

Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedures for Resecting DNA Samples and Aging for Finfish

Version 1.1

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Signatures on File.

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 Ecology uses an alternative methodology, procedure, or process.

SOP Revision History.

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
08/30/06	V1.0	SOP Publication		
10/19/10	V1.0	Three year review, minor changes		
10/21/10	V1.0	QA approval, recertified		
03/13/14	V1.0	Three year review, minor Changes		
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Environmental Assessment Program

Standard Operating Procedure for Resecting DNA Samples and Aging Structures.

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 DNA samples and structures for determining fish age in fish fin. The Washington State Department of Ecology (Ecology) works cooperatively with the Washington Department of Fish and Wildlife (WDFW) in the collection of fish DNA sample tissue and age structures. Age structures include scales, otoliths, operculum and spines from pectoral and/or dorsal fins.

2.0 Applicability

2.1 This procedure is to be followed by Ecology person(s) conducting fish tissue processing and at the time of processing the fish in part or as a whole.

3.0 Definitions

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

3.2 Caudal fin – The tail of fishes.

3.3 DNA – Deoxyribonucleic acid. A nucleic acid that carries the genetic information in the cell and is capable of self-replication and synthesis of RNA. DNA consists of two long chains of nucleotides twisted into a double helix and joined by hydrogen bonds between the complementary bases adenine and thymine or cytosine and guanine. The sequence of nucleotides determines individual hereditary characteristics (Wikipedia, 2014a).

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

3.5 EAP – Environmental Assessment Program.

3.6 Ecology – Washington State Department of Ecology.

3.7 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 3).

3.8 MSDS – Material Safety Data Sheets provides both workers and emergency personnel with the proper procedures for handling or working with a particular substance. MSDS's 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.

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

- 3.10 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.
- 3.11 Polymerase Chain Reaction (PCR) amplification – This is a molecular biological technique for amplifying (creating multiple copies of) DNA without using a living organism. The technique allows a small sample of DNA to be copied multiple times so it can be used for analysis. As the PCR reaction proceeds, the number of newly synthesized DNA strands increases exponentially so that after 20 to 30 cycles, the initial template is replicated several million-fold, thus facilitating its manipulation for further analysis such as sequencing, genotyping or probe labeling (Wikipedia, 2014b).
- 3.12 Pectoral fin – Either of the anterior pair of fins attached to the pectoral (mid) girdle of fishes.
- 3.13 Resecting – Surgical removal of all or part of an organ, tissue or structure.
- 3.14 USFWS – United States Fish and Wildlife Service
- 3.15 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 Ecology Chemical Hygiene Plan and Hazardous Materials Management Plan (Section 1) (WA State Department of Ecology, 2011), which includes a Laboratory Safety Orientation, Job-Specific Orientation and must know Chemical Safety Procedures and follow the Standard Operating Procedures (Section 16).
- 4.2 Read WDFW handout “Tissue Sampling for DNA Analysis” prior to processing. Should also work with experienced individual for hands on training.

5.0 Equipment, Reagents, and Supplies

- 5.1 DNA sampling supplies are obtained from the WDFW Genetics Laboratory by contacting Todd Kassler 360-902-2722. Please see Section 6.0 for more details. These supplies include:
 - 5.1.1 2ml screw-cap cryovials.
 - 5.1.2 Sample boxes.
 - 5.1.3 DNA preservative solution of ethanol 100 proof – NO SUBSTITUTES ACCEPTED. See Attachment 5 for MSDS and concentration grade. See Section 4.1 for safety requirements.

- 5.1.4 Labels for vials. Labels are laser printed on acid resistant paper and contain a four-digit WDFW code (e.g., “CD05”). Never use labels for a sample set different from the originally assigned set. Unused labels should be destroyed or returned to the Genetics Lab. New labels are required for each processing season.
- 5.1.5 WDFW scale cards.
- 5.1.6 Aging structure envelopes supplied by WDFW or a commercial supplier.
- 5.1.7 Otolith sample trays.
- 5.1.8 Small dissecting scissors.
- 5.1.9 Fine point, flat and rounded forceps.
- 5.1.10 Butcher knife with 6-8 inch stainless steel blade.
- 5.1.11 Marking pens, pencils, pencil sharpener, permanent markers.
- 5.1.12 Talc-free nitrile exam gloves.
- 5.1.13 Spine resection tools.
- 5.1.14 Needle nose pliers for spine resection.
- 5.1.15 Pliers for spine resection.
- 5.1.16 Wire clippers for spine or large fish opercle resection.
- 5.1.17 Cleaning equipment for age structures.
- 5.1.18 Microwave.
- 5.1.19 Casserole dish with glass lid.
- 5.1.20 Toothbrushes.
- 5.1.21 Forceps.
- 5.1.22 Paper towels.
- 5.1.23 Garbage can.
- 5.1.24 Processing Bench Sheet – A table, usually created in Excel®, used to plan and document sample processing data for each fish collected (Attachment 2).
- 5.1.25 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 3).

6.0 Procedure

6.1 DNA Sampling.

- 6.1.1 DNA Sampling Preparation. In an effort to streamline Ecology's fish processing, DNA samples are only collected when requested by WDFW, USFWS, or stated in permit conditions. Individual project managers typically decide if timeframes and staffing allow for this extra step in processing.
- 6.1.1.1 BEFORE BEGINNING DNA SAMPLING, Please talk to:
Todd Kassler (office phone: 360-902-2722; email: todd.kassler@dfw.wa.gov) or;
Sewall Young (office phone: 360-902-2773; email: sewall.young@dfw.wa.gov).
- 6.1.1.2 Inform them of the anticipated workload and discuss potential costs involved. Make arrangements for supplies.
- 6.1.1.3 READ WDFW DNA Sampling Summary document entitled "Tissue Sampling for DNA Analysis" (Attachment 1).
- 6.1.1.4 Formulate processing plan: timing, location, staff resources and equipment.
- 6.1.1.5 Prepare and print Processing Bench Sheet to record DNA sample data for fish to be processed. See Ecology's SOP #007 *Procedures for Resecting Finfish Whole Body, Body Parts, or Tissue Samples* Section 7.0 for record keeping details, and Attachment 2 of this SOP for an example of the Bench Sheet.
- 6.1.1.6 Prepare and print Lab Analysis & Tracking Plan spreadsheet for samples to be processed. See Ecology's SOP #007 *Procedures for Resecting Finfish Whole Body, Body Parts, or Tissue Samples* Section 7.0 for record keeping details, and Attachment 3 of this SOP for an example of the Lab Analysis & Tracking Plan.
- 6.1.1.7 Clean dissecting areas, sampling instruments and hands with mild soap and water before beginning DNA collection process. Rinse hands and sampling equipment with DI water between each specimen or as frequently as necessary to avoid sample-to-sample contamination. It is also important to wipe dry sampling equipment and hands after rinsing. This prevents sample to sample contamination and avoids tissue deterioration from water. Because all the DNA analyses involve Polymerase Chain Reaction (PCR) amplification of the DNA extracted from the tissue samples, sample-to-sample contamination can be a problem and must be avoided. Nevertheless, it is not necessary to wear gloves during the dissection process to avoid contamination of the samples unless part of another more restrictive process. However, gloves are recommended as this procedure to date has always been part of another sampling process which requires that gloves be worn. If DNA resection is part of another process, see Ecology's SOP #007 *Procedures for Resecting Finfish Whole Body, Body Parts, or Tissue Samples*.

6.1.1.8 Prepare sample box of 100 (or as many needed) 2ml screw-cap cryovials by inserting a preprinted label in each vial and filling each vial with the DNA preservative ethanol solution using a small dispensing bottle. Gloves and safety glasses should be worn when handling preservative solution (See Attachment 5 for MSDS and concentration grade. See Section 4.1 for safety requirements). **DO NOT USE INK ON ANY LABELS** inside or outside of the vials; the preservative solution will dissolve the ink. Pencil on “write-in-the-rain” paper identifying the sample may be used in an emergency.

6.1.2 DNA Sample Resection.

6.1.2.1 Clip approximately 1-2cm² (about the size of your little finger nail) piece of tissue from the distal end of the caudal, dorsal or pectoral fin (Figure 1). Although a sample collected from an opercle may also be used, within a given study, it is best to use the same tissue source for all samples. For fin-clip or opercle samples, the DNA will actually be extracted primarily from the epithelial cells covering the surface; therefore, it is imperative that there is a reasonably intact layer of skin covering the tissue sample available. Avoid significant abrasion or freezer-burned tissue for sampling. By cutting further into a fin and taking an inside clip rather than on the tip of the fin, the chances of skin covering damage may be minimized.



Figure 1. Fin clip for DNA analysis.

6.1.2.2 Place tissue sample from each specimen in a 2 ml screw-cap cryovial filled with DNA preservative solution, (ethanol), and a label immediately after dissection (Figure 2). Caps should be securely tightened on the vials, but not over-tightened. It is critical that the volume ratio of tissue to preservative not exceed 1:4 (20% tissue: 80% preservative). An excess of preservative is okay.



