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Methods and Equipment

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Collection and storage

The primary sources of age samples processed by the Investigation are Northeast Fisheries Center (NEFC) bottom trawl and shellfish resource surveys and commercial landings. Additional samples are periodically collected during various state-conducted research surveys and by fisheries observers who serve on foreign fishing vessels.

Scales and otoliths are the anatomical structures most frequently collected from finfish. Scales are preferred because they are easier to collect and process, providing, of course, that clearly defined growth patterns are consistently formed. Young-of-year specimens and samples of certain species with fragile or difficult-to-remove age structures, e.g., Atlantic herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*), are frozen whole for later dissection and processing at the laboratory. Other anatomical structures, such as fin rays or vertebrae, may be collected and used for special studies, such as age validation.

While scales are the easiest structure to collect, they must be taken from an area on the fish known to exhibit complete and clear growth patterns. For gadids and flounders, this area is on either side of the lateral line anterior to the caudal peduncle, the area where the first and largest scales develop. For other species, such as bluefish (*Pomatomus saltatrix*), black sea bass (*Centropristis striata*), striped bass (*Morone saxatilis*) and scup (*Stenotomus chrysops*), scales are removed from the area behind the pectoral fin where the largest scales are located. The area is first scraped with a blunt knife from the head towards the tail of the fish to remove adhering slime and dirt. The knife is then cleaned and used to remove a sample of scales by scraping firmly towards the head of the fish. The knife blade with adhering scales is then placed between the sheets of a folded absorbent paper liner in a coin envelope and wiped clean of the scales.

Otoliths are removed by dissection of the head of the fish with a sharp knife or a bone saw. Only the sagittal otoliths, the largest of the three pairs found in the sacculi of the inner ear located posterior to the brain, are removed for examination. Otoliths to be stored dry are removed from the sacculi (enveloping membranes) before being placed in an envelope. For some species (e.g., Atlantic mackerel, alewife (*Alosa pseudoharengus*) and Atlantic herring), otoliths are stored for one or two days in water-filled vials after dissection, since they require careful cleaning under a microscope at the laboratory.

The following information is recorded on the envelope for each specimen sampled on resource surveys: cruise, station, species, length, sex, and maturity. Corresponding information for specimens collected from commercial sources includes: vessel name, date, statistical area, latitude and longitude, port, depth, gear, species, market category, sampling method, length, and sex.

Surf clams (*Spisula solidissima*) and ocean quahogs (*Arctica islandica*) collected during NEFC surveys are shucked at sea. Whole paired valves are stored in cloth bags with labels referring to station location, date, and number of specimens. To minimize valve damage, small specimens are frozen whole for later processing at the laboratory.

Preparation of age structures

The following are general descriptions of methods for preparation of structures commonly used for age determination in the Fishery Biology Investigation. Other techniques, such as staining, may be used for special studies but are not presented in detail here. Modifications of certain techniques for particular species are described where appropriate. A complete list of equipment used, with specifications and possible commercial suppliers, is given in Appendix A.

Most of the procedures currently in use for finfish were developed in the early 1970's under the direction of Mr. Fred Nichy, then head of the Age and Growth Unit. From 1970 to 1975, he conducted numerous experiments to enhance otolith growth patterns by heating (e.g., burning, "deep frying" in hot oil) and baking in toaster and microwave ovens. Baking otoliths proved to be best for our purposes. His experiments with various plastics from 1972 to 1974 led to the use of laminated plastic for making scale impressions. Other experiments from 1972 to 1974 with low-speed saws and different types of blades led to development of procedures for thin-sectioning otoliths.

Scales

Age determinations using scales may be made from direct observations, scale impressions, or photographs. Actual fish scales are rarely used, however, because they are often covered with dirt or dried and pigmented residue. In addition, they are generally translucent because of their thickness, internal structure, and coloration, rendering growth zones difficult to interpret with transmitted light. Also, scales are not flat, resulting in uneven light diffraction and distortion of the image during microscopic examination.

Scale impressions in laminated plastic film usually avoid the above mentioned problems so that age marks are more detectable. Cellulose acetate plastics are avoided since they require heat or chemicals to soften the surface for adequate scale impressions. However, studies of fish species with thick scales (e.g., striped bass) may require the use of the heavier cellulose acetate plastic. Several types of laminated plastic film, consisting of a thin, soft polyethylene or surlyn layer over a thicker, harder vinyl or polyester substrate, are simpler to use and produce consistent results for most species. Representative compositions are as follows (Dery 1983):

Substrate	Middle Layer	Surface Layer
0.203 mm (0.0080 inch) semi-rigid polyester	0.002 mm (0.0001 inch) saran	0.032 mm (0.0013 inch) polyethylene
0.203 mm (0.0080 inch) semi-rigid polyester	0.002 mm (0.0001 inch) saran	0.019 mm (0.0008 inch) surlyn
0.190 mm (0.0075 inch) polyvinyl chloride	none	0.051 mm (0.0020 inch) surlyn
0.190 mm (0.0075 inch) polyvinyl chloride	none	0.051 mm (0.0020 inch) polyethylene
0.185 mm (0.0073 inch) vinyl chloride	0.018 mm (0.0007 inch) saran	0.051 mm (0.0020 inch) polyethylene

Any of the above laminated plastics produce impressions of scale surface features, but those having a middle layer of saran and a surface layer of polyethylene are generally superior.

Scale impressions have several advantages over the direct use of scales. They may be viewed by either transmitted or reflected light, and several scales may be impressed at the same time on one slide allowing for the selection of scales with the clearest features. The impressions are clean, even if the original scale used was not, and are easily stored and handled. The image of the scale is also flatter than the original scale and causes minimal depth-of-focus problems at high magnification. Impressions are fairly easy to prepare and simplify the handling of a large number of specimens.

Scale impressions are prepared by placing several scales, sculptured side up, on a heavy base slide of 1-mm thick (0.040 inch) cellulose acetate plastic. A laminated plastic slide, with the soft side down, is then placed over the scales (Fig. 1). Another heavy plastic slide (0.65-1 mm thick) is placed on top of the laminated plastic slide, and the whole "sandwich" of slides is rolled through a jeweler's press (Fig. 2). The two heavy acetate slides act as cushions to help equalize pressure over the thin and thick areas of the scales, resulting in a more uniform impression. The scales are then removed from the plastic slide and the resulting impression is stored in the original specimen envelope. The impressing procedure must be done in one smooth, continuous motion to avoid distortion of the finished impression. The two rollers of the press must be carefully adjusted to obtain a complete, clear impression. The upper roller of the press is usually canted slightly for impressing ctenoid scales. This applies slightly more pressure to the thin anterior edge of the scale. Also, two laminated slides may be used to "sandwich" very small scales, if it is difficult to distinguish between the sculptured and smooth sides.

