

# SALMON OTOLITH SAMPLING GUIDE

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## BACKGROUND

Hatchery-released salmonids can be identified and managed using thermal marks on their otoliths, or “ear stones.” Salmonid otoliths are naturally marked by altering their growth before they are released from the hatchery. When embryonic fish are in incubators at the hatchery, the water temperature is altered according to a pre-determined schedule that leaves a predictable sequence of visible growth increments or “thermal rings” on their otoliths. The appearance of thermal marks can be compared to the growth rings on a tree. Several thermal rings grouped closely together make up a “band.” Different groupings can create multiple unique marks. 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 also six thermal rings, but they are grouped into two bands each composed of three rings.

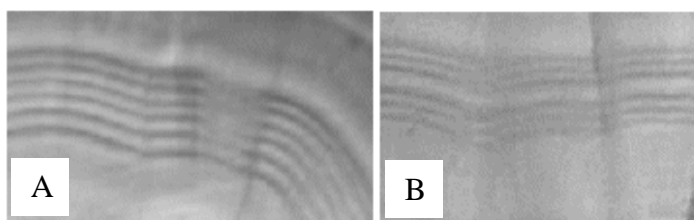


Figure 1. Two thermal markings on sockeye sagittal otoliths: (A) one band with six rings and (B) two bands with three rings in each band.

Fish have 3 pairs of otoliths – the sagittae, lapillae, and asteriscae. The sagittae are the largest and are commonly referred to as “otoliths.” There are left and right otoliths that make up each pair, and both the left and the right sides are sampled for thermal marks. The left sagittal otolith is glued to a glass slide and then ground 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.

## OTOLITH DISSECTION

### Equipment

Provided by Thermal Mark Lab	Not provided
Fine tip forceps	6-8” butcher knives
Tray labels	Pencils
5x7” anti-skid matting	Paper towels
96-cell otolith trays, compression plates, lids	Latex gloves
Brightly colored beads	

## Tray preparation

Adhere a tray label to the bottom of each tray used for otolith sampling. To avoid spilling otoliths from a tray, adhere the label(s) before filling the tray with otoliths. **Note: Do not place sample labels on the tray lid because the lids can be separated from the sample tray.**

*Field recommendation:* Sample information can be written directly on the side of the tray or on laboratory tape on the side of the tray using a permanent marker. Then sample information can be transcribed to the tray label later in a controlled environment.

Fill out each tray label with the appropriate fishery information (Figure 2). Use a soft lead pencil on the label. Alcohol and water dissolve ink, thus sample information will be lost. **Note: Information on each tray label is critical to maintaining the integrity of each sample.**

<b>Alaska Department of Fish and Game - Comm Fish</b>	
Species: _____	Tray <u>794</u>
Sample Date: Month ____ Day ____	Year <u>2005</u>
Port/Location: _____	
District/Subdistricts: _____	
Collectors: _____	
Comments: _____	
ADF&G MTA Lab, Juneau - (907) 465-2306	
10107 Bentwood Place, Juneau, AK 99801	




Figure 2. Otolith tray label.

Each tray number is unique and is shown on the upper right of the tray label. Only otoliths from fish in a single sample should be included in a tray. For example, if samples are separated by statistical week and area, then otoliths from multiple statistical weeks or areas should not be included in the same tray. In addition different types of gear usually distinguish a different fishery and are noted in the database. Please include gear type in the comments field on the tray label (e.g.—drift gillnet, purse seine). It is better to have a few otoliths in one tray (one pair of otoliths is okay) than to have multiple samples in a tray. Otoliths from different samples (e.g. multiple statistical weeks, areas, or gear) in one tray will later need to be moved into separate trays.

Fill each cell of the otolith tray half way with water prior to otolith dissection; this makes it easier for otoliths to stay in their cells.

## Otolith dissection from head only

Hold the fish in front of you, place the knife on the top of the head above the eyes and cut down towards the back of the head (Figure 3).

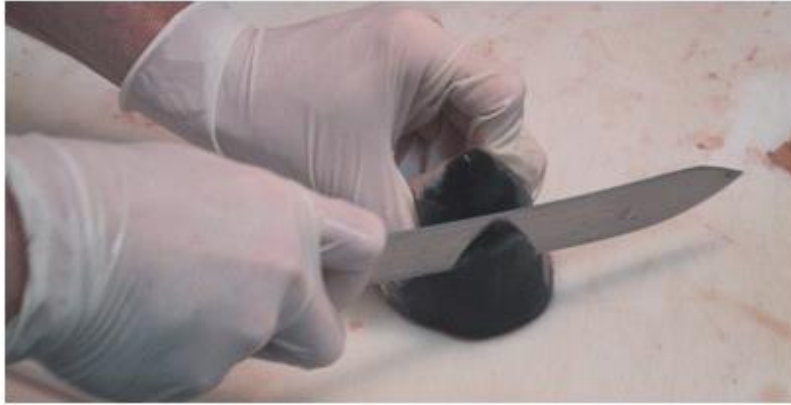


Figure 3. Initial cut on salmon head.