Figure 2. Placing clipped fin tissue into ethanol solution in sequenced cryovial.

- 6.1.2.3 Begin loading vials in the storage boxes in the back left corner cell (A1) and proceed from left to right and back to front forward to the front right corner cell (J10). Thus, for a collection of 100 fish DNA samples consecutively numbered from 1-100: sample #1 should be placed in cell A1, sample #2 should be in cell A2, ... sample #10 should be in cell A10, sample #11 should be in cell B1, ... sample #20 should be in cell B10, sample #91 should be in cell J1, ... sample #100 should be in cell J10. Note that one collection of up to 100 samples or two collections of up to 50 samples each (or several smaller collections) can be stored in a single box. Also note that Ecology fish collection projects have typically collected DNA under one sampling plan for WDFW, therefore, all collections of DNA samples can and should be combined consecutively rather than separate boxes per project unless otherwise instructed.
- 6.1.2.4 Document that each DNA sample was collected at the time of each resection on the lab bench sheet. Under the field titled “WDFW DNA ID (code),” enter the number of the vial for each DNA sample matching the correct “Waterbody” and “ECY Field ID.” In the field titled “DNA Taken,” enter “Y” for yes. Sampling data should be cross-referenced to the location of the site where the fish was collected and field identification.
- 6.1.2.5 Verify documentation of samples to location, that each vial is filled with DNA preservative solution and that the caps are secured so the solution won’t evaporate causing sample deterioration.
- 6.1.2.6 Store all vials containing DNA samples in the storage boxes provided by WDFW at room temperature in the Hazardous Chemical Storage Room (room # OL-14). Do not put tape on the individual vials or write on them. If you need to add a label, write it in pencil on a piece of paper and put it inside the top of the storage box.
- 6.2 Aging Structure Resection
- 6.2.1 Preparation for Aging Structure Resection: **CONTACT WDFW BIOLOGISTS WHO DO FISH AGING SEVERAL MONTHS BEFORE FISH COLLECTIONS.** Discuss your sampling plans, target numbers and species of fish. Also discuss timeframes for sampling, getting age structures to them, and when you need the age data returned.

- 6.2.2 Prior to fish collections, please contact:
 Lucinda Morrow (office phone: 360-902-2763; Lab phone: 360-902-2859; email: lucinda.morrow@dfw.wa.gov) or;
 Lance Campbell (office phone: 360-902-2725; email: lance.campbell@dfw.wa.gov).
- 6.2.2.1 Clean dissecting areas and sampling instruments with mild soap and water before you begin. Rinse hands and sampling equipment between specimens or as frequently as necessary to avoid sample-to-sample contamination. If aging structures resection is part of another process, see Ecology's SOP #007 *Procedures for Resecting Finfish Whole Body, Body Parts, or Tissue Samples*.
- 6.2.2.2 Refer to the "Fish Aging Structure" spreadsheet for the type of age structure to resect per fish species (Attachment 4). Aging structures indicated by an "X" need collected per fish sample. Please pay close attention to footnotes regarding "first choice" structures vs. secondary (back up) structures.
- 6.2.2.3 Pre-label aging structure collection containers.
- 6.2.2.3.1 Label scale cards using a pencil with fish identification (ID) numbers, collection date, species, length (mm), weight (g), collection site water name (write out full name of water body or site of collection), collection month and year (i.e. 11/05), and card number, (which is the consecutive number for the season starting with the number 1) (Figure 3). The information needed to fill out scale cards can be found on the projects current Processing Bench Sheet. If the sample length and weight data is absent from the Processing Bench Sheet, the individual fish will need to be measured and weighed prior to processing. Clip prepared scale cards together in consecutive order and store in zip lock bag. Label bag with project name and year of collection.

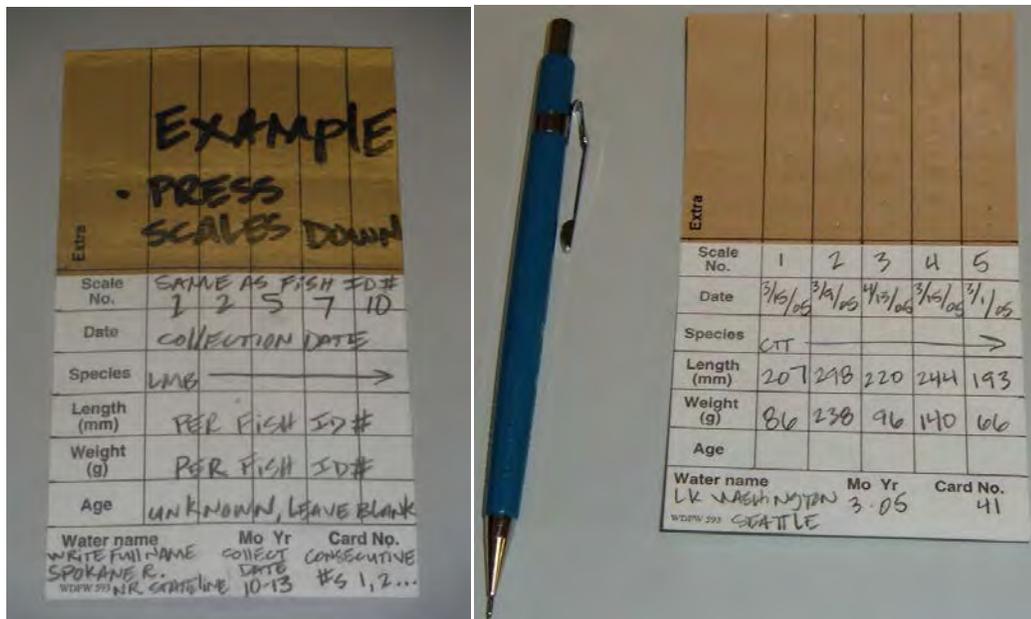


Figure 3. Fish scale card instructions and example.

- 6.2.2.3.2 Label aging structure envelopes with the collection site name (water body name), species and fish ID number (abbreviated), collection date, type of aging structures collected (spines or operculum), and sample number (Figure 4). See current bench sheet for fish type abbreviation. Label a quart size zip lock bag with the project name, site name, collection date, species and structures collected. Keep structures organized together by collection site and composite groups. Place structure envelopes in bag and store in freezer.

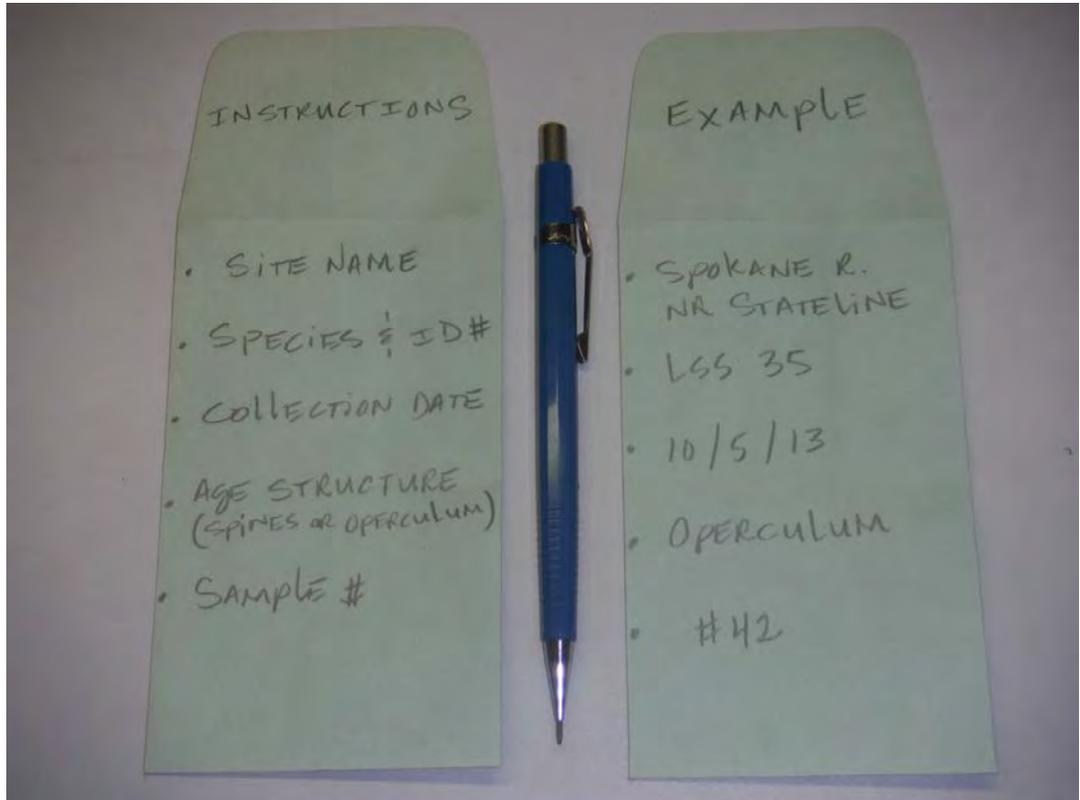


Figure 4. Fish aging structure envelope instructions and example.