Otoliths

Otolith preparation for microscopic examination includes whole, baked and broken, or thin cross-sectioned specimens. Species-specific methodology has been developed and is described more fully in the individual species sections of the manual.

Whole otoliths are microscopically viewed individually in ethyl alcohol or placed in depressions of black plastic trays. Embedding in resin improves contrast and enhances detection of growth zones under reflected light. Whole otoliths of some species, such as the short-lived butterfish (*Peprilus triacanthus*), are examined in ethyl alcohol in the unmounted condition, since they are thin enough for detection of early annuli and prominent, widely spaced later annuli. Pairs of otoliths from such species as Atlantic mackerel, Atlantic herring, and alewives are positioned in circular depressions in black molded plastic trays (Watson 1965) and embedded in Permount or clear fiberglass resin (Fig. 3). Permount may in time react with the molded plastic, producing air bubbles, "yellowing," or crystallization. Application of a few drops of solvent (xylene) after several months of storage removes air bubbles and stabilizes the resin for permanent storage in a sealed bag. Fiberglass casting resin will not adhere to molded plastic, but may be used with other materials such as plexiglass (a high-density acrylic). Other resins ("Eukitt," used in Europe, and Canadian balsam) are either prohibitively expensive or difficult to procure.

Currently, only Atlantic cod (*Gadus morhua*) otoliths are baked, a process that takes from 3 to 6 minutes in a scientific radiant heat oven at about 275°C (525°F). Small otoliths tend to require more baking than large otoliths, possibly as a result of their more rapid growth and greater diffusion of protein. Properly baked otoliths

are a caramel color; a grey or ashy color is an indication of overbaking, which may cause the otolith to crumble when broken in half at the nucleus. Visibility of the annuli is enhanced by baking, since the hyaline zones turn brown in contrast to the white opaque zones. Burned otoliths may fade with time, but baked otoliths remain unchanged even after storage for several years.

Dry otoliths are thin-sectioned on an Isomet low-speed saw (Nichy 1977) using a pair of fine-grit diamond-impregnated blades separated by a spacer approximating the desired thickness of the section (Fig. 4). Carborundum blades are an alternative, but they tend to break quite easily. Sectioning is accomplished by mounting the otolith on a small cardboard tag bearing crosslines that facilitate proper alignment. Otoliths of most species are positioned to obtain a transverse cross-section across the collum of the sulcus (Fig. 5). A small piece of double-sided tape covers the crosslines at the center of the tag and secures the otolith in the proper alignment for sectioning. Some species have fragile otoliths and the sections break easily. For these, a bed of molten wax is flooded onto the tag and the otolith is positioned on the wax bed before it hardens completely. The wax is heated in a double boiler or egg poacher, with glycerin in the base, on an adjustable-temperature hot plate at approximately 115°C. The otolith is then completely embedded in a mixture of four parts molten paraffin wax, one part decolorizing carbon (enough to just turn the wax black), and three parts calcium oxide powder (enough bulk to prevent the wax from running and to provide additional abrasive action during sectioning). Only a thin layer of wax covering the otolith is required. After the wax has hardened, the tag is inserted in a custom-machined slotted holder on the saw which aligns the otolith for cross-sectioning by the blades (Fig. 6). The saw's micrometer adjustment may be used for final alignment to produce a precision cut. Two 7.6-cm (3-inch) diameter blades separated by a 6.35-cm (2.5-inch) plastic or metal spacer are mounted on the saw unit to produce a thin-section with one cut. Spacer thickness varies from 0.015 to 0.030 mm (0.006 to 0.012 inch) depending on the viewing requirements for age marks in the section. The diameter of the spacer is normally the same, or slightly smaller, than the flanges supporting the two blades.

The saw is operated at maximum rpm (300) and the otolith is gently lowered onto the spinning blades with their rims immersed in a lubricating/cooling solution of 15 parts cold water to 1 part clear, liquid dishwashing detergent. (If foaming is excessive, the amount of detergent may be decreased.) The detergent solution also washes away particles of wax from the blade surfaces. A balancing weight is used on the saw arm that is light enough to keep sectioning time between 1 and 2 minutes. This also avoids warping the blades. The automatic shut-off on the saw is adjusted to allow the blades to just begin cutting through the double-stick tape, but not into the tag itself. After completing a section, the tag is removed from the slotted holder and bent along the cuts (Fig. 7). This exposes the section for removal and placement on a small square of black construction paper. It is folded inside a protective piece of paper, and returned to the specimen envelope with the cut otolith remaining on the tag. Preparation and sectioning of an otolith generally takes about 2 to 3 minutes.

Bivalves

Age determinations of surf clams are made from thin sections of chondrophores (Ropes and O'Brien 1979). For large surf clams, a portion of the chondrophore is first excised using a pair of 25.4 cm (10 inch) diameter diamond-impregnated sawblades spaced 4 mm apart and mounted in a high-speed (1725 rpm) saw unit (Fig. 8); those of small surf clams are excised using 10-13 cm (4-5 inch) diameter blades and a low-speed (300 rpm) saw to minimize breakage. The excised portion is broken away from the valve by finger pressure. Accurate age determination depends upon careful orientation of the valve during excision of the chondrophore so that the excised portion contains the earliest formed portion of the valve, termed the umbo (Fig. 9).

After minor polishing on carbide paper to flatten the surface and remove saw marks, the excised portion is glued to a glass slide using epoxy cement and thin-sectioned by a single blade on the low-speed saw (Fig. 10). An acceptable section is about 0.25 mm thick and takes less than 15 minutes to cut. Surf clams have a chondrophore in each valve of a pair, so the second valve may be processed if the first does not produce a suitable thin-section. Photographically enlarged prints may also be obtained by using the section as a negative in a photographic enlarger. At maximum lens aperture opening of a photographic enlarger, a 5-second exposure of the image onto photographic paper usually produces a suitable print.

