduce the possibility of cross contamination.

6.2.6.8 For station 2, set up on foil the DNA sample vials, scale cards, aging structure envelopes and otolith tray – depending on WDFW aging structure requirements (See Ecology's SOP #008 Resecting DNA Samples and Aging for Finfish). Possible equipment needed at this station includes a fillet knife, scalpel, scissors, forceps, pliers and access to DI water to rinse equipment. The equipment at this station does not need decontamination with acid and solvents. A bench light and magnifier are also handy for intricate maneuvers.

6.2.6.9 For station 3, set up on foil, over chopping board, the decontaminated fillet knife and scalpel for fillets or other instruments such as a butcher knife for whole fish and carcasses. Tear off new foil large enough to place each fish on for filleting procedure to be done after station 1 and 2's resecting, scaling, and DI rinsing.

6.2.6.10 For station 4, set up on foil: the clean Kitchen Aid, decontaminated Kitchen Aid grinding unit, 3-quart bowl and large (approximately 8 inch) mixing spoon for fillets. Alternatively, set up the clean Hobart (decontaminated Hobart grinding unit including large pan that funnels tissue into grinder), 13-quart bowl and extra-large (approximately 15 inch) mixing spoon for whole fish and carcasses. Note: Make sure when assembling the Hobart grinding unit that the grinding blade's cutting edge (one-sided) sits flat against the holed-strainer plate. The Kitchen Aid blades are on both sides so either side can sit against the strainer plate. If compositing, tear off foil pieces large enough to wrap each ground sample mixture and then set these pieces aside near station 4. Also, make sure there is a label for each of these wrapped mixtures that identifies the sample, using the original fish ID.

6.2.6.11 For station 5, set out and review Lab Analysis & Tracking Plan and Processing Benchsheet. Label DNA vials, scale cards, aging structure envelopes and otolith trays as described in Ecology's SOP #008 Resecting DNA Samples and Aging for Finfish.

6.2.6.12 Pre-label fish tissue sample jars either before set up or at station 5. Label jars by using custom printed labels or labels provided with sample jars. If using labels provided with sample jars use a ballpoint pen or fine-tipped permanent marker to fill out needed info – allow time for ink to dry so it does not smear or run when wet. For composite samples, fill in the labels according to the "Sample Tag Labeling" illustration in Attachment 4 and stick on the side of the appropriate jar (Figure 7). For composites, refer to "Label example for past PBDE project" as illustrated in Attachment 4. For individual fish, refer to "Label example for mercury (Hg) Trends analysis on individual fish" label as illustrated in Attachment 4. For all jars, write the abbreviated Field ID (listed in the Lab Analysis & Tracking Plan) and last two digits of sample number on the jar lids (Figure 8), using a fine tipped permanent marker.

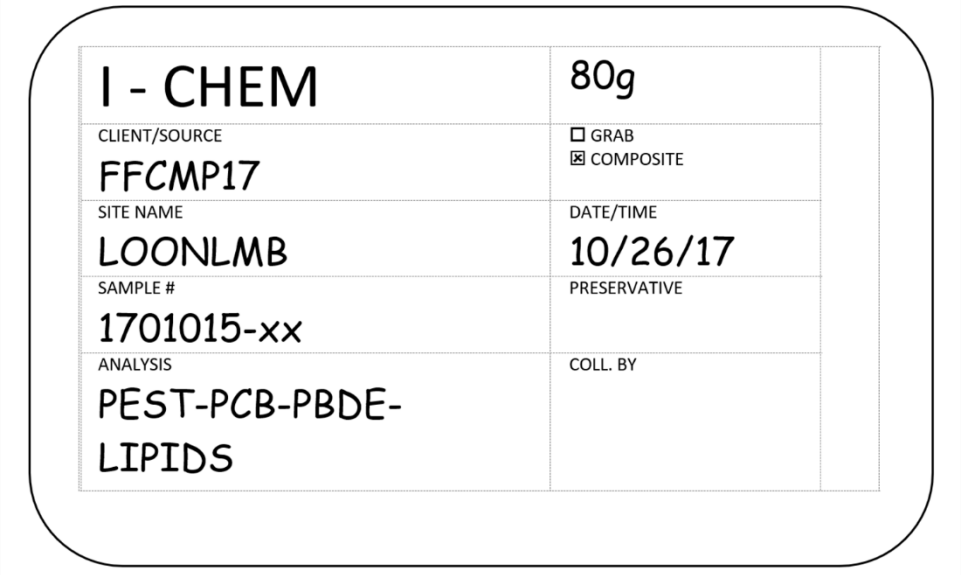


Figure 7. Freshwater Fish Contaminant Monitoring Program example label for composite fish tissue jar

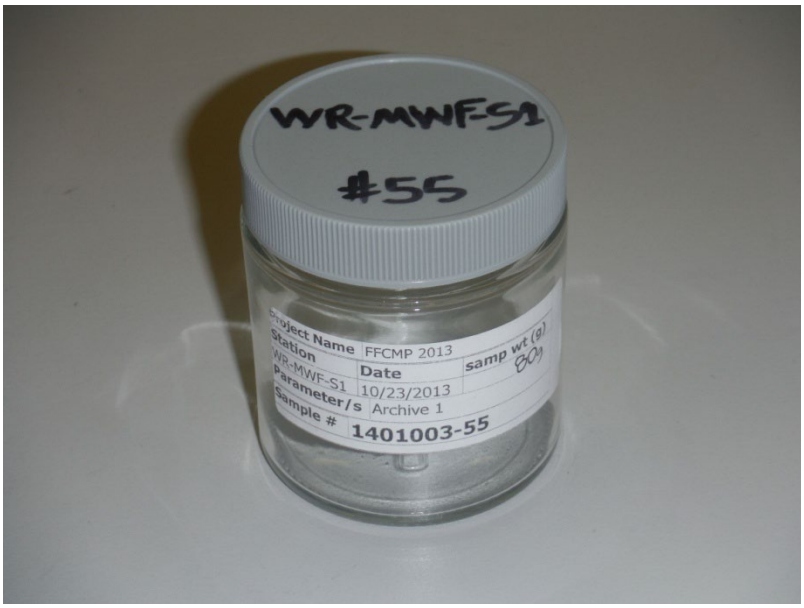


Figure 8. Example of sample jar lid information and custom printed label

6.3 Fish Processing Resection.

6.3.1 The resection process steps described below are complete within each category of fillet, whole and carcass. Turn to the appropriate subheading of either “Individual fish resection” or “Preparation of composite sample fish resection” and read the brief introduction. Then follow the procedures outlined for fillet, whole or carcass, depending on the project’s objectives for each fish sample or group of samples collected.

6.4 Individual Fish Resection.

6.4.1 It is essential that the weights of individual homogenates be sufficient to perform all necessary analyses. Generally, lab analyses for groups of organic compounds (e.g. PCBs, lipids, chlorinated pesticides) require a minimum of 30 grams of homogenized tissue. Lab analyses for mercury and other metals generally require a minimum of 5 grams. Check with project officer and/or laboratory for minimum amounts needed for each analysis or group of analyses.

6.4.2 Although there is a 30-gram minimum weight for organics and a 5-gram minimum weight for mercury, the weights may need to be doubled or more. The extra weight is intended to provide sufficient sample material to analyze for all recommended target analytes at required detection limits, meet minimum QC requirements for the analyses of laboratory duplicate, matrix spike and matrix spike duplicate samples, and allow for reanalysis if the QC control limits are not met or if the sample is lost.

6.4.3 Therefore, check the Lab Analysis & Tracking Plan to determine for analytes tested and the number of jars needed. The decision for whether to use one fillet side or both fillet sides of the individual fish in order to have enough tissue for homogenizing is depended on the number of analytes tested and jars needed.