Cut towards the body continuing the cut through the top of the head. This exposes the cranial cavity (Figure 4).

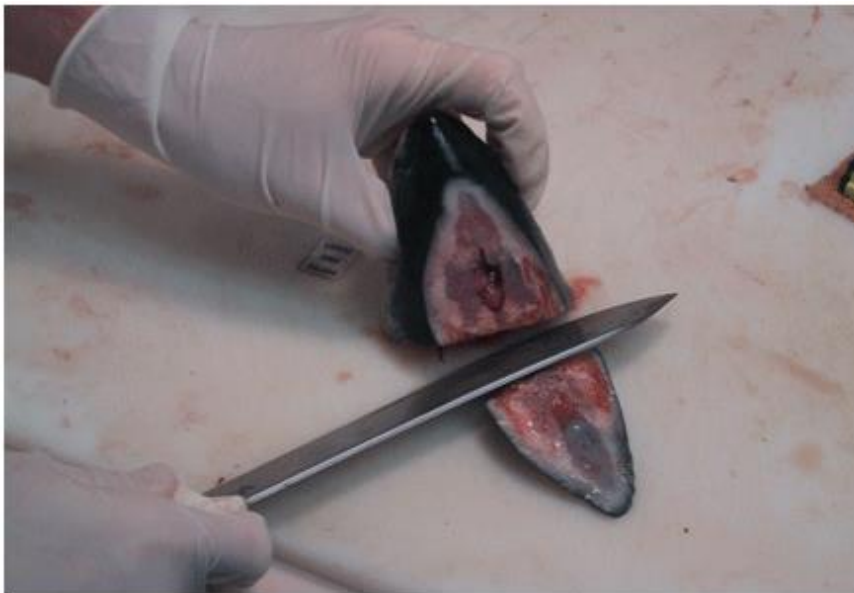


Figure 4. Cranial cavity and brain are exposed.

Remove the brain so that the left and right wells are exposed (Figure 5). The left and right otoliths are found in small depressions, the sagittal wells, on the left and right side of the head.

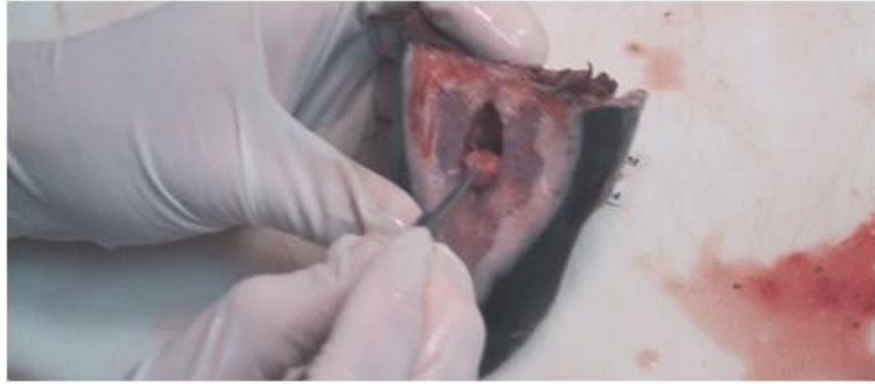


Figure 5. Brain is removed from the head using forceps.

Use forceps to remove the left then right otoliths from each well. If an otolith is accidentally cut in half, place both halves in the well. Halves of otoliths can be useful, thermal marks can sometimes be seen on partial otoliths.

Clean the otolith by removing the surrounding sac and any blood or tissue.

### **Otolith dissection from whole fish**

Hold the fish in front of you and cut down at the top of the head behind the mouth and above the gill covers (Figure 6).



Figure 6. Example of where to make the incision for collecting otoliths from a whole fish.

The otoliths will be located in the sagittal wells located towards the body and beneath the brain (Figure 7).



Figure 7. Right otolith is exposed towards the body behind the brain.

Use forceps to remove each otolith from its well (Figure 8).



Figure 8. Left otolith is being removed from left sagittal well.

Clean the otolith by removing the surrounding sac and any blood or tissue from the otolith.