Age determinations for ocean quahogs are made from acetate peels (Ropes 1987). The left valve is used, since it has a single prominent tooth in the hinge containing annuli which can be exposed by sectioning. The valve is marked on the ventral margin at a point from the posterior end equal to one-third of the valve length (Fig. 11). This orients sectioning through the umbo and parallel to the broadest tooth surface. The valve is fastened to the adjustable arm holder of an Isomet low-speed saw with its concave, inner surface toward a diamond-impregnated sawblade. The valve is oriented so that the cut is made from the ventral margin through the middle of the tooth, or beside the posterior edge of the tooth for small valves (Fig. 12). The anterior end of the valve is saved for subsequent processing. The cut surfaces are immersed in full-strength household bleach for removal of the periostracum.

An epoxy resin with a colored pigment is used to support the valves during subsequent polishing. The mixed epoxy is poured into molds to a depth of about ½ cm and the sectioned valve is lowered cut-surface-down into a mold (Fig. 13). After an overnight hardening period, three successively finer grits (240, 400, and 600) of carbide paper are used for grinding to expose the cut valve surface, followed by polishing with a vibrating lap machine, to obtain a blemish-free high-gloss surface. Treatment with a 1% hydrochloric acid solution for 1 minute etches the valve surface. Acetate peels are then made by applying an acetate sheet (0.013 mm (0.005 inch) thick) over the etched block surface after flooding it with acetone. After a 1-hour drying period, the acetate is peeled off and sandwiched between glass slides for examination under a light compound microscope.

Methods of examination

Appendix B contains detailed information on equipment mentioned in this section and possible commercial suppliers.

Scale impressions are viewed using microprojectors (Figs. 14 and 15) and microfiche readers (Fig. 16) with transmitted light at magnifications of 20× to 52×, depending on the size of the scales.

Otoliths are examined under binocular stereomicroscopes with a reflected light illuminator inclined at 45-60 degrees at magnifications of 10× to 65× (Fig. 17). Under reflected light, winter (hyaline) zones appear dark and summer (opaque) zones are white. Polarizing filters may be used to reduce glare and enhance contrast. Growth patterns of otoliths or sections are also enhanced by applying wetting agents, such as Kodak Photo-Flo 200, alcohol,

glycerin, or clove oil. Whole, individual otoliths are placed in black plastic or clear glass holders with a black base or background and then immersed in the wetting agent for viewing. Embedded otoliths are viewed in the trays with no additional preparation. Broken otolith halves are either hand-held under the microscope or are temporarily mounted in a piece of soft, black plasticene. Otolith thin-sections may be placed on small squares of black construction paper for examination. A wetting agent flooded onto the surface soaks into the paper providing the necessary background contrast.

Thin-sectioned chondrophores from surf clams are examined microscopically using transmitted light. The annuli are translucent and in sharp contrast to opaque growth increments. Photographically enlarged prints of the sections show annuli appearing as dark zones alternating with white growth zones (the opposite of the image seen under transmitted light).

Acetate peels of ocean quahogs are sandwiched between glass slides for examination under a light compound microscope with transmitted light. Annuli appear as dark lines curving down from exit locations at the valve surface toward the umbo; growth increments, which are most evident in the early ontogeny of a specimen, have a lightly textured, granular and homogeneous appearance.

A TV camera monitor connected to a binocular stereomicroscope or dual-viewing heads on binocular stereomicroscopes may be used for viewing various age structures. Such systems are very useful for training personnel and resolving age determination of difficult specimens.

Interpretation and conventions

In temperate-zone waters, both fish and shellfish species exhibit seasonal growth patterns indicative of age. Generally, growth is fast during warm "summer" months, and slow during cold "winter" months. One year of growth consists of one summer zone plus one winter zone. The annulus is usually defined as the winter zone. Summer and winter growth zones differ in appearance, thus providing the basis for age determinations. Increase in length is proportional to the growth of the age structure being used and is a basis for empirical relationships.

By convention, a birthdate of 1 January is assigned for almost all species in the Northern Hemisphere (exceptions to this rule are given in the individual species descriptions). This means that a winter growth zone forming on the edge of the age structure is designated as an annulus on 1 January, even though the zone is not complete.

Growth patterns on age structures and growth rates often vary geographically. Growth is generally faster in more southern areas and slower in more northern areas. Sedentary species tend to show greater geographical variations than migratory species. Geographical patterns are discussed in greater detail under the individual species descriptions.

Certain areas on age structures are preferred for interpretation because the annuli are more distinct or have fewer visible checks. The best area is dependent on the species being examined. Regenerated scales (Fig. 18) or crystallized otoliths (Fig. 19) cannot be used for age determination.

Problems in age determination occur because of deviations in growth. These may result from checks or split annuli occurring in the age structure. Such accessory zones must be recognized as anomalies when assigning an age to a specimen. A knowledge of typical growth patterns helps in distinguishing checks from annuli. Checks tend to be discontinuous, weak or diffuse, and inconsistent with the general growth pattern of true annular zones.

Checks most often occur during periods of rapid growth and are especially common at younger ages. Some may be due to changes in food habits (e.g., a "settling check" forms when some young fish settle to the bottom and begin feeding). Maturation, migration, summer aestivation, or spawning may also cause checks. Some species exhibit distinctive checks typical for certain geographical locations.

Another common problem occurs because of atypical edge growth. For a specimen showing winter edge type in August, a determination must be made as to whether summer growth is retarded (in which case the winter edge zone would be counted as an annulus) or winter growth is advanced (in which case the zone would not be counted), or a check is being formed.

A major problem in assigning age is the determination of the first annulus. Knowledge of the geographic spawning times of a given species helps in determining if the first annulus is expected to be very small. Such annuli consist of minimal growth around the nucleus of an otolith or focus of a scale. For other fish, the first winter zone is expected to be some distance from the nucleus or focus.

Whenever possible, each specimen is examined independently by two age readers, to prevent long-term deviations in results. In general, percent agreement between the two readings has been maintained at better than 85% and exceeds 90% for many species. Comparisons of summaries of age-length data from one season to the next also help maintain consistency in age determinations.

Citations

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1983. Use of laminated plastic to impress fish scales. Prog. Fish-Cult. 45(2): 88-89.

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1977. Thin sectioning fish ear bones. Sea Technol. 2:27.

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1979. A unique method of ageing surf clams. Bull. Am. Malacol. Union Inc., p. 58-61.

Watson, J.E.
1965. A technique for mounting and storing herring otoliths. Trans. Am. Fish. Soc. 94(3):267-268.

