2005 SALMON OTOLITH PORT SAMPLING GUIDE

PURPOSE

Thermal marking is one of the methods being utilized to identify and manage hatchery-released salmonids. Thermal marking places a recognizable mark on the otoliths of a fish. This marking technique makes use of the natural growth tendencies of a developing otolith. When the embryonic fish are incubating in hatcheries, the water temperature is raised and lowered according to a pre-determined schedule that results in a predictable sequence of visibly enhanced growth increments or "thermal rings." The appearance of thermal marks can be likened to the growth rings on a tree. Several thermal rings grouped closely together make up a "band." Increasing the distance between these groups can create multiple bands. A discrete thermal mark is identified by counting the number of thermal bands present, and then counting how many thermal rings compose each band. For example in Figure 1a, there are 6 closely grouped rings that make up one band. In Figure 1b, there are still only six thermal rings, but this time they are grouped into two bands each composed of three rings.

Mark # 1	Mark # 2
IIIIII	III III
Figure 1a	**Figure 1b**

Fish have 3 pairs of otoliths – the sagittae, lapillae, and asteriscae. The sagittae are the largest and are what most people refer to as "otoliths." When a sample of fish is collected, the sagittal otoliths are removed and sent to ADF&G's Thermal Mark Laboratory for processing. The left sagittal otolith is glued to a glass slide and then ground down on fine grit sandpaper. When the center of the otolith is reached, it is examined under a microscope for the presence of a thermal mark.

EQUIPMENT

DISSECTION TOOLS

1) Butcher knives with deep 6-8" blade
2) Forceps (fine point)
3) Cotton gloves
4) 96-cell otolith trays with compression plates & lids*
5) Sample labels*
6) Pencils
7) 5x7" Anti-skid matting
8) Paper towels
9) Brightly colored beads*

OTOLITH CLEANING SUPPLIES

1) 1.0% Chlorine solution*
2) De-chlor solution (0.7% sodium thiosulfate) *
3) Water
4) Rubber bands (size 62)*
5) 1000ml Nalgene bottles*
6) 125ml Nalgene squirt bottles*

SHIPPING SUPPLIES

1) Ziploc plastic bags
2) Packing tape
3) Packing boxes
4) Pre-addressed and numbered shipping labels*

*** Provided by ADF&G Thermal Mark Laboratory**

DISSECTION

1. Collect a sample of fish heads from the appropriate fishing district.

2. Assemble the sample and dissection equipment in the location identified by processing plant personnel.

3. Fill out and place the adhesive tray label to the bottom of the 96-cell otolith sampling tray before it is filled with otoliths. **This step is critically important to maintaining the integrity of each sample!**

In the Port Sampling Supplies shipped to you, you will have several adhesive Tray Labels. Each label has a pre-assigned sample number printed in the upper right-hand corner (see diagram below). We prefer that you keep the tray numbers in sequential order throughout a given stat week and sampling season. **It is critical that each and every tray have a tray label attached**.

Because the sample number on each tray label is unique, you cannot use a label to identify more than one tray of 96 otoliths. Fill out each tray label completely with the appropriate fishery information. Use soft lead pencil – not a pen because alcohol and water will dissolve ink! To avoid spilling otoliths from a tray, affix the label to the bottom of a tray ***before*** filling them with otoliths. **Do not place sample labels on the otolith tray lid because the lids can get separated from the sample tray!**

Never place otoliths from more than a one stat-week in a single tray. Otoliths from multiple stat-weeks should not be included in the same tray (e.g. one stat-week per tray)! Mixing otoliths from various statistical weeks results in massive confusion and an irrevocable loss of data. Even if you have a tray containing only ONE otolith for a Stat Week or to complete a Stat Week, send it that way.

Alaska Department of Fish and Game - Comm Fish

Species: ____________________ Tray 794

Sample Date: Month____ Day____ Year 2005

Port/Location: ____________________

District/Subdistricts: ____________________

Collectors: ____________________

Comments: ____________________

ADF&G MTA Lab, Juneau - (907) 465-2306
10107 Bentwood Place, Juneau, AK 99801

TRAY NUMBER: provides a unique sample number for each tray.

