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# Chapter 16
# AGE DETERMINATION

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# Chapter 16

# Age Determination

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## 16.1 INTRODUCTION

"Why age fish?" is a question often asked by fisheries students. "How can fish be aged?" was the question asked by the scientists who first began utilizing fishery resources. Both questions are important and serve as the foundation of this chapter. Age determination is necessary for fisheries scientists and fisheries managers working with fish populations. A basic knowledge of how quickly fish grow and the relative numbers of juvenile and mature fish in a population is required to help answer questions about how the numbers of fish caught affect the population. It is helpful to know at what size and age a particular species reaches sexual maturity. Then fishing can be restricted so that sufficient numbers of fish are allowed to reproduce before being exposed to sustained fishing pressure. When stocking fish it is also often necessary to hold the fish until they reach an age capable of reproducing. Knowledge of normal size variation at different ages over several years is also important for basic comparison studies. Changes in size ranges at a given age over several years may be the result of normal variation or may reflect a change in the suitability of the environment. This is either for the worse, as is the case with the addition of some contaminants, or for the better, as may be true after clean-up efforts have taken effect. So with some species a change in the average size attained by a certain age may be used to indicate a corresponding change in the environment of that species. These are some reasons age determination is an important part of any management effort.

Three basic approaches to age determination have evolved. They can be categorized as follows: (1) an empirical aproach relying on direct observation of individual fish held in confinement and/or fish marked and recaptured, (2) a statistical approach based on the utilization of length-frequency distributions, (3) an anatomical approach based on aging individual fish.

### 16.1.1 History

A complete review of the history of fish age determination is beyond the scope of this chapter. What follows is a brief review and explanation of the three methodologies mentioned above.

The empirical approach to aging was initially utilized by the fish culturist and is by far the oldest method of age determination. It relies on measuring the size of fish at different ages using fish held in confinement. A logical extension of this method was the discovery that individual fish could be marked, released, and recaptured. The sizes of the fish when first captured could be recorded and compared with the size at recapture. Both the mark and recapture and the holding in confinement methods depend on observation of individuals and extrapolation to populations. Many techniques have evolved for marking fish. Fish have been marked by fin clipping, tattooing, attaching external and internal tags, and by injecting a chemical, e.g., tetracycline, into the fish which causes a mark to form on calcareous structures (Weber and Ridgway, 1967).

The empirical approach to age determination is the least used method of the three basic approaches. As an aging technique the cost-benefit ratio is very high. In confinement studies the amount of space needed for holding several individuals is a limiting factor. Also, fish held in confinement are seldom exposed to the exact conditions of temperature, day length, food, etc. that fish in natural conditions experience. As a result, captive fish often have different sizes at a given age than wild fish. Furthermore, processes of capture, handling and recapture of marked individuals are time consuming and risky. Many fish do not survive being marked and those that do survive may not behave normally or achieve the same size as unmarked fish. However, the benefits of the empirical method often outweigh the costs in studies concerned with growth, migration, and stock identification.

Another method, the statistical analysis of length-frequency distributions, has been used to estimate the age of fish since the late 19th century. In 1892 the Danish biologist, C.G. John Petersen, showed that when the fish in a large sample are separated by size and the number of fish of each size plotted, distinct peaks emerge. The number of different age groups were determined by counting the number of peaks. Since Petersen's work more sophisticated methods of modal (peak) analysis have evolved.

The first serious account of the theoretical and empirical basis of the aging of fish utilizing body parts (the anatomical approach) was published in 1759 by the Reverend Hans Hederstrom. He was able to demonstrate that the age of a fish may be discerned from the marks on its vertebrae. This short but important article was overlooked for almost two centuries. It was republished in the bicentenary of its first publication (1959) in Sweden.

Before the early 1900's most age determination was done by the Petersen length-frequency method. In an early review, Dahl (1909) gives credit for the discovery of age marks on hard fish parts to C. Hoffbauer and J. Rebisch. Hoffbauer utilized scales of the common carp and Rebisch utilized otoliths (ear bones) of the plaice in 1898 and 1899, respectively. Dahl's review made it clear that the discovery of the anatomical method made it possible to examine the age composition of unfished (natural) and fished (exploited) populations.

Basic to the anatomical method of aging fish is the recognition of regular periodic growth markings in hard body parts to which a regular time scale can be assigned. This is a concept analogous to the technique of determining the age of a tree by counting annual rings in a cross section through the base of the trunk. As in trees, seasonal changes in the growth of fishes in temperate waters are generally reflected by contrasting bands in body parts such as scales, otoliths, finrays, spines, and bones. In bivalves such as clams the contrasting bands can be found in the shells.

The annulus (year mark) is the result of a slowing of the growth rate in response to such factors as colder winter temperatures. Environmental and physiological factors cause variations in time of annulus formation. The response to these factors (change in growth rate) may vary among individuals, populations and species. This results in some complications which will be discussed in following sections.

Since Dahl's review, many independent studies and reviews have been published (Bagenal and Tesch, 1978; Carlander, 1973; Chuqunova, 1959; Graham, 1929; Lee, 1920; Lux, 1971; Menon, 1953; Ricker, 1979). A quick tabulation of various studies clearly shows that the anatomical approach is the most widely used and preferred method of age determination. The advantages and disadvantages of this approach to determine age will be discussed in subsequent sections.

Many recent developments in fish age determination are concentrated on the anatomical approach. Considerable effort is being placed on developing a technique for automatic age determination of fish. The technique basically will depend on sophisticated image analysis instrumentation coupled with a computer. The most promising structure is the fish scale and, to a lesser degree, the otolith. Basic to this technique is the assumption that light sensitive marks on anatomical parts can be recognized and will appear in a predictable pattern. Advantages of the technique include increased objectivity, quicker assessment of many time consuming measurements useful to fishery analysis, and rapid aging of large volumes of age samples. See Fawell (1974) for a discussion of some aspects of image analysis.