6.5 Fillet.

6.5.1 Unwrap an individual fish, keeping it on foil at station 2, and perform a simple inspection to make sure the specimen will not compromise the representativeness and accuracy of the data in any way. Examine that the sample was properly preserved during shipment, tissue was not destroyed, and correct species were identified.Note any gross morphological abnormalities. Double check to make sure that the fish field identification matches Processing Benchsheet and Lab Analysis & Tracking Plan identification. Be sure to document any findings on the Processing Benchsheet. If the field weight and/or length were not recorded, weigh and measure the fish during this inspection time, writing the measurements on the Processing Benchsheet (See Ecology’s SOP #009 Field Collection, Processing and Preservation of Finfish Sample).

6.5.2 If needed, remove DNA samples prior to continuing next steps to ensure intact genetic sample is collected. Remove aging structures such as otoliths, operculum, and spines after resecting the fillet sample to prevent contamination of sample tissue when removing fillets (See Ecology’s SOP #008 Resecting DNA Samples and Aging for Finfish).

6.5.3 At station 1, remove slime and scales from fish that have scales by scraping from the tail to the head using the blade edge or the backside of a large knife, and rinsing periodically under cold running water. Be careful not to thaw the fish too much. Small specimens thaw very quickly, especially in water. After descaling the fish, rinse it with DI water and, at station 3, place on a new piece of foil (dull side up) with the accompanying field ID tag. Rinse the scaling equipment with DI water between fish if other individual fish are to be resected.

6.5.4 Perform skin removal at station 3 if appropriate for the species and project objectives. To remove the skin, first outline the area where skin is to be removed by making an incision just through the skin. Then loosen skin just behind the gills and pull it off with pliers, pulling from the head towards the tail. Rinse the skinning equipment with DI water between fish samples, if resecting other fish. At this point, the fish can be staged temporarily by wrapping the fish in aluminum foil with the accompanying field ID tag and placing it in a cooler on ice. This can be used for short-term delays between cleaning, descaling, and filleting to keep the fish from thawing completely.

6.5.5 Fillet fish at station 3 while ice crystals are still present in the muscle tissue, using the illustrated procedure in Attachment 5 and/or under the direction of an experienced person. Do not separate any dark muscle tissue near the lateral line from the light muscle tissue that constitutes the rest of the muscle tissue mass. Include the belly flap portion if possible. Avoid bones while filleting.

6.5.5.1 Be careful to avoid contaminating fillet tissues with material and fluid released from inadvertent slicing into the internal organ cavity. If materials released from the internal cavity and/or organs during resection contaminate the fillet tissue, eliminate the contaminated tissue pieces as part of the sample or, alternatively, rinse the fillet tissue in contaminant-free, DI water. Make a note on the Processing Benchsheet if this occurs.

6.5.5.2 Cut fillets into smaller pieces to facilitate homogenization in the Kitchen Aid grinder. Smaller pieces may be cut as the fillet is removed from the fish. Prior to grinding tissue, set up a lab scale with a small piece of foil, dull side up. Tare the scale so it reads zero. Place the cut up pieces of fillet tissue on the foil. Weight the fillet to the nearest gram and record the weight. In addition, record the side of the fish filleted (i.e. right, left, or both) and whether the fish was processed with skin on or off.

6.5.5.3 Totally wrap the tissue within the foil and place it in a freezer bag with a secured sample identification label attached to it. Place the packet in the freezer (≤ -20 °C) for temporary (less than one day) storage to keep the tissue free from contaminants and frozen until homogenization is done. Return the carcass to station 2, ("dirty" area), for age structure removal and for determining the sex.

6.5.6 Homogenize the fish tissue at station 4 using the previously decontaminated Kitchen Aid grinder, stainless steel bowl and spoon. Grind the fish tissue. Then mix the ground tissue until it appears to be of a consistent color and texture. If there is a large amount of tissue , divide the ground tissue into quarters, mix the opposite quarters together, and then mix the two halves together. Repeat the grinding and mixing two more times. No chunks of tissue or skin should remain in the sample homogenate. Poorly homogenized tissue may not be extracted or digested efficiently and could bias the analytical results. If necessary, repeat the grinding and mixing again. Each sample should be ground and mixed three times at minimum.

6.5.7 Fill sample jars with adequate amounts of homogenate. Do not pack the jars too full because the homogenate expands when it freezes, and increases the risk of losing the sample due to jar breakage. Eighty (80) grams maximum is enough for the 4 oz. jars and roughly 50 grams or ¾ full maximum is enough for the 2 oz. jars. Record the homogenate weight on the jar label and on the lab tracking sheet. If only using the individual fish tissue for one process, then move on to the next step. Otherwise, weigh the rest of the ground tissue, note this on the sample identification label, rewrap the tissue in foil, and return it to the freezer for using with other processes such as compositing.

6.5.8 At station 2, remove aging structures (otoliths, operculum and spines) appropriate for the species. Record appropriate information on the Processing Benchsheet per Ecology's SOP #008 Resecting DNA Samples and Aging for Finfish.

6.5.9 Determine the sex of fish by making an incision on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pectoral fins. If necessary, make a second incision on the left (or right) side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap should be folded back to observe the gonads. Ovaries have a granular texture and depending on the species can range from orange/red to dark green/blue or even whitish in color. Testes appear creamy off-white and have a smooth texture. Record the sex of each fish on the Processing Benchsheet using M for male, F for female, U for unknown. Add a question mark (?) after M or F when unsure.

6.5.10 Verify and document that the Lab Analysis & Tracking Plan, Processing Benchsheet, and all labeling is complete and accurate. Be sure to write down the process date and crew name initials.

6.5.11 Store jars in freezer (≤ -20 °C) with lids secure for transport to lab. See MEL's Manchester Environmental Laboratory Lab Users Manual for instructions for shipping samples to the lab (MEL, 2016).

6.6 Whole Fish.

6.6.1 Unwrap an individual fish, keeping it on foil at station 2, and perform a simple inspection to make sure the specimen will not compromise the representativeness and accuracy of the data in any way. Look to see that the sample was properly preserved during shipment, tissue was not destroyed, and correct species identified, and note any gross morphological abnormalities. Double check to make sure that the fish field identification matches the Processing Benchsheet and the Lab Analysis & Tracking Plan identification. Be sure to document any findings on the Processing Benchsheet. If the field weight and/or length were not recorded, weigh and measure the fish during this inspection time, writing the measurements on the Processing Benchsheet (See Ecology's SOP #009 Field Collection, Processing and Preservation of Finfish Samples for weighing and measuring techniques).

6.6.2 If needed, remove DNA sample and aging structures appropriate for the species (See Ecology's SOP #008 Resecting DNA Samples and Aging for Finfish).

6.6.3 Slime, scales and/or skin might be included in whole fish analysis. Therefore, processors must check with the project officer and/or the Lab Analysis & Tracking Plan and Processing Benchsheet. If removing scales, remove slime from fish that have scales at station 1 by scraping from the tail to the head using the blade edge or the backside of a large knife. Be careful not to thaw the fish too much. Small specimens thaw very quickly, especially in water. After descaling the fish, rinse the fish with DI water. At station 3, place the fish on a new piece of foil (dull side up) with the accompanying field ID tag. Rinse the scaling equipment with DI water between fish, if other individual fish are to be resected.

6.6.4 Perform skin removal at station 3 if appropriate for the species and project objectives. To remove the skin, loosen skin just behind the gills and pull it off with pliers, pulling from the head towards the tail. Rinse the skinning equipment with DI water between fish samples, if resecting other fish.

6.6.5 For fish where scales or skin is not removed, remove slime at station 1 by scrubbing then rinsing with DI water. Then place the fish on new piece of foil (dull side up), with accompanying field ID tag at station 3.

6.6.6 At station 2, remove structures for aging (otoliths, operculum and/or spines) appropriate for the species. Record appropriate information on the Processing Benchsheet per Ecology's SOP #008 Resecting DNA Samples and Aging for Finfish.

