CHAPTER 5

Cleaning, Handling, and Storage

Otoliths should be cleaned before preparation or storage to reduce analytical error, insure good infiltration of embedding plastics and mounting media, and improve the "shelf life" of the specimen.

5.1. Otoliths greater than 300 μm

We use forceps for routine handling of larger otoliths. Following removal, clean otoliths with dilute (10%) bleach (sodium hypochlorite). After immersing otoliths in bleach draw the bleach off and rinse the otoliths with distilled water so that bleach crystals do not form on the surface of the otolith. Draw off the water and just cover the otolith with 95% ethanol to remove water within the otolith. Following cleaning, completely dry otoliths in a low temperature oven or by exposure to air. Then store them in vials or tissue culture plates ("otolith trays") (Fig. 8). Otoliths should not be stored in paper envelopes because curved otoliths can easily fracture when envelopes are wrapped with rubber bands, slipped into pockets, or placed in notebooks. We use culture plates, since they permit otolith cleaning without any handling, and safe and convenient storage. Code otoliths by writing information on the tray. Dry otoliths can be stored indefinitely, but take care not to invert or overturn the trays. For long-term storage of whole otoliths, place wax paper under the lid of each tray, thus sealing the individual wells, and tape the trays shut. Embed very large otoliths (>10 mm) directly, and store them in blocks of Spurr or Epon (see Chapter 6.1). Mark the blocks for future identification.

![Fig. 8. Otolith tray (tissue culture plate) used for storing otoliths. Information is written on the tray’s lid.](image)

5.2. Otoliths less than 300 μm

Otoliths less than 300 μm cannot be easily manipulated with forceps. Too little pressure, and the otolith may be dropped. Too much pressure might cause it to be crushed, especially if there are any flanges or irregular surface features. Also, if the otolith is removed from an aqueous solution, it may be difficult to overcome the surface tension of the liquid. We recommend that investigators treat otoliths much smaller than 300 μm with special care. We also recommend setting up procedures that reduce otolith handling as much as possible.

Separate small otoliths from adhering tissue by teasing away the vestibule and macula with fine tools such as forceps and dissecting needles while the otoliths remain in some aqueous medium (e.g., water, ethanol). Several methods are available for the transfer of small otoliths. 1) Using a micropipette, remove otoliths with a small amount of the dissection medium. Under a dissecting microscope, transfer the otoliths to a clean slide or storage container. A small mouth pippette can increase control. 2) Shunt the otoliths over to the side of the media with a dissecting needle, thus
separating them from the tissue of the fish. Once the dissecting media has dried (if water or ethanol), press a finger down on top of the otolith. Otoliths less than 300 μm will fit nicely into the epidermal ridges of a (clean) finger. Scrape the otoliths from the ridges using a dissecting needle. 3) Pick up otoliths with a wetted dissecting needle. This works well for picking up dry otoliths and placing them in aqueous media. The reverse method of transferring otoliths from a wet medium to a dry surface is more difficult because of the surface tension of the medium. 5) Use small brushes, bacterial loops, or invertebrate forceps to transfer small otoliths.

Give handling steps some emphasis in deciding on protocols for small otolith examination. Considerations include: How were otoliths removed? Are otolith weights desired? Will sectioning and polishing be necessary? How many times will each otolith be examined? How will otoliths be randomized between increment counts? Is any SEM work going to be done? One important decision is which dissecting medium. We use bleach, water, or 95% ethanol if otoliths will be weighed. Otherwise, we remove otoliths of larvae in immersion oil (since it clears the fish) and leave them there. If sectioning and polishing are necessary (e.g., for SEM work or more detailed microstructural study), use bleach, water, or 95% ethanol as dissecting media. Alternatively, embed entire larvae (see Chapter 4.2).

We prefer to store smaller otoliths (< 300 μm) on slides. If otoliths are dissected in an aqueous media, then it is possible to mount small otoliths in a variety of media (e.g., Canada balsam, Spurr, Epon, epoxy resins, cyanoacrylate glues, clear fingernail polish). If the otolith is dissected in viscous media such as immersion oil, clove oil, or glycerol, then contain it on the slide by mounting a coverslip over it. Do not leave viscous media uncovered since dust will quickly collect in the media. Draw a bead of cyanoacrylate glue or fingernail polish around the oil that contains the otoliths. Allow it to dry partially and then place a cover slip on top of the bead, without resting it on the otolith. Another method is to place a strand of hair on either side of the otolith to prevent crushed otoliths. Always mark a spot on the cover slip or on the back of the slide to demarcate the position(s) of small otoliths. Store otoliths mounted in oil flat.

There is some evidence that long-term storage in immersion oil or glycerine (and presumably other oils) can cause degradation of otoliths. The rate of degradation may be significant over weeks, months, or years. Therefore, promptly examine and process otoliths mounted in these media.