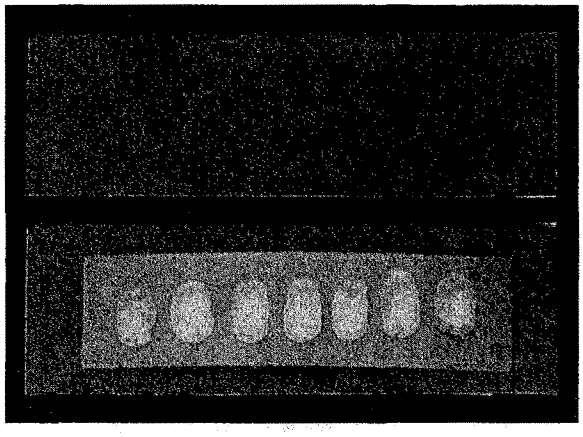


Figure 1
Scales arranged on a base of heavy plastic, sculptured-side-up, and a slide of laminated plastic, soft-side-down, placed over the scales.

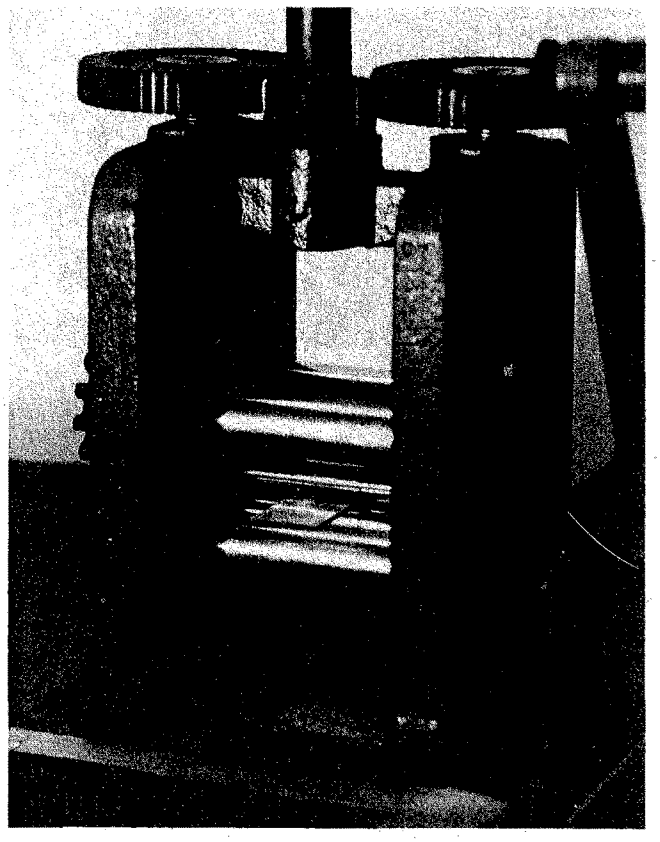


Figure 2
Scales "sandwiched" between plastic slides are passed through a jeweler's press.

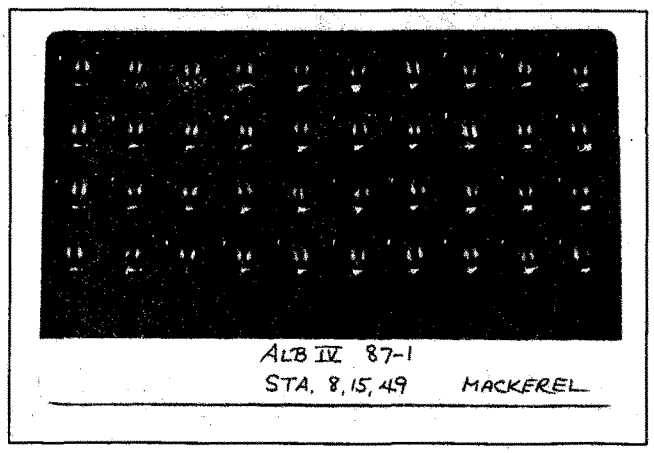


Figure 3
Tray of embedded Atlantic mackerel otoliths.

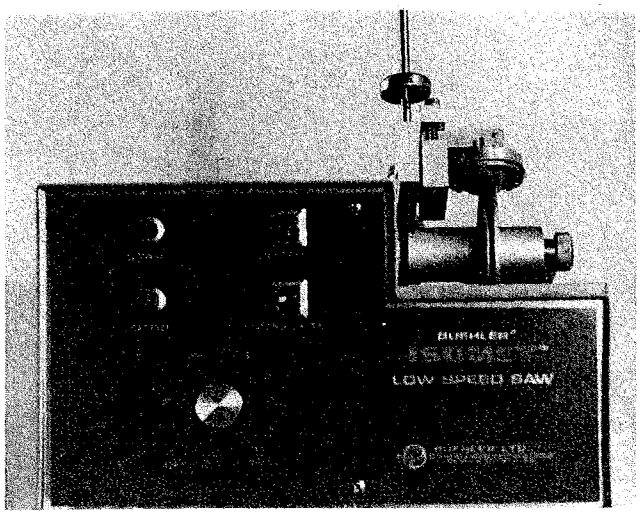


Figure 4
Otolith being sectioned on an Isomet low-speed saw.

