

FRED Reports

Juvenile Salmonid Otolith Extraction and
Preparation Techniques for Microscopic Examination

by
W. J. Glick and P. A. Shields

Number 132



Alaska Department of Fish & Game
Division of Fisheries Rehabilitation,
Enhancement and Development

**Juvenile Salmonid Otolith Extraction and
Preparation Techniques for Microscopic Examination**

by
W. J. Glick and P. A. Shields

Number 132

Alaska Department of Fish and Game
Division of Commercial Fisheries
Management and Development
(merged Division of Fisheries Rehabilitation,
Enhancement, and Development)

Carl L. Rosier
Commissioner

Jeffery P. Koenings
Director

P. O. Box 25526
Juneau, Alaska 99802-5526

July 1993

The Alaska Department of Fish and Game conducts all programs and activities free from discrimination on the basis of sex, color, race, religion, national origin, age, marital status, pregnancy, parenthood, or disability. For information on alternative formats available for this and other department publications, please contact the department ADA Coordinator at (voice) 907-465-4120, (TDD) 1-800-478-3648, or (fax) 907-568-6596. Any person who believes he or she has been discriminated against should write to:

ADF&G
P.O. Box 25526,
Juneau, AK 99802-5526
or
O.E.O.
U.S. Department of the Interior
Washington, DC 20240

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
ABSTRACT.	1
INTRODUCTION.	2
METHODS AND MATERIALS.	3
Sample Preservation.	3
Extraction.	5
Molding and Embedding	7
Grinding the Medial Side.	8
Polishing the Medial Side	10
Grinding the Lateral Side	10
Polishing the Lateral Side.	11
Epoxy Mounting.	11
Cutting Off Excess Resin	12
Determining Cessation of Grinding.	13
DISCUSSION	13
Extraction.	13
Molding.	14
Otolith Grinding	14
Otolith Polishing	16
Adhesives	16
Resin Cutting.	16
Preparation Time	17
SUMMARY AND CONCLUSIONS	17
ACKNOWLEDGEMENTS.	18
REFERENCES.	19

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Lateral (A) and posterior (B) view of a typical salmon otolith. Note the downward positioning of the sulcus acusticus on the glass plate.	4
2. Schematic showing the dissection of the skull cap (A) and exposure of the sagittae under the brain cavity (B).	6
3. Schematic of the proper otolith grinding angle (A) and the depth of grinding the otolith relative to the surface of the primordia (B).	9

ABSTRACT

The use of fish otoliths for purposes of management and research is becoming more common. In Alaska, the use of otoliths for hatchery stock identification and life history investigations have been in use for less than five years. Otoliths are inorganic structures that form early in the development of juvenile fish, and thus provide a means whereby physical and environmental events throughout a fish's life history are recorded. Microscopic examination of otoliths requires a carefully-prepared sample to yield such detailed information. The following paper describes in detail each process involved in the preparation of juvenile otoliths. The described technique allowed one person to prepare 30 samples per day that were ready for computer-image analysis.

INTRODUCTION

Determining the number and allocation of salmon for harvest are difficult enough for management of fisheries in Alaska; however, the identification of stocks in a mixed fishery is an even greater challenge. Harvested fish are often a mixture of individual wild and/or hatchery stocks; thus it is often necessary to evaluate the strength of such stocks to protect the most vulnerable one from overexploitation. In essence, knowing stock proportions of the harvested population and migrational timing allows fish managers to provide recommendations regarding fishery regulations.

For hatchery stocks, marks such as coded wire tags and fin clips have been used for identification. However, these methods are laborious, expensive, and usually are done on a subsample of the population. A technique that allows marking of the entire population is the use of oxytetracycline (Koenings et al. 1983; Stekoll and Smoker 1986; Paragamian et al. 1992;), but identification of the mark can be problematic. A more recently developed technique used in hatcheries to mass-mark stocks is through thermal manipulation to produce recognizable and individual patterns on otoliths for identification (Volk 1987; Volk et al. 1990). Thermal marks become part of the otoliths by cyclical episodes of varied water temperatures. Changes in temperature, feeding irregularities, and other stress factors (e.g., egg shocking, hatchery transport) inhibit or alter the deposition of calcium on the otolith surface (Paragamian et al. 1992). Wild fish also exhibit unique marks on their otoliths through naturally-occurring temperature fluctuations. In addition, trace elements that deposit on otoliths during the juvenile stage may also provide a history of the environment in which the fish stocks reared, and may be useful in separating stocks (Edmonds 1988).

Besides identifying separate stocks, otoliths provide a host of other early life history data, including daily and annual growth determinations. As the otolith microstructure is distinctly formed at the embryonic and early stages, and persist throughout the life of the fish (Brothers 1985); daily and yearly annuli recorded on otoliths are generally more accurate than scales, and because of their early development, accessibility, and structure, otoliths are well suited for early life-history studies (Casselman 1983).

There are three mirror-image pairs of otoliths known as the sagittae, lapilli, and asterisci. These bones probably provide sound perception for fish, though the method is obscure (Pannella 1980). Otoliths are among the first bones to calcify in early development, and comprise of calcium carbonate and protein materials (Degens et al. 1969). Salmon sagittae grow on a daily basis in flattened concentric layers (Figure 1A), which appear as rings, bands, and opaque zones under light microscopy (Marshall and Parker 1979; 1982). The general shape of the sagittae is usually concavoconvex or subelliptical, with a middle focal point of growth known as the primordia. Each sagitta has a sulcus acusticus (a corrugation) through which auditory canals and nerves pass (Figure 1A), and is encapsulated within a thin membranous sack, called the sacculus, which rests in the neurocranium.