## Otolith placement in trays

The first pair of otoliths should go in cell “A1” (Figure 9). The tray should be filled left to right by row (like reading a book): cell A1 to cell A12, then cell B1 to B12 and the last row to fill is H1 through H12. **Do not fill cells in columns!**

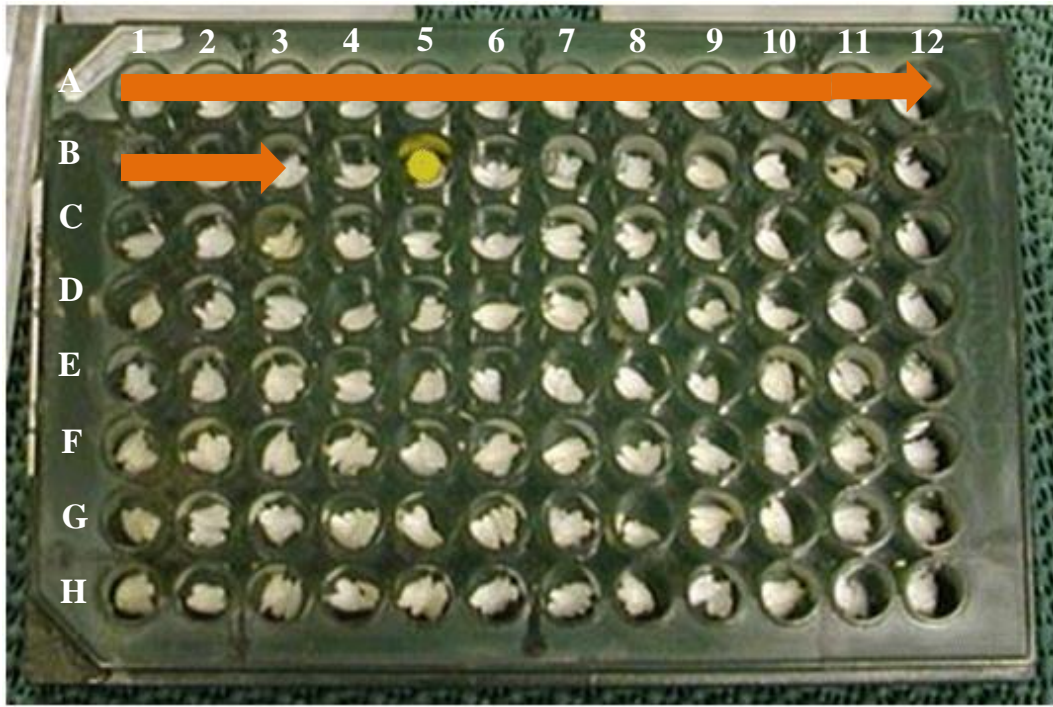


Figure 9. 96-well otolith tray.

If one of the otoliths is lost, put a colored bead in place of the missing otolith. If no otoliths can be recovered from the head, then place two beads in the corresponding cell.

## Otolith cleaning

Fill each well with 5% chlorine solution. Be careful not to fill the wells with too much pressure, too much pressure might cause the otoliths to jump out of the well and mix (we discourage inter-otolith socializing). Let the tray sit for five minutes.

After five minutes, wash each well with de-chlorination solution (“de-clor”, 0.7% sodium thiosulfate), again, be careful not to use too much pressure when applying the solution. Let the tray sit for five minutes.

Rinse the wells with water using the squeeze bottle, letting the excess run out of the tray. **Note: Be careful to not lose the otoliths while rinsing!**

The tray can be air dried to remove the remaining water, or each cell can be siphoned with a pipette. If a pipette is used, take care not to remove any otolith pieces so that otoliths are not transferred to other cells. Let the cleaned and rinsed otolith tray dry.

## SAMPLE SHIPMENT

Place *two* compression plates (acetates) that are taped together between the tray and the lid (Figure 10). Secure the tray, compression plates, and lid with *three* rubber bands!



Figure 10. Tray prepared for shipment with compression plates and three rubber bands.

Please ship vis Fed Ex, UPS, or Air Cargo to:

Alaska Department of Fish and Game  
Division of Commercial Fisheries  
Mark, Tag, and Age Laboratory  
Attn: Thermal Mark Lab  
10107 Bentwood Place  
Juneau, AK 99801

Or you can ship via US Postal Service to (slower):

Alaska Department of Fish and Game  
Division of Commercial Fisheries  
Mark, Tag, and Age Laboratory  
Attn: Thermal Mark Lab  
PO Box 115526  
Juneau, AK 99811-5526

If you have any further questions, please call:

MTA Mark lab	(907) 465-2306
Lab supervisor	(907) 465-3498
Assistant lab supervisor	(907) 465-2424
Crew leader	(907) 465-5972

Thank you for your cooperation and happy sampling!