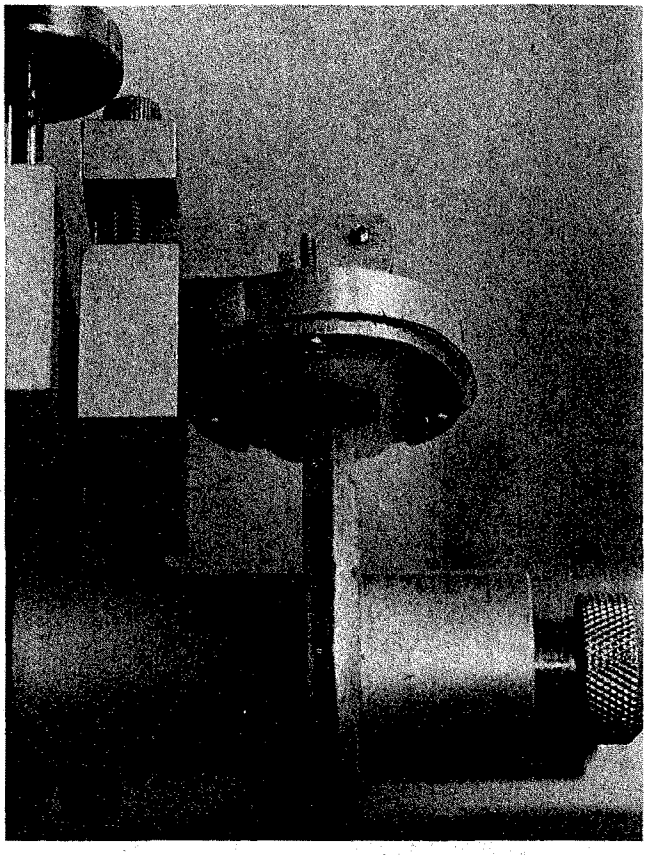
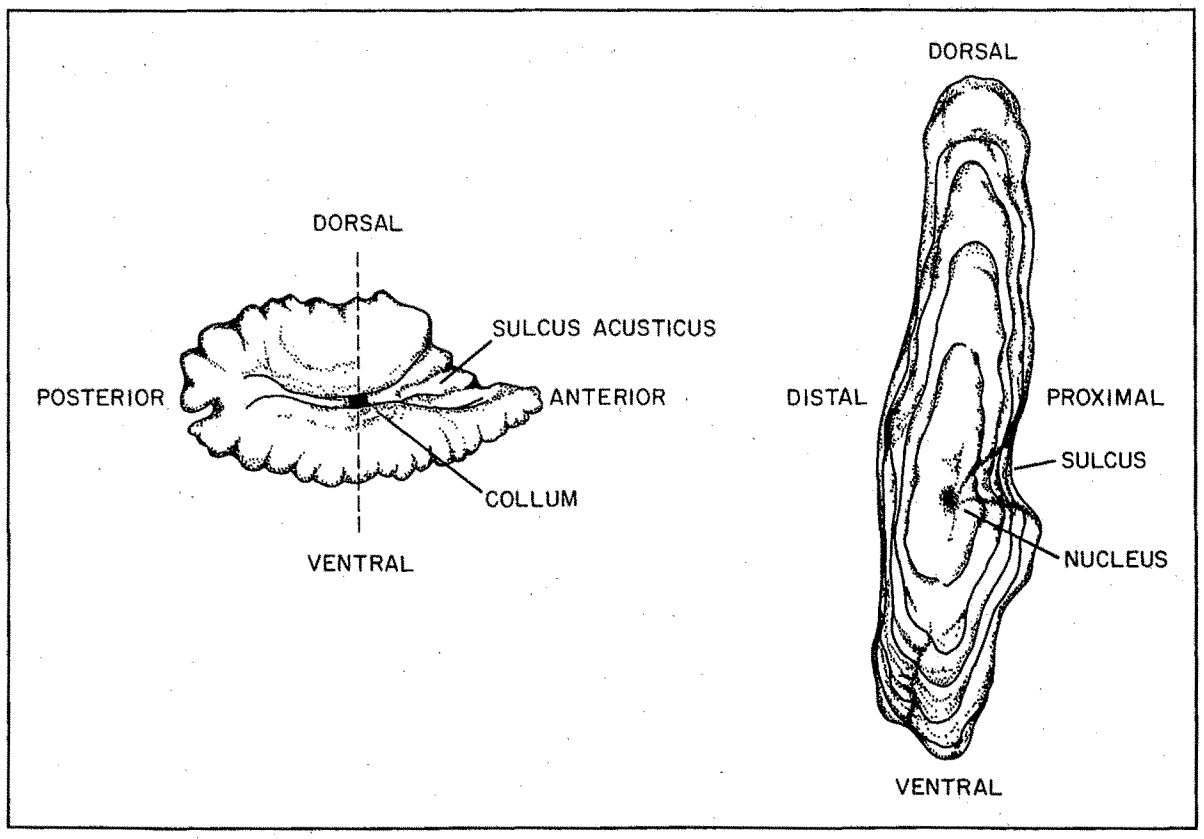


Figure 6
Close-up view of an otolith mounted in the chuck of the low-speed saw being sectioned by two diamond blades.

Figure 5
Sketch of an otolith and cross-section from a demersal species with descriptive terms and direction of cut (dashed line) for removing a thin-section. Proximal side of the whole otolith, with sulcus acusticus and collum, is shown.



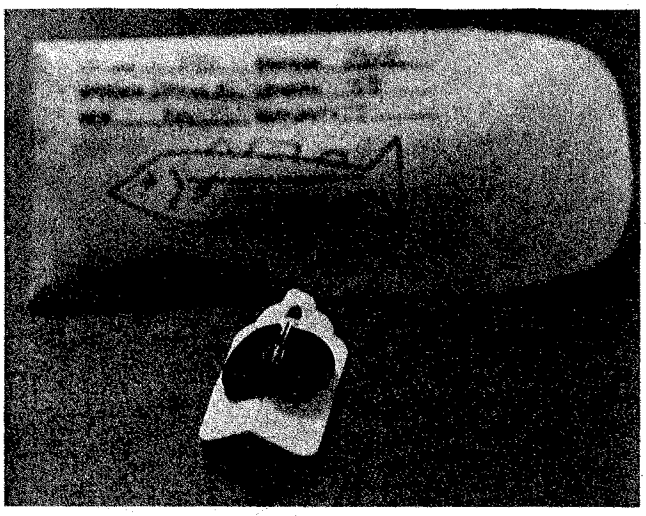


Figure 7
Tag bent open to reveal the thin-section. A printed coin envelope used for specimen storage is in the background.

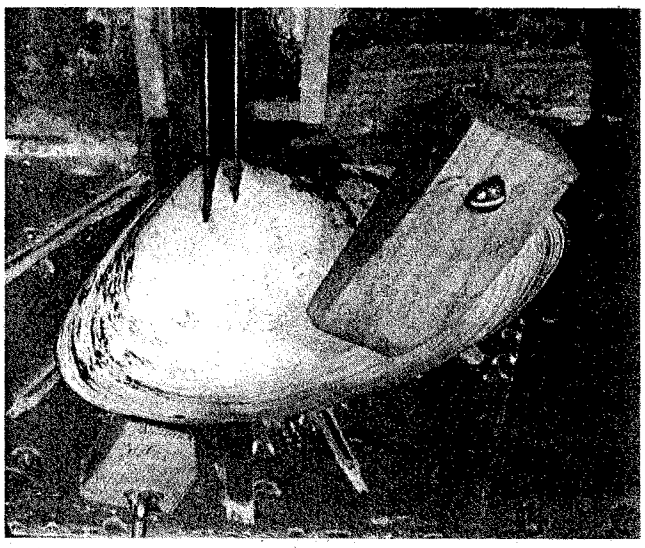


Figure 8
High-speed saw excising a portion of a chondrophore from a large surf clam.