4. To separate the messy job of cutting heads from the removal of otoliths (where some cleanliness is desired) cut the heads in batches of 20-40 using the following guidelines (Figs. 2 – 3):

a) Hold the fish head in front of you on its severed end, with the dorsal surface (top of the head) facing you. Flare out the gill plates to help stabilize it;
b) With left hand cupped against left side of fish face to stabilize it, and with knife in right hand (or opposite for lefties), begin a cut, down to about 1” deep, through the nose and such that if this cut line were to continue, it would go equilaterally between the left and right eye.
c) With your gloved left hand, grab the lower jaw and insert the point of the knife into the mouth and just below the thumb of your left hand (which is crosswise in the fish’s mouth). Let the knife find your initial starter cut, then bear down on the knife so that the tip finds the table surface first, and then pivot the cut from this point down through the rest of the fish head.

d) The end product should be two halves split top-to-bottom between the eyes and still connected by the lower jaw. This allows for a fast way to cut heads and make their otoliths readily accessible.

Figure 2. Begin the first cut through the nose. Leave a small area attached at the base of the two halves.

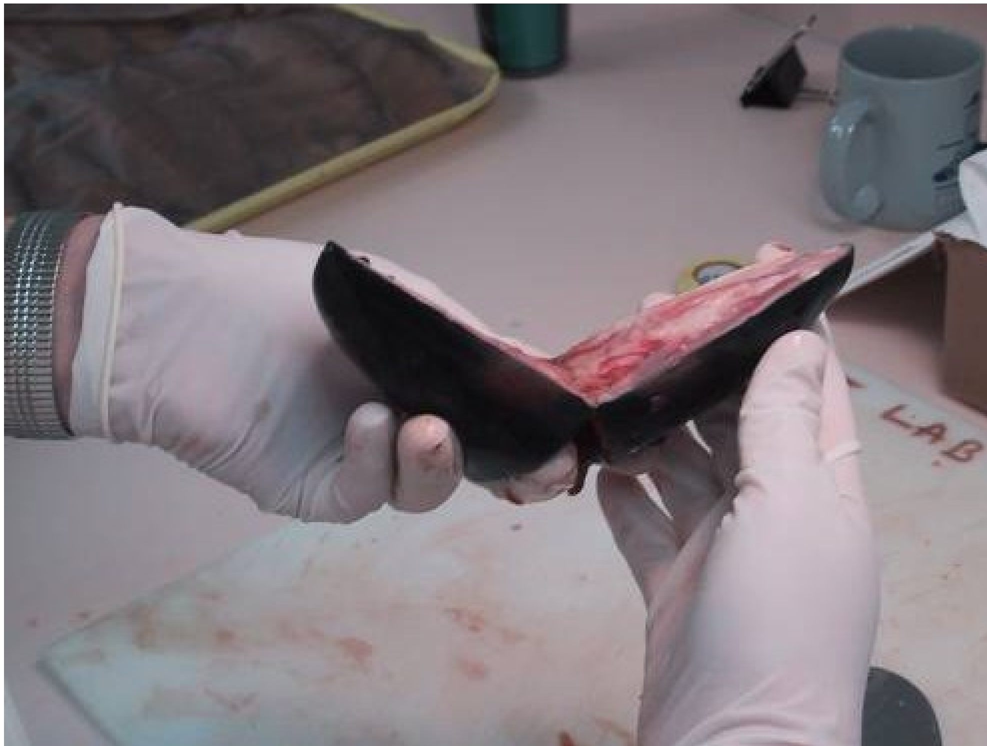

Figure 3. Allow the halves to open up, exposing the otolith cavities on either side.

5. After all of the fish heads have been cut, briefly clean the area and set up all the tools needed for otolith removal: 96-cell tray with pre-printed label, forceps, water squirt bottle, and towels.

6. Just before removal, fill each cell of the otolith tray with water so that residual blood and slime does not back into the crevices of the otolith.

7. Pick up a fish head and let the halves flop "open." With forceps, remove the left sagittal otolith from its "sagittal well". The sagittal well is a depression underneath the brain in the most posterior-ventral portion of the brain cavity. Place the otolith onto the back of your hand and then recover the right otolith from the right sagittal well (Fig. 4).