6.6.7 If needed, determine the sex of fish at station 2 or station 3 by making an incision on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pectoral fins. If necessary, make a second incision on the other left (or right) side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap should be folded back to observe the gonads. Ovaries have a granular texture and depending on the species can range from orange/red to dark green/blue or even whitish in color. Testes appear creamy off-white and have a smooth texture. Record the sex of each fish on the Processing Benchsheet using M for male, F for female, U for unknown. Add a question mark (?) after M or F when unsure.

6.6.8 Ensure intact whole fish will fit down Hobart grinder chute. If too large, chop fish into pieces small enough to facilitate homogenization in the Kitchen Aid and Hobart grinders. Chop, cut or saw fish using appropriate tools (cleaver, knives, and hacksaw) at station 3 while ice crystals are still present in the muscle tissue. Wear safety glassesor a facial shield. Place the fish pieces on aluminum foil that is dull side up.

6.6.9 Totally wrap the tissue within the foil and secure a sample identification label to it, place the wrapped tissue in the freezer (≤ -20 °C) for temporary (less than one day) storage to keep the tissue free from contaminants and frozen until ready for homogenization. Write on the Processing Benchsheet whether the fish is processed with the skin on or off.

6.6.10 Homogenize fish tissue at station 4 using a previously decontaminated grinder, stainless steel bowl and spoon. Whole fish are typically ground using a larger grinder such as the Hobart grinder. Grind the fish tissue. Then mix the ground tissue until the tissue appears to be of a consistent color and texture. If there is a large amount of tissue, divide the ground tissue into quarters, mix the opposite quarters together and then mix the two halves together. Repeat the grinding and mixing two more times. No chunks of tissue or skin should remain in the sample homogenate. Poorly homogenized tissue may not be extracted or digested efficiently and could bias the analytical results. If necessary, repeat the grinding and mixing again. Each sample should be ground and mixed three times at minimum.

6.6.11 Fill sample jars with adequate amounts of homogenate. Do not pack the jars too full because the homogenate expands when it freezes, which increases the risk of losing the sample due to jar breakage. Eighty (80) grams maximum is enough for the 4 oz. jars, and 50 grams or ¾ full maximum is enough for the 2 oz. jars. Record the homogenate weight on jar label and on the Lab Analysis & Tracking Plan. If only using the individual fish for one process, then move on to the next step. Otherwise, weigh the rest of the ground tissue, note this on the sample identification label, rewrap the tissue in foil, and return it to the freezer for using with other processes such as compositing.

6.6.12 Verify and document that the Lab Analysis & Tracking Plan, Processing Benchsheet, and all labeling is complete and accurate. Be sure to write down the process date and crew name initials.

6.6.13 Store jars in freezer (≤ -20 °C) with lids secure for staging transport to lab. See MEL Manchester Environmental Laboratory Lab Users Manual for instructions for shipping samples to the lab (MEL, 2016).

6.7 Carcasses.

6.7.1 The purpose of processing fish carcasses and fillets separately is to determine the ratio of contaminant concentration in fillets to whole fish, in order to estimate concentrations in fillet tissue when only whole-fish data are available. Determining this ratio requires a mass balance approach using concentration and weight data from fillets and the remaining carcass. To account for changes in weight of each fish during processing, (e.g. weight loss from scale removal), the weight of each fish and its various tissues need recorded at selected steps in the processing procedure.

6.7.2 Treat the fillets and carcasses from the same fish sample as two separate samples. Therefore, cleaned and decontaminated equipment for each sample. Refer to Ecology's Chemical Hygiene Plan and Hazardous Materials Management Plan Section 16 Standard Operating Procedures for decontamination procedures (Ecology, 2016).

6.7.3 Prepare the Supplemental Benchsheet for processing fillet and carcass tissue, in addition to the Lab Analysis & Tracking Plan and regular Processing Benchsheet. Fill in the site location name, fish species, fish field ID and weight for matching individual fish (See Section 7.3 and Attachment 3 in this document for the Supplemental Benchsheet for processing fillet and carcass tissue instructions and example).

6.7.4 At station 2, unwrap an individual fish, keeping it on foil, and perform a simple inspection to make sure the specimen will not compromise the representativeness and accuracy of the data in any way. Look to see that the sample was properly preserved during shipment, that tissue was not destroyed and that correct species were identified. Note any gross morphological abnormalities. Double check to make sure that the fish field identification matches the Processing Benchsheet and the Lab Analysis & Tracking Plan identification. Be sure to document any findings on the Processing Benchsheet. If the field length was not recorded, measure the fish during this inspection time, documenting the measurements on the Processing Benchsheet (See Ecology's SOP #009 Field Collection, Processing and Preservation of Finfish Samples for measuring techniques).

6.7.5 Place new foil, dull side up, on scale and tare bench scale. Weigh whole fish on the new foil on the tared bench scale. Record weight to the nearest gram on the Supplemental Benchsheet for processing fillet and carcass tissue.

6.7.6 Remove DNA sample and aging structures appropriate for the species, except the otoliths. Remove otoliths after resecting the fillet sample to prevent contamination of sample tissue when removing fillets (See Ecology's SOP #008 Resecting DNA Samples and Aging for Finfish).

6.7.7 At station 1, scale and remove slime from fish that have scales, by scraping from the tail to the head using the blade edge or the backside of a large knife and rinsing periodically under cold running water. Be careful not to thaw the fish too much. Small specimens thaw very quickly especially in water. After descaling the fish, rinse the fish with DI water and place on a new piece of foil (dull side up) with the accompanying field ID tag over at station 3. Rinse the scaling equipment with DI water between fish if resecting other individual fish. At this point, the fish can be staged temporarily by wrapping the fish in aluminum foil with the accompanying field ID tag and placing it in a cooler on ice. This can be used for short-term delays between cleaning, descaling, and filleting to keep the fish from thawing completely.

6.7.8 Weigh whole fish again after removing scales and DNA sample. Record the weight to the nearest gram on the Supplemental Benchsheet for processing fillet and carcass tissue. Use new foil dull side up, or the same foil used before scale and DNA resection.

6.7.9 Fillet fish at station 3 while ice crystals are still present in the muscle tissue, using the illustrated procedure in Attachment 5 and/or under the direction of an experienced person. Do not separate any dark muscle tissue near the lateral line from the light muscle tissue that constitutes the rest of the muscle tissue mass. Include the belly flap portion if possible. Avoid bones while filleting.

6.7.10 Be careful to avoid contaminating fillet tissues with material released from inadvertent slicing into the internal organ cavity. If materials released from the internal cavity and/or organs during resection contaminate the fillet tissue, eliminate the contaminated tissue pieces as part of the sample or, alternatively, rinse the fillet tissue in contaminant-free, DI . Blotted dry. Make a note on the Processing Benchsheet.

6.7.11 Cut fillets into smaller pieces to facilitate homogenization of the Kitchen Aid grinder. Prior to grinding tissue, set up the lab scale with a small piece of foil dull side up on the scale. Tare the scale so it reads zero. Place the cut up pieces of fillet tissue on the foil. Weigh the fillet to the nearest gram and record the weight of both fillets on the Supplemental Benchsheet for processing fillet and carcass tissue.

6.7.12 Totally wrap the fillets within the foil and place in a freezer bag with a secured sample identification label attached to it. Place the packet in the freezer (≤ -20 °C) for temporary (less than one day) storage to keep the tissue free from contaminants and frozen until homogenization.

6.7.13 Remove otolith and aging structures (i.e. operculum/spines if not already resected) from the carcass appropriate for the species at station 2 (See Ecology's SOP EAP008 Resecting DNA Samples and Aging for Finfish). Record otolith tray number, cell number or aging structure type and ID number in Processing Benchsheet.

6.7.14 Weigh otoliths and other aging structures (i.e. operculum/spines). Record any measurable weight on the Supplemental Benchsheet for processing fillet and carcass tissue.