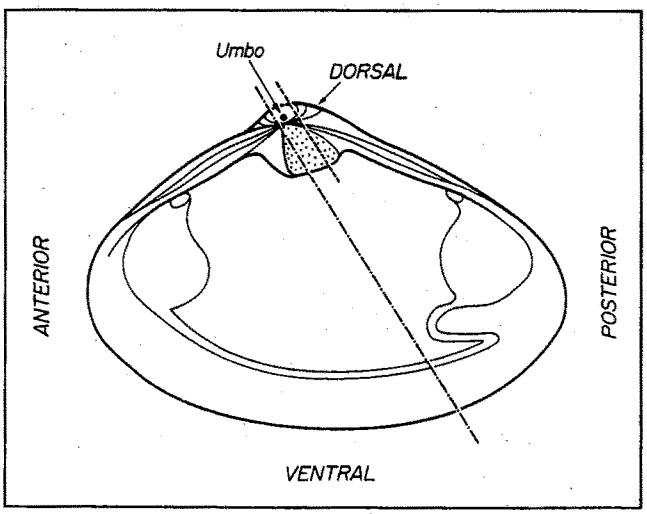


Figure 9
Internal valve features and direction of cuts (dashed line) required to excise the chondrophore from a surf clam.

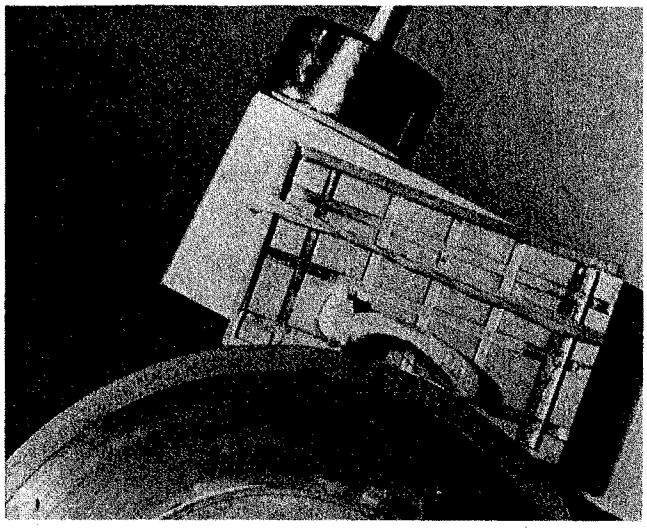


Figure 10
Excised chondrophore portion being thin-sectioned by a single blade on a low-speed saw.

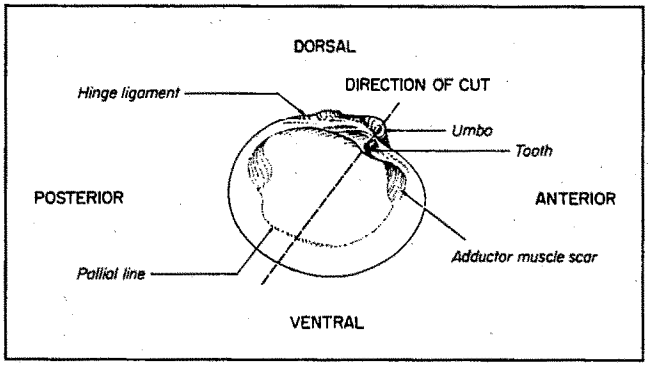


Figure 11
Internal valve features and direction of cut (dashed line) required to completely section the left valve of an ocean quahog.

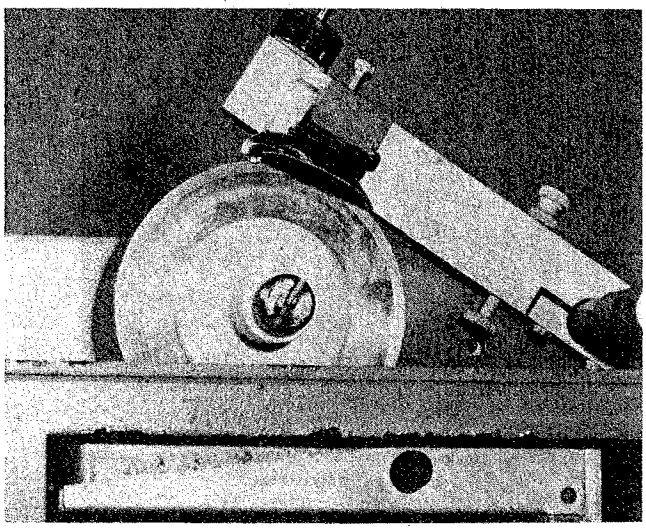


Figure 12
Close-up view of left valve of an ocean quahog fastened to the adjustable arm of a saw unit and oriented with the tooth beside the diamond blade.

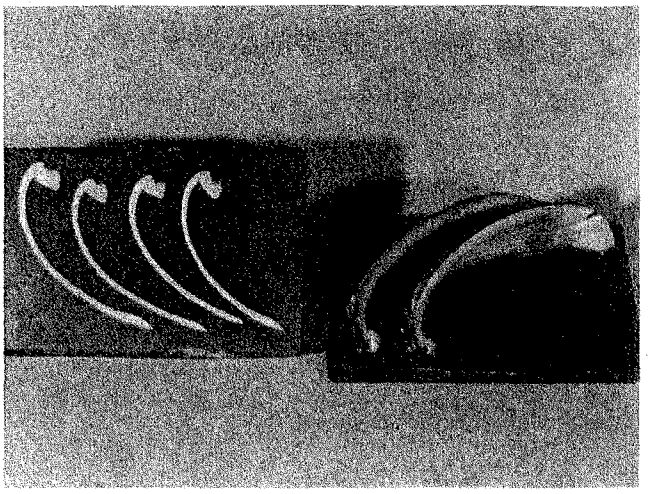


Figure 13
Ocean quahog valves embedded in epoxy molds.