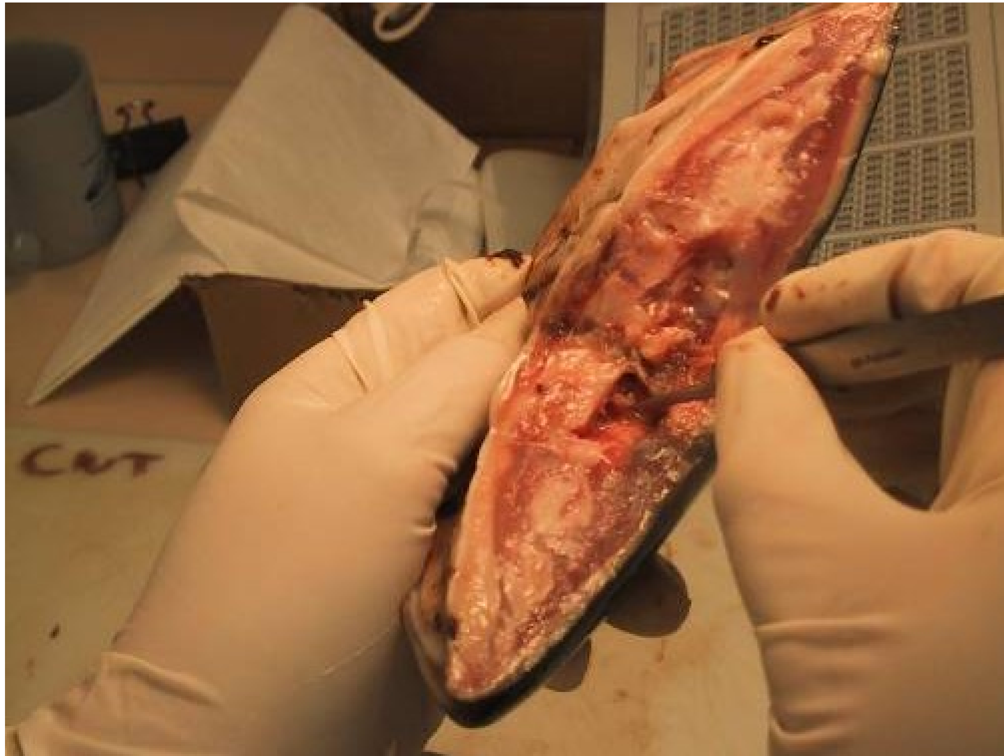

Figure 4. Remove the otoliths from the sagittal wells with forceps, clean, and place in the tray.

8. Each otolith is encased in tissue. Tweeze the tissue off of each otolith, gently wipe off the blood, and place the pair of otoliths into the appropriate cell of the otolith tray. Note that the first specimen for every tray should go into the highlighted cell, "A1". Cells in a tray should be filled left to right by row (like reading a book): A1-A12, B1-B12…H1-H12. Do not fill cells in columns!

9. Repeat the dissection procedure until the tray(s) is (are) full and the sample is finished.

10. If one of the two otoliths is lost during the process, place one bead into the cell with the recovered otolith. If no otoliths are recovered from a fish head, discard that head. Do not skip a cell or place any beads into that cell unless there are AWL data matched to that fish head. If the otoliths are to be matched with other data in the sample collection, then place two beads in the cell to indicate that both otoliths are missing.

ALTERNATIVE DISSECTION METHOD

A second methods used to dissect and removal the otoliths is the “flip top” approach. In this method, the top of the head is removed by starting to cut at the top of both eyes. Then you slice back towards the body, but not beyond a line extending above the gill cover. With a twist of the knife, cut back towards the top of the head removing a wedge of tissue and bone. This will expose the cranial cavity. Remove the brain tissues so that both pairs of otoliths of can be extracted with forceps. The flip top method takes practice to obtain consistent cuts but tends to be less messy than the first approach (Figs. 5 – 7).

Figure 5. Make the cut just above eye-level.

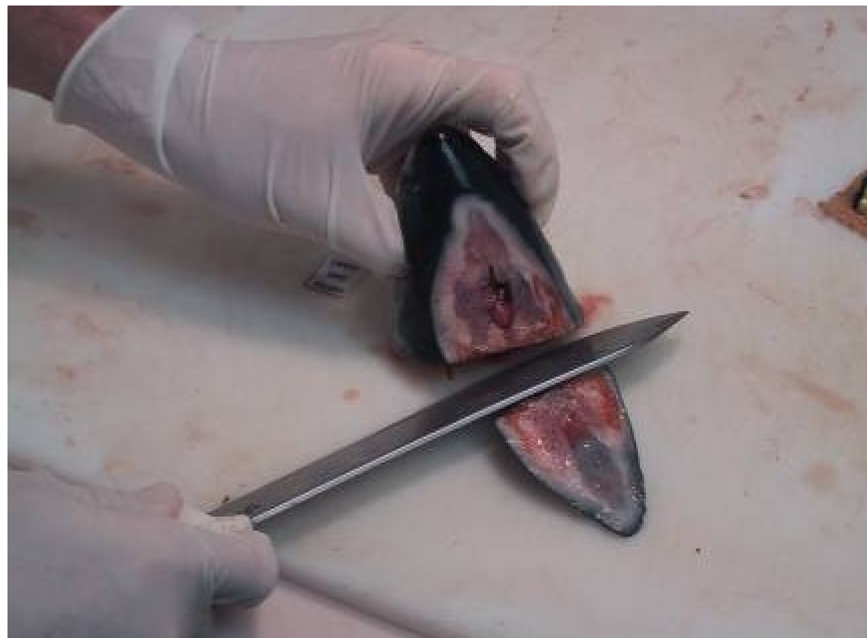

Figure 6. Cut all the way through and remove the skull-cap.