6.7.15 Weigh remaining carcass on new foil dull side up. Record weight to the nearest gram in the Supplemental Benchsheet before processing the fillet and carcass tissue.

6.7.16 Determine sex of fish by making an incision on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pectoral fins. If necessary, make a second incision on the left (or right) side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap should be folded back to observe the gonads. Ovaries have a granular texture and depending on the specie can range from orange/red to dark green/blue or even whitish in color. Testes appear creamy off-white and have a smooth texture. Record the sex of each fish on the Processing Benchsheet using M for male, F for female, U for unknown. Add a question mark (?) after M or F for unsure.

6.7.17 Ensure fish carcass will fit down grinder chute. If too large, chop carcass at station 3 using appropriate tools (e.g. knives, saws) while ice crystals are still present in the muscle tissue. Wear safety glasses or a facial shield are recommended. Chop fish pieces small enough to facilitate homogenization of the Hobart grinder. Place the fish pieces on a piece of aluminum foil that is dull side up.

6.7.18 Totally wrap the tissue within the foil and secure a sample identification label to it, place the packet in the freezer (≤ -20 °C) for temporary (less than one day) storage to keep the tissue free from contaminants and frozen until homogenization.

6.7.19 Homogenize fish carcass pieces at station 4 using a previously decontaminated grinder, stainless steel bowl and spoon. Grind the carcass pieces. Then mix the ground carcass tissue until the tissue appears to be of a consistent color and texture. If there is a large amount of ground carcass tissue, divide it into quarters, mix the opposite quarters together and then mix the two halves together. Repeat the grinding and mixing two more times. No chunks of tissue or skin should remain in the sample homogenate. Poorly homogenized tissue may not be extracted or digested efficiently and could bias the analytical results. If necessary, repeat the grinding and mixing again. Each sample should be ground and mixed three times at minimum.

6.7.20 Fill sample jars with adequate amount of homogenate. Do not pack the jars too full because the homogenate expands when it freezes, which increases the risk of losing the sample due to jar breakage. Eighty (80) grams maximum is enough for the 4 oz. jars and 50 grams or ¾ full maximum is enough for the 2 oz. jars. Record the homogenate weight on the jar label and on the Lab Analysis & Tracking Plan. If only using the individual fish for one process, then move on to the next step. Otherwise, weigh the rest of the ground tissue, note this on the sample identification label, rewrap the tissue in foil and return to the freezer to be used with other processes such as compositing.

6.7.21 Verify and document that the Lab Analysis & Tracking Plan, Processing Benchsheet, Supplemental Benchsheet and all labeling is complete and accurate. Be sure to write down the process date and crew name initials.

6.7.22 Store jars in freezer (≤ -20 °C) with lids secure for staging transport to lab. See MEL's Manchester Environmental Laboratory Lab Users Manual for instructions for shipping samples to the lab (MEL, 2016).

6.7.23 Homogenize fish fillets at station 4 using the previously decontaminated Kitchen Aid grinder, stainless steel bowl and spoon. Grind the fillet tissue. Then mix until the ground tissue appears to be of a consistent color and texture. If there is a large amount of tissue, divide the ground tissue into quarters, mix the opposite quarters together and then mix the two halves together. Repeat the grinding and mixing two more times. No chunks of tissue or skin should remain in the sample homogenate. Poorly homogenized tissue may not be extracted or digested efficiently and could bias the analytical results. If necessary, repeat the grinding and mixing again. Each sample should be ground and mixed three times at minimum.

6.7.24 Fill sample jars with adequate amounts of homogenate. Do not pack the jars too full because the homogenate expands when it freezes, which increases the risk of losing the sample due to jar breakage. Eighty (80) grams maximum is enough for the 4 oz. jars and 50 grams or ¾ full maximum is enough for the 2 oz. jars. Record homogenate weight on the jar label and on the Lab Analysis & Tracking Plan. If only using the individual fish for one process, then move on to the next step. Otherwise, weigh the rest of the ground tissue, note this on the sample identification label, rewrap the tissue in foil, and return to the freezer to be used with other processes.

6.7.25 Verify and document that the Lab Analysis & Tracking Plan, Processing Benchsheet, Supplemental Benchsheet and all labeling is complete and accurate. Be sure to write down the process date and crew name initials.

6.7.26 Store jars in freezer (≤ -20 °C) with lids secure for transport to lab. See MEL's Manchester Environmental Laboratory Lab Users Manual for instructions for shipping samples to the lab (MEL, 2016).

6.8 Preparation of composite sample.

6.8.1 Composite samples are prepared using equal-weight aliquots of processed tissue from one or more individual fish. When using equal-weight aliquots from each fish, the amount of tissue available from the smallest fish determines the size, or weight, of the aliquot from each fish making up the composite sample. When using small fish to create a composite sample, reevaluate if there is adequate tissue combined from all fish designated for the composite to meet analytical needs. Measure or estimate the amount of tissue available from the smallest fish then multiply by the number of fish in composite to find the amount of tissue available for the required analyses. There should be sufficient sample material to analyze for all recommended target analytes at required detection limits; meet minimum QC requirements for the analyses of laboratory duplicate, matrix spike and matrix spike duplicate samples; and allow for reanalysis if the QC control limits are not met or if the sample is lost. If the combined amount available is greater than or equal to the amount of tissue needed for analyses, then proceed with processing individual samples and creating the composite sample as described below. If there is not enough tissue, then determine whether to increase the number of fish used in the composite sample or limit the number of lab analyses.

6.9 Fillet, Whole and/or Carcass Tissue Compositing.

6.9.1 Follow procedures for individual fish tissue resection above (fillet, whole fish, carcasses), but grind and mix tissue from each fish only twice before wrapping in foil, marking the weight of the tissue on the sample identification label and storing it in the freezer. (The third homogenization and mixing will be done as the homogenates from individual fish are combined, ground, and mixed together).

6.9.2 Combine equal weights of tissue from individual homogenates (fillet, whole or carcass). Partially thaw and rehomogenize individual homogenates samples if frozen prior to compositing. Maintain any associated liquid as a part of the sample. Record the aliquot weight to the nearest gram on the Lab Analysis & Tracking Plan.

6.9.3 Mix all individual homogenate aliquot portions together until completely homogenized. Grind and mix one more time. The tissue should be ground three times and mixed four times at minimum by the time tissue from the individual fish are composited and ready to put in containers for sending to the lab.

6.9.4 Continue following the instructions for processing individual fillet, whole fish or carcasses.

7.0 Records Management

7.1 Lab Analysis & Tracking Plan – This spreadsheet tracks information about tissue samples from field collection through final lab analysis. Use this tool for organizeing, planning, coordinating, and tracking sample characteristics for single or multiple projects. The spreadsheet is used to: determine which lab analyses are done on which samples, determine costs for analyses and QA/QC requirements, document cross-reference lab and field identification codes, and document other sample and plan characteristics. The structure of the spreadsheet may vary depending on the type of project(s) and the objectives, and can be tailored to suit the user. Table 1 lists the fields used in this form. Some fields are required for all projects (R), while others are suggested (S) or optional (O). See Attachment 1 for an example.

7.1.1 The Lab Analysis & Tracking Plan form is prepared at the beginning of field collections for each project or season. During fish collection for the project, enter information into the form for the project, site, species, collection date, and number of fish available for each species. Populate the fields indicating the desired lab analyses as sampling and planning progresses. Doing so produces numbers and costs of analyses, allowing project staff to track anticipated costs and make changes as needed.

7.1.2 A hardcopy of the spreadsheet is used to record data while processing fish. As samples are processed, record sample information on the spreadsheet (e.g. the sample process date, aliquot used per fish, etc.). Transfere these handwritten data to the electronic version at regular intervals so project staff can determine the status of fish collections and sample processing. When entering documentation from the hard copy into the master electronic Lab Analysis & Tracking Plan, write the word *Entered, the date, and your initials* on each hard copy of the Lab Analysis & Tracking Plan.