Since Panella (1971) discovered that the otoliths of some tropical and temperate fishes contained daily growth rings (or "d.g.i.'s," daily growth increments), there has been an increasing interest in the use of these rings for age determination. In temperate regions, most work by this method has been done with larvae. Examination of these tiny otoliths requires the use of high magnification or scanning electron microscopy (SEM) to reveal the fine rings. Preparation for SEM studies may include grinding, polishing, and etching with decalcifiers (see Radtke and Dean, 1981). Otherwise the whole otolith is viewed in resin at 600x-1000x.

Because d.g.i.'s may become evident at different points in larval development depending on species (e.g., at hatching or at yolk sac absorption) a full time series from hatching should be studied. When the time of first d.g.i. formation is established the daily nature of the rings formed thereafter can be verified (examples can be found in Barkman, 1978; Brothers et al., 1976; Lough et al., 1982). Information gained from accurately aging wild larvae can be applied to studies of the growth, mortality, and hatch dates of wild populations.

The study of daily growth is also useful for adult fish in tropical regions. D.g.i.'s and rings formed in response to lunar activity have been noted on their otoliths. The structures of tropical species do not exhibit distinct seasonal zones as the environment remains relatively stable during the year. Annual or subannual slow growth zones may form, however, as the result of spawning activity (as is also true for temperate species) or possibly in response to changing conditions during rainy and dry seasons (Panella, 1974). The age and growth of tropical species remains a frontier area of research due to the difficulties in age determination and the increasing importance of fishery science in developing countries.

### 16.1.2 Age Terminology

Biologists interested in age determination of fish and shellfish often discover important disagreements and conflicting information between fellow workers. Some of the confusion is due to inconsistent terminology used to report results. Careful consideration must be given by age readers to standard terminology and notation. See Bagenal and Tesch (1978), pp. 105-106; and Jensen (1965) for a discussion of terminology used among fish age investigators. Also see Box 16.1.

Box 16.1 near here.

There are intra- and interspecific differences as well as geographical differences in the time of year when an annulus appears. Since biologists must be able to compare their findings it has become necessary to standardize the birth date of fish. There is an internationally accepted convention (International Commission for the Northwest Atlantic Fisheries) of designating January 1 for the North Atlantic bottom dwelling species. Hile (1948) and others working with freshwater species proposed that January 1 be designated as the birth date for fish in the Northern Hemisphere. Therefore, a winter growth zone forming on the edge of scales, otoliths, finrays, spines, etc. is designated as an annulus on January 1, even if the zone is not complete. July 1 would be the corresponding date for year mark formation on hard parts of fish in the Southern Hemisphere. The assignment of an arbitrary birth date, other than the biological birth date, may not be the best system in some instances. In some fish, annuli may appear months after an assigned birth date and may be misinterpreted by different age readers.

Reporting the age of fish can also be confusing. By convention, some fishery biologists designate the age of a fish in Roman numerals, e.g., age I for a one-year-old; age II for a two-year-old, etc. However, more recently the less cumbersome procedure of designating the age of a fish by using Arabic numberals is becoming more prevalent (Ricker, 1975). In either case it is consistency that is important.

Fish in their first year of life, before their first January 1st birth date (whose calcareous structures are without an annulus), are designated as members of the 0-age group. Terminology for members of the 0-age group depends upon stage of development and taxa. They may be called larvae, fry, elvers (eel), alevins (salmon), fingerlings (catfish), or young-of-the-year. Balon (1975) presents an excellent proposal for standardizing terminology for periods in fish development.

Ages of older fish are expressed by numerals corresponding to the number of annuli or completed years of life. A fish in age group I(1) has completed one year or less of growth from time of hatching to the January 1st birth date and has entered its second growth season. During the growth season after annulus formation, any growth of the age structure between the last annulus and the edge is termed "+" growth. If the age group I(1) fish is collected in the summer or fall, its age will therefore be I+(1+). If the same fish is caught during the next winter, after January 1st but before the second annulus is complete, it will be assigned to age group II(2) even if the edge still only indicates "+" growth. Using a conventional birth date regardless of minor variation in time of annulus formation ensures that fish hatched in the same calendar year are members of the same age group (also called year class, brood, or cohort). For example, the 1980 V cohort, the 1981 VI cohort, and the 1983 VIII cohort are all members of the 1975 year class.

Much diversity is found in the designation of ages and the methods of designating age groups for fish that migrate between fresh and salt waters. Special notation is used to designate the time in each environment. However, here too, there is no standard notation. In the United States salmon age designation may follow two methods, the European method or the Gilbert and Rick (1927) method.

The Gilbert and Rick method is more complicated than the more widely used European method for salmonids. Under the European method, 2.3 indicates two years of freshwater life and three years of saltwater life. With the Gilbert and Rick method the notation $5_3$ would indicate a fish who had lived 5 winters from the time its parents spawned to the time it was captured. The subscript $_3$ indicates the number of winters between the time the parents spawned and the fish migrated seaward. Many other terminologies have been used in the past and much confusion still exists. The special problems associated with aging salmon and using appropriate terminologies is thoroughly discussed in Koo (1962) and Mosher (1968).

## 16.2 SELECTING AN AGE STRUCTURE

The value of a thorough literature review on the life history of the species selected for investigation cannot be overemphasized. This tool alone will help orient you along the path of least resistance. The literature will indicate: (1) whether age determination has or has not been established for the species in question, (2) which age determination approach(es) was utilized, and (3) which hard structures were used.

