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SUM NOTES

Preparation of Otolith Cross Sections

Introduction

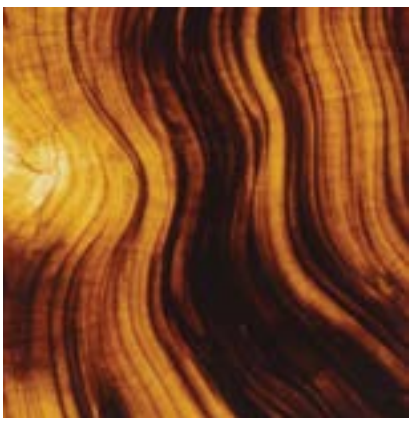
Otolith microstructure analysis is an important tool in determining the growth pattern and age of a fish. This information can then be applied to the larger study of the overall fish population, species dynamics and understanding the influence of environmental changes.

The age of a fish is generally determined by examining and counting growth rings on scales or ring like structures found in otoliths (small bones of the inner ear). The rings correspond to seasonal changes in the environment and can be compared to the annual rings of tree trunks. When the rings grow fast, relatively wide separations are observed; slower growth is indicated by narrow separations between rings.

Three pairs of otoliths are present and are most commonly termed the lapilli, sagittae and astersci. They each differ in location, function, size, shape and ultrastructure. These differences influence preparation decisions such as the polishing plane and the amount of preparation necessary.

Size is of particular significance since larger otoliths tend to have more three-dimensional depth and irregularity that limits external microstructural observations. On the other hand, it is possible to examine the microstructure of smaller otoliths without sectioning or polishing.

In most cases the otoliths are sectioned into very thin sections, cleanly and precisely. The rings then become visible. For otoliths greater than 100µm, it may also be advantageous to polish the specimen. This will further improve the resolution when observing the rings.



Otolith thin section with growth rings evident. 50x.

In order to continue developing an understanding of the fish population dynamics, it is critical that the techniques for removal, preparation, and analysis of otoliths are well refined with appropriate attention to quality control. In many cases, researchers have developed species specific approaches. The method shown here is fairly generic.

Preparation Procedure

1. Record data from the fish sample. Then remove the otoliths from the fish. Properly clean in bleach and rinse with distilled water. Remove any remaining moisture using an alcohol solution, then weigh and measure the selected otolith.

2. Select an appropriate embedding medium for the specimen. For specimens that will be complete after cross sectioning, wax is routinely used. If the section is to be polished, a hard media with low viscosity such as EpoHeat™ is recommended.
3. Prepare a mold, applying Release Agent to increase the ease of specimen removal. Mix the mounting media according to the manufacturer's directions. Fill the mold half full and allow the epoxy to cure.

4. Place the otolith on the half filled mold. Adhere the otolith to the cured epoxy with a drop of cyanoacrylate glue at the appropriate orientation. This will keep the specimen from shifting when the remainder of the mold is filled with epoxy.

5. Finish filling the mold with the mounting media, making certain to completely cover the otolith. Allow the epoxy to cure.

6. Trim excess mounting media around the otolith using an IsoMet® Precision Saw with a 15LC blade. This will save preparation time by reducing the surface area of epoxy that needs to be removed in the grinding process. A parallel cut will also insure that the correct polishing plane is established.

Blade selection can influence the quality of the results. The calcium based otolith tends to be fairly soft and can be sectioned with a conventional abrasive blade. However, the rigidity of the diamond blade offers more control and typically produces a straighter cut.

7. Attach the mounted otolith to a glass slide with Crystalbond Mounting Wax. You will have to preheat the hot plate to above the softening point of the glue (85-100 °C). Orient the mount on the slide such that the surface to be initially polished faces up and is parallel to the plane of interest.

8. Grind using a series of CarbiMet® Abrasive Discs starting with 240 grit (P280) and continuing thru 1200 grit (P2500). Holding the slide parallel to the grinding surface, apply even pressure

across the surface. After grinding into the otolith, inspect the section frequently using a light microscope. Check for the presence and position of the core.

The core is a circular area surrounded by concentric increments (rings) of relatively high contrast typically less than 20µm in diameter. As you approach the core, switch to the finer abrasive papers.

9. Once the core has been reached, polish the surface with MicroPolish® II 0.3µm Deagglomerated Alumina on a MicroCloth® Polishing Cloth.

10. Clean and examine the otolith surface. The single sided preparation may create sufficient resolution of the rings. However, for larger otoliths, higher resolution may be obtained by polishing both sides

11. Place the slide back on the hot plate, just long enough to soften the Crystalbond Wax. Remove the slide from the polished otolith mount. Add more wax to the slide and place a small piece of glass on the wax. Add more wax on top of this glass to attach the mount.

The additional glass is introduced to increase the working distance for the preparation process. This should make it easier to hold onto the slide as the cross section becomes even thinner. An alternative approach is to use a thin section slide holder (69-1583) in combination with a MiniMet® 100 Grinder-Polisher.

Automating the preparation process can save time. Grinding by hand also tends to favor one side or another of the thin section, eventually making one side thinner.

12. 12. Remove from the heat and place the polished surface onto the glass. Apply pressure to prevent air bubbles from forming between the glass and the mount.

13. 13. Polish as before, closely monitoring progress until a thin section, typically 10 to 50µm thick, is achieved. Visually watch for the core and incremental rings to reach sufficient contrast. Perform a final polish with the MicroPolish® II and MicroCloth®.

Note: The morphology of the otolith depends on the species and age of the fish. In some cases, a more complex morphology may be present. For example, the otolith may have growth axes which will cross over several planes. The result is that a single plane does not contain the core and all of the increments. In order to create complete documentation, the structure is alternately polished and photographed. Then a montage is constructed to observe the entire microstructure. Regardless of the complexity and planes of observation, the preparation procedure itself does remain essentially the same.

Bibliography

D.H. Secor, J.M. Dean, and E.H. Laban, "Otolith Removal and Preparation for Microstructural Examination: A Users Manual", Baruch Inst. 1991.

Equipment*
IsoMet® Family of Linear Precision Saws
MiniMet® 1000 Grinder- Polisher

Consumables*
IsoMet® Diamond Wafering Blade
EpoHeat™
Release Agent
Crystalbond Mounting Wax
CarbiMet® Abrasive Discs
MicriPolish® II 0.3µm Deagglomerated Alumina
MicroCloth® Polishing Cloth

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