7.1.3 A template of the Lab Analysis & Tracking Plan is located in Ecology's Shared files. The name of the file should include the project, sampling year, the words or abbreviation indicating "Lab Tracking Plan", and the version number, (e.g. 2021 FFCMP Lab Analysis & Tracking Plan.xlsx).

Table 1. Lab Analysis & Tracking Plan field titles and descriptions. Field entries include required (R), suggested (S) or optional (O). Use additional fields as needed per study or user preference.

Need	Field Title	Field Description
R	Project 1 pre-assigned work order #	Pre-assigned 7 digit MEL work order (example: FFCMP work order #**1401003**-xx).
R	Project 2 pre-assigned work order #	Pre-assigned 7 digit MEL work order (example: Hg Trends work order #**1401009**-xx).
R	Site	Name of the waterbody fish was collected from.
R	Specie	Abbreviated species of fish. Example: LMB (largemouth bass), MWF (mountain whitefish).
R	Collect Date	Date fish was collected, m/d/yy.
R	# Fish in Comp	Number of fish in composite.
R	LAR Field Station ID	Unique identification given to each sample. Name used on LAR (Laboratory Analysis Required) form.
R	MEL Lab #	Unique 2 digit sample ID added on end of work order # during fish processing (typically #s 01-99, example #1401003-**01)**.
R	Process Date	Date the fish was processed, m/d/yy.
R	Aliquot per Fish (g)	Weight in grams per fish in equal aliquots for composite.
R	Skin: off or on	Skin is either removed or left on when processing.
R	Comment	Additional observations, instructions and procedure documentation.
S	Project 1	Example: FFCMP (Freshwater Fish Contaminant Monitoring Program).
S	Project 2	Example: Hg Trends.
S	Analyte Group 1	Example: Pest PCB PBDE lipid.
S	Analyte Group 2	Example: PCB congener.
S	Analyte Group 3	Example: Hg (mercury).
S	Analyte Group 4	Example: PCDD/F
S	MEL Lab Dup	MEL lab duplicates indicated for project.
S	Contract Lab Dups	Contract lab duplicates indicated for project.
S	MS/MSD	MEL lab matrix spikes and standards indicated for project.
S	# Fish Avail	Total number of fish collected.
O	Sort	A column allowed for entries from which to sort specifications as needed.
O	Analytical Group 1 sample weight	Weight in grams of sample sent to MEL for these analytes. Example: Pest PCB PBDE lipid (g).
O	Analytical Group 2 sample weight	Weight in grams of sample sent to MEL for these analytes. Example: PCB congener (g).
O	Analytical Group 3 sample weight	Weight in grams of sample sent to MEL for these analytes. Example: Hg (g).
O	Analytical Group 4 sample weight	Weight in grams of sample sent to MEL for these analytes. Example: PCDD/F (g).
O	Archive	Weight in grams of sample archived. Ocassionally a second archive jar is retained for future needs.
O	Samples to Lab date	Date processed fish samples were sent to MEL.
O	Fillet+Carcass	Special process indicated when marked with an F or C, (Fillet/ Carcass).
O	Process Batch (H-Hg trends; F-FFCMP)	Example of study/user specific documentation. This was created for prioritizing analysis by study type.

Processing Benchsheet – Use this spreadsheet to document field and processing data for individual fish. Such information includes individual fish identification coding, composite sample designation, fillet weights, sex, age structure cross references, and general comments about the fish or its processing. The structure of the spreadsheet may vary depending on project(s) and objectives and can be tailored to suit the user. Table 2 lists fields used in this form. Some fields are required for all projects (R), while others are suggested (S) or optional (O). See Attachment 2 for an example.

7.1.4 A hardcopy of the spreadsheet is used to record data while processing fish. Record information on the spreadsheet as samples are processed. Transfer these handwritten data to the electronic version at regular intervals, so project staff can determine the status of fish collections and sample processing. When entering information from the hard copy into the master electronic Processing Benchsheet, write the word *Entered, the date, and your initials* on each hard copy of the Processing Benchsheet.

7.1.5 A template of the Processing Benchsheet is located in Ecology's shared files and SharePoint. Rename of the file to include the project, sampling year, the words or abbreviation indicating "Benchsheet," and the version number, (e.g. 2021 FFCMP Fish Processing Benchsheet.xlsx).

Table 2. Processing Benchsheet field titles and descriptions.

Need	Field Title	Field Description
R	Site	Name of the waterbody fish was collected from.
R	ECY Field ID	Identification (number or combination given to fish when collected in the field).
R	Species Code	Abbreviated species of fish. Example: LMB (largemouth bass), MWF (mountain whitefish).
R[1]	WDFW DNA ID	Identification of the vial that the DNA sample clip is stored, (i.e. CD05-##).
R	Total Length (mm)	Total length of fish in millimeters.
R	Weight (g)	Weight of fish in grams.
R	Collect Date	Date fish was collected.
R	Process Date	Date the fish was processed.
R	Fillet Weight (g)	Fillet weight of fish in grams if filleted.
R	L, R, or B fillet	L, R, or B for left, right, or both side(s) of fish that was filleted.
R	Skin Status	On or off for skin when processing.
R	Sex	Sex of fish - Male = M, Female = F, Unidentified = U.
R[2]	Scale Card #	Number of scale card.
R[2]	Scale #	Individual fish identification, which can be the same as the Field ID.
R[2]	Otolith Tray #	Number of otolith tray.
R[2]	Otolith Cell #	Otolith cell number.
R[2]	Opercle or Spines taken Y/N	Indicates if operculum or spines were collected.
R	Comment	Additional observations, instructions and procedure documentation.
S	WDFW Field ID	WDFW field ID if collected by WDFW (if available).
S	DNA Taken?	Yes or No (Y or N) confirmation that the DNA tissue sample was collected.
S	Collect Method	Abbreviated collection method. See Collection Methods Abbreviations[a].
S	Fish Age	Blank until WDFW results return indicating age of fish.
S	Fin Clipped?	For ID as hatchery origin. Usually salmonids, rainbow trout, cutthroat trout.
S	LAR Field Station ID	Unique identification given to each sample. Name used on LAR (Lab Analysis Required) form.
S	MEL Lab ID #	Unique 2 digit sample ID added to end of work order # during fish processing (typically #s 01-99. example: 1401003-25).
O	Composit Group	Group of fish that were processed together to create a composite sample.
O	Fork Length	Length of fish from nose to crescent in tail.

The appended Table 3 Collection Method Abbreviations describes abbreviations for fish collection methods to enter in the Collect Method field of Processing Benchsheet. Field entries are designated as

required (R), required only if DNA sample is collected (R1), required only if fish is aged (R2), suggested (S) or optional (O). Use additional fields as needed per study or user preference.

Combine the agency code below with the collection method code to create "collection method code" (e.g. "ecy-E").

Table 3. Collection Methods Abbreviations

Code	Agency or Collection Method
ecy	Ecology
dfw	WA Dept of Fish and Wildlife
epa	U.S. Environmental Protection Agency
Tri	Tribe
UW	University of Washington
E	Boat electroshock
G	Gillnet
A	Angling
T	Trolling
BP	Backpack electrofishing
BS	Beach seine
NPMP	Northern Pike minnow Program

7.2 Supplemental Benchsheet – is for documenting the weights of the different tissue parts while processing fillet and carcass tissue, in order to determine the ratio of contaminant concentration in fillets to whole fish for estimating concentrations of contaminants in fillet tissue when only whole-fish data are available.

7.3.1 The Supplemental Benchsheet is located in Ecology's shared drives under "Forms for Fish." A copy may also be created from Attachment 3 of this document. Enter all hard-copy documentation into the master electronic Supplemental Benchsheet for processing fillet and carcass tissue. Write the word "Entered," the date, and your initials on each hard copy of the Supplemental Benchsheet.