Common to otolith preparations is embedding them in a firm substrate, and subsequent grinding by hand or machine. Maceina (1988) describes a simple method for grinding large otoliths using a hand drill and sandpaper, which is useful for identifying gross otolith features. Another hand-grinding procedure (Schultz 1987) suggests placing the otolith in epoxy, attaching the otolith to a sheet of acetate, then grinding with fine sandpaper. However, frequent fracturing of the otoliths may occur, and detailed information recorded on otoliths requires extensive clarification. The method described herein is an efficient procedure to produce consistent and high-resolution otoliths for qualitative as well as quantitative analyses.

METHODS AND MATERIALS

Sample Preservation

Much of the research on salmonid otolith microstructure is done on juveniles and smolt; however, a few have focused on fish during the egg-incubation stage. Collection of otoliths is most often done by preserving the whole fish (or at least its head) previous to transport to an appropriate facility. Preservation of otoliths themselves is not necessary due to their bony composition, but there is need to preserve the surrounding tissue to facilitate extraction of these structures at a later date. Formalin is not an acceptable preservative due to the fact that it dissolves bone material. Ethyl alcohol (95%) is most commonly used because of its abilities to

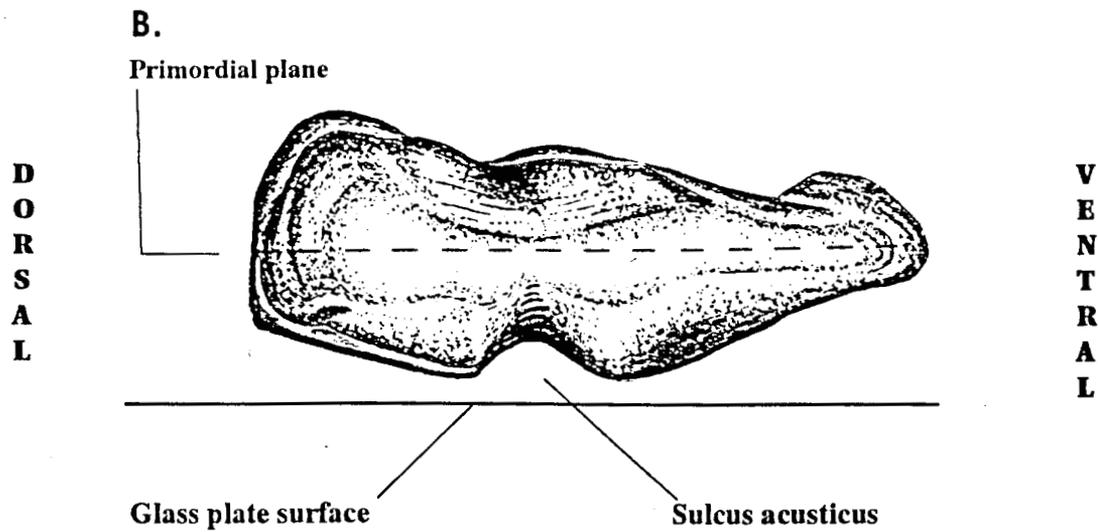
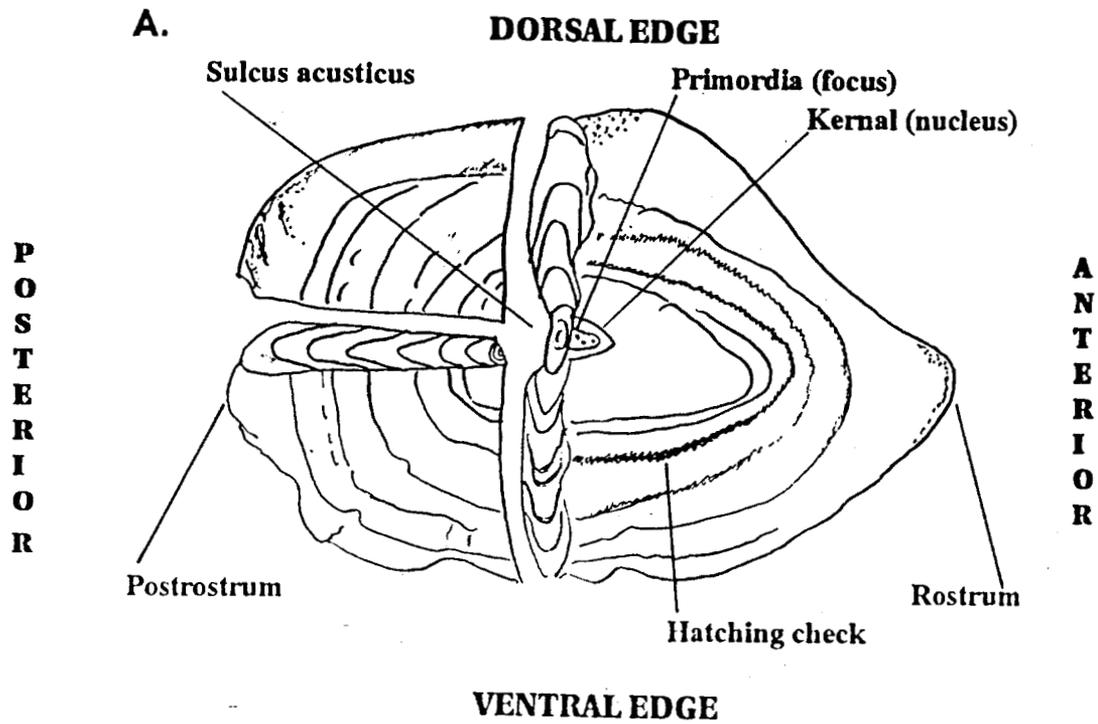


