CHAPTER 1

Introduction

1.1. Pannella's Rosetta Stone

Investigators have long made use of the annuli deposited in the calcified structures of fishes in age determination. The discovery of daily increments within the otolith's microstructure (Pannella 1971) provides intriguing subtlety and exciting avenues for research. Otolith microstructure analysis has permitted new levels of precision in daily, seasonal, and yearly age estimates. The quantification of early growth and age structure will enable biologists to make better predictions about fisheries dynamics. Early life history paradigms like the critical period concept can be better tested.

The increments first described by Pannella record both pattern and process (Mugiya et al. 1981; Dean et al. 1983; Mugiya 1987a). In this they are analogous to the hieroglyphs of ancient Egypt which also show consistent and repeated forms and patterns. The hieroglyphs represented a wealth of information on the culture of ancient Egypt. However, it was not possible to "read" the glyphs correctly until the discovery and interpretation of the Rosetta Stone which contained parallel symbols in hieroglyphs and Coptic language.

The breakthrough in interpreting the Rosetta Stone came from extensive scholarship on the grammar of the Coptic language. Without knowledge of process, the French scholar Champollion would have not been able to decipher the inscriptions.

The analogy of the Rosetta Stone is that, with Pannella's observation of daily increments within otoliths, we too are at a point of discovery. We may be confident that the structure of otoliths contains an abundance of information on age, growth, physiology, and ecology of fishes. However, the exciting challenge before us is to do the detailed scholarship that was demonstrated by Champollion. We need to undertake rigorous, detailed, controlled, and carefully interpreted experiments on the process of otolith growth. Our hope is that this manual will encourage fish ecologists to employ information gained through microstructural analysis in their studies. By enlarging the field of otolith analysis, we who study fish age and growth can only benefit from new perspectives, new applications, and broadened scholarship.

1.2. Manual objectives

This manual presents techniques used to efficiently and precisely prepare otoliths for microstructural analysis. To make our manual easy to use, we apply most technical descriptions to striped bass, *Morone saxatilis*. Although no species can be said to be typical, the approaches we use in otolith preparation for striped bass are representative of our laboratory's protocols. We do not suggest that our techniques are unique or the best for all applications (see Wild and Foreman 1980; unpubl. man.; Pannella 1980a; Haake et al. 1982; Campana and Neilson 1985; Brothers 1987). Indeed, in a literature review (Appendix I), we found dozens of different techniques, most yielding sufficient resolution for microstructural analysis.

Table 1. Glossary of otolith and microstructure terms.

Accretion zone: Component of increment comprised predominantly of aragonitic calcium carbonate and intralamellar matrix. Ultrastructural examination has shown elongate crystals in this zone which are perpendicular to the periphery of the otolith.

Antirostrum: Anterior "thumb-like" projection of the sagitta, located dorsal to the rostrum (Fig.3).

Core: Calcified area occurring within the earliest deposited increment (area contained within the first discontinuous zone).

Discontinuous zone: Component of increment comprised predominately of organic matrix. Zone preferentially dissolves when weak acids (e.g., low concentration HCl or EDTA) are applied, resulting in narrow grooves which are observed in SEM examination.

Growth axes: Axes within the otolith along which proportionately rapid rates of deposition occur. Increment widths are greatest along these axes. Otoliths can have more than one growth axis in which case axes are sometimes referred to as major and minor. Growth axes have been demonstrated by Ca-45 incorporation.

Increment: Bipartite concentric ring comprised of alternating accretion and discontinuous zones. Increments validated to occur at a daily rate are termed daily increments.

Increment width: Linear measure of increment, comprised of one accretion zone + one discontinuous zone. Usually measured along a major growth axis.

Macula: Sensory epithelium composed of sensory hair cells and supporting cells. Cilia bundles of the macula serve as mechanoreceptors in hearing. The macula is located along the sulcus of the sagitta in the vestibule.

Otolithic membrane: Noncellular membrane adhering to portions of the otolith (see Dunkelberger et al. 1980).

Primordia: Initial deposition sites of organic matrix and calcium carbonate. Usually located in the core, primordia may fuse or remain separate, forming multiple cores. Peripheral primordia (areas beyond the core) have been described for the juvenile transition of pleuronectids (Campana 1983).

Postrostrum: Posterior-most projection of the sagitta. Can also refer to the entire posterior margin of the sagitta (Fig. 3).

Rostrum: Anterior-most projection of the sagitta (Fig. 3).

Sulcus: Sculptured groove along the sagitta's medial face (Fig. 3). Sulcus rests on the macula.

Vestibule: Sac structure which contains the otolith. Composed of epithelial tissue. The utricular vestibule contains the lapillus, the saccular vestibule contains the sagitta, and the lagenar vestibule contains the astericus.