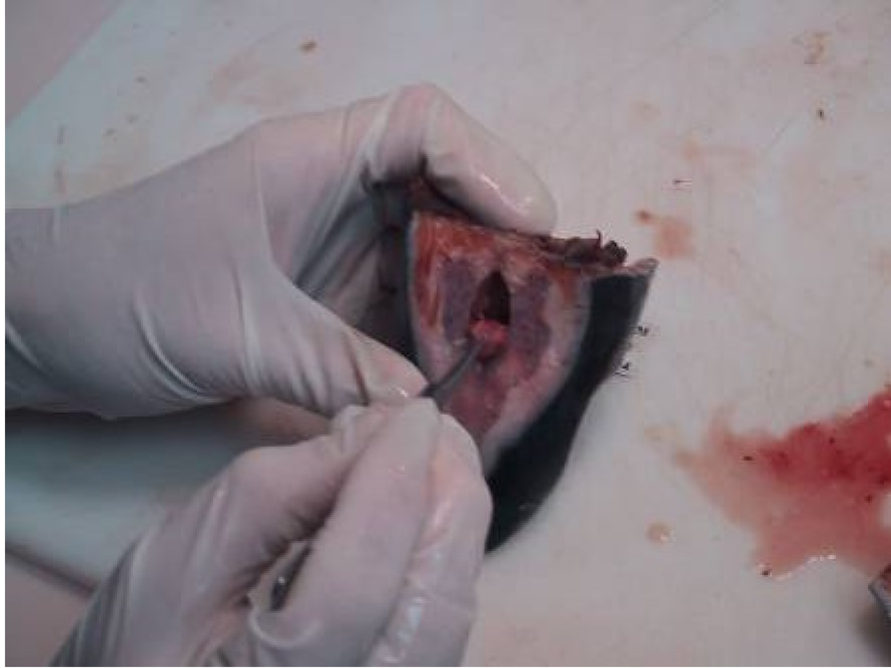

Figure 7. Remove otoliths through a single opening into the brain cavity. They will be located on opposite sides of the opening.

CLEANING

1. Gently squirt the 1.0% chlorine solution into each cell with an otolith, filling each cell approx 1/2 full. Let sit for10 minutes. This helps to clear away blood and tissue. ***DO NOT*** let this sit for more than 30 minutes! Use caution when using squirt bottles of any solution: a forceful stream of water will cause the otoliths to jump from their cells and mix up the data!

2. Gently squirt the De-chlor solution to fill the cell. This deactivates the chlorine solution. You may move on to the next step without waiting because the de-chlorination is immediate.

3. Rinse with tap water from a squirt bottle. First, tap the tray gently against the counter to settle the otoliths in the very bottom of their cells. Check that they've all fallen down. For those that don't, simply push them down with some forceps. Tip the tray 45-80 degrees and gently wipe the surface several times with the palm of your hand. Be careful not to "draw" the otoliths out of the cells by too fast a motion. This will help remove the excess De-chlor solution. Put the tray back on the counter and gently squirt tap water into the cells from the squirt bottle. Do some final wiping passes to remove water from the final rinse.

4. Dry the otoliths by letting them sit uncovered.

5. **Place *two* compression plates -- aligned with each other and so that they cover all 96-cells – then place a lid on the tray, and secure this with *three* rubber bands**!

SHIPPING

1. If there is any moisture left in the tray(s), wrap a few paper towels around them and place them inside a sealable plastic bag (Ziploc). Place the individual bags inside one more sealable plastic bag.

2. Pack the trays into a box, cushioning them with packing material.

3. Seal the box with tape and affix with the adhesive shipping labels provided:

SHIP TO: Alaska Department of Fish and Game
Otolith Processing Lab
ATTN: Kray Van Kirk
10107 Bentwood Place
JUNEAU, ALASKA 99801

4. Ship "C.O.D." via ALASKA AIRLINES. If sending via the postal service, affix the correct postage.