- 6.2.2.3.3 Label otolith trays using a permanent felt-tip marker on the end of tray. Make sure the tray is right side up and include the following information: project name, type of fish (abbreviated), year (i.e. 13) and otolith tray number. See current bench sheet for fish type abbreviation.
- 6.2.2 Aging Structure Sample Resection.
- 6.2.2.1 Scales resection.
- 6.2.2.1.1 Locate scale removal area per fish species on fish diagram in Attachment 6.
- 6.2.2.1.2 Wipe off excessive slime. Using forceps grab scale and pull toward caudal fin to remove.

- 6.2.2.1.3 Mount on scale card in the row matching fish ID. Situate scales so the outside (side exposed to elements) of scale is facing up and the posterior end of scale is toward the top of card. **PRESS DOWN ON THE SCALE** to secure the scale to the acetate card. Scales tend to curl when dried and therefore detach themselves from the card and fall off. Sometimes adding a bit of moisture (a wet finger) to the scale when pressing them down will help to secure them.
- 6.2.2.1.4 Repeat process for a minimum of five to seven scales per fish. If there is room on the scale card, collect up to ten scales per fish for comparison by the fish biologist.
- 6.2.2.1.5 Place prepared scale card with other cards in numerical order with the most current (largest number) on top in a pre-labeled zip seal bag. This exposes the last number done and is used as a reference starting number for the next scale card to be processed.
- 6.2.2.1.6 Verify & document that the scales per fish on the scale card match the same fish ID on the Processing Bench Sheet.
- 6.2.2.1.7 Record scale card number and scale numbers on Processing Bench Sheet.
- 6.2.2.2 Opercle resection.
 - 6.2.2.2.1 Bend opercle towards head of fish to break then tear off using your fingers. Larger fish may need to be cut off using cutters. Collect operculum on both sides of fish.
 - 6.2.2.2.2 Place operculum in pre-labeled envelope matching the fish ID. All samples for one site can be placed in a pre-labeled zip seal bag and placed in freezer to prevent decomposition until cleaned.
 - 6.2.2.2.3 Verify & document that the operculum per fish in the envelope match the same fish ID on the Processing Bench Sheet.
- 6.2.2.3 Spine resection.
 - 6.2.2.3.1 Determine spine resection type per fish species by referring to the “Fish Aging Structure” spreadsheet for aging structure type (Attachment 4).
 - 6.2.2.3.2 When pulling spines it is important to get the whole spine intact, including the joint articulation below tissue surface. Using a pair of wire clippers and scalpel, cut around the base of spine until joint articulation and spine become free from body. This process should be performed by an experienced individual.
 - 6.2.2.3.3 Place spines in pre-labeled envelope matching the fish ID. All envelopes for one site can be placed in a pre-labeled zip seal bag and placed in freezer to prevent decomposition until cleaned.

6.2.2.3.4 Verify & document that the spines per fish in the envelope match the same fish ID on the Processing Bench Sheet.

6.2.2.4 Otolith resection. There are several techniques used for otolith removal. Ecology employees have found the following techniques to be very predictable at retrieving otoliths from a variety of species.

6.2.2.4.1 Using a sharp fillet knife, the first incision should be a vertical cut in line with the posterior end of gill plate cover. This incision should stop at eye level (Figure 5B). The second incision should be a parallel cut just above the eyes, starting where first incision ended (Figure 5C). This incision should create a flap of tissue that can be folded aside to expose the brain. The largest of typically 3 pairs of otoliths, (the saggital otoliths), are located just below the brain on each side of the brain cavity. These otoliths are situated in a protective area that resembles apple seed pits (Figure 5A). A rough landmark for otoliths location is mid-way between the eye and posterior end of the opercula.

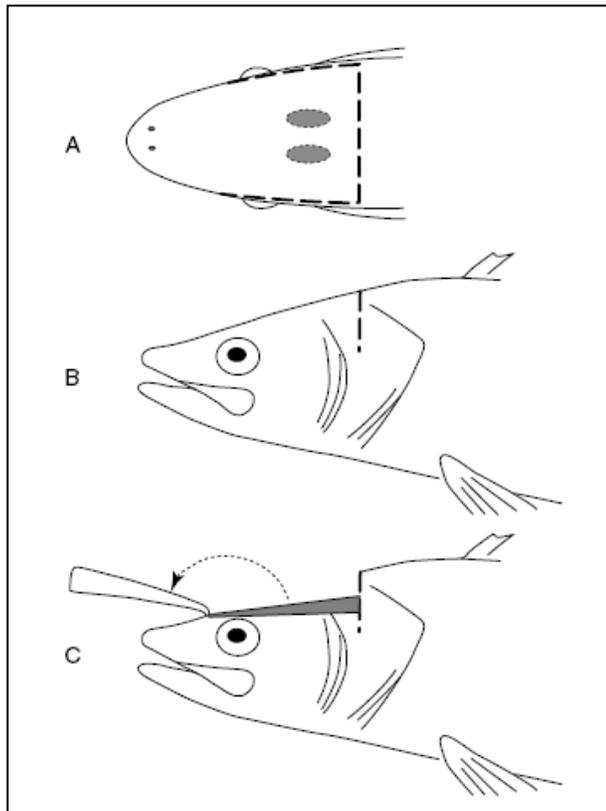


Figure 5. Extraction procedure for walleye sagittal otoliths. A. Positioning of otoliths and location of cuttings. B. Initial cut. C. Second cut to expose brain, otoliths lie beneath brain in fleshy cavities (Fieler, 2002).

6.2.2.4.2 Remove the brain tissue with forceps carefully so that both otoliths are exposed towards the back of the brain. They may appear sitting in a divot much like apple seeds sit when an apple core is sliced in half lengthwise (Figure 6).



Figure 6. Otolith removal from a cod (Miljolare.no, 2006).

- 6.2.2.4.3 Extract the otoliths using fine-tipped forceps. Be very careful not to force the otolith out as otoliths tend to break easily. If frozen or unable to see clearly, gently squirt some water into the brain cavity to thaw and clean. A bowl of water can be useful in thawing and cleaning the head. Also, if the otolith cannot be found and the “sockets” are clearly empty or destroyed, rinsing the head in a bowl of water and then “panning” for the otoliths has brought some retrieval success. Remove any tissue so that otoliths are clean.
- 6.2.2.4.4 Place both otoliths from the same fish sample in the same otolith tray cell.
- 6.2.2.4.5 Record the cell number and otolith tray number on the Processing Benchsheet for each sample. Validate that each otolith sample recorded on the Processing Benchsheet matches the correct fish sample ID tag.
- 6.2.3 Cleaning Operculum and Spines.
 - 6.2.3.1 Cut corners off envelope. Be careful not to cut aging structure. Keep holes small enough so structures do not come out of envelope while cooking.
 - 6.2.3.2 Fill casserole dish $\frac{1}{2}$ to $\frac{3}{4}$ full of water.
 - 6.2.3.3 Place 10 – 15 envelopes in casserole dish and cover with glass lid.
 - 6.2.3.4 Place casserole dish with contents in microwave and cook for roughly 4-5 minutes on high power. May need to adjust cook time up or down depending on ability to remove tissue from structure.
 - 6.2.3.5 When cooking is complete, pull casserole dish out of microwave. **BE CAREFUL, VERY HOT.** Place casserole dish next to sink. Leave envelopes in hot water to soak. Work on one envelope at a time.

- 6.2.3.6 Scrub operculum and spines with toothbrush. Make sure to get ALL tissue off structure. Be careful not to break structure while scrubbing.
- 6.2.3.7 Once structure is cleaned and rinsed off, place envelope and structure on counter top to dry. It is important to keep structure with assigned envelope as to keep identification correct.
- 6.2.3.8 Repeat Sections 6.2.3.1 through 6.2.3.7 until all structures are cleaned.
- 6.2.3.9 Place structures back into their original envelope once structures and envelopes are approximately half dry.
- 6.2.3.10 Keep samples together in foil box. Label foil box with project name and age structures (e.g. FFCMP13 AGE STRUCTURES). No need to put structures in freezer once they are cleaned.
- 6.2.3.11 Store structures in bottle room (room # OL-17) until ready for delivery to WDFW.

6.3 DNA and Aging Structure Samples Delivery to the lab.

6.3.1 It is important to keep in contact with WDFW to coordinate and communicate project needs. As collections are completed or at the end of the sampling season, personally deliver the DNA and aging structure samples to the WDFW Genetic Laboratory and Ecological Services respectively. Prior to delivery, set up a drop off appointment and include the following items: otolith trays, scale cards, spine/operculum envelopes, DNA sample boxes and vials containing tissue, all unused DNA sampling equipment and appropriate spreadsheets with needed information to process samples.