Figure 1. Lateral (A) and posterior view (B) of a typical sockeye salmon otolith. Note the downward positioning of the sulcus acusticus on the glass plate.

preserve the surrounding tissue while not altering the composition of the otolith. To insure proper preservation of surrounding tissue, we used a ratio of three parts preservative to one part sample by volume. This ratio allowed easy removal of otoliths by solidifying the brain tissue surrounding the otoliths. A lower ratio of preservative left the tissue mushy, which made it difficult to separate brain material from the otoliths. For long-term preservation, replacing or adding to the original alcohol was done when the alcohol became yellowish in color or excessive evaporation of alcohol in the containers was observed.

Extraction

Extracting otoliths (sagittae) from a salmon in the egg or alevin stage was accomplished by using a dissecting microscope on low power (3x) and fine forceps (no. 5). The first step was to peel the egg shell, or membrane surrounding the head of an alevin, away from the region directly over the otoliths. Next, the developing skull cap was removed with fine forceps, and the sagittae were excised from their pockets located at the bottom of the brain cavity. Removal of the sacculus was accomplished by brushing each otolith against the head of the embryo. Polarized light was used to highlight otoliths, especially in instances where the brain material was poorly preserved.

For larger fry (>40 mm) and smolts, different methods of opening the brain cavity were utilized, depending upon solidification of the skull cap. A posterior-to-anterior transverse cut was made using a razor blade to detach the skull cap (Figure 2A). If the skull cap was particularly hard, the two lateral halves were cut away separately. Ideally, while cutting the skull cap, the brain and optic lobes were also transversely cut. When necessary, excess cartilage was removed to expose the brain stem before the stem was severed to remove the brain (Figure 2B). Once the brain was removed, the sagittae which appear as 'lobes' under a tissue layer lining the brain cavity near where the vertebral column joins the cranium, were singularly removed from their 'pockets' with forceps. The sacculus and clinging tissues were removed by rubbing or brushing the otolith on the juvenile against the dissection plate or between two fingers of the dissector. With practice, it became easy to maneuver the forceps between the brain cavity lateral wall and the brain to grasp and remove the otoliths. Alternatively, sagittae can be

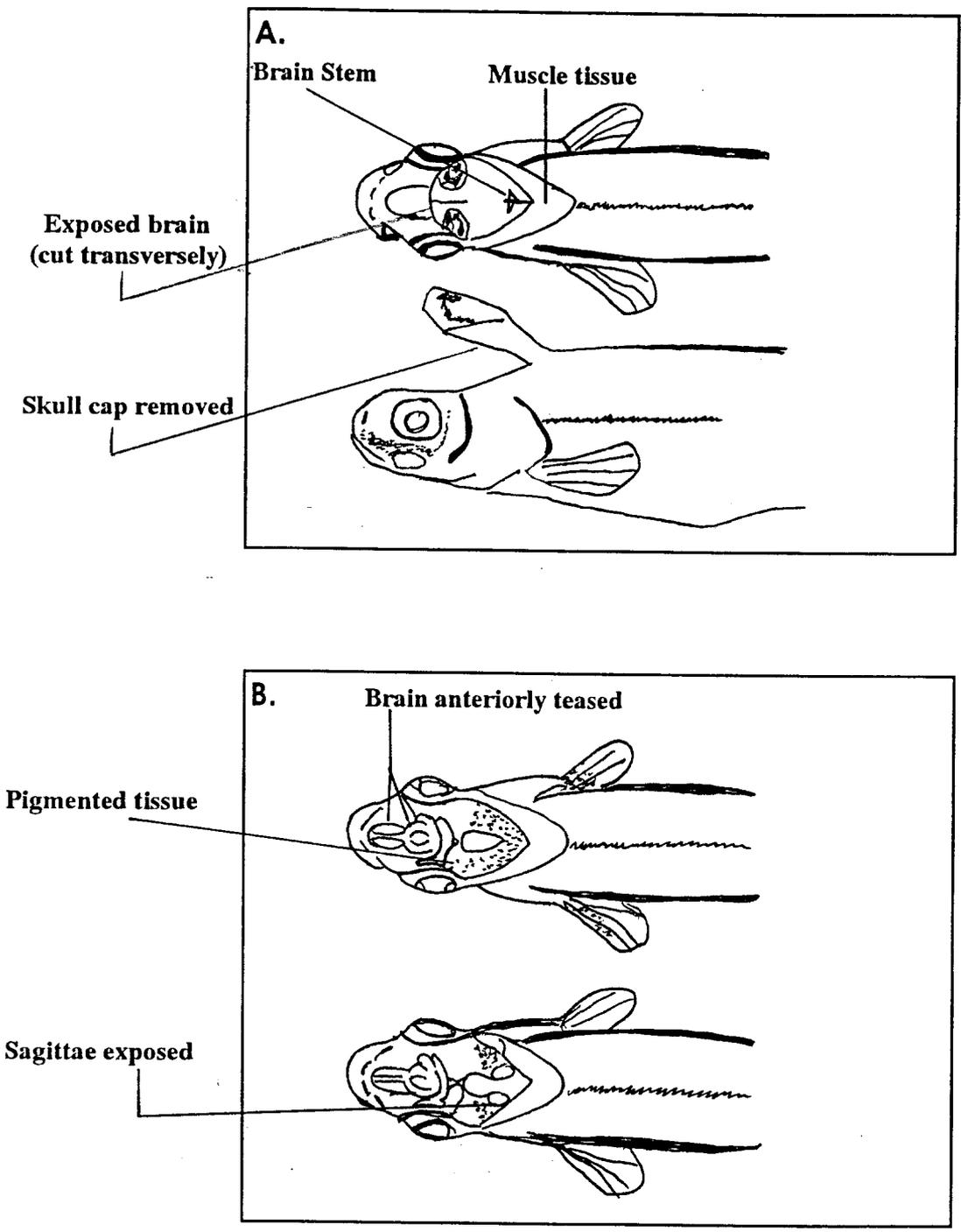


Figure 2. Schematic showing the dissection of the skull cap (A) and exposure of the sagittae under the brain (B).

