

Method for Preparing Otoliths for Microstructure Examination

Since Pannella (1971) described increments of daily growth from marine fish otoliths, research on fish otolith microstructure has proliferated. Use of otoliths for age determination or assessment of other aspects of life history of fishes typically involves use of light or scanning electron microscopy. The latter method offers greater depth of field and resolution than is possible with light microscopy, and has been used in several recent studies of otolith microstructure (Pannella 1971, 1974; Liew 1974; Popper 1978). Light microscopy has the advantage of greater convenience and availability of equipment. However, use of this method usually requires that the rather opaque otoliths be ground to improve light transmission. Previous workers (Pannella 1971; Taubert and Coble 1977; Wilson and Larkin 1980) have ground otoliths by hand-held techniques, but this method may result in an uneven plane through the otolith and consequent loss of detail. The new apparatus-assisted method of grinding we describe eliminates that shortcoming, and

has been used successfully with otoliths of juvenile salmon (*Oncorhynchus tshawytscha*) up to an age of 200 days.

The apparatus described here is similar in principle to the metallurgical jigs often used when planar grinds or polishes of metal surfaces are required. Our adaptation of the metallurgical jig is shown in Fig. 1. A microscope slide with the mounted otolith is attached to the jig with two set screws (Fig. 2). The jig is then placed on a sheet of metallurgical lapping film secured to a piece of plate glass. The spring-loaded central shaft is in the fully depressed position (Fig. 1), allowing the otolith to contact the abrasive. The operator then grasps the plexiglass plate and moves the jig in a circular fashion, thus obtaining a planar ground surface on the otolith.

The jig we used has a 24-cm diameter plexiglass plate and is supported by three machined aluminum legs. The spring-loaded shaft at the center of the plate moves freely through the collar shown in Fig. 2. The

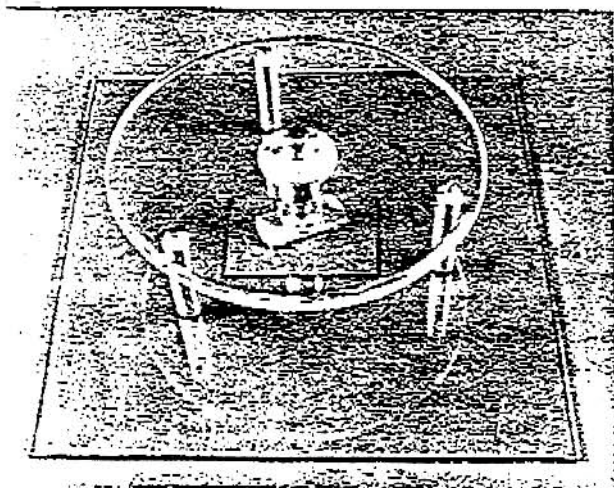


Fig. 1. Fish otolith grinding and polishing jig.

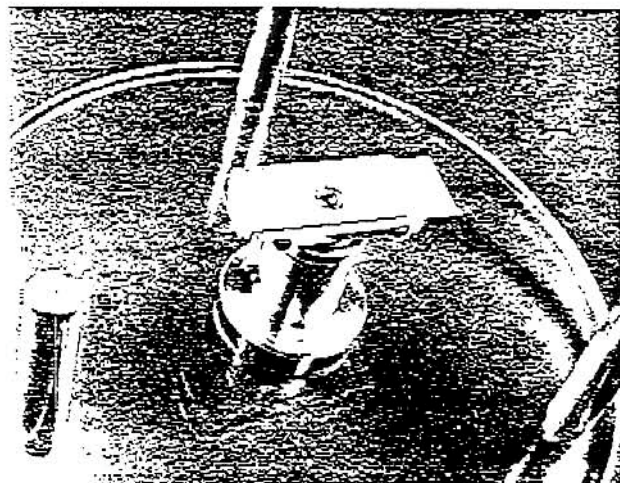


Fig. 2. Detail of microscope slide attachment.

shaft, when fully depressed, is flush with the base of the three supporting legs. The loading weight on the shaft was 185 g. Later modifications of this jig have included a provision for adjusting the loading weight on the central shaft, which allows the rate of grinding to be matched to the size of otolith.

The otolith is secured to the microscope slide by using a thermosetting plastic resin (Crystalbond, Aremco Products Inc., New York). Properties of this material enable easy removal of the otoliths by heating the slide (on a hot plate) if both sides of the otolith are to be ground or if the material is to be examined with a scanning electron microscope. The abrasives we used were "Imperial" Brand Lapping Film (from 3M Canada, Inc.); films with particle sizes of 30 and 0.3 μm were used in the initial and final grinds, respectively.

Acknowledgments

We thank Jim Oxtor of the Simon Fraser University Physics Department, who demonstrated the metallurgical grinding techniques that stimulated this work. Frank Wick of the University Machine Shop built the jig and Ron Long, Department of Biological Sciences, took the photographs. The lapping films used in this study were provided by 3M of Canada.

References

- Liew, P.K.L. 1974. Age determination of American eels based on the structure of their otoliths. Pages 124-137 in T.B. Bagenal, ed. Ageing of fish. Unwin Brothers Ltd., Surrey, England.
- Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. *Science* 173:1124-1126.
- _____. 1974. Otolith growth patterns: an aid in age determination in temperate and tropical species. Pages 28-39 in T.B. Bagenal, ed. Ageing of fish. Unwin Brothers Ltd., Surrey, England.
- Popper, A.N. 1978. A comparative study of the otolithic organs in fishes. *Scanning Electron Microsc.* 2:405-415.
- Taubert, B., and D.W. Coble. 1977. Daily rings in otoliths of three species of *Lepomis* and *Tilapia mossambica*. *J. Fish. Res. Board Can.* 34:332-340.
- Wilson, K.H., and P.A. Larkin. 1980. Daily growth rings in the otoliths of juvenile sockeye salmon (*Oncorhynchus nerka*). *Can. J. Fish. Aquat. Sci.* 37:1495-1498.
- J.D. Neilson and G.H. Geen. *Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada, V5A 1S6.*

Accepted 9 December 1980