6.3.2 Deliver the DNA structure samples to WDFW Genetic Laboratory in Olympia, WA at:

WDFW Genetics Laboratory
Natural Resource Building, Rm 665
1111 Washington Street SE
Olympia, WA 98504
Attn. Todd Kassler

6.3.3 Deliver the aging structures to Ecological Services in Olympia, WA at:

Ecological Services
Natural Resource Building
1111 Washington Street SE
Olympia, WA 98504
Attn. Lucinda Morrow

6.3.4 It is best to hand-deliver the samples rather than shipping. This eliminates the possibility of samples being damaged or lost during shipping. If personal delivery is not possible, contact the WDFW Lab and set up shipping arrangements prior to sending samples. WDFW contact information for aging structures is:

Lucinda Morrow
360-902-2763

7.0 Records Management

7.1 The Lab Analysis & Tracking Plan and Processing Benchsheet are tables, usually created in Excel®, used to document and coordinate all activities and data for single or multiple projects per collection and sampling time period and for documenting the tissue sample preparation for lab analysis. See Ecology's SOP #007 *Procedures for Resecting Finfish Whole Body, Body Parts, or Tissue Samples* Section 7.0 for more information about these two forms.

7.1.1 Bench sheet - A template of the Processing Benchsheet is located on the Ecology Intranet at Y:\SHARED Files\TSU Fish\SOP forms for Fish\filename of template (See Attachment 2). The name of the file should include the project sampling year, the

words or abbreviation indicating “Bench Sheet” and the version number, (e.g. 2005 Bench Sheet 2.xls). Enter all hard copy documentation into the master electronic Processing Benchsheet. It is important to ensure that the electronic data is valid for future use. The individual responsible for entering Processing Benchsheet updates must verify the accuracy of the data and sign off on each hard copy Processing Benchsheet (e.g. write the word *Entered, the date and individual’s initials*). By signing the hard copy, this states data is correct and entered into the master electronic Processing Benchsheet.

- 7.1.2 Lab Analysis & Tracking Plan - A template of the lab tracking plan is located on Ecology Intranet at Y:\SHARED Files\ TSU Fish\SOP forms for Fish\filename of template (See Attachment 3). 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. 2005 fish labplan tracking 2.xls). Enter all hard copy documentation into the master electronic Lab Analysis & Tracking Plan. As with the Processing Benchsheet, it is important to ensure that the electronic data is valid for future use. The individual responsible for entering Lab Analysis & Tracking Plan updates must verify the accuracy of the data and sign off on each hard copy Lab Analysis & Tracking Plan (e.g. write the word *Entered, the date and individual’s initials*). By signing the hard copy, this states data is correct and entered into the master electronic Lab Analysis & Tracking Plan.

8.0 Quality Control and Quality Assurance Section

- 8.1 Verify that all information is filled out on the hard copy bench sheet. There should not be any blank cells under fields labeled with WDFW DNA ID, DNA Taken, scale card #, scale #, otolith tray #, otolith cell #, opercle or spine taken. Be sure to document the process date and crew initials.
- 8.2 Verify sample number, species, date of collection and any other pertinent information on the scale card, aging structure envelopes, zip seal bags and otolith tray.
- 8.3 Check scales on scale card making sure they are attached. If starting to curl and become loose, rewet the scale with wet finger and press them down again on the card.
- 8.4 Verify all hard copy and electronic documentation entered on the Processing Benchsheet and Lab Analysis & Tracking Plan for completeness and accuracy.

9.0 Safety

- 9.1 Wear gloves and safety glasses when handling the DNA preservative solution.

- 9.2 Preferably, gloves should be worn to avoid exposure to bacterial pathogens when handling fish samples for DNA resection. Gloves are required for aging structure resection. Hands should be cleaned using soap and clean water after completing work or any time hands become soiled during the process. Gloves should be changed whenever they get torn or punctured, anytime used gloves are removed from hands, and between individual or composite samples.
- 9.3 Extreme care must be taken when using all knives, not only for you but for others that may be working in close proximity. Verify that the first aid kit is available in the lab room and that the contents are complete. Contact the room supervisor and/or the safety officer if any accident occurs, first aid supplies are inadequate, chemical spills or any other need or questions. The names and numbers of the room supervisor are posted in the room. Work with a “buddy” if possible, or at least notify a coworker of your lab work plans to and put them on your calendar.

10.0 References

- 10.1 Aaper Alcohol. 2006. Aaper Alcohol and Chemical Company (Note this business no longer exists, although MSDS still applies in Attachment 5).
- 10.2 American Fisheries Society (AFS). 1983. *Fisheries Techniques*. Edited by Nielsen, L.A and D. L. Johnson. Columbus, Ohio.
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- 10.9 Sneva, J. 2005. WDFW biologist. Personal communication with B. Era-Miller, Dept. of Ecology.
- 10.10 University of Washington (UW). 2006. *Fish Collection*.
<http://www.burkemuseum.org/ichthyology/FishKey/diagram1.gif>. Accessed April 2014.
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Considerations & Guidelines for Collecting Representative Samples for Genetic Analysis

The two most important goals of field sampling for genetic analysis are: 1) to obtain tissue samples with little or no biochemical degradation, so that the genetic markers to be screened can be successfully analyzed and 2) to obtain samples from individuals that are representative of the target population in terms of age, sex, location, and timing.

If the samples are so degraded because of post-mortem tissue decomposition that they cannot be successfully analyzed to provide reliable genetic data, the time and money spent collecting and analyzing the samples will be wasted. At the same time, if the collection of samples that is obtained is not representative of the population as a whole, the genetic data collected may not provide an accurate profile of the target population and may, therefore, be misleading or useless.

Tissue Quality

Tissue samples obtained from live fish (or fish that have just been sacrificed) are of the highest quality, while those from relatively fresh carcasses are also suitable for genetic analysis. However, tissue samples from animals that have been dead for a considerable period of time and have experienced significant decomposition invariably yield little or no useful data. Poor quality carcasses should NOT be genetically sampled.

Individuals separately IDed (to retain association of individual-specific genetic & biological data):

Wherever possible, samples should consist of a piece of fin or opercle tissue from each fish approximately 1-2 cm² in size). [*Note that the tissue sample can be a fin clip sample obtained with scissors, a series of 2-4 punches obtained using a standard 1/4" diameter paper punch, or a sample of a tissue such as muscle, heart, or liver*]. Within a given study, it is best to use the same tissue source for all samples. For fin-clip or opercle samples, the DNA will actually be extracted primarily from the epithelial cells covering the surface, therefore, it is imperative that there is a reasonably intact layer of skin covering the tissue sample -- it should not be significantly abraded. If fin will be sampled and survival of the fish is not an issue, we recommend sampling the distal end of the caudal, dorsal, or pectoral fin. When it is not feasible to obtain samples as large as 1-2 cm² (e.g., non-lethal sampling of fry or pre-smolts), a smaller piece of fin (perhaps as small as 0.5 cm x 0.5cm) should be adequate. Because partial fin clips regenerate, whereas total fin amputations typically do not, we recommend obtaining partial fin clips from two fins (if necessary to get the desired amount of tissue) rather than complete removal of one fin, for non-lethal sampling of small fish.

The tissue sample from each specimen should be placed in a 2 mL screw-cap cryovial (filled with DNA preservative solution and a label) immediately after dissection. Caps should be securely tightened on the vials (but not over-tightened) and the sample vials should be stored upright at room temperature (do not freeze).

Each cryovial should contain a small laser-printed label on **write-in-the-rain paper** that gives a 4-digit WDFW collection code issued to each species and location each year (e.g., "03CY") and the individual fish number [or, in an emergency, a pencil label identifying the sample]. Printed labels are

provided by the WDFW Genetics Lab. **NOTE: DO NOT USE INK ON ANY LABELS inside or outside of the vials; the preservative solution will dissolve the ink.**

Each set of tissue samples from a single locality/date should be accompanied by collection data (completed WDFW scale cards, WDFW Genetic Sampling Field Data Sheets, or another suitable form). Sampling data should be cross-referenced either to a map annotated with sampling locations or to GPS coordinates (for the site of collection of each fish, if possible) wherever possible.

Please store all vials containing DNA samples in the plastic sample storage boxes provided. Before placing vials containing tissue samples in the storage boxes, please verify that the vials are filled with

DNA preservative solution and that the caps are securely tightened; but do not over tighten.

Begin loading vials in the storage boxes in the back left corner cell (A1) and proceed from left to right and back to front to the front right corner cell (J10). Thus, for a collection of 100 fish, consecutively numbered from 1-100: sample #1 should be placed in cell A1, sample #2 should be in cell A2, ... sample #10 should be in cell A10, sample #11 should be in cell B1, ... sample #20 should be in cell B10 sample #91 should be in cell J1, ... sample #100 should be in cell J10). Note that one collection of up to 100 samples or two collections of up to 50 samples each (or several smaller collections) can be stored in a single box. The storage boxes should always be stored upright at room temperature until they are returned to the Genetics Lab in Olympia (as per instructions). Do not put tape on the boxes or on the individual vials or write on them. If you need to add a label, write it (in pencil) on a piece of paper and put it inside the top of the storage box.