removed from juveniles ventrally (Winter 1985), in which the lower jaw is removed to expose the otolith 'lobes' under the brain.

Molding and Embedding

After extraction, the otoliths were placed in pairs on a glass plate (2.4 cm x 2.4 cm and 4.8 mm thick), which was used as the embedding foundation. The glass plates were pre-cleaned with alcohol, and otolith pairs were identified (sample number) by writing on the plates with a standard alcohol-based laboratory marker. Otoliths have an inconsistent shape and lack uniform surfaces; however, the sulcus acusticus appeared to provide the most level plane of reference upon which to grind, and therefore was placed downward on the glass slide (Figure 1B).

Various molding materials were used including 25.4-mm diameter silicon, 19-mm diameter heater hose, 25.4-mm diameter laboratory tubing, and square vulcanized rubber. Several embedding resins were tried including polyester casting resin, epoxy resin, and thermoplastic resin. We found casting resin to be superior to the others. A well-ventilated area or ventilation fume hood was required to mix and mold the casting resin. The mixture consisted of 50 ml resin and 10 drops of hardener. All pouring of resin was completed within 10-15 minute before the resin began to harden. A small drop of resin was adequate to bond the otolith to the glass slide. Generally, positioning of the sulcus side of the otolith required the use of a dissecting microscope, and air bubbles trapped under the otoliths were easily removed with extra-fine probes or by tapping the glass plate on a table top. Most of the molds we used were tubular and open-ended, leaving the top open for pouring the resin. Resin was gently poured into the mold beside the otoliths to prevent disturbance until the resin was ~ 10 mm deep.

Next, the otoliths were placed in a pre-heated oven at 65-85°C. Heating the resin hastens the curing time to one hour (as opposed to 24 hrs at room temperature). Otoliths were usually ground individually because it was difficult to manually grind large surface areas and maintain a specific plane angle. Consequently, the otolith pairs were ground separately, and usually one otolith was kept as a backup for later preparation. We used a metallurgic cutoff saw to split the otolith pairs after the resin was fully cured. However, otolith pairs could be easily separated

by cutting the resin while it was still soft with a razor blade. Cutting pre-cured resin was accomplished by removing the otoliths from the oven after 25 minutes and lifting the molds from the resin blocks. A razor blade was passed between the pair of otoliths, and both halves were returned to the oven for curing completion. Resin can be cured at room temperature, but we found that it was usually difficult to remove from the glass plates. Cooling the resin in a freezer (10-15 minutes) further separated the blocks from the glass plates because the resin cooled faster than the glass.

Grinding the Medial Side

Manual grinding of otoliths was tried, but grinding efficiency was augmented by using a variable speed, eight-inch grinder/polisher equipped with a lubricating water spout. Initial grinding began with 400 grit paper discs, unless grinding small fry and embryo otoliths, then 600 grit paper discs were used. Slow to medium rotations (180-220 rpm) were used in conjunction with moderate amounts of water for lubrication. The embedding process created irregular surfaces on the top side of the resin blocks, which were removed by a rasping file or by the grinder. It was necessary to smooth the top of the block to provide a stable surface to place on a microscope stage. It was also essential to grind the bottom (where the otolith was located) flat and evenly (Figure 3A), as minute changes in the angle of grinding was exaggerated under the microscope. Further precision in maintaining an even grind was obtained by changing the direction that the block was held after each grinding. The otolith mounted on a glass slide was placed on a light microcopy stage and inspected using 10x magnification. Primordias were used as the primary reference point to identify the nuclear plane of the otolith. Repeated grinding and subsequent scope viewing were needed to slowly remove the otolith matrix until the reference point was reached.

Judging the depth of material left before reaching the primordia was accomplished by focusing first on the surface of the otolith matrix, then downward to the primordia (Figure 3B). Mistakenly, observers may only focus on the primordial reference and miss the semi-transparent material that separates the surface from the primordia; however, we found that it was not critical to grind down into the primordia to reduce optical density for the final preparation. More