8.0 Quality Control and Quality Assurance

8.1 Verify all information for completeness on the Processing Bench Sheet. Make sure there are no blank cells under any fields except fish age. WDFW will determine fish age and fill in later.

8.2 Verify that the values for weight of homogenate sample, date of processing, and Field ID are complete in the Lab Analysis & Tracking Plan.

8.3 Verify all the information for completeness on the Supplemental Bench Sheet for processing fillet and carcass tissue. There should not be any blank cells, except possibly for the field labeled "other."

8.4 Verify all hard-copy documentation on the Processing Bench Sheet and Supplemental Bench Sheet for processing fillet and carcass tissue accuracy. Cross check hard copies against electronic versions to verify data.

9.0 Safety

9.1 Conduct fish processing only by or under the supervision of someone with experience.

9.2 Gloves are required for fish processing, in order to avoid exposure to pathogens and chemicals and to avoid sample contamination. Hands should be cleaned using soap and clean water after completing work or any time hands become soiled during the process. Change gloves whenever they get torn, punctured, or removed from hands.

9.3 The use of nitric acid, acetone, and hexane requires training as per the *Chemical Hygiene Plan and Hazardous Materials Management Plan* (Section 1) (Ecology, 2019), which includes a Laboratory Safety Orientation, Job-Specific Orientation, and must know Chemical Safety Procedures ; and follow the "Basic Lab Rules" (Section 2). Gloves, safety glasses, and a full-body protective apron is required.

9.4 Material Safety Data Sheets for the chemicals used in these procedures are stored on the Toxics Technical Coordination Team (TTCT) SharePoint site. Review prior to field activities using this SOP.

9.5 Take extreme care when using all knives and grinding equipment, not only for your safety, but also for others that may be working in proximity. Verify that the first aid kit is available in the lab room and the contents are complete. Contact the room supervisor and/or the safety officer if any accident occurs, if first aid supplies are inadequate, if chemical spills occur, or for any other need or questions. The name and number of the room supervisor are posted in the room. In extreme emergency, call 911. Work with a "buddy," if possible, or notify a coworker of your lab-work plans, and put them on your office calendar.

10.0 References

10.1 Ecology. 2019. Chemical Hygiene Plan and Hazardous Materials Management Plan. Olympia, WA.

10.2 MEL, 2016. Manchester Environmental Laboratory Lab User's Manual, Tenth Edition. Manchester Environmental Laboratory, Washington State Department of Ecology, Manchester, WA.

10.3 U.S. EPA (Environmental Protection Agency). 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1 Fish Sampling and Analysis. Third Edition. Office of Science and Technology. Office of Water, Washington D.C.https://epa.gov/fish-tech/epa-guidance-developing-fish-advisories. Accessed April 2017.

10.4 U.S. EPA (Environmental Protection Agency). 1992. Specifications and Guidance for Contaminant-Free Sample Containers. Office of Solid Waste and Emergency Response, Washington D.C. Pub. # 540R93051. https://www.epa.gov/nscep

Attachment 1. Lab Analysis & Tracking Plan, (example only)

Note: A Lab Analysis & Tracking Plan may look different, due to different fields and requirements of the project(s) involved, but fields will be available for documentation and cross reference of each sample's collection and processing information.

Lab Analysis & Tracking Plan for FFCMP: 2016

FFCMP Work Order # 1701015

SIC DST03

updated: 1/17/17 NM

								DE THESE FIELDS WHEN PRINTIN								jar size ->		2 oz	4 oz	4 oz	4 oz	4 oz	4 oz
						min amount needed per analysis -->		5 g	40 g	40 g	60 g				min amount needed per jar -->			5 g	80 g	60 g	60 g	80 g	80 g
						cost/sample -->		$ 50	$ 379	$ 242	$ 675	$ 531						Use these fields for sample jar LABEL. Record amount of tissue in each sample jar.					
FFCMP (f)	Lab Dup MEL	MS/MSD MEL	Lab Dup CL	Site 1	Site 2	Species	suffix for LAR Field ID	collect date	# fish in comp	Hg	CP, PCBa (PEST2PCB)	PBDE, lipid	PCB congnr	PCDD/F	FFCMP LAR Field ID	FFCMP MEL Lab # 1701015-nn	FFCMP fish IDs	process date	aliquot per fish (g)	skin: off or on	space	Hg	PEST2PCB, PBDE, lipid	MEL QC	PCB congener, PCDD/F	Archive 1	Archive 2
f				Cowlitz R: Castle	CR	LSS	LSS-1	8/29/16	5		1	1			CR-LSS-1	**01**	6,8,15,24,30	12/28/16	300	ON			80			80	80
f				Cowlitz R: Castle	CR	LSS	LSS-2	8/29/16	5		1	1			CR-LSS-2	**02**	9,11,13,19,23	12/28/16	300	ON			80			80	80
f				Cowlitz R: Castle	CR	LSS	LSS-3	8/29/16	5		1	1			CR-LSS-3	**03**	2,18,22,27,33	12/28/16	300	ON			80			80	80
f				Cowlitz R: Castle	CR	MWF	MWF-1	8/29/16	5	1	1	1	1	1	CR-MWF-1	**04**	6,9,10,13,17	1/6/17	79	ON		5	80		60	80	80
f				Cowlitz R: Castle	CR	MWF	MWF-2	8/29/16	5	1	1	1	1	1	CR-MWF-2	**05**	8,15,18,22,23	1/6/17	89	ON		6	80		60	80	80
f				Cowlitz R: Castle	CR	MWF	MWF-3	8/29/16	5	1	1	1	1	1	CR-MWF-3	**06**	5,14,26,27,28	1/6/17	64	ON		5	80		60	60	60
f	a	a	a	Cowlitz R: Castle	CR	NPM	NPM-A	8/29/16	5	1	1	1	1	1	CR-NPM-A	**07**	1,2,3,4,5	1/4/17	90	ON		5	80	60	60	80	80
f	p	p	p	Cowlitz R: Castle	OL	CTT	CTT-1	8/30/16	5	1	1	1	1	1	OL-CTT-1	**08**	7,13,14,17,18	1/11/17	83	ON		5	80	60	60	80	80
f	a	a	a	Cowlitz R: Castle	OL	CTT	CTT-2	8/30/16	5	1	1	1	1	1	OL-CTT-2	**09**	5,6,9,10,16	1/11/17	94	ON		5	80	60	60	80	80
f				Cowlitz R: Castle	OL	CTT	CTT-3	8/30/16	5	1	1	1	1	1	OL-CTT-3	**10**	3,8,11,12,15	1/10/17	51	ON		5	80		60	62	44
f				Cowlitz R: Castle	OL	LSS	LSS-1	8/30/16	5		1	1			OL-LSS-1	**11**	16,18,21,29,35	12/29/16	300	ON			80			80	80
f				Cowlitz R: Castle	OL	LSS	LSS-2	8/30/16	5		1	1			OL-LSS-2	**12**	6,7,14,27,40	12/29/16	300	ON			80			80	80
f				Cowlitz R: Castle	OL	LSS	LSS-3	8/30/16	5		1	1			OL-LSS-3	**13**	8,10,17,32,37	12/29/16	300	ON			80			80	80
f				Cowlitz R: Olequa	OL	MWF	MWF-1	8/30/16	5	1	1	1	1	1	OL-MWF-1	**14**	13,15,25,32,37	12/28/16	91	ON		5	80		80	80	80
f	p	p	p	Cowlitz R: Olequa	OL	MWF	MWF-2	8/30/16	5	1	1	1	1	1	OL-MWF-2	**15**	23,28,29,33,34	12/28/16	80	ON		5	80	60	60	80	75
f				Cowlitz R: Olequa	OL	MWF	MWF-3	8/30/16	5	1	1	1	1	1	OL-MWF-3	**16**	14,20,24,30,40	12/28/16	77	ON		5	80		60	80	80
f				Cowlitz R: Olequa	OL	MWF	MWF-L1	8/30/16	3	1	1	1	1	1	OL-MWF-L1	**17**	3,4,17	1/5/17	103	ON		5	81		80	80	32
f				Cowlitz R: Olequa	OL	MWF	MWF-L2	8/30/16	3	1	1	1	1	1	OL-MWF-L2	**18**	6,10,19	1/5/17	108	ON		5	80		80	80	44
f				Cowlitz R: Olequa	OL	MWF	MWF-L3	8/30/16	3	1	1	1	1	1	OL-MWF-L3	**19**	7,9,16	1/5/17	115	ON		6	80		80	80	75
f				Cowlitz R: Olequa	OL	NPM	NPM-1	8/30/16	3	1	1	1	1	1	OL-NPM-1	**20**	7,10,14	1/5/17	105	ON		5	80		60	80	80
f	p	p	p	Cowlitz R: Olequa	OL	NPM	NPM-2	8/30/16	3	1	1	1	1	1	OL-NPM-2	**21**	8,9,11	1/5/17	102	ON		5	80	60	60	80	19
f				Cowlitz R: Olequa	OL	NPM	NPM-3	8/30/16	3	1	1	1	1	1	OL-NPM-3	**22**	6,12,13	1/5/17	115	ON		5	80		60	80	80
f				Mayfield L	ML	LSS	LSS-1	8/31/16	5		1	1			ML-LSS-1	**23**	1,11,27,30,32	12/27/16	300	ON			80			80	80
f				Mayfield L	ML	LSS	LSS-2	8/31/16	5		1	1			ML-LSS-2	**24**	6,23,31,33,34	12/27/16	300	ON			80			80	80
f				Mayfield L	ML	LSS	LSS-3	8/31/16	5		1	1			ML-LSS-3	**25**	3,12,16,37,40	12/27/16	300	ON			80			80	80
f				Mayfield L	ML	NPM	NPM-1	8/31/16	5	1	1	1	1	1	ML-NPM-1	**26**	12,16,18,30,32	1/4/17	83	ON		5	80		60	80	80
f				Mayfield L	ML	NPM	NPM-2	8/31/16	5	1	1	1	1	1	ML-NPM-2	**27**	4,11,15,20,31	1/4/17	84	ON		5	80		60	80	80
f				Mayfield L	ML	NPM	NPM-3	8/31/16	5	1	1	1	1	1	ML-NPM-3	**28**	13,17,22,26,29	1/4/17	88	ON		5	80		60	80	80