Tissue Preservation - DNA Analysis

Whatever the state of the animal to be sampled is, it is imperative to be sure to preserve the tissue samples as soon as possible after they are taken. In the case of tissue samples for DNA analysis, we use a special ethanol preservative solution. This preservative, which is a *poison* and is *flammable*, should be obtained from the WDFW Genetics Lab. The solution preserves the DNA by dehydrating the tissue. Thus, **it is critical that the volume ratio of tissue to preservative not exceed 4 parts of preservative for every 1 part of tissue.** One approach to achieve this is to fill the sampling vial 4/5 full of preservative solution and then add small pieces of tissue until the solution reaches the top of the vial. Several small pieces of tissue are preferable to a single large piece because the preservative solution can penetrate the small pieces more quickly, thereby achieving better preservation. Note, an excess of preservative solution is okay; not using enough preservative is a problem. Once in this preservative, tissue samples can be stored at room temperature. Be aware that the DNA preservative solution will dissolve ink; therefore labels in, on, and around the DNA sample vials should either be printed on a laser printer or written in pencil!

Sampling instruments, dissecting areas, and your hands should be kept clean (rinsed in fresh water between specimens or as frequently as necessary) to avoid sample-to-sample contamination and blotted dry after rinsing to minimize dilution of the DNA preservative solution. Because all our DNA analyses involve PCR amplification of the DNA extracted from the tissue samples, sample-to-sample contamination can be a problem and must be avoided. Nevertheless, it is not necessary to wear gloves during the dissection process to avoid contamination of the samples -- just keep your hands, the sampling instruments, and the work area clean.

Representative Sampling

Obtaining samples that are representative of the target population is a very important, but sometimes difficult goal to achieve.

Adult Fish - from a single population

The goal here should be to obtain a collection of samples (approx. 100 fish to provide adequate statistical power) that is representative of the target population (e.g., with regard to sex, age, location, and return/spawn timing). Sampling adults on the spawning grounds at, or immediately after, spawning is the surest way to obtain a collection that is not contaminated with individuals from other populations.

Because most populations have an approximate 50:50 sex ratio, collections should consist of about 50% males and 50% females. Similarly, if a population consists of 3, 4, and 5 year old spawners, the collection should have all of these age classes represented in approximately the same proportions as they occur in the population at large. In the same way, if a population spawns throughout a 10 mile stretch of river over approximately 1 month, the collection should ideally include approximately proportional samples over the same geographic range and time frame. Because of cost and logistical constraints, this might actually translate into obtaining samples on 3 different days at four locations distributed throughout the 10 miles of spawning habitat. A collection of samples as described above provides the best opportunity to obtain an accurate genetic profile of the target population.

Representative (proportional) sampling of spawning adults from a hatchery (or natural) population over time is schematically shown in Figures 1A & B below.

Smolts, Juveniles, and Fry - from a single population

Although the goal here is also to obtain a collection of samples that is representative of the target population, the approach and concerns are somewhat different than those described above for adult sampling. Here there are four, sometimes conflicting sampling concerns. One is to obtain a sample that is representative of the entire population. A second is to be sure to avoid sampling a mixture of fish from two or more populations. A third is to avoid sampling sibs (offspring from a single mating), and half-sibs. A fourth, for DNA-based investigations, is often to sample non-lethally. As a result, the actual sampling design is usually an attempt to balance these concerns. Sampling small, newly emerged fry from locations where adult spawning occurred maximizes the likelihood of obtaining a collection from the target population (and only that population) but this approach maximizes the likelihood that the collection will contain sibs or half-sibs and that the tissue sampling will be lethal or at least detrimental to survival (because of the small size of the fish). Sampling larger juveniles, which have presumably moved around substantially after hatching and emergence from the gravel reduces the likelihood of sampling sibs and, for DNA studies, increases the likelihood that the sampling will not affect subsequent survival (because the fish are larger), but increases the likelihood of obtaining a mixed collection of fish from multiple populations.

Sampling fry or pre-smolts from a hatchery should be designed to include representative samples of progeny from all egg takes/parents. Taking proportional subsamples from each of the rearing vessels used for the population is one aspect of this. Sampling outmigrating smolts to characterize a single

population should be stratified over space and time to achieve a representative collection (see Figure 1A & B below).

Sampling Mixtures (e.g., fishery sampling)

Again, the ultimate goal is to obtain a collection of samples (mixture sample sizes of several hundred are often needed to provide the desired accuracy and precision) that is representative of the mixture you are trying to characterize (whether this be a mixed-stock fishery sample of adult salmon or a sample of a mixture of outmigrating smolts from multiple populations). The goal is to obtain representative numbers of the various components of the mixture. For a fishery, some of the key factors may be: location and date of catch, type of gear or proportions of gear types, and size and sex of fish. For a collection of smolts, the key objectives may be to sample proportionally over the entire period of outmigration, to sample all size classes, and to sample during both day and night.

DNA

When obtaining a non-lethal sample from a small fish, a small sample should be taken. The **minimum amount of tissue** that is needed for DNA analysis is approximately the size of this circle: ● (e.g., a piece of tissue with the same approximate surface area as a 1.5mm diameter disc). The recommended sources of such a tissue sample are any of the following:

- 1) A distal portion of the dorsal lobe of the caudal fin
- 2) A distal portion of one of the pelvic fins
- 3) Smaller distal portions of both pelvic fins
- 4) One entire pelvic fin

By sampling only the distal portion of a fin, we expect that the fish will successfully regenerate the entire fin over time. In contrast, removing an entire fin often results in little or no fin regeneration, presumably leaving the fish at a selective disadvantage.

Larger samples are preferred whenever possible (e.g., a piece of tissue approximately the size of this circle: ●); or even as much as approximately 1-2 cm² area), because this will provide more material (DNA). The “extra” tissue provides a reserve that can be used to overcome some types of analytical problems in the lab by repeated analysis and/or it also provides material that can be used for subsequent analyses (for example to examine additional loci at a future date) or to share with other laboratories/agencies.

BEFORE BEGINNING SAMPLING, please talk to:

Genetics Lab (360-902-2775)

Todd Kassler (office phone: 360-902-2722; email: todd.kassler@dfw.wa.gov)

Obtaining Sampling Supplies & Sampling Kits:

For WDFW projects or collaboratives, supplies such as cryovials & screw-caps, sample boxes, DNA preservative solution, labels, WDFW genetics-style scale cards and/or WDFW Genetic Field Collection Data Sheets, and complete sampling kits can be obtained from the WDFW Genetics

Laboratory (see below) by contacting Todd Kassler or Genetics Lab. **Never use labels for a collection/stock different from the one the labels were originally assigned to. Do not retain labels for use in future years. Unused labels should be destroyed or returned to the Genetics Lab.**

Delivery of Samples to the WDFW Genetics Laboratory:

Whenever possible, it is best to hand deliver samples rather than ship them, both because this eliminates the possibility of loss of the samples and because there are restrictions on shipping the preservative solution. As collections are completed, or at the end of the sampling season, samples including accompanying scale cards, field collection data sheets, locality information appropriate to the samples, and **all unused sampling supplies** should be delivered to the lab at:

WDFW Genetics Laboratory
Natural Resources Building, Rm 665
1111 Washington Street SE
Olympia, WA 98504

Before shipping any samples, please contact the lab so they will expect the shipment and can initiate a search with the shipper if the samples do not arrive on time.

Attachment 2. Processing Bench Sheet, (example only)

Note: The Bench Sheet 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.