If the literature search shows that no age determination process yet exists for the species in question, then you are faced with making an independent decision. Traditionally age determination of an unaged species has started with the anatomical approach. The next step involves deciding which of the hard body structures (e.g., scales, otoliths, vertebrae, spines, fin rays, cleithra) best shows the periodic changes in growth and most clearly reflects the age of the fish. Each structure must be examined to determine whether it shows a recognizable pattern and if a regular time scale can be assigned to that pattern, beginning as early in the life of the individual as possible. Actual discussion of how each body structure is collected and prepared will take place in later sections. One assumption here is that a random sample of the fish has been obtained using methods discussed in Chapter 1 of this manual. From this sample, choose various hard structures from several (5-10) fish of each age group (larvae, young-of-the-year, yearlings and older juveniles, young adults, and old adults). When deciding which hard structure is most suitable consideration must be given to the time and effort involved in collection and preparation.

If the literature search indicates previous aging studies have already been done on the species of interest, then a thorough review of these papers should reveal the best method for the purposes required. For many taxa of fish certain body parts have become accepted by people working in the field as the most useful for age determination. Table 16.1 gives a brief summary of some major taxa and the body part most widely used for aging.

## 16.2.1 Collection and Preparation of Scales

### Collection.

Although scale samples are the easiest hard structure to collect from fish and, therefore, the most popular, removal must be done carefully and by a standard procedure. Some useful instruments to have are a pair of forceps, a blunt knife (table variety), and a sharp knife (preferred by some studying freshwater and tropical species).

Only scales from particular areas on a fish are suitable for aging. The area generally used for scale sampling is the middle region of the side of the body (Bagenal and Tesch, 1978). For some species, however, the area preferred is below the lateral line near the point of the pectoral fin when the fin is pressed to the body (Carlander, 1982). See Figure 16.1 for preferred scale collection sites for sunfish, bass, perch, flounder, and other species.. It is advisable to experimentally select and examine scales from several areas to determine where consistently large and symmetrical specimens occur. An area likely to shed scales or that has irregularly shaped scales is a poor site choice for collecting scale samples. For example, trout in rivers and streams undergo as much as 90% scale regeneration whereas fish from lakes, ponds, and open marine environments have much less regeneration. See Lagler (1956) and Bagenal and Tesch (1978) for additional discussions of scale sample collection methods.

Figure 16.1 near here.

Before collecting scales, the area should be gently but thoroughly wiped in the direction of the tail with a blunt edged knife to remove the mucus, dirt, and epidermis. The scales are loosened by a quick, firm scraping motion in the direction of the head and removed on the blade of the knife, which is then inserted between the liners of a scale (coin) envelope. Envelopes without glue on the flap are preferred. An alternate method is to place the point of a sharp knife firmly on a scale and push posteriorly to remove the scale. As many scales as desired may be collected one at a time without scales being lost (i.e., flying all over as in scaling fish). With either method, be sure to clean instruments of all scales from one fish before you start on another.

Preparation for age analysis.

Samples for age analysis may take two primary forms: raw scales or scale impressions. Raw scales are generally the least desirable for aging because they: (1) are covered with dried and pigmented residue from the fish and miscellaneous dirt picked up through handling; (2) are generally translucent rather than transparent and thus interfere with viewing under transmitted light; and (3) are not flat, which causes problems of illumination during microscopic examinations if any degree of magnification is required. In situations where you must use raw scales you can mount 6 to 10, sculptured side up, between glass slides labeled and held together with masking tape.

A more satisfactory method is to prepare an impression of the outer surface of the scale onto plastic by using manual or power-driven roller presses. Clear cellulose acetate slides approximately 1 mm thick have been used by some workers (Nesbit, 1934; Smith, 1954; Redkozubov, 1966). The NMFS Woods Hole Laboratory currently uses a double plastic film (laminated plastic) with a thin (2 mils. thick) soft polyethylene layer over a thick (6-8 mils.)

harder vinyl substrate. This material results in very reproducible scale impressions. It is soft enough to allow impressions without use of heat, heavy pressure, or softening chemicals. Scale impressions may be magnified and viewed with transmitted light and have several advantages over direct use of scales. Several scales may be impressed at the same time on one slide, and the impressions affording the clearest scale features can be selected. The impressions are clean, even if the original scale was not, and they are easy to store and handle (one option is to simply slip the plastic impression into the already properly labeled coin envelope). The image of the scale is generally flatter than the original scale, although it does retain enough depth features to cause depth-of-focus problems at higher magnifications. Other disadvantages of using cellulose acetate are: (1) the technique can be time consuming, and (2) scales having delicate and shallow sculpturing (e.g., yellowtail flounder) can be problematic. If laminated plastic is used thick scales can cause distorted images.

### 16.2.2 Collection and Preparation of Otoliths

Otoliths, or earstones, of which there are three pairs of varying size, are flat oval to spindle-shaped structures found in the heads of bony fishes. They are associated with the brain and function as part of the organs of balance, auditory, and mechanical reception. The largest pair, called the sagittae or saccular otoliths, is preferred for determination of annual year marks. More recently all three pairs have been used for counting daily age marks.

Collection.

The collection of otoliths for age determination necessitates removing the otoliths from behind the brain. For most fish this may be done in the following manner. Grip the head firmly by the eye sockets with one hand and cut the top of the skull slightly behind the eyes, down and back to the upper edge of the gill cover (Figure 16.2). A strong, sharp knife may be sufficient for small to large fish, while for larger fish and/or hard-headed ones such as pollock and flathead catfish, a saw is sometimes necessary. The head is then opened by pressing down quickly on the nose. If the angle of the cut was correct the large sacculus otoliths should be plainly visible behind the brain Cuts that do not result in the otolith being revealed require searching.