Attachment 2. Processing Benchsheet, (example only)

Note: The Processing Benchsheet used during lab processing may look different due to different fields and requirements of the processes involved, but fields will be available for documentation and cross reference of each sample's information.

Field Data for Fish Tissue Samples: FFCMP 2016

updated: 12/21/16 PS

Notes: 1. ND = Not Determined; U = checked but could not determine.

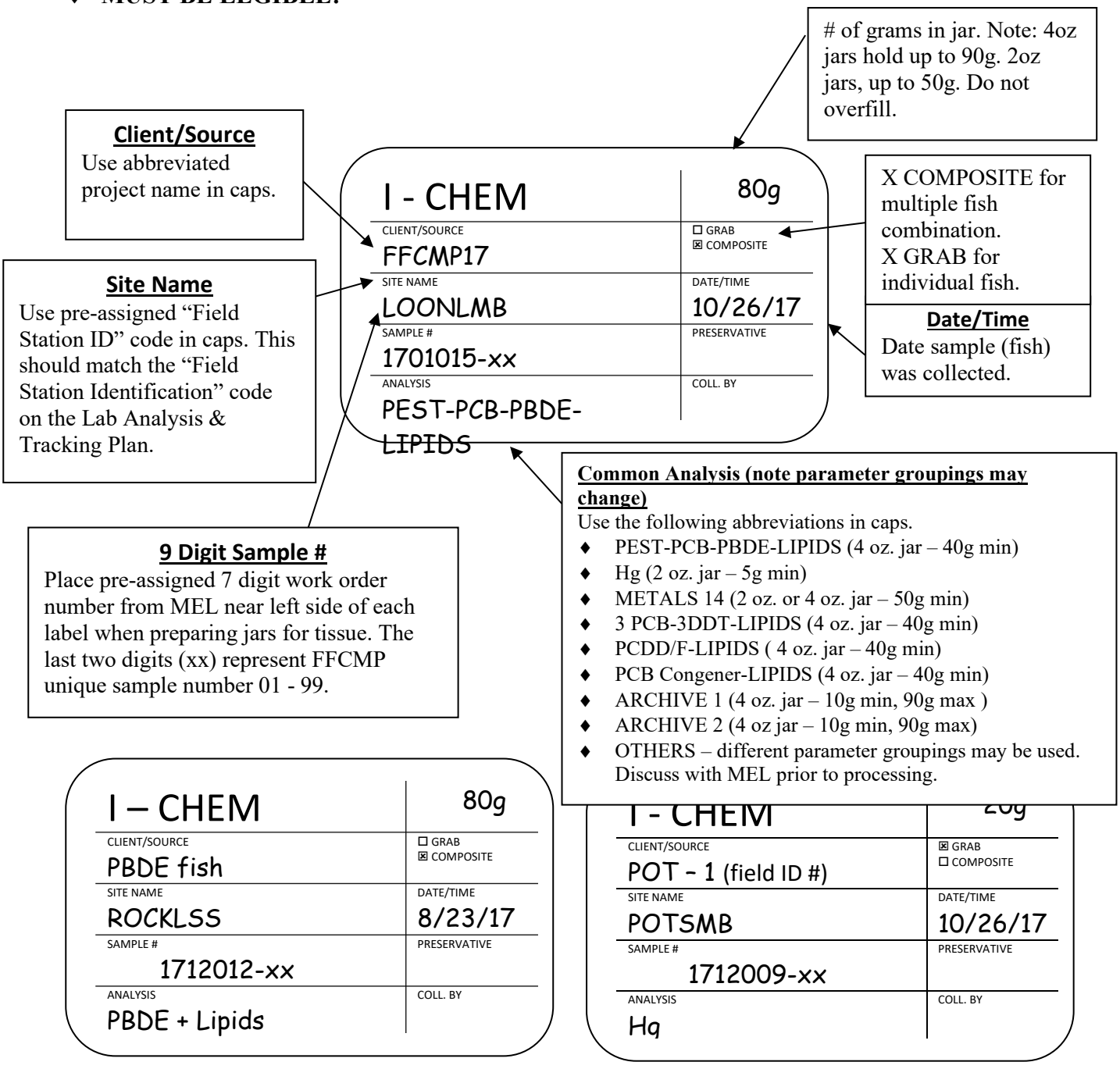
															Age Structures							
Site 1	Site 2	Species	ECY Field ID	Total Length (mm)	Field Weight (gm)	Collect Date	Collect Method	Process date	Processing crew	FFCMP fillet weight (gm)	FFCMP L, R, or B fillet	FFCMP skin status On/Off	sex M/F [1]	fish age	scale card #	scale #	otolith tray #	otolith cell #	Pull Opercula Spines Y/N	Comment	FFCMP LAR Field ID	FFCMP MEL ID: 1701015-nn
Castle Rock (CR)	CR	NPM	1	344	365	8/29/2016	E	1/4/17	PS	139	B	ON	F						Y		CR-NPM-A	**07**
Castle Rock (CR)	CR	NPM	2	367	454	8/29/2016	E	1/4/17	PS	166	B	ON	F						Y		CR-NPM-A	**07**
Castle Rock (CR)	CR	NPM	3	330	286	8/29/2016	E	1/4/17	PS	113	B	ON	F						Y		CR-NPM-A	**07**
Castle Rock (CR)	CR	NPM	4	311	258	8/29/2016	E	1/4/17	PS	105	B	ON	M						Y		CR-NPM-A	**07**
Castle Rock (CR)	CR	NPM	5	327	292	8/29/2016	E	1/4/17	PS	114	B	ON	F						Y		CR-NPM-A	**07**
Olequa (OL)	OL	CTT	7	350	498	8/30/2016	E	1/10/17	PS, NM, JM	103	L	ON	M		4				N	NO ADIPOSE FIN PRESENT	OL-CTT-1	**08**
Olequa (OL)	OL	CTT	13	316	355	8/30/2016	E	1/10/17	PS, NM, JM	105	B	ON	F		4				N	NO ADIPOSE FIN PRESENT	OL-CTT-1	**08**
Olequa (OL)	OL	CTT	14	318	362	8/30/2016	E	1/10/17	PS, NM, JM	103	B	ON	F		4				N	NO ADIPOSE FIN PRESENT	OL-CTT-1	**08**
Olequa (OL)	OL	CTT	17	300	262	8/30/2016	E	1/10/17	PS, NM, JM	100		ON	F		4				N	ADIPOSE FIN PRESENT	OL-CTT-1	**08**
Olequa (OL)	OL	CTT	18	308	302	8/30/2016	E	1/10/17	PS, NM, JM	100	B	ON	F		4				N	ADIPOSE FIN PRESENT	OL-CTT-1	**08**
Olequa (OL)	OL	CTT	5	340	487	8/30/2016	E	1/10/17	PS, JM	101	L	ON	M		5	5			N	NO ADIPOSE FIN PRESENT	OL-CTT-2	**09**
Olequa (OL)	OL	CTT	6	332	435	8/30/2016	E	1/10/17	PS, JM	126	B	ON	F		5	6			N	NO ADIPOSE FIN PRESENT	OL-CTT-2	**09**
Olequa (OL)	OL	CTT	9	337	410	8/30/2016	E	1/10/17	PS, JM	137	B	ON	F		5	9			N	NO ADIPOSE FIN PRESENT	OL-CTT-2	**09**
Olequa (OL)	OL	CTT	10	326	422	8/30/2016	E	1/10/17	PS, JM	100	L	ON	F		5	10			N	NO ADIPOSE FIN PRESENT	OL-CTT-2	**09**
Olequa (OL)	OL	CTT	16	333	452	8/30/2016	E	1/10/17	PS, JM	104	B	ON	M		5	16			N	NO ADIPOSE FIN PRESENT	OL-CTT-2	**09**
Olequa (OL)	OL	LSS	6	416	720	8/30/2016	E	12/29/16	KS, NM	whole	whole	ON							Y		OL-LSS-2	**12**
Olequa (OL)	OL	LSS	7	485	1122	8/30/2016	E	12/29/16	KS, NM	whole	whole	ON							Y		OL-LSS-2	**12**
Olequa (OL)	OL	LSS	14	463	1021	8/30/2016	E	12/29/16	KS, NM	whole	whole	ON							Y		OL-LSS-2	**12**
Olequa (OL)	OL	LSS	27	486	1171	8/30/2016	E	12/29/16	KS, NM	whole	whole	ON							Y		OL-LSS-2	**12**
Olequa (OL)	OL	LSS	40	485	1057	8/30/2016	E	12/29/16	KS, NM	whole	whole	ON							Y		OL-LSS-2	**12**

