Specimen Preparation System for Analyzing Fish Otoliths

Purpose

Sample preparation of fish otolith bones for age determination has been used for many years in the fishery industry. The study of otolith microstructure has become increasingly more important in recent years to help determine fish age, growth rates, environmental factors, and other important aspects to fish microstructure (Campana, 1985). Related to this increase in study is the sample preparation techniques implemented for the study of otolith microstructure. Improving the existing methods and reducing the tedium of sample preparation is important to improve the amount of collected data used in fish studies.

A new preparation system has been produced which allows for precise cutting, semi-automatic polishing, and precise removal of material of individually mounted otolith samples. This report will briefly describe the basic preparation method used for producing thin, polished otolith specimens for optical microscopy and laser chemical analysis.

Procedure

There are several requirements for the specimen preparation process during which the specimen is subjected to a wide variety of environments. Initial cutting of samples is generally done, depending upon the size of the otolith and the encapsulated geometry. In general, samples are mounted into an epoxy resin and cut into a block, similar to the methods used in ultramicrotomy. Using the Model 650 Low Speed Diamond Saw and the Model 65014 Ball Joint Holder, cutting otolith sections at virtually any plane is possible. The ability of the model 650 and it's accessories to adjust the cutting plane allows the section to be completed parallel to the growth planes, enabling precise polishing for accurate growth ring determination. Figure 1 illustrates the basic setup used for holding these samples during the cutting process.

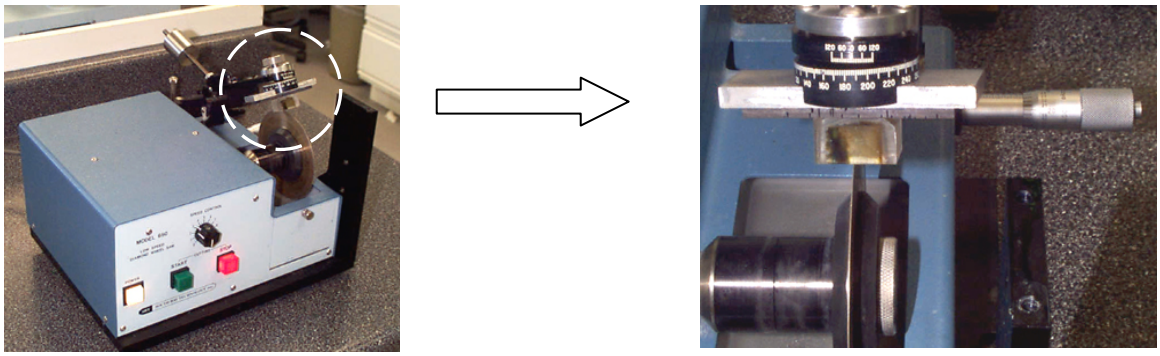

Figure 1: Image of the Model 650 Low Speed Diamond Saw with the Model 65014 Ball Joint Holder attachment. An encapsulated otolith is shown as mounted to the Model 65001 Single Axis Goniometer and oriented above the diamond wheel for sectioning. Adjustment of the cutting angle can be done in any direction up to 10°.

After cutting otolith sections to aproximately 200µm in thickness, the specimens must be mounted for polishing. Ideally, each individual section can be mounted to the polishing fixture and controlled via micrometers for accurate specimen thickness determination. To accomplish this, the Model 195 MultiLap™ allows individual mounting of samples on 6 micrometer controlled piston assemblies. Each piston assembly is equipped with a dial micrometer capable of removing material in 5 micron increments. A magnetic sample holder with a 15mm diameter glass slide is used for holding the samples onto the Model 195. To mount the specimens each is initially exposed to an adhesive similar to super glue in which the specimen is mounted onto a glass cover slip. The glass cover slip is waxed to the magnetic specimen mount of the Model 195 polishing fixture and held into place.

Following specimen mounting, each specimen is zeroed with respect to the fixture using the dial indicator at the top of each piston assembly. This is completed by placing the Model 195 onto a hard, glass plate and each micrometer is adjusted accordingly. After the zero process, each piston assembly is adjusted to remove the desired amount of material. Sanding down each sample is done using diamond lapping films attached to an 8" diameter polishing machine. The Model 920 Lapping and Polishing Machine combined with the Model 92002 Workstation (for rotation of the Model 195 fixture) is used to semi-automatically lap and polish the otolith samples. The diamond lapping films are applied to the plate of the Model 920 Lapping and Polishing machine using water, and specimens are polished using 6 , 3 , and 1 micron films in progressive steps until the specimen is polished to less than 10 microns in thickness. Below is an illustration of the setup used for holding the fixture onto the lapping machine.

Figure 2: Schematic illustration of the arrangment used for holding the specimens in place during lapping. The Model 195 MultiLap is held into place using a Model 92002 workstation which both holds the fixture in place and rotates it during the polishing process. The specimens are affixed to six different, individually controlled pistons which allow precise control over each individual specimen. Specimens are polished to within about 10 microns thickness and then observed under transmitted light.

When the polishing process has been completed, the specimens are then subjected to an etching process to remove some of the unwanted material from the specimen. The etching is done using a mild bleach for a period of about 24 hours, followed by a boiling water rinse and then compressed air drying. Each specimen is inspected under the light microscope for age determination via counting of the rings. The samples are then placed into a laser chemical analyzer and a full chemical analysis is done to help determine factors such as environmental conditions, diet, and other important aspects of the fish.

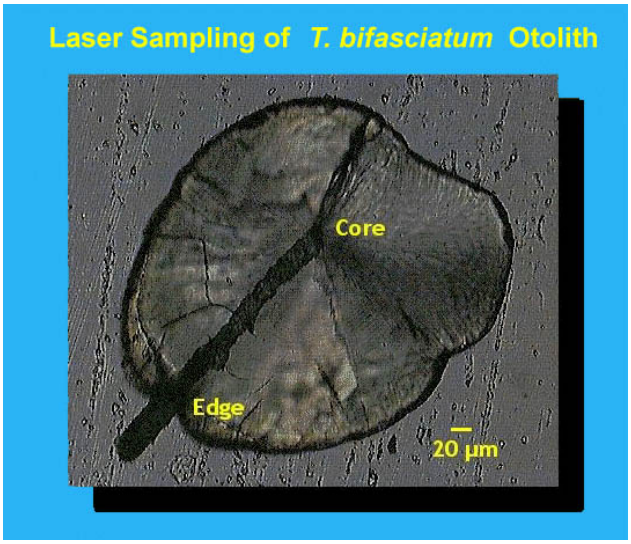

Figure 3: Image showing the otolith sample after polishing and laser ablation. The large trench extending from the core to the edge was created by the laser ablation process and produced the required data for the chemical analysis. Polishing was done using the Model 920/195 system. (Image courtesy S. Swearer, UCSB Dept. of Ecol., Evol., & Mar. Biol.)

Conclusion

A complete sample preparation system for producing high quality otolith sections has been developed by SBT. Combining high precision, reduced user interface, and compatible equipment and consumables, otolith section can easily be obtained using this system. The increased number of otolith samples that can be prepared simultaneously will enhance the throughput of the laboratory environment and enable a larger sampling of fishes to be studied.

References

1. S. Campana, et al, 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42; 1014-1032.
2. S. Swearer, UCSB, Dept. of Ecol., Evol., & Mar. Biol.