1.3. How to use this manual

Chapters are written in manual format. Terms listed in Table 1 follow those defined by Wilson et al. (1987). All steps of our protocol are presented in Table 2. Detail on individual steps is given in the text and sections have been organized so that they can be read independently. We recommend that Chapter 3 be read as it should be useful in developing laboratory protocols. We also suggest reference to Appendix I for a review of techniques which have been applied to over 140 different species. Literature review and collaboration will prevent redundant efforts. Finally, supplies and equipment used in our protocols are listed in appendices II, III, and IV.

Table 2. Laboratory protocol for processing sagittae from juvenile striped bass. Material and equipment lists are given in appendices II, III, and IV.

A. Removal and Cleaning

1) Record data from the fish sample (i.e., length and weight).
2) Remove otoliths from fish (see Chapter 4).
3) Place otolith in a marked storage well of an otolith tray, or other storage container.
4) Soak otolith in bleach until clean (overnight or longer if necessary).
5) Rinse with distilled water.
6) Cover otolith with 95% ETOH, and allow the alcohol to evaporate.
7) Measure otolith length using a light micrometer or calipers, depending on size.
8) Weigh otolith using an appropriate balance.

B. Embedding

1) Mix only enough Spurr as needed, according to directions. Follow safety precautions.
2) While Spurr is being stirred, spray front and back of embedding molds with silicon lubricant.
3) After mixing Spurr, use a disposable pipet to fill molds half full. Bake in an oven for at least 12 h at 60°C.
4) Remove molds from the oven and store in clean container. Half-molds can be retained indefinitely as long as they are kept clean of dust. The polymerized Spurr of each mold (blank) can be removed and also retained for later use.
5) Draw a diagram of the molds to record individual otolith positions. This prevents later confusion of samples.
6) Place otolith on half-filled mold blank and record otolith number in the corresponding blank in the diagram.
7) Place a small drop of super glue, so as not to cover the otolith, on the blank of Spurr.
8) Orient the otolith in the glue drop and watch until the glue has set (only a few seconds). If there is too much glue, the orientation may shift, making polishing difficult.
9) Prepare another batch of Spurr. (Step 9 can be performed while working on steps 5–8.)
10) Finish filling the molds with liquid Spurr, being sure to cover the otolith completely.
11) Place the molds in the oven and bake for at least 12 h.
12) After the Spurr has polymerized, remove the molds from the oven, remove the individual Spurr blocks from the molds, and place in the appropriate storage cell.

Table 2. Continued.

C. Polishing

1) Trim excess Spurr around the otolith using an Isomet saw. This can save time by reducing the amount of Spurr that needs to be removed by polishing. Also, it insures that polishing will commence on a face parallel to the desired polishing plane.

2) Prepare a polishing board. It should have regions of wet/dry sandpaper of various grits: 220 grit for rapid polishing through the Spurr; 400 grit for initial polishing into the otolith; 600 grit for polishing to the core; 1200, 1500, and 2000 grits for fine polishing. The size and position of each region is dependent on how often it is used. The polishing paper is affixed to a plexiglass sheet (30 x 20 cm) with rubber cement or spray adhesive.

3) Place a polishing cloth on a separate sheet of plexiglass (not much larger the polishing cloth), and wet with water. Add polishing alumina (0.3 µm) as needed during polishing.

4) Preheat the hot plate to above the softening point of the Crystal Bond glue (65–100°C).

5) Place a glass slide on the hot plate.

6) Place a small piece of hard Crystal Bond glue on the slide and allow it to melt (Fig. 12A).

7) Remove the Spurr block (which contains an otolith) from its storage cell. Orient it on the glue so the region to be polished initially faces up (Fig. 12B). (Generally the otolith is oriented so that the antirostrum faces away from the glued face of the block.)

8) Wet the polishing board. Holding the slide edges and surface with the fingertips. The slide should be held parallel to the polishing board. Polish in a circular motion to insure equal polishing to all parts of the section (Fig. 12C).

9) After polishing into the otolith, inspect the section frequently using a light microscope to check for the presence and position of the core (see Chapter 6.3). As the core is approached, change to finer grit paper.

10) After the core has been reached and fine-polished with the polishing cloth, clean the slide and section with water or ultrasonicator and dry completely (Figs. 12D,E). Place the slide back on the hot plate just long enough to loosen the glue. Remove the slide from the heat and remove the polished section from the slide with forceps (Fig. 12F).

11) Place the slide back on the hot plate and add more glue.

12) Place a small piece of glass (ca. 1 cm^2) on the glue. Add more glue on top of the glass.

13) Remove from heat. Place the polished side of the Spurr block on the piece of glass, using pressure to prevent air bubbles from forming between the glass and the otolith (Fig. 12G).

14) Polish as before until a "paper-thin" section is achieved. Increment contrast should be monitored (see Chapter 6.3).

15) After the core and increments are all of sufficient contrast, perform a final polish using the polishing board.

16) Carefully label and store the slide and section in a slide box for later retrieval and examination.