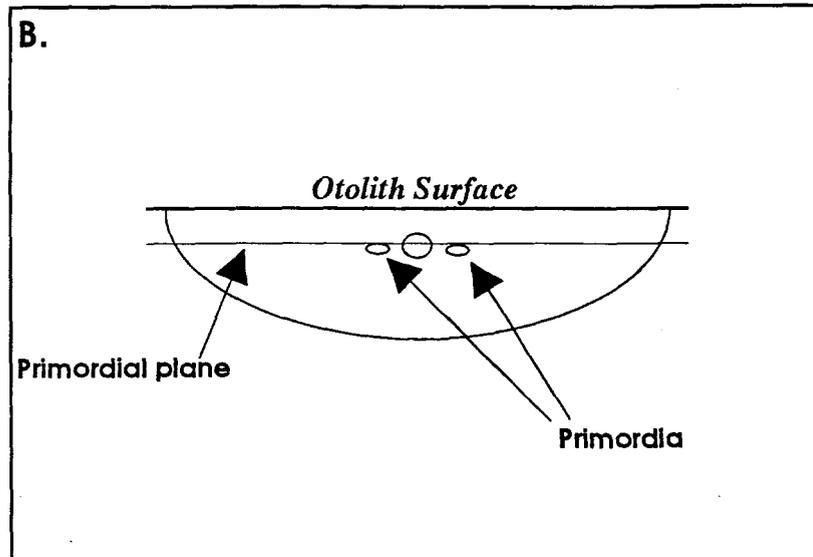
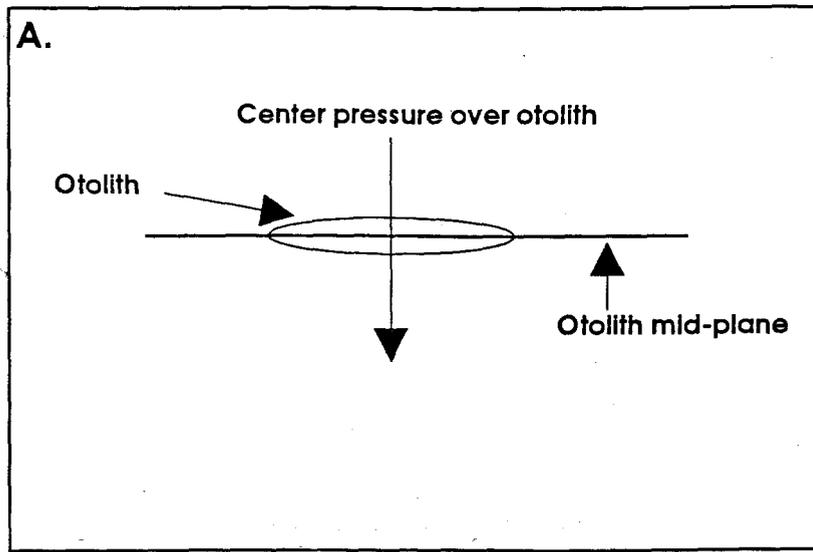


Figure 3. Schematic of the proper otolith grinding angle (A) and the depth of grinding the otolith relative to the surface of the primordia (B).

accurate determination of grinding was accomplished by using 40x on the scope and switching to 600-grit paper after the majority of otolith material was ground. Primordia nearest the rostrum frequently came into focus first, possibly suggesting an uneven grind or, that the otolith did not set evenly on it's sulcus. When this occurred, grinding stopped to preserve the rostrum region where most of the information was located.

Polishing the Medial Side

Polishing was performed using the identical grinder/polisher and eight-inch discs that were used for grinding. The discs were covered with either Microcloth® or Texmet polishing cloths. Deagglomerated alpha alumina one-micron Micropolish compound was used to augment polishing. Water and 50-75 ml of polishing composite were agitated in a 1000-ml squirt bottle, and spread on the polishing fabric until it was initially saturated. Additional compound was added during the process as necessary. The polisher was adjusted at moderate speeds of 200-240 rpm to confine compound spray. Polishing compound accumulation was rinsed out of the grinder tray by leaving the water on during the procedure. The blocks were polished until the obvious scratches were eliminated from the base.

Grinding the Lateral Side

Grinding the otolith's lateral side properly was crucial to obtain a quality preparation for detailed analysis. The process was analogous to grinding the distal side, but more difficult because the otolith was now mounted on a glass slide rather than a resin block. Best leveling and pressure control of the slide for grinding were achieved by using a two-handed grip; using two fingers from one hand to grip a suction cup that was used to hold the slide, and one finger from the other hand to press down on the leading edge of the slide. First, the trailing edge of the slide, relative to the direction of rotation, was gently placed onto the paper disc to prevent catching the leading edge. Next, the remainder of the slide was evenly eased down to the grinding disc, and light to moderated pressure was applied for grinding. A vacuum chuck was also tried as

®Mention of commercial products and trade names does not constitute endorsement by ADF&G.

an appropriate tool to hold slides, but our preference was to utilize a suction cup attached to the back of the slide while grinding. To preclude excessive wear of the paper, rotation velocity was set at slow to medium speed (180 rpm). For full lubrication and cleansing of the paper, a moderate volume of water was used, and the grinding was done directly in the water stream. Generally, grinding began with 400 grit sand paper to rid the otolith of excess resin left behind after the cutting stage. Once the otolith surface was reached, the paper was changed to 600 grit paper for better control.

Polishing the Lateral Side

The same polisher and components that were used for previous grinding steps were also used to complete the polishing of the lateral side. Moderate rotation speeds of 200-240 rpm were used for this polishing step. Using a suction cup and one hand, the slide could be firmly secured and held in place during the otolith's final polishing. Micropolish compound was applied to the grinding cloth until the napt was wet and saturated with compound. Periodically, water or compound was added to the cloth to maintain moisture and compound coverage. The trailing edge of the slide was placed on the polishing cloth first, followed by the leading edge so cloth snagging was prevented. Firm and steady pressure were applied until all visible scratches were eliminated. The slide was rinsed, dried, and examined with a microscope for grinding scratches, which when found dictated further polishing.