Making an accurate cut for removing otoliths from flatfish (flounder, plaice) is more difficult but is easily mastered with practice. A bony ridge between the eye and the edge of the gill cover should be opened along a line extending from the end of this ridge (Figure 16.3) by pressing down on the bone with a sharp blade until the bone is penetrated. The knife should not be pressed down too far or the otoliths will be shattered. After the head is opened by bending down the nose of the fish one otolith should be visible; the other one will be found underneath by probing with tweezers.

Figure 16.3 near here.

For fish with poisonous spines, e.g., redfish, catfish, and some tropical fish, it is advisable that a heavy glove be used to hold the fish firmly by the head while the cut is being made for otolith removal. Upon removal of the otoliths, store them dry in a coin envelope or in alcohol or glycerin. The use of a storing medium of 2:3 glycerin:alcohol will clear thicker otoliths, enabling the viewing of rings at a later date. Do not store them in formalin or acid. These chemicals will dissolve otoliths.

Advantages of using otoliths for aging are: (1) they form during the embryonic period and, therefore, are good coders of life history events; (2) in some cases they show age more clearly than scales and are often considered better than scales for determining the age of older fish; (3) a rather small sample size can be used; and (4) all fish of a species have otoliths that are similar in shape.

A major disadvantage of using otoliths is that their removal requires killing the fish. This could be a problem when fish are valuable as commercial fish, sport fish, trophies, or endangered species. There are only two large otoliths per fish, they crack easily, and require relatively more skill and time than scales for collecting.

Preparation for age analysis.

The technique for preparing otoliths is variable and in most instances it is modified for the convenience of the investigator. Some otoliths may be viewed whole in glycerin or alcohol while others must be sectioned. Tiny otoliths such as those of herring, mackerel, and many freshwater species with very small otoliths can be imbedded in resin using molded black plastic trays with rows of circular depressions. Assuming that the ring structure on the whole otolith can be seen, a resin will enhance the contrast between summer and winter zones to an extent not possible with the simple use of alcohol.

Sectioning technique.

With increasing age, earlier-formed annuli on otoliths may be obscured by subsequent calcium deposition. Therefore, it may be necessary to examine a cross-section of the structure. Sectioning techniques range from rough to delicate. For some species with large otoliths (e.g., cod and haddock), the process is to simply break the otolith in two. Break the otolith at the sulcus (nucleus center) by applying pressure with the thumb or with a pair of

Figure 16.4 near here.

nipper pliers. A variation to this technique is to bake the otolith at a determined temperature and time before breaking. Baking enhances the annual marks.

More involved sectioning techniques require the use of sandpaper and/or a saw (e.g., a jeweler's saw). Otoliths from many species may be mounted to a glass slide with a thermoplastic cement. Otoliths that do not clearly reveal all annuli under low power magnification using reflected light can simply be ground down with fine sandpaper or a dentist's drill to a favorable plane.

Many otoliths with complex growth patterns must be accurately and precisely cut into very thin sections for easy age determination (Figure 16.4). Nichy (1977) developed a method that takes 30 seconds to 2 minutes to thin section an otolith using a low speed diamond blade saw. Transverse sections as thin as 0.175 mm can be obtained by using this lapping machine to cut through otoliths mounted in wax on cardboard tags. Other reliable techniques for sectioning otoliths are available. Discussions are reported in Bedford (1973), Rauck (1975, 1976), and Bagenal and Tesch (1978).

### 16.2.3 Collection and Preparation of Spines and Finrays

In bony fish without scales or satisfactory otoliths for age determination, some other structure must be selected if possible. In catfish, for example, the choice is usually made between the pectoral spines (dorsal in some studies) and the vertebrae (Marzolf, 1955).

<u>Collection.</u>

Pectoral spines, unlike vertebrae, do not require killing the fish, are easier bones to collect in the field and laboratory, and involve less preparation time.

Spines can usually be removed free of tissue except for a thin layer of skin and blood and require little special treatment or preparation. To detach the spine (left or right consistently) one of two methods or a combination of the two may be used. Physical separation of the spine may be accomplished by: (1) simultanteously twisting and depressing the spine toward the body of the fish at the articulating process, or (2) grasping the spine with a pair of pliers for large fish (and/or forceps for small fish), pulling outward to loosen the joint, and then rotating clockwise for left spines and counter-clockwise for right spines. For larger fish it may be necessary to cut the muscles surrounding the spine. Air drying before storing in a serially numbered coin envelope is advisable.

In some fishes, the suckers and sturgeons for example, the best structure for age determination is the fin ray. In bony fishes one function of the fin ray is internal support for fins.

Select the fin ray to be used (e.g., pectoral) and remove it just below the point of articulation with a scissor, knife, or pliers. Remove excess membranous tissue by scraping, followed by soaking in household bleach to remove any remaining tissue traces. Let dry and store as for spines.

As compared with other aging structures, the disadvantages of using the pectoral spine are few and minor. Marzolf (1955) sometimes found the first annulus obscured in older fish.

Sectioning spines and fin rays.

For all spines, the position of cut is critical (Figures 16.5 and 16.6). Reliable age determination is most efficient, accurate, and precise when spines are cut to retain all the annual rings. Channel catfish pectoral spines should be sectioned at the distal end of the basal groove (Figure 16.5) (Snead, 1951). Sections cut from the articulating region will be helpful in

Figures 16.5 and 16.6 near here.

distinguishing annual rings in the peripheral region of basal groove sections. For flathead catfish, the articulating process of the pectoral spine was the preferred cut (Tunner, 1980). Instruments used for sectioning may be similar to that described by Witt (1961). Section thickness depends on many factors, but in general the thinnest sections are most useful if transmitted light is to be used. Some cross sections may be examined under water in a shallow dish. Other cross sections will require grinding and polishing before microscopic examination and/or measurement. Grinding and polishing may be performed on an individual cross section held with the fingers or several cross sections mounted on glass or plastic slides. Sneed (1950) includes an excellent discussion of spine methodology. He also states that pectoral spines have fewer false annuli than the more cumbersome to work with vertebrae.