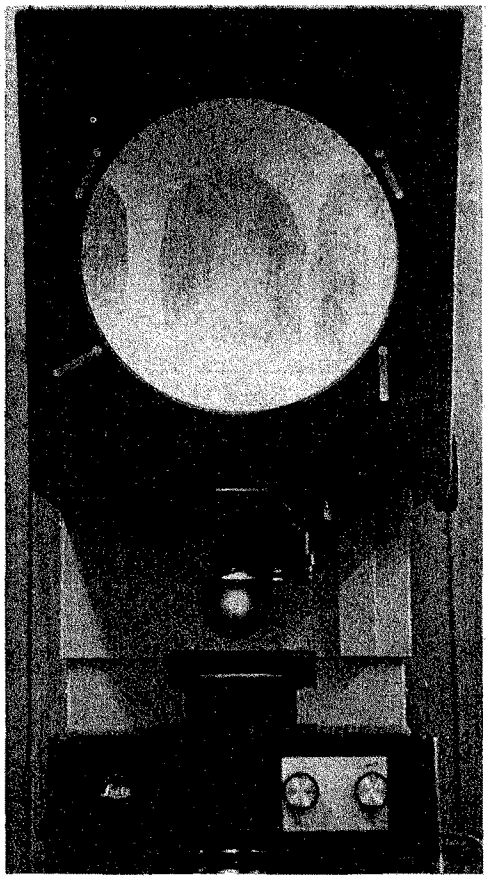


Figure 14
Microprojector (contour bench projector) used for viewing scale impressions.

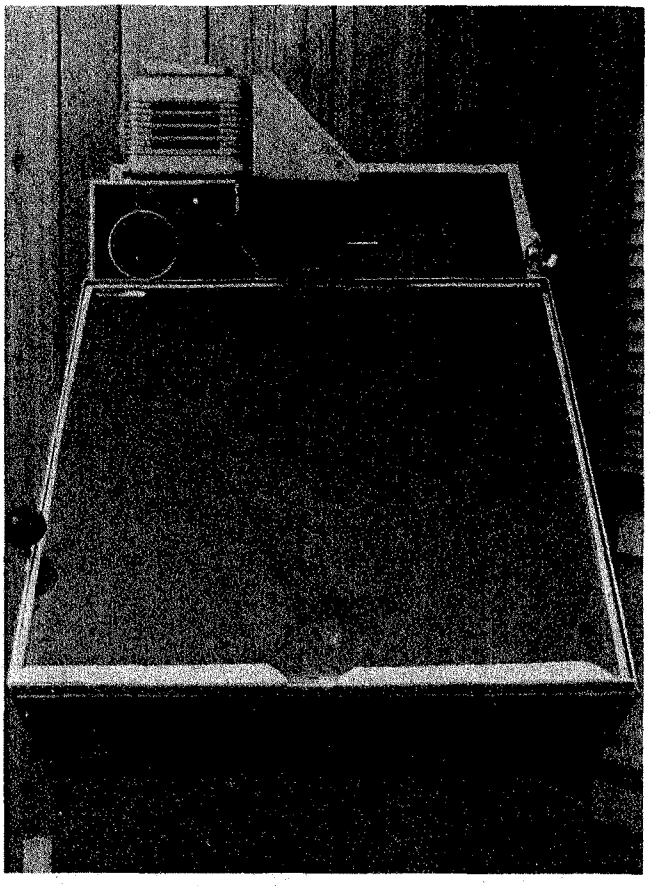


Figure 15
Older type of microprojector used for viewing scale impressions.

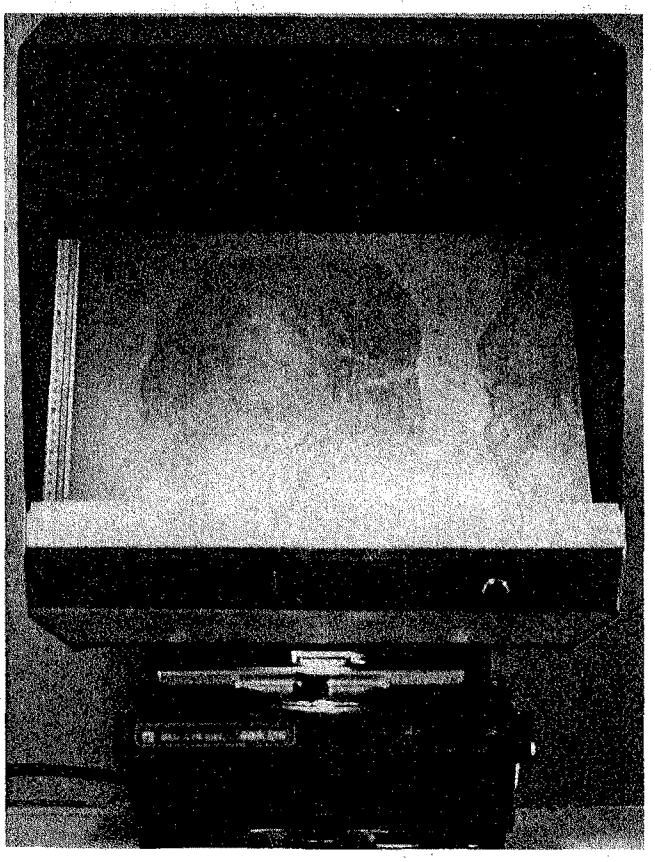


Figure 16
Microfiche reader used for viewing scale impressions.