2009 WSTMP Fish Field and Bench Processing Data																				Processing crew:				
Sort #1: Waterbody, Species, TL descending or ascending										Sort #2: Waterbody, Species, ECY Field ID														
Waterbody	ECY Field ID	Species	WDFW Field ID	WDFW DNA ID (CD10-)	DNA Taken Y/N ¹	Total Length (mm)	Weight (gm)	FCI	Collect Date	Collect Method	Process date	Fillet weight (gm)	L, R, or B fillet	Skin status On/Off	Sex M/F	Fish age	Scale card #	Scale #	otolith tray #	otolith cell #	Opercula or Spines taken Y/N	Comment	WSTMP LAR Field ID	WSTMP MEL Lab #
Whatcom Lk	1	SMB	-	652	Y	443	1420	1.63	10/6/09	ecy-G	12/3/09	203	L	ON	F	7	9	1	3	11	N	Do as indiv for HG Trend. Also do as 5 fish comp for WSTMP. All caught in SW end of Lk. See LTS for more details.	WHATSMB	12
Whatcom Lk	2	SMB	-	646	Y	372	764	1.48	10/6/09	ecy-G	12/3/09	136	L	ON	F	5	9	2	3	12	N	Do as indiv for HG Trend. Also do as 5 fish comp for WSTMP. All caught in SW end of Lk. See LTS for more details.	WHATSMB	12
Whatcom Lk	3	SMB	-	647	Y	375	790	1.50	10/6/09	ecy-G	12/3/09	124	L	ON	F	5	9	3	3	13	N	Do as indiv for HG Trend. Also do as 5 fish comp for WSTMP. All caught in SW end of Lk. See LTS for more details.	WHATSMB	12
Whatcom Lk	4	SMB	-	-	N	340	588	1.50	10/6/09	ecy-G	12/3/09	97	L	ON	M	4	9	4	3	14	N	Do as indiv for HG Trend. Also do as 5 fish comp for WSTMP. All caught in SW end of Lk. See LTS for more details.	WHATSMB	12
Whatcom Lk	5	SMB	-	648	Y	330	573	1.59	10/6/09	ecy-G	12/3/09	105	L	ON	M	4	9	5	3	15	N	Do as indiv for HG Trend. All caught in SW end of Lk. See LTS for more details.	-	-
Whatcom Lk	6	SMB	-	649	Y	284	337	1.47	10/6/09	ecy-G	12/3/09	74	L	ON	M	3	10	6	3	16	N	Do as indiv for HG Trend. All caught in SW end of Lk. See LTS for more details.	-	-
Whatcom Lk	7	SMB	-	650	Y	400	1166	1.82	10/7/09	ecy-G	12/3/09	215	L	ON	M	5	10	7	3	17	N	Do as indiv for HG Trend. Also do as 5 fish comp for WSTMP. All caught in SW end of Lk. See LTS for more details.	WHATSMB	12
Whatcom Lk	8	SMB	-	653	Y	340	565	1.44	10/7/09	ecy-G	12/3/09	119	L	ON	F	4	10	8	3	18	N	Do as indiv for HG Trend. All caught in SW end of Lk. See LTS for more details.	-	-
Whatcom Lk	9	SMB	-	654	Y	306	411	1.43	10/7/09	ecy-G	12/3/09	85	L	ON	F	3	10	9	3	19	N	Do as indiv for HG Trend. All caught in SW end of Lk. See LTS for more details.	-	-
Whatcom Lk	10	SMB	-	655	Y	285	314	1.36	10/7/09	ecy-G	12/3/09	71	L	ON	U	3	10	10	3	20	N	Do as indiv for HG Trend. All caught in SW end of Lk. See LTS for more details.	-	-
Notes:																								
1. In an effort to streamline Ecology's fish processing, DNA samples are only collected when requested by WDFW, USFWS, or stated in permit conditions. Individual project managers typically decide if timeframes and staffing allow for this extra processing step.																								

Attachment 3. Lab Analysis & Tracking Plan, (example only)

Note: The 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.

2009 Lab Analysis & Tracking Plan for Fish Samples																													
7 digit MEL Work Order # for WSTMP: 1001015-(xx)																													
jar size -> 2 oz 4 oz 4 oz 4 oz 4 oz																													
cost est/sample -> \$40 \$246 \$701 \$694 min amount of tissue needed per jar -> 5 g 40 g 40 g 40 g up to 90 g																													
sort	WSTMP Exp 09 (X)	WSTMP LT 09 (L)	Hg Trends Fish 09 (M)	Lab Dup MEL	Contract	MS/MSD	Sites	Specie	Collect date	# fish avail	# fish in comp	Hg	3 PCB, 3 DDT, lipids	Pest, PCB, PBDE, lipids	PCDD /Fs, lipids	Field ID (for multiple composites use suffix 1,2,3 to indicate groups A,B,C, respectively)	WSTMP MEL Lab # (# assigned during fish processing)	Hg Trends MEL Lab #	Process date	Process batch for MEL ^a	aliquot per fish (g)	skin: off or on	Hg wt (g)	3 PCB, 3 DDT, lipids (g)	Pest, PCB, PBDE, lipids (g)	PCDD/F lipids (g)	Archive wt (g)	COMMENTS (Bold = WSTMP; unbold = Hg Trends)	
X							Amber L (S of Cheney)	RBT	10/15/09	10	5	1	1	1	1	AMBERRBT	28	-	-	1/12/10	a	150	ON	17	-	90	88	90	Process 3,4,6,7,10. Tossed extra fish.
X							Black L (nr Colville)	TT	9/30/09	15	5	1	1	1	1	BLACKTT	08	-	-	1/6/10	a	90	ON	12	-	90	-	89	Process 1,4,6,8,11. Tossed extra fish.
X							Duck L, Ocean Shores	LMB	9/21/09	10	5	1	1	1	1	DUCKLMB	19	-	-	1/11/10	a	90	ON	30	-	90	90	90	Process 1,2,3,9,10. Tossed extra fish.
X							Duck L, Ocean Shores	YP	9/21/09	10	5	1	1	1	1	DUCKYP	18	-	-	1/11/10	a	21	ON	10	-	46	-	46	Process 1,2,3,6,9
X							Duck L, Ocean Shores	BC	9/21/09	6	5	1	1	1	1	DUCKBC	17	-	-	1/11/10	a	37	ON	35	-	90	-	59	Process 1,2,3,4,5
X	M						Failor L	LMB	9/22/09	13	5	1	1	1	1	FAILLMB	69	FLHGLMB 1-13	11-23	11/10/09	a	75	ON	28	-	84	-	90	Hg Process Individuals 1-13. WSTMP wants 1,2,3,4,5 as 5-fish composite. CF/MF processed earlier in season. See BS for more info.
X	M						Failor L	CTT	9/22/09	17	5	1	1	1	1	FAILCTT	70	FLHGCTT1 FLHGCTT2 FLHGCTT3	24 25 26	11/12/09	a	47	ON	33	-	50	50	50	FLHGCTT1 - Process 15, 16, 17. FLHGCTT2 - Process 10, 11, 9, 8. FLHGCTT3 - Process 3, 4, 7. For FLHGCTT3 - WSTMP wants 3,4,7,2,5 as 5-fish composite. Spare PCDD/F, lipids jar stored with arcs. CF/MF processed earlier in season.
X							Leo L (nr Colville)	YP	10/1/09	10	5	1	1	1	1	LEOYP	29	-	-	1/12/10	a	37	ON	11	-	72	-	42	Process 1,2,3,5,9. Tossed extra fish.
X							Leo L (nr Colville)	PMP	10/1/09	12	5	1	1	1	1	LEOPMP	30	-	-	1/12/10	a	28-33	ON	16	-	74	-	67	Process 3,5,6,7,9. Tossed extra fish.
X							Leo L (nr Colville)	BC	10/1/09	10	5	1	1	1	1	LEOBC	31	-	-	1/12/10	a	40	ON	10	-	62	-	66	Process 3,6,7,8,9. Tossed extra fish.
X	M						Pierre L	SMB	9/29/09	10	5	1	1	1	1	PIERSMB	68	PRHGSMB 1-10	1-10	11/10/09	a	107	ON	29	-	81	84	82	Hg process Individuals 1-10. WSTMP wants 1,2,3,4,5 as 5-fish composite. Spare PCDD/F, lipids jar stored with arcs. CF/MF processed earlier in season.
X	L						Snake R, Central Ferry	CC	10/28/09	13	5	1	1	1	1	SRCFCC1	09	-	-	1/8/10	a	118	off	10	-	90	91	92	A- 3,8,11,12,13
X	L						Snake R, Central Ferry	CC	10/28/09	13	4	1	1	1	1	SRCFCC2	10	-	-	1/8/10	a	106	off	40	90	-	90	90	B- 4,6,9,10
X	L						Snake R, Central Ferry	CC	10/28/09	13	4	1	1	1	1	SRCFCC3	11	-	-	1/8/10	a	137	off	40	85	-	85	85	C- 1,2,5,7
X	L						Snake R, Central Ferry	CCP	10/28/09	11	3	1	1	1	1	SRCFCCP1	63	-	-	1/21/10	a	150	ON	32	-	85	85	85	A- 3,9,10
X	L						Snake R, Central Ferry	CCP	10/28/09	11	3	1	1	1	1	SRCFCCP2	64	-	-	1/22/10	a	140	ON	40	90	-	-	90	B- 1,2,8
X	L						Snake R, Central Ferry	CCP	10/28/09	11	3	1	1	1	1	SRCFCCP3	65	-	-	1/21/10	a	150	ON	30	85	-	-	85	C- 5,6,7
Notes:																													
a = All samples were sent to MEL together (one large batch) on 1/28/10.																													