Fin rays should be sectioned near the base with a jeweler's saw. Those to be read without grinding should be between 0.4 and 0.6 mm thick (Figure 16.7). Sections can be mounted on glass or plastic slides with a cement. Thicker sections, e.g., 1.0 mm or less, can be polished with a fine grit sandpaper. A technique applicable to spines and fin rays is to oven-dry before sectioning. Dried structures are then soaked in Axion (household detergent with enzyme) and rinsed in ammonia (to stop enzyme action). This procedure completely cleans the structures, removes grease and oil, and speeds sectioning time (previewer communication). See Cuerrier (1951), Scidmore and Glass (1953), and Pycha (1955) for more information on preparing fin rays for age determination.

Figure 16.7 near here

## 16.3 COLLECTION AND PREPARATION OF BIVALVE SHELLS

Shellfish age determination has evolved from a relatively simple visual examination of the external shells of bivalves to rather complex microstructural examinations (Lutz and Rhoads, 1980). Similar to the finfish, seasonal changes in growth rate are often reflected in zones or bands in shell structures of clams, oysters, scallops, and mussels. Zonation is similar to that found in finfish hard structures, with a light band forming in the early part of the growing season followed by a narrow, dark band in the winter. As in finfish the first annulus is difficult to discern or lacking and accuracy of age determination is less reliable in very old molluscs.

Because bivalves spend most of their lives as benthic organisms, they are usually collected with dredging apparatus. Subsequently, they must be cleansed before age can be determined. For storage prior to aging, shells shucked of their meats can be placed in a household bleach solution. The period can range from a brief dip for relatively clean shells to a lengthy soak. Longer soaking time requires frequent examination to minimize over-bleaching annual marks. Bleaching is followed by rinsing in tap water and, for some shells, scrubbing with a brush. Let shells dry before storing for later sectioning. Both halves of the shells are usually cupped together for storage. This allows for reference to both halves if necessary for aging purposes as well as reducing volume and breakage.

Examination of the internal shell structure requires that it be sectioned. Ropes and O'Brien (1979) found a correspondence between the number of annuli in the chondrophore and in the shell of surf clams (Figure 16.8).

Figure 16.8 near here.

Their findings formed the basis for a unique and expedient means of accumulating age data on this species. Briefly, the methodology for preparing thin sections of a chondrophore is as follows:

1. Using a pair of diamond blades, excise a thick piece of the chondrophore from the right valve hinge.
2. Use wettable silicon carbide paper to grind and polish the anterior cut surface of the piece down to the umbo.
3. Air dry the piece and mount on a glass slide by applying a two-part epoxy glue to the polished surface.
4. Using a low speed (0-300 rpm) saw, cut a section about 0.02 mm thick.

## 16.4 AGING PROCEDURES

Anatomical samples having been collected and prepared, the next step is to determine their age. One important consideration is time of annulus formation. In order to establish this time, it is helpful to supplement interpretation of the structure with information on spawning, migration, and feeding habits of the sampled fish population as well as environmental data such as latitude and water temperature range. All of these factors influence growth rate and, therefore, zone formation on hard structures. Depending on latitude and environmental conditions, seasonal growth may shift from the general pattern and result in annuli forming in the spring or fall, rather than in the winter months. For species of the Temperate Zone where there are distinct seasonal changes, clear annular zones form during the colder months of the winter. This is especially true for species in freshwater environments where changes in water temperature and chemistry are more radical than in marine environments. Time of annulus formation may also vary with age. Very typically, the younger fish of a population resume growth earlier than older individuals and may begin annulus formation earlier as well.

Another consideration in aging hard structures is the formation of anomolous rings such as checks or split annuli. Checks are generally formed during rapid growth periods whereas split annuli are associated with slow growth periods. Both of these occur in response to physiological changes or stresses that slow growth. Often these accessory marks can be difficult to distinguish from true annuli and can lead to overinterpretation of age. This is one reason validation is important: to verify which age marks are true annuli.

To minimize confusion I will present separate discussion of procedures for scales and otoliths and fewer comments for other structures. Biases common to all structures used to determine the age of a fish are errors associated with, (1) missing the first annulus; and (2) the crowding of annuli with increasing age; (3) overinterpretation of age due to the presence of anomolous rings; and (4) loss of peripheral annuli due to resorbtion or erosion.

### 16.4.1 Aging Procedure for Scales

To determine age you will need to magnify the scale. Scales or scale impressions may be examined under a low-power microscope or by use of a microprojector or microfiche reader.

Depending upon your preparation methods and objectives, other items necessary might include microscope slides, forceps, measuring instruments, and forms for recording data.

To project a scale, select a proper magnification which will accommodate all scales of a sample. Scales or impressions should be oriented with the sculpted surface toward the light source. Be sure the scale or impression is held flat during projection and measurement.

Pattern recognition.

The age of a fish is determined from scales by counting the number of annuli. Usually the preferred orientation is anterior field up, posterior field down (Figures 16.9 and 16.10).

Figures 16.9 and 16.10 near here.

Scale types of most fish are either cycloid or ctenoid (Figures 16.9 and 16.10). Cycloid scales have circuli which extend completely around the scale edge as growth continues. The anterior field of the scale is embedded in the skin and comprises most of the surface area. This part of the scale is usually used for age determination. Some examples of fish with cycloid scales are: cod, haddock, salmon, trout, whitefish, pike, minnows, and most soft-finned fish. Ctenoid scales differ from cycloid scales, with the field posterior to the focus appearing devoid of clearly defined circulus ridges. The area may be obscured by prominent spines or ctenii. The anterior field is usually used for age determination. Examples of fish with ctenoid scales are: some flounders, bass, sunfish, perch, and most spiny-finned fishes.