Epoxy Mounting

Mounting the otolith/blocks onto glass slides was necessary to thin-section the otoliths for detailed analysis. At this point, only one face of the otolith has been polished for examination. Before eliminating superfluous resin and thin sectioning, the otoliths were affixed to glass slides measuring 26 x 46 mm using epoxy, super glue, or thermoplastic resin. Because of its durability, epoxy was preferred. Epoxy consists of two parts and must be mixed to catalyze the glue. Since modest quantities of glue adhere numerous blocks, the epoxy was measured in drops. Usually several blocks were glued as a unit and eight drops of epoxy base for every four drops of hardener were sufficient to adhere the otoliths. The slides and the adhering base of the

blocks were cleaned with alcohol to assure a firm connection. After placing a small drop of epoxy on a mixing rod tip (metal mixing rods preferred) on the immediate otolith region, the block was eased directly onto the slide. Pressure was applied for approximately ten seconds to eliminate air bubbles and to distribute the epoxy into a thin layer. The epoxy took twelve hours at room temperature to cure. Curing time was shortened by using an oven at 100-110°F for 45 minutes and three hours at room temperature. When the epoxy was dry, the slides were permanently labelled using a diamond or tungsten-carbide inscriber.

Cutting Off Excess Resin

Once the epoxy was dry, the excess resin material was cut off using a metallographic saw to expose the distal side of the otolith for grinding. The saw was equipped with a vacuum chuck to hold glass slides, an adjustable lateral micrometer, variable speed motor, water cooling spouts, circulating water reservoir, and a diamond or aluminum oxide blade. In addition, a scientific vacuum pump with a capacity of 10-15 in. Hg was used to generate vacuum for the chuck. The first step was to attach the vacuum chuck on the table mount and to direct the water spouts toward the center of the blade to fully disperse lubricating water onto the edge of the cutting blade. A clean and dry slide was then placed on the vacuum chuck and the micrometer adjusted, bringing the slide to within 0.5 mm of the cut-off blade. Thickness of the cut was determined by the size of the otolith encased within the block. Most juvenile otoliths necessitated a cut of only 0.5- 1.0 mm. A thicker layer of resin required more grinding to reach the otolith surface. Caution in making adjustments was necessary to avoid cutting otoliths because of the possibility of cracking when cut. The chuck may be rotated to adapt vertical or horizontal cuts without any appreciable difference. The forward speed of the table was set to move slowly and the blade rotation was set to 2,500 rpm (diamond blade). If equipped, the auto shut off knob should be set to prevent damage to the blade axle. When the cut was completed, the table was slowly returned to the start position to prevent damage to the hydraulic and spring works. After approximately seventy-five plastic resin cuts, the reservoir water was exchanged and the vacuum pump's filter drained.

Determining Cessation of Grinding

Thickness of the resin material left on the slide was a discriminate indicator of further grinding. During the grinding process, the slide was subjectively inspected as to the thickness of remaining resin material, and was ground until very little of the resin was left (approximately 0.25 mm or less). Each slide was wiped dry and set aside after initial grinding. When several otoliths had accumulated, a disc with 600-grit sandpaper was exchanged for more precise grinding. Before proceeding with additional grinding, the otoliths were inspected with a microscope to assess the amount of otolith material separating the primordia from the surface. Primordias were used for reference points to identify the center plane of the otolith. Frequent observation of the slide under a microscope revealed the need for further grinding. Initial observations began with the 10x objective and later observations required 40x magnification for finer detail. Grinding and microscope viewing was repeated until the primordias were at the surface. Up to four repetitions were completed before reaching the primordial plane. As grinding proceeds, small fry and alevin otoliths appear very transparent, thus, extreme caution was necessary to avoid over-grinding .

DISCUSSION

Extraction

Fry began to desiccate if exposed to air for longer than five minutes, which caused the brain tissue to become tacky and adhere to the otoliths and forceps. When the fry became dry we dampened them with either water or alcohol. In addition, poor preservation resulted in 'mushy' or loose brain tissue, which hampered locating the otoliths. The use of polarized light to isolate otoliths was cumbersome and time consuming; we preferred to physically locate the otoliths, and found this method to be more efficient. Fry otoliths may be rubbed between two fingers to remove adhering tissues; however, otoliths were easily dropped using this method. We found that brushing the otoliths against the specimen's head or a dissection plate reduced loss of otoliths and assured a cleaner otolith. Also, it is beneficial to have a dark surface around the dissecting

area to contrast with the white-colored otoliths. We always removed the left otolith before the right one to prevent mixing of otoliths.

Molding

We found that keeping the surface of the mounting glass clean and cleaning the interfacing surface of the mold with alcohol assured a tight seal between the two. We preferred the silicon molds because the resin did not stick to this mold and they sealed well with glass. The silicon molds were readily available, but were designed as cups and must be modified to tubes. In contrast, the vulcanized rubber also worked well for molds, but were not readily available, and usually must be designed and shaped to fit this application. Heater hose did not provide an adequate seal, did not have sufficient durability, and the resin stuck to the surface of the hose. Laboratory tubing was not firm enough to maintain its form for molding, it did not endure the oven temperatures, and the resin also stuck to the surface of the tubing.

Mixing the resin in a ratio of 50 ml resin to 10 drops hardener was decided after some trial and error. The resin was poured directly onto the otoliths after they had been positioned with the sulcus side down, which helped maintain their orientation. Separating pairs or multiple sets of otoliths from the same block of resin was readily accomplished by cutting the soft resin with a razor blade. Use of a cutoff saw was more precise, but more time consuming. We found that 25 minutes was sufficient time before cutting with a razor blade. If the resin crumbles when cut it needs further curing, and if it was cured too long the razor blade would not easily slice the resin.