Attachment 4. Fish Aging Structures

Fish Aging Structures					Updated 11/28/12, per WDFW aging dept recommendations			
Spines	Otoliths	Opercula	Scales	Cleithra	Ecology Species Code	Common name	Scientific name	Family name
	X		X		BC ¹	Black crappie	<i>Pomoxis nigromaculatus</i>	Centrarchidae
	X		X		BG ¹	Bluegill	<i>Lepomis macrochirus</i>	Centrarchidae
		X	X		BLS ²	Bridgelp sucker	<i>Catostomus columbianus</i>	Catostomidae
	X		X		BKT ³	Brook trout	<i>Salvelinus fontinalis</i>	Salmonidae
X					BBH ⁴	Brown bullhead	<i>Ameiurus nebulosus</i>	Ictaluridae
	X		X		BNT ³	Brown trout	<i>Salmo trutta</i>	Salmonidae
	X		X		BLT ³	Bull trout	<i>Salvelinus confluentus</i>	Salmonidae
	X				BUR	Burbot	<i>Lota lota</i>	Gadidae
X					CC ⁴	Channel catfish	<i>Ictalurus punctatus</i>	Ictaluridae
		X	X		CLM ²	Chiselmouth	<i>Arocheilus alutaceus</i>	Cyprinidae
X		X			CCP ⁵	Common carp	<i>Cyprinus carpio</i>	Cyprinidae
X		X			GC ⁵	Grass carp	<i>Ctenopharyngodon idella</i>	Cyprinidae
	X		X		CTT ³	Cutthroat trout	<i>Oncorhynchus clarki</i>	Salmonidae
	X				ENS	English Sole	-	-
					GST ⁶	Green sturgeon	<i>Acipenser medirostrus</i>	Acipenseridae
	X		X		GS ¹	Green sunfish	<i>Lepomis cyanellus</i>	Centrarchidae
	X		X		KOK ³	Kokanee salmon	<i>Oncorhynchus nerka</i>	Salmonidae
	X		X		LT ⁹	Lake trout	<i>Salvelinus namaycush</i>	Salmonidae
	X		X		LWF ⁹	Lake whitefish	<i>Coregonus clupeaformis</i>	Salmonidae
	X		X		LMB ¹	Largemouth bass	<i>Micropterus salmoides</i>	Centrarchidae
		X	X		LSS ²	Largescale sucker	<i>Catostomus macrocheilus</i>	Catostomidae
		X	X		LNS ²	Longnose sucker	<i>Catostomus catostomus</i>	Catostomidae
		X	X		MS ²	Mountain sucker	<i>Catostomus platyrhynchus</i>	Catostomidae
	X		X		MWF ⁷	Mountain whitefish	<i>Prosopium williamsoni</i>	Salmonidae
				X	NOP	Nothern pike	<i>Esox lucius</i>	Esocidae
		X	X		NPM ²	Northern pikeminnow	<i>Ptychocheilus oregonensis</i>	Cyprinidae
		X	X		PEA ²	Peamouth	<i>Mylocheilus caurinus</i>	Cyprinidae
	X		X		PMP ¹	Pumpkinseed	<i>Lepomis gibbosus</i>	Centrarchidae
	X		X		RBT ³	Rainbow trout	<i>Oncorhynchus mykiss</i>	Salmonidae
	X		X		RKB ¹	Rock bass	<i>Ambloplites rupestris</i>	Centrarchidae
	X				RKS	Rock Sole	-	-
	X				COT	Sculpins	<i>Cottus sp.</i>	Cottidae
	X		X		SMB ¹	Smallmouth bass	<i>Micropterus dolomieu</i>	Centrarchidae
	X				STF	Starry flounder	<i>Platichthys stellatus</i>	Pleuronectidae
	X		X		WAL ⁹	Walleye	<i>Stizostedion vitreum</i>	Percidae
	X		X		WM ¹	Warmouth	<i>Lepomis gulosus</i>	Centrarchidae
	X		X		WC ¹	White crappie	<i>Pomoxis annularis</i>	Centrarchidae
					WST ⁶	White sturgeon	<i>Acipenser transmontanus</i>	Acipenseridae
X					YBH ⁴	Yellow bullhead	<i>Ameiurus natalis</i>	Ictaluridae
	X		X		YP ⁸	Yellow perch	<i>Perca flavescens</i>	Percidae

Notes:

- 1 - Otoliths first choice structure if >300mm. Fish <300mm OK to pull scales only.
- 2 - Opercula first choice structure in all size classes, scales back-up structure if opercula damaged.
- 3 - Scales first choice structure in all size classes, otoliths back-up structure if unable to pull scales (note: if KOK are spawning stage, pull otoliths).
- 4 - Pectoral spines (including articulating joint).
- 5 - Dorsal spine first choice structure, opercula back-up structure if dorsal spine is damaged.
- 6 - Pull pectoral fin ray. (Mike Wall ages sturgeon for WDFW # 906-6727).
- 7 - Otoliths first choice structure if >350mm. Fish <350mm OK to pull scales only.
- 8 - Otoliths first choice structure if >200mm. Fish <200mm OK to pull scales only.
- 9 - Otoliths first choice structure in all size classes, scales back-up structure if otoliths not retrieved.

For trout and salmon, add comment to bench sheet for presence or absence of adipose fin.

Aging Questions??? Call Lucinda Morrow @ WDFW #902-2763, or marine lab #902-2859.

Attachment 5. MSDS for Ethanol (Aaper Alcohol 2006)

MATERIAL SAFETY DATA SHEET

ETHYL ALCOHOL USP - 200 PROOF

AAPER MSDS NUMBER: E200

EFFECTIVE DATE: May 2005

AAPER Alcohol and Chemical Company
1101 Isaac Shelby Drive, P. O. Box 339
Shelbyville, Kentucky 40066-0339
Telephone: (502) 633-0650

For chemical emergency – spill, leak, fire, exposure, or accident, call CHEMTREC at 1-800-424-9300 day or night.
Outside the continental United States, call CHEMTREC at 1-703-527-3887 (collect calls accepted).

AAPER Alcohol and Chemical Company urges the customer receiving this Material Safety Data Sheet (MSDS) to study it carefully to become aware of hazards, if any, of the product involved. In the interest of safety, you should: (1) notify your employees, agents, and contractors of the information on this sheet, and (2) furnish a copy to each of your customers to inform their employees and customers as well.

SECTION I – IDENTIFICATION

PRODUCT NAME: Alcohol USP, Ethyl Alcohol, 200 proof
SYNONYMS: Anhydrous Ethyl Alcohol, Dehydrated Alcohol
CHEMICAL FAMILY: Alcohol
MOLECULAR WEIGHT: 46.07
FORMULA: C₂H₅OH

Section II – INGREDIENTS

<u>COMPOSITION</u>	<u>CAS RN.</u>	<u>NOMINAL WT/WT%</u>	<u>PEL/TLV</u>	<u>HAZARD</u>
Ethyl Alcohol	64-17-5	100.0	1000 ppm	Flammable/Nervous System Depressant

SECTION III – HEALTH INFORMATION

INHALATION: Exposure to over 1000 ppm may cause headache, drowsiness and lassitude, loss of appetite, and inability to concentrate. Irritation of the throat.
INGESTION: Can cause depression of central nervous system, nausea, vomiting, and diarrhea.
EYE CONTACT: Liquid or vapor may cause irritation.
SKIN CONTACT: May cause irritation and defatting of skin on prolonged contact.

SECTION IV – OCCUPATIONAL EXPOSURE LIMITS

PEL (OSHA Permissible Exposure Limit): Mixture – See Section II
TLV (ACGIH Threshold Limit Value): Mixture – See Section II

SECTION V – EMERGENCY FIRST AID PROCEDURE

FOR OVEREXPOSURE BY:

SWALLOWING: If victim is conscious and able to swallow, have victim drink water or milk to dilute. Never give anything by mouth if victim is unconscious or having convulsions. CALL A PHYSICIAN OR CHEMTREC (POISON CONTROL) IMMEDIATELY. Induce vomiting only if advised by physician or Chemtrec (Poison Control).
INHALATION: Immediately remove victim to fresh air. If victim has stopped breathing, give artificial respiration, preferably mouth-to-mouth. GET MEDICAL ATTENTION IMMEDIATELY.
CONTACT WITH EYES OR SKIN: Immediately flush affected area with plenty of cool water. Eyes should be flushed for at least 15 minutes. Remove and wash contaminated clothing before reuse. GET MEDICAL ATTENTION IMMEDIATELY.

SECTION VI – PHYSICAL DATA

BOILING POINT: 173° F
FREEZING POINT: -173° F
VAPOR PRESSURE: 44.6 mm Hg @ 68° F
SPECIFIC GRAVITY: 0.7940 @ 60°/60° F
VAPOR DENSITY (AIR = 1): 1.59
SOLUBILITY IN WATER: Complete
APPEARANCE AND COLOR: Clear and colorless

SECTION VII – FIRE AND EXPLOSIVE HAZARDS

FLASH POINT: 56° F ASTM D-56 (Tag Closed Cup)
AUTO-IGNITION TEMPERATURE: 685° F
FLAMMABLE LIMITS IN AIR, % BY VOLUME: LOWER: 3.3 UPPER: 19
NFPA (National Fire Protection Association) RATING: HEALTH (0) FIRE (3) REACTIVITY (0)
(Does not apply to exposure hazards other than during a fire.)