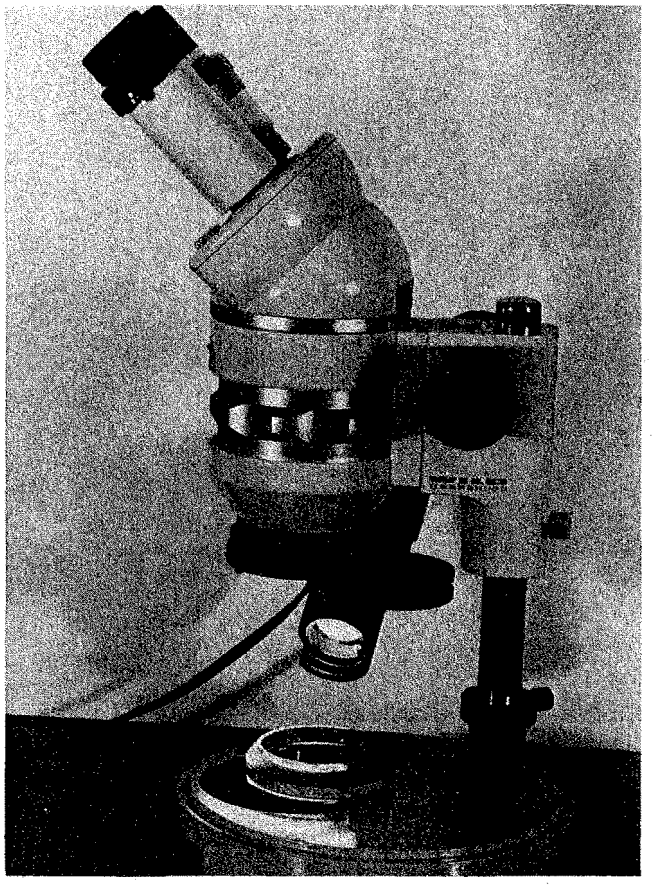


Figure 17
Binocular microscope used for viewing thin-sections.

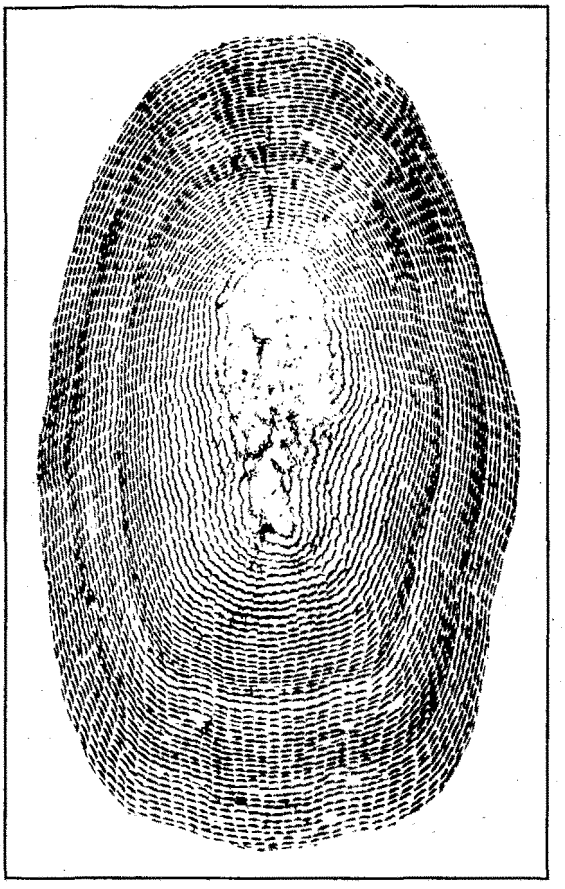


Figure 18
Regenerated scale from a haddock.

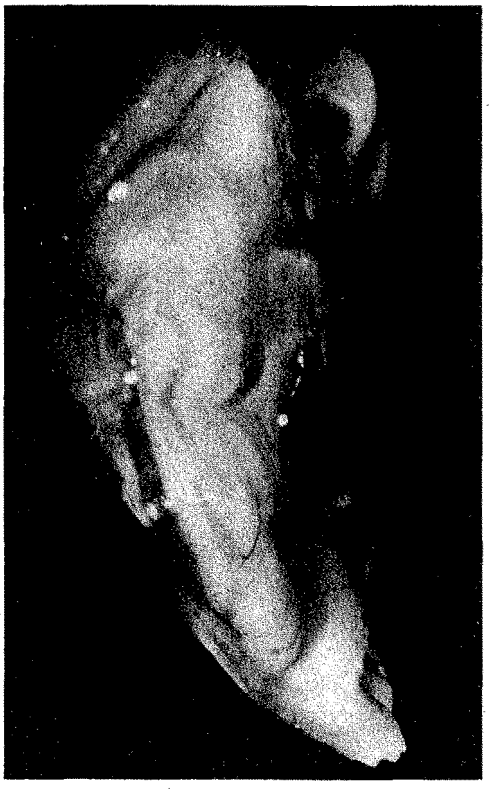


Figure 19
Crystallized Atlantic cod otolith broken in half at the nucleus.

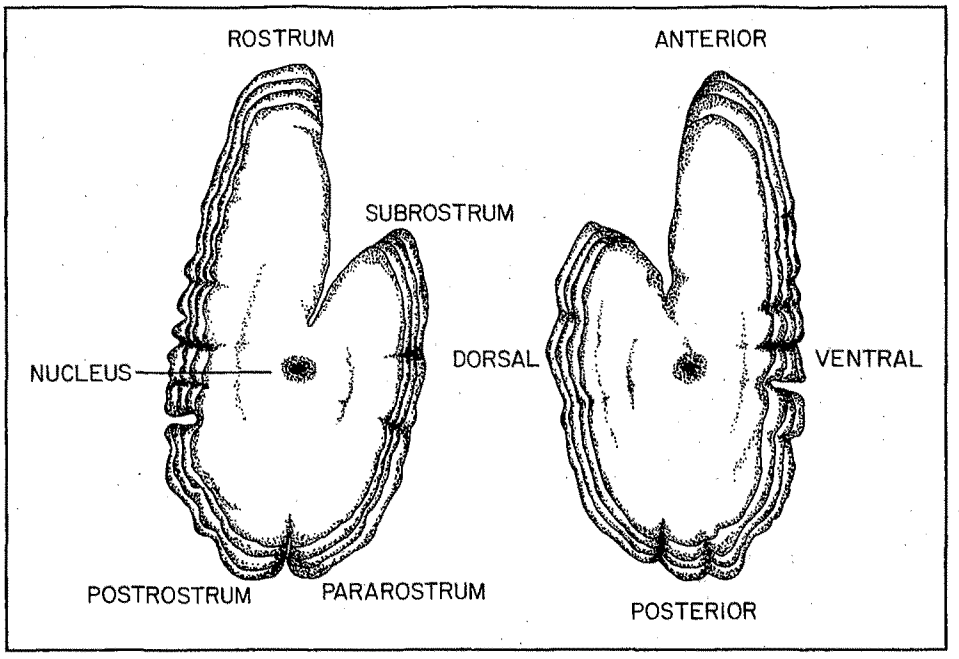


Figure 20

Sketch of a pair of otoliths from a pelagic species with descriptive terms. Distal side of the otoliths is shown.