Attachment 3. Supplemental Benchsheet for Processing Fillet and Carcass Tissue

Supplemental benchsheet for processing fillet and carcass tissue on selected samples.					
The ratio of contaminant concentration in fillets to whole fish is needed to estimate concentrations of contaminants in fillet tissue when only whole-fish data are available. Determining this ratio requires a mass balance approach using concentration and weight data from fillets and the remaining carcass. To account for changes in weight of each fish during processing (e.g. weight loss from scale removal), the weight of each fish and its various tissues should be recorded at selected steps in the processing procedure. Record the following weights to nearest gram for each fish processed within a composite sample for fillet/carcass analysis. Remember that the composite of fillets and composite of carcass tissue from the same group of fish are treated as two separate samples - so clean equipment is needed for each sample.					
Site					
Species					
Fish ID					
Field Weight (from field notes)					
Process Date					
Lab Weight (weigh fish prior to scale and slime removal)					
Whole fish after scales and DNA sample removed (ready to fillet)					
Fillet tissue ready for grinder (both fillets)					
Operculum					
Otoliths					
Spines					
Remaining carcass ready for grinder					
other					

Attachment 4. Sample Tag Labeling for Processed Fish Tissue Container.

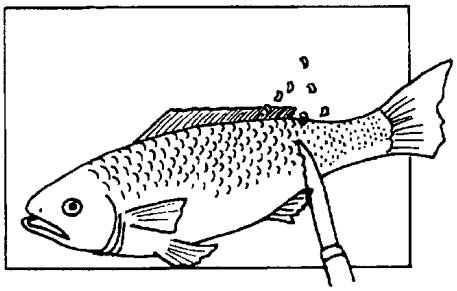
- Use custom printed labels or labels supplied with sample jars. If hand-writing labels use ballpoint pen or fine-tipped permanent marker (ink that will not run when wet).
- **MUST BE LEGIBLE!**



Attachment 5. Illustration of Basic Fish Filleting Procedure (U.S. EPA, 2000).

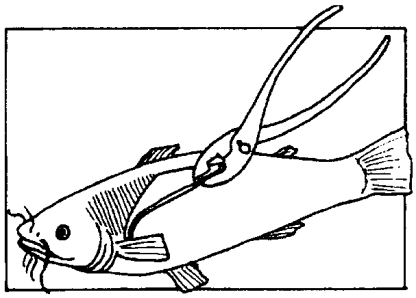
1 **Scaled Fish**

After removing the scales (by scraping with the edge of a knife) and rinsing the fish:



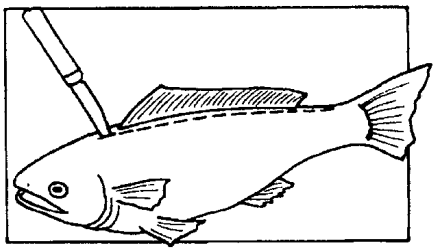
1b **Scaleless Fish**

Grasp the skin at the base of the head (preferably with pliers) and pull toward the tail.



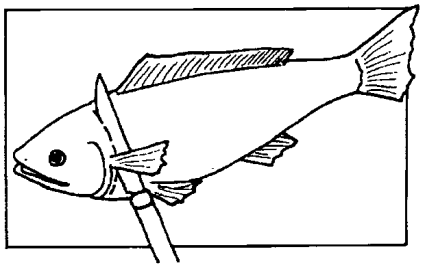
Note: This step applies only for catfish and other scaleless species.

2



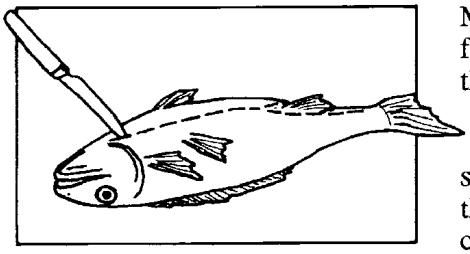
Make a shallow cut through the skin (on either side of the dorsal fin) from the top of the head to the base of the tail.

3



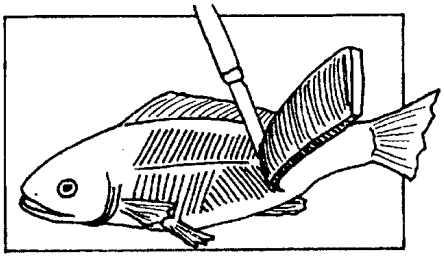
Make a cut behind the entire length of the gill cover, cutting through the skin and flesh to the bone.

4



Make a shallow cut along the belly from the base of the pectoral fin to the tail. A single cut is made from behind the gill cover to the anus and then a cut is made on both sides of the anal fin. Do not cut into the gut cavity as this may contaminate fillet tissues.

5



Remove the fillet.