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FIRE FIGHTING PROCEDURES: (Note: Individuals should perform only those fire-fighting procedures for which they have been trained.) Use dry chemical, "alcohol" foam, or carbon dioxide; water may be ineffective, but water should be used to keep fire-exposed containers cool. If a leak or spill has not ignited, use water spray to disperse the vapors and to protect men attempting to stop a leak. Water spray may be used to flush spills away from exposures and to dilute spills to nonflammable mixtures. (NFPA 49)

UNUSUAL FIRE & EXPLOSION HAZARDS: Firefighters should wear self-contained breathing apparatuses in the positive pressure mode with a full face piece when there is a possibility of exposure to smoke, fumes, or hazardous decomposition products.

SECTION VIII – REACTIVITY

STABILITY: Generally stable.
HAZARDOUS POLYMERIZATION: Not likely.
CONDITIONS & MATERIALS TO AVOID: Contact with acetyl chloride and a wide range of oxidizing agents may react violently.

SECTION IX – EMPLOYEE PROTECTION

CONTROL MEASURES: Handle in the presence of adequate ventilation.
RESPIRATORY PROTECTION: Where exposure is likely to exceed acceptable criteria, use NIOSH/MSHA approved respiratory protection equipment. Respirators should be selected based on the form and concentration of contaminant in air and in accordance with OSHA (29 CFR 1910.134).
PROTECTIVE CLOTHING: Wear gloves and protective clothing which are impervious to the product for the duration of the anticipated exposure if there is potential for prolonged or repeated skin contact.
EYE PROTECTION: Wear safety glasses meeting the specifications of ANSI Standard Z87.1 where no contact with the eye is anticipated. Chemical safety goggles meeting the specifications of ANSI Standard Z87.1 should be worn whenever there is the possibility of splashing or other contact with the eyes.

SECTION X – ENVIRONMENTAL PROTECTION

ENVIRONMENTAL PRECAUTIONS: Avoid uncontrolled releases of this material. Where spills are possible, a comprehensive spill response plan should be developed and implemented.
SPILL OR LEAK PROCEDURES: Wear appropriate respiratory protection and protective clothing as described in Section IX. Contain spilled material. Transfer to secure containers. Where necessary, collect using absorbent media. In the event of an uncontrolled release of this material, the user should determine if the release is reportable under applicable laws and regulations.
WASTE DISPOSAL: All recovered material should be packaged, labeled, transported, and disposed of, or reclaimed in conformance with applicable laws and regulations and in conformance with good engineering practices.

SECTION XI – REGULATORY CONTROLS

DEPARTMENT OF TRANSPORTATION (DOT):		<u>For Pint packages Domestic Shipments Only</u>
DOT CLASSIFICATION:	3 (Flammable Liquid)	ORM-D
DOT PROPER SHIPPING NAME:	Ethyl Alcohol	Consumer Commodity ORM-D
OTHER DOT INFORMATION:	Identification No. UN1170 P.G. II Emergency Response Guide No. 127	
TTB DISTILLED SPIRITS ACT:	Use of ethyl alcohol without prior payment of applicable excise tax is strictly controlled by regulation promulgated and enforced by the Tax and Treasury Bureau (TTB). Governing regulations have been defined in Title 27, Code of Federal Regulations.	
TOXIC SUBSTANCE CONTROL ACT (TSCA):	This product is listed in the TSCA Inventory of Chemical Substances.	

SECTION XII – PRECAUTIONS: HANDLING, STORAGE, AND USAGE

Protect container against physical damage. Detached or outside storage is preferred. Inside storage should be in an NFPA approved flammable liquid storage room or cabinet. All ignition sources should be eliminated. Smoking should be prohibited in the storage and usage areas. Electrical installations should be in accordance with Article 501 of the National Electrical Code. NFPA 30, Flammable and Combustible Liquids Code, should be followed for all storage and handling. Frequent careful leakage inspections should be done. An automatic sprinkler system should be provided. Isolate from oxidizers, chemicals capable of spontaneous heating, materials reacting with air or moisture to liberate heat, ignition sources and explosives. Consult local fire codes for additional storage information.

When contents are being transferred, containers must be bonded to the receiving container and grounded to avoid static discharges. Never use pressure to empty. Replace closure securely after each opening.

Keep material packaged in bottles out of sun and away from heat. Remove closure carefully; internal pressure may be present. Keep closure on to prevent leakage.

Container hazardous when empty. Since emptied containers retain residual product (vapor and liquid), all precautions described on this MSDS must be observed.

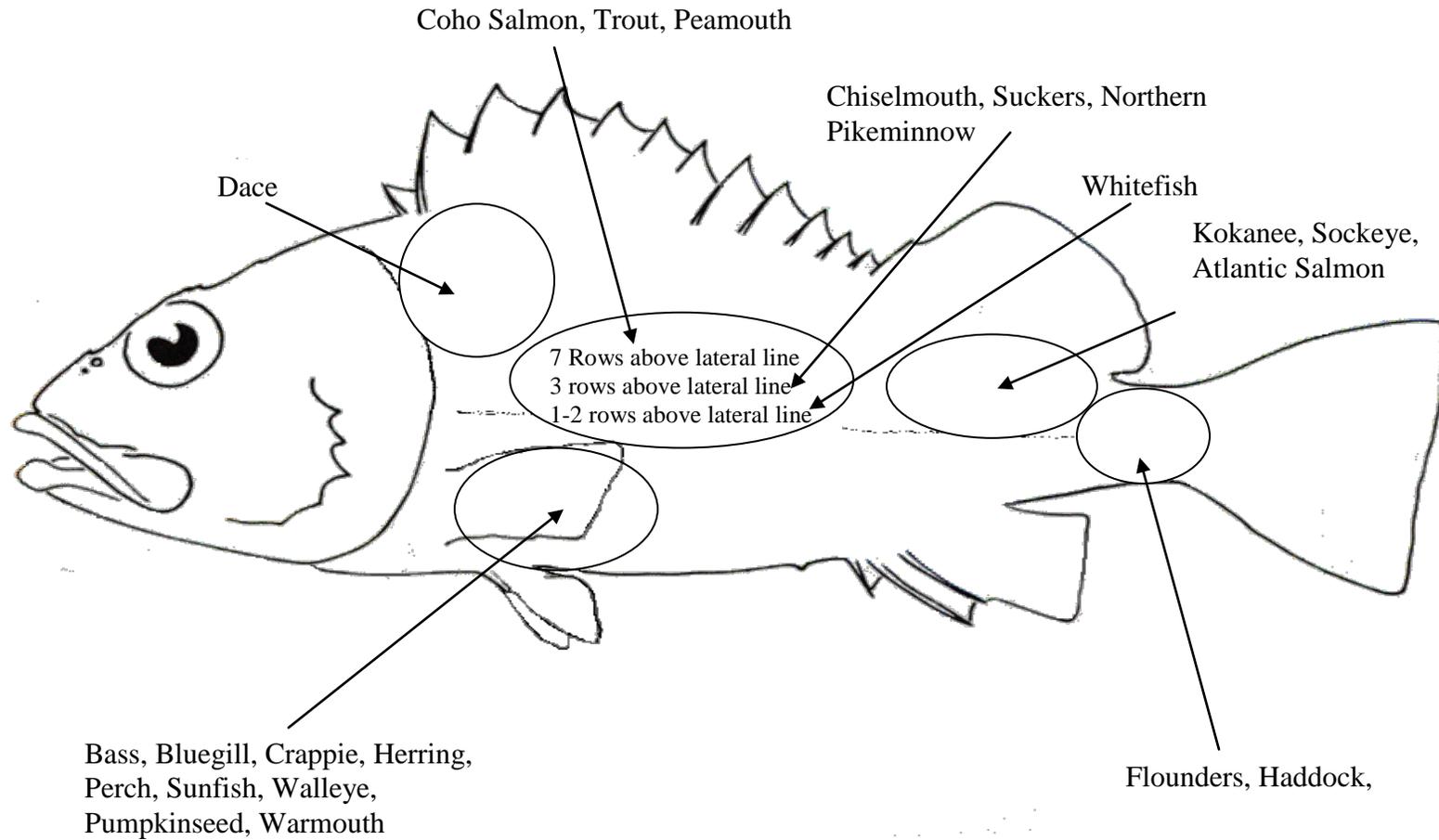
CAUTION: For manufacturing, processing, repackaging, or industrial use.

The information contained herein is furnished without warranty of any kind. Employers should use this information only as a supplement to other information gathered by them and must conduct testing and/or make independent determinations of suitability and completeness of information from all sources to assure proper use of these materials and the safety and health of employees.

AAPER ALCOHOL AND CHEMICAL COMPANY
 May 2005

Ethyl USP 200 proof
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Attachment 6. General Areas of Scale Removal



Scales taken for aging samples should be around the same size per fish for a more consistent analysis. Use undamaged scales only. When measuring Walleye scales in addition to aging, take scale samples above the lateral line, otherwise the preferred scales for aging Walleye are located below the lateral line near the pectoral fins. (AFS, 1983; Morrow, 2006; Sneva et. al., 2003; Sneva, 2005; and UW, 2006).