Otolith Grinding

A re-occurring problem in otolith grinding was the rapid use of grinding paper. Faster rotations appeared to smear the plastic resin and clogged the grinding paper. Also, forcefully placing blocks onto the disc congested the paper with resin. In contrast, slowing the speed of rotation and careful positioning of the blocks helped to preserve the grinding paper and decreased grinding time. Knowing how long to grind the blocks during each repetition was difficult at first

and required considerable practice. Because different size otoliths have inconsistent depths of primordial planes they could not be ground together. Also, pairs of otoliths from the same fish were not always regular in size, which sometimes dictated separate grinding preparations. As otoliths from two and three year old smolts lose some of their uniformity on the outer edges as they mature, the fringes (primarily the ventral and rostrum edges) were often ground excessively before the central plane was reached. If the primordial area was ground excessively, the most useful information on the otolith was considered lost and the otolith was discarded.

Experimentation with diverse slide gripping devices revealed that a suction cup applied to the back of the slide offered the best control and practicality. However, if the suction cup became scratched or was applied wet, it lost suction during the grinding procedure. Bare fingers or fingers shielded with rubber tips, did not allow gripping control of the slide. Likewise, vacuum chucks sufficiently coupled to the glass slide, but were found to be quite cumbersome.

Processing the lateral side of the otolith did not require water or any other clearing agent to enhance the primordial image if the otolith was sufficiently thin-sectioned by the distal grinding to allow light penetration. Surplus resin material over the surface of the otolith did not deter primordial inspections, since it was clear. The otolith matrix was improved when the suction cup was placed on the slide directly over the otolith block. If the resin or otolith was ground unevenly, the glass slide was also sanded, which resulted in an unusable otolith.

Deciding when to stop grinding the otolith was important since short intervals of grinding may completely granulate the otolith. Initially, feeling the thickness of the remaining resin was functional, but repetitive grinding and scope investigations were predominantly used during grinding. Cessation of the grinding process through inspection of the otolith by visual transparency required practice and patience. Also, practice was necessary to determine judgment of pressure to apply to the block to produce adequate preparations. Excessive pressures applied during the grinding or polishing created air bubbles through otolith fissures which distorted analyzing procedures. Air bubbles were also introduced through the epoxy when the blocks were cemented to the slides.

Otolith Polishing

Equal results were obtained for hand-polishing the otolith blocks using polishing cloths or using the grinder/polisher. It was important to eliminate all major abrasions on the block to prevent them from showing up on the final preparation. Polishing was improved if the compound bottle was shaken each time before use such that the alumina was well mixed. The polishing cloths were rinsed thoroughly after use to restore napt. If the otoliths were not too optically dense and only gross information was needed, no further preparation was required. When the lateral edge of an otolith was ground, a small amount of plastic resin or otolith structure usually remained, but did not appear to hamper balancing the slide on the polisher. Polishing was enhanced by periodically turning the slide in different positions, perpendicular to the direction of the cloth rotation. Further enhancement was achieved by keeping the polishing cloth moist. Also, we found that excessive amounts of polishing compound were not needed to produce fine polishes; as a moderate distribution of compound provided equal results.

Adhesives

Super glues were far more simple to use compared to epoxy procedures, but the epoxies were superior in strength, viscosity, and optical clarity. The super glue frequently lost its ability to hold during the rigorous grinding procedure, and air bubbles were encountered more often. The higher viscosity of the epoxy reduced the amount of adhesive needed and adequately permeated minor scratches. In addition, the epoxy allowed one hour before hardening, cleaned easily with alcohol, and only occasionally caused air bubbles (which was due more to excessive force in applying the block to the glass slide). On the down side, the epoxy took several hours to cure, and although heating shorten the curing time, it was our experience that accelerated curing caused the block sides to warp.

Resin Cutting

To save preparation time, block cutting and grinding of the otolith's lateral side may be completed simultaneously. Free-flowing water (lubricant) was necessary to generate undeviating

cuts and prevent deterioration of the blade. The spouts were aimed near the center of the blade to disperse the water evenly, but not touching the blade. Aluminum oxide blades were found to cut favorably, but did not last as long as diamond blades. In addition, diamond blades were operated at higher rpm's, which cut the resin easier. Feeding the block at a faster speed through the rotating blade did not improve cutting precision.

Preparation Time

Detailed analysis of juvenile salmon otoliths demand a sample that has been very carefully prepared. We experimented with grinding multiple otoliths in one resin block, but this was unsatisfactory because otoliths were not uniform in size and shape, and they often did not lie in the same horizontal plane when positioned prior to preparation. Therefore, otoliths were prepared one at a time. After spending a few months learning the above technique, up to thirty samples/day was achieved. These preparations were ground and polished on both sides (medial and lateral) and were available for detailed microscopic examination. For preparations requiring less optical clarity (medial side preparations), up to fifty samples/day were observed. Finally, we have conducted limited experimentation with preparing adult salmon otoliths. We found that with a multiple-specimen holder we could prepare 200-300 adult samples per day for gross examination (i.e., thermal mark recognition).

SUMMARY AND CONCLUSIONS

Otoliths have the potential to benefit a variety of studies. For example, otoliths can be used to chronicle the life history of wild and hatchery stocks for comparative purposes, and to distinguish patterns of growth for purposes of stock identification. As fishery researchers and managers are looking for efficient ways to study and regulate stocks, the development of a preparation procedure for otolith analysis is an essential factor in the practical use of otoliths for salmon studies. The described technique provides for a method by which fishery researchers can use for both gross and detail otolith studies. This method describes embedding the otoliths into a resinous plug, which provides stable mounting of the otolith for planar sectioning. Otoliths are then positioned on a glass plate and a silicon cast is placed over them which are

filled with polyester resin. After the resin cures, the medial surface of the otolith is ground and glued to a glass slide, the spare resin is cut from the lateral surface, and the otolith is finally ground for fine details. Quality of the preparation is proven when grinding reaches the primordial plane and the final polish produces an otolith capable of providing detailed growth and age data.

Production time improves as experience was gained in the dissection and preparation of the otoliths. For example, it was found that when the otoliths were placed sulcus side down onto the mounting plate, air bubbles were eliminated when the resin was poured. In addition, separating otoliths before the resin cures reduced preparation time, and the use of epoxy to secure otoliths to slides saved time because otoliths did not become unfastened which necessitated re-gluing. Also, precisely cutting the excess resin from the lateral side reduced grinding time and excessive wear of the grinding paper. Positioning the slides and resin plugs onto the grinding surface evenly conserved time and improved planar sectioning.

To comprehend the technique and begin preparation with some degree of confidence required approximately 2-4 weeks. However, consistent and detailed preparations may take up to six months, depending on practice and proficiency. Presently, this method allows for the preparation of 45-50 medially-ground samples per day and 25-30 medially/laterally ground samples per day.

ACKNOWLEDGEMENTS

We thank Erik Volk of the Washington Department of Fisheries for training, advice, and expertise in developing our techniques of otolith preparation. We also thank Gary Kyle for editorial review of an early draft of this report.

REFERENCES

- Brothers, E. 1985. Otolith marking techniques for the early life history stages of lake trout. Great Lakes Fishery Commission Research Completion Report. 1 Oct. 1985.
- Casselman, J. M. 1983. Age and growth assessment of fish from their calcified structures-techniques and tools. Pages 1-18 in E.D. Prince and L. M. Pulos, (eds). Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks. NOAA Technical Report NMFS 8.
- Degens, E. T., W. G. Deuser, and R. L. Haedrich. 1969. Molecular structure and composition of fish otoliths. Mar. Biol., 2:105-113.
- Edmonds, J. S., M. J. Moran, and N. Caputi. 1988. Trace element analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in western Australian waters. Can. J. Fish. Aquat. Sci. 46:50-54.
- Koenings, J. P., J. Lipton, and P. McKay. 1983. The fluorometric determination of the uptake and retention of the antibiotic oxytetracycline in sockeye salmon (*Oncorhynchus nerka*) fry: a quantitative approach to tetracycline marking. Alaska Department of Fish and Game, FRED Technical Report Series 19:22 p.
- Maceina, J. J. 1988. Simple grinding procedure to section otoliths. North American Journal of Fisheries Management 8:141-143.
- Marshall, S. L. and S. S. Parker. 1982. Pattern identification in the microstructure of sockeye salmon (*Oncorhynchus nerka*) otoliths. Can. J. Fish. Aquat. Sci. 39:542-547.
- Marshall, S. L. and S. S. Parker. 1979. Chignik sockeye studies: Daily growth patterns of sockeye salmon otoliths. Fisheries Research Institute. College of Fisheries, Univ. of Wash.

- Pannella, G. 1980. Growth patterns in fish sagittae. Pages 519-560 in D.C. Rhoads and R. A. Lutz (eds.). Skeletal growth of aquatic organisms. Plenum, New York.
- Paragamian, V. L., E. C. Bowles, and B. Hoelscher. 1992. Use of growth increments on otoliths to assess stockings of hatchery-reared kokanee. Trans. Am. Fish. Soc. 121:785-791.
- Schultz, D. L. and R. S. Taylor. 1987. Preparation of small otoliths for microscopic examination. N. Am. J. Fish. Management 7:309-311.
- Stekoll, M. S. and W. W. Smoker. 1986. Extraction and analysis of oxytetracycline tags in salmonids. Proposal to Alaska Dept. of Fish and Game. SFS-UAJ 8601. Univ. of Alaska School of Fisheries and Ocean Sciences, Juneau, Alaska.
- Volk, E. C. 1987. Inducement of banding patterns of the otoliths of juvenile chum salmon (*Oncorhynchus keta*). Proceedings of the 1987 Northeast Pacific Pink and Chum Salmon Workshop, Anchorage, Alaska.
- Volk, E. C., S. L. Schroder, and K. L. Fresh. 1990. Inducement of unique otolith banding patterns as a practical means to mass-mark juvenile Pacific salmon. Am. Fish. Soc. Symposium 7:203-215.
- Winter, B. D. 1985. A method for the efficient removal of juvenile salmon otoliths. California Fish and Game, March 1985. 63-64 p.

The Alaska Department of Fish and Game administers all programs and activities free from discrimination based on race, color, national origin, age, sex, religion, marital status, pregnancy, parenthood, or disability. The department administers all programs and activities in compliance with Title VI of the Civil Rights Act of 1964, Section 504 of the Rehabilitation Act of 1973, Title II of the Americans with Disabilities Act of 1990, the Age Discrimination Act of 1975, and Title IX of the Education Amendments of 1972.

If you believe you have been discriminated against in any program, activity, or facility, or if you desire further information please write to ADF&G, P.O. Box 25526, Juneau, AK 99802-5526; U.S. Fish and Wildlife Service, 4040 N. Fairfax Drive, Suite 300 Webb, Arlington, VA 22203 or O.E.O., U.S. Department of the Interior, Washington DC 20240.

For information on alternative formats for this and other department publications, please contact the department ADA Coordinator at (voice) 907-465-6077, (TDD) 907-465-3646, or (FAX) 907-465-6078.