INTRODUCTION

In the inner ear of fish, three pairs of otoliths are formed by daily calcium carbonate (aragonite) deposition. Otoliths can provide aging information and chemical analyses of the otolith can be used to infer habitat information of the fish at different ages (Fig. 1) (4). The largest pair of Sagittal otoliths is usually used for age determination. The otolith starts out as a core “nugget” (Fig. 2, 4-6), and each day a new layer of calcium carbonate material is added, so that otoliths observed cleared or sectioned reveal rings (which really represent growth layers) (Fig. 3).

It can be very difficult to count these rings with light microscopy. SEM has been used to age fish, whereby the otolith is embedded in epoxy plastic like Spurrs, and then ground down on a diamond grinding wheel to the core (2). Then etching fluids like HCL and EDTA preferentially remove calcium carbonate between the layers (2) (because of easier access) thereby better displaying the rings.

The purpose of this study is to develop an optimal method of counting “rings” with the SEM, particularly with Low Vacuum SEM which requires no special specimen preparation like heavy metal coating. Otoliths in this study came from juvenile striped bass from field collections and hatchery rearings (John Mohan, ECU Dept. of Biology).

METHODS

- Each sample was etched using 5% EDTA or 1% HCL, or a mixture of the two
- Times varied on the sample and agent used
- HCL was used for no longer than 3 minutes (by itself and in mixture)
- EDTA had a range of 3-6 minutes depending on each individual sample
- The sample was then rinsed with distilled water and dried using a hair dryer on a cool setting
- Samples were observed in the Low Vacuum mode (LV) at 1 Torr in a Quanta 200 Scanning Electron Microscope.
- Photoshop CS4 was an essential tool for optimizing contrast of daily growth layers

RESULTS / DISCUSSION

- EDTA and EDTA/HCL were successful etching agents, but did not produce as clear an image as HCL by itself.
- This is in contrast to the paper by Correia, which found EDTA “…to be the most efficient and effective etching agent…” (1).
- Another paper suggested using proteinase K buffer which removes the proteins to produce “…better-contrasted daily increments and reducing ambiguous areas…” (3).
- HCL reacts with CaCO3, subsequently producing calcium chloride, water and carbon dioxide whereas EDTA chelates calcium and removes the ions from otoliths (3).
- At 3 minutes, HCL showed signs of damaging the otolith sample (Fig. 9).
- At 5 minutes, HCL made the sample unusable (Fig. 7 & 8)
- Each sample was not uniform.
- The samples should have been monitored more closely under the light microscope shortening the time increments the otoliths are exposed to the etching agents. This will be standard procedure in the future.
- Both secondary (SEI) and backscatter (BEI) images were used to count daily growth rings.

RESULTS / IMAGES

- Fig. 1: Whole Striped Bass Otolith
- Fig. 2: Sectioned otolith 4 minutes of 5% EDTA / 1% HCL
- Fig. 3: Sectioned otolith 4 minutes of 5% EDTA / 1% HCL
- Fig. 4: core after 3 minutes 1% HCL
- Fig. 5: core after 3 minutes 1% HCL
- Fig. 6: core after 3 minutes 1% HCL
- Fig. 7: 5 minutes of 1% HCL
- Fig. 8: 5 minutes of 1% HCL
- Fig. 9: 3 minutes of 1% HCL
- Fig. 10: 3 minutes of 5% EDTA
- Fig. 11: 6 minutes of 5% EDTA

ACKNOWLEDGEMENTS

I would like to thank Dr. Thomas Fink from East Carolina University for his help with the methods part of the experiment. It was a better project for having a sounding board. Also for his help in the SEM imaging to get the best picture possible. Also, I would like to thank John Mohan, a graduate student at East Carolina University, who provided the otoliths, and took the time to explain his research.

LITERATURE CITED