Scale features to look for are fine ridges called circuli (dark lines). Circuli are laid down in a circular pattern around the scale center or focus. Several circuli are added to the scale each year.

During the warm months when fish growth is rapid the ridges of circuli are widely spaced, while in the colder months growth is slow and ridges are laid down close together. Fish continue to grow throughout their life; therefore, this pattern is repeated each year. The outer edge of the closely spaced circuli indicate the termination of that year's growth and this point is called the year mark or annulus (Figures 16.9 and 16.11). See Box 16.2 for a fuller discussion of scale pattern recognition.

Figure 16.11 near here.

In older scales, age determination becomes more difficult. With difficult scales the value of careful preparation techniques becomes

Box 16.2 near here.

evident. Experience will allow you to identify which scales to keep and which ones to discard. For example, regenerated scales should not be aged. Such scales develop as the result of prior scale loss. They are devoid of the circulus ridges formed by the original scales. The central part of regenerated scales contains a clear window-like area surrounded by irregular circulus markings (Figure 16.15).

Figure 16.15 near here.

16.4.2 Aging Procedures for Otoliths

Otoliths of many species requires only low magnification (15-20x) for viewing. Depending upon the degree of enhancement needed to distinguish age marks clearly, improvise with lighting, magnification, and immersion in various clearing fluids (e.g., Fotoflow, clove oil, alcohol, or glycerin). Clove oil will enhance contrast between opaque and translucent zones if the opaque zones are weakly defined. As mentioned earlier, the use of a resin will also enhance this contrast.

Otoliths from older fish and otoliths which cannot be aged whole require more experimentation. A recommended technique for microscopic examination of thin (e.g., 0.2 mm thick) transverse sections of otoliths is as follows: mount the section on a dark background (e.g., black paper) and moisten with ethyl alcohol unless clove oil is required to enhance the contrast between opaque (summer) and translucent zones. Interpret annular zones on the part of the otolith where rings seem most distinct and condensed, at an appropriate magnfication using reflected light. Sections requiring transmitted light for best resolution would be placed on a transparent or translucent surface.

Pattern recognition.

Otolith form is species-specific and varies from a flat oval to a spindle shape. The most prominent external feature of the otolith is a central groove. It extends from the anterior to the posterior end of the inner (concave) surface of the otolith. This groove is very useful in locating the center or nucleus of the otolith (Figure 16.4). A more detailed description of the otolith can be found in Blacker (1974).

The formation of growth zones in otoliths and other bones follows the general pattern outlined earlier for scales. Growth, as in the scales, is concentric around a central nucleus. The age of the fish is determined from the banding that results. When whole otoliths or transverse sections are viewed under a microscope the layers making up spring and summer months of active growth appear as white opaque bands (zones) under reflected light. Layers laid down during slow growth periods (usually the fall and winter months) appear as dark or translucent hyaline bands (zones). A light and dark band together represents one year of growth. Age in years is usually determined by counting the number of dark bands or annuli (Figures 16.16 and 16.17). The following criteria should be considered when making age determinations for otoliths: (1) count the widest, strongest, and most distinct hyaline zones on the otolith--the width decreases with age; (2) hyaline zones should be consistent in formation around the periphery of the whole otolith; (3) the hyaline zones are spaced increasingly close together from the nucleus to the edge; and (4) on transverse sections true annular zones are usually continuous through the sulcus groove.

As with scales, accessory markings such as checks, splits and false annuli are common. Checks may appear as thin and/or discontinuous hyaline zones around the otolith periphery. Abnormally shaped crystalline otoliths should not be used for age determination. On these otoliths either calcium has been reabsorbed or disruption of the otolith membrane has occurred (Bilton, 1974).

### 16.4.3 Aging Procedures for Other Hard Structures

Various bones other than the popular otolith have been used for age determination in different fishes (Menon, 1950). Certain of these structures either in whole or in cross sections have been demonstrated to show seasonal zonation associated with age. However, the time and materials required for a satisfactory preparation of permanent study sections has been a serious objection to use of these structures. There are, however, researchers who have found many of these structures to be significantly superior to scale or otolith preparations for certain fish. Harrison and Hadley (1979) favored the cleithra of muskellunge over scales, Quinn and Ross (1982) found the fin ray of the white sucker to be a more accurate determinant of age than scales (see Figure 16.14), and Morzoff (1955) found both pectoral spines and vertebrae useful for determining age and growth of the channel catfish.

Annuli are visible in the whole structure of the cleithrum (Figure 16.18) and opercular bones, or in cross section from the fin ray (Figures 16.6, 16.7 and 16.14), vertebrae Figure 16.19), and spines (Figure 16.5). Structures may be examined with transmitted or reflected light. As with otoliths, a fluid may be required to enhance the resolution of the opaque and translucent bands. Annual bands in channel catfish spines appear as concentric rings around the lumen (Figure 16.5). Zonation in vertebrae is concentric around the core of the centrum. Both opercular and dentary bones show banding along their growing edge.

Figures 16.14 through 16.19 near here.

As in spines, the first ring on the fin ray in older fish can be faint and in the white sucker (Quinn and Ross, 1982) annuli may be missing in older fish. There can also be a problem in finding a fixed central point for making measurements. In cross sections made too far above the base, the first annulus can be difficult to discern or may be missing. Another drawback is that different rays of the pectoral fin may not show as sharply defined a growth pattern as others. Therefore, considerable practice is necessary when selecting the best fin ray for age determination.

Accessory checks may be evident on these structures. These, as well as abnormalities in bone formation should be looked for and avoided. Bones are particularly prone to extra protrusions and growths resulting from diseases and/or injuries.

Elasmobranches

Most anatomical structures used for bony fishes are not applicable to elasmobranches. In this group spines are uncommon, teeth are constantly renewed, scales are unsatisfactory, and most of the skeleton is cartilage. Mineralized vertebral centra are the only hard tissue components consistently present among elasmobranches (Gilbert, Mathewson, and Rall, 1967) and these are not well-calcified (Figure 16.19). For studies including validation see Daiber (1960); Haskell (1949);Holden and Vince (1973); Ishiyama (1951); Richards, Merriman, and Calhoun (1963); and Waring (1980).

Zonation in the centrum of elasmobranches is similar to that found in the vertebrae of bony fishes. Annual marks are laid down as concentric rings reflecting fast or slow growth. A year zone consists of a lightly calcified opaque zone and a dark translucent zone when the centrum is viewed under reflected light. Accessory checks are frequently observed in cross section.

## 16.5 VALIDATION

Validation involves using several independent techniques to age the same fish. This provides a verification so that the final age can be assigned with some confidence. Although using hard structures in age determination is the most commonly used method, other methods can verify or challenge the original ages assigned. The usefulness of a thorough literature search for validation of age marks cannot be overstated. Since Graham (1929) and van Oosten (1929) presented the first comprehensive criteria for validating age marks on hard structures, most changes have been in the area of technologies available to enhance growth pattern recognition. For an excellent coverage of validation methods the serious student should read at least one of the publications by Brothers (1979, 1982).

Some standard methods for age validation are: (1) length-frequency analysis; (2) modal-progression analysis (e.g., following the relative abundance of a dominant year class from year to year to serve as a landmark); (3) counting the number of annual marks in known-age fish (marked and recaptured or grown in confinement); (4) determination of periodicity of annual zone formation (following edge formation in a sample taken at different times of the year); (5) comparison of ages derived from different hard structures [(e.g., scales vs. otoliths, scales vs. cliethra, scales v. fin rays (Figure 16.14)], (Adams, 1942; Harrison and Hadley, 1979; Kohler and Clark, 1958; Marzolf, 1955); (6) comparison of back-calculated lengths-at-age determined from hard structure(s) with lengths calculated from mark-recapture or length frequencies; and (7) comparison of young of the year and yearlings from different sources to validate the interpretation of the first annulus (see Box 16.3 for examples). A more recent validation technique is to count the number of daily rings between successive annuli.

Box 16.3 near here.

Of course the reliability of any aging method is greatest when it has been validated. Statistical comparisons of age readings using different hard structures (see item (5) above) can measure relative consistency of methods. The results of validation studies are most useful in establishing aging criteria for interpretation of annuli, as well as for pinpointing significant life history events. Occurrence of accessory marks (e.g., false checks) between validated annual marks may be excellent indicators of spawning, extreme temperature changes (e.g., thermal discharge into a reservoir), lack of food, change in habitat, or the presence of pollution.

The strongest validation, but not always the most cost-effective, is to test a questionable method and/or age structure scale against results obtained from known-age fish (Taubert and Tranqueli, 1982).

## 16.6 BACK CALCULATION

Thus far the discussion on the use of fish body structures has concentrated on their reflection of the age of the fish at capture. Another use of these structures involves measuring annual rings on the body parts to estimate the size of the individual fish at earlier ages. This process is called back-calculation. The assumption being made is that there is a proportionate relationship between how much the fish increases in length and how much the hard structure increases in size. Analysis of this relationship can be used to determine the past growth history of individual fish. Knowledge of past growth history can be used to describe how the fish grew under past environmental conditions.

Calculations using the length or radius of hard structures and the distance between their annuli were first done by Lea and Dahl (1910). Since then a variety of methods have arisen. Lagler (1956) reports a summary of earlier methods. More recent discussions can be found in Bagenal and Tesch (1978), Bryuzgian and Chuqunova (1963), Carlander (1981), and Hile (1970). Two primary methods are the proportional and regression techniques. All methods depend upon knowledge of the correlation between body length and age structure size.

The whole otolith rather than a section is preferred for back-calculation analyses because of the difficulty of sectioning otoliths precisely at the nucleus. It is also important that sections of fin rays and spines expose the center of the structure. Measurements for back-calculations from otolith, spines, fin rays, and bones are made with an ocular micrometer. When scales are used, measurements are easiest using a ruler attached to a microprojector or a nomograph may be used (Carlander and Smith, 1944). In general, measure to the outer edge of each annulus from the center, or the total diameter of each ring through the center (focus, nucleus, or lumen in spines). The radius or diameter should be measured along the longest axis (e.g., largest lateral lobe in spines), assuming that there are no irregularities on that part of the structure (Figure 16.20). Indentations, erosion (crystalline areas), or unusual protuberances that are likely to interrupt growth and/or measurements should be avoided. Scales are usually measured from the focus to the anterior edge along a consistent line. Standardizing the direction of measurement and plane of sectioning cannot be overemphasized. Variation in the point at which the section is taken results in variations in measurements which can invalidate any calculations. Scales taken from several points on the fish can also result in variations in back-calculated lengths.

Figure 16.20 near here.

Once measurements of large numbers of structures have been made, the exact relationship between the length of the fish and the size of the structure must be determined. A literature search will be useful in revealing the type of relationship other workers found. However, the relationship between fish length and structure size varies between species, and in some cases even between populations. An adequate sample size is essential. The sample must include fish of all lengths in order to describe the entire relationship. The total size of the structure is plotted ageinst fish length. A line fitted to the points may be linear, curvilinear, or a combination of the two.

The relationship usually assumed by proportion methods is a linear one. In the case of scales, the y-intercept is not at zero since scales do not appear until some time after hatching. It is not always necessary to calculate this intercept value (a) for the back-calculation formulas. In some instances standard a values can be obtained from the literature for each species. This would eliminate the variance caused by poor sampling techniques. However, a standard a value is not accepted by some workers because of the differences between populations.

Carlander (1981) noted that the true relationship between body lengths and scale size is probably curvilinear but deviations from a straight line are usually so small over the size ranges of interest that they can be disregarded. If regression methods are used to fit a curve to the data, 90-99% of the variation in body length may be related to age structure size. Although this method of curve-fitting may be satisfactory if derived from a large enough sample size, according to Carlander (1981) traditional methods reduce variance more. The fish length to otolith length relationship is more often significantly curvilinear over at least part of the length range (especially for smaller fish).

There are many methods of calculating fish length at successive annuli. The earlier cited references, as well as Carlander (1950), Whitney and

Carlander (1956), and Winsor (1946) provide formulas and information on the methods to use. It is important to note that unless the age determinations are correct the back-calculations will be misleading. Box 16.4 illustrates the use of a simple regression method, based on a linear relationship.

Box 16.4 near here.

Back-calculations are useful to derive information on growth of various cohorts or as a method of age validation. However, the process is tedious and not necessary for routine aging.

## 16.7 AGE DETERMINATION FROM SIZE FREQUENCY DISTRIBUTION

In the event that individual fish cannot be reliably aged, population size structure may be analysed for indications of age groupings. This alternative method of age determination began with Petersen's (1891) speculation that peaks in size-frequency distribution plots represented the modes of year classes (cohorts). Fish hatched in the same year tend to be in the same size range, with most fish being close to an average size. There tends to be a statistically "normal" distribution of sizes around a modal (most frequent) size. There should be recognizable peaks in the size distribution of a population sample as long as the sample is unbiased and some other assumptions are met, as are discussed later in this section.

An example from Lux (1971) illustrates this graphical method (Figure 16.21). This curve was drawn from a fall catch sampling a haddock population, and shows that the larger of the young of the year fish (YOY) were just beginning to be caught in otter trawl nets. The peaks correspond to modal sizes of about 6" for the YOY, 11" for $1^+$-year-olds, and 16" for $2^+$-year-olds. Size overlap begins to obscure the peak at age 2, and beyond that prevents the appearance of any obvious peaks (called the "damping" of modes).

Figure 16.21 near here.

The damping of modes due to overlapping sizes of different cohorts restricts the usefulness of the basic Petersen method to the first 2-4 years for most fish populations. More sophisticated graphical procedures have since been developed to separate cohort size distributions from population size distributions (see Table 16.2). These procedures may be utilized without computer assistance, which can be an advantage in some fishery facilities. However, these graphical techniques are often not reproducible. Different conclusions may result from analysis of data by different methods or authors (Frechette and Parsons, 1981; Pauly, 1980), especially if the size frequency distribution is not clearly polymodal (MacDonald and Pitcher, 1979).

Table 16.2 near here.

Several computer-assisted statistical procedures have recently been adapted to separate mixes of normal size-frequency distributions in fishery research (see Table 16.3). These methods are statistically superior to graphical ones but require large sample sizes to be used effectively. Also, the estimates which result may still have large errors in cases of overlapping modes (McNew and Summerfelt, 1978).

Table 16.3 near here.

All graphical and most statistical methods assume that size distribution within each cohort is normally distributed, and that there is some discernible separation between year class size distributions. The usefulness of either type of age determination can be limited if growth is largely uniform throughout the year (as in tropical fish), if spawning seasons are prolonged or intermittent, if individuals of a particular species tend to school according to size or maturity, or if size variability among individuals or cohorts is extreme. Older age classes with smaller sample sizes and higher degrees of overlap will be especially difficult to differentiate. Furthermore, none of these methods are free from subjectivity; different techniques for grouping and analysing data can produce different results.

Within their limitations, however, these methods are useful for aging populations if individuals cannot be aged easily or reliably, if validation of other aging techniques is desired, or if catch statistics are the only data available for analysis.

## 16.8 CONCLUSION

Every fisheries biologist considers how accurately the age data presented represents the age composition (structure) of the fish population or stock being examined. Therefore, accurate age determination is a challenge to the inexperienced and the expert fish age reader. Historically, the interpretation and counting of growth zones on calcareous structures is the preferred method of fish age determination. This method, in spite of modern computer technologies, is still a subjective process dependent on the interpretation of the human age reader. This condition makes it necessary for us as age readers to contemplate the relative nature of our visual evaluation of time markers on fish hard parts. A similar charge holds true when using the statistical and empirical approaches. These approaches are not without biases and error resulting from subjectivity.

Much needs to be done to standardize the nomenclature use by fisheries biolgists to record and communicate their age determinations. Standardization of terminology would remove an immense obstacle to communication of age results as well as provide better comparisons between fish studies.

Incumbent upon us as fish age readers is to make sure that we make every effort possible to improve the quality of our age determinations.

# CHAPTER 16 BOXES

16.1 TERMINOLOGY OF AGING

16.2 SCALE GROWTH PATTERN RECOGNITION

16.3 AGE VALIDATION

16.4 BACK-CALCULATION USING PROPORTION METHOD

BOX 16.1: TERMINOLOGY OF AGING

| TERM | SYNONYMS | DEFINITION |
|---|---|---|
| Age group | -age class<br>-cohort<br>-year class | Fish of the same calendar age, hatched in the same year. |
| Annulus | -band<br>-ring<br>-year or age mark<br>-zone (winter zone) | Slow-growth zone on age structure, considered to form annually and counted for age determinations. |
| Center | -central kernel<br>-nucleus (otolith)<br>-origin<br>-focus (scale)<br>-central lumen (spine) | Point of origin of age structure. |
| Check | -accessory ring or mark<br>-false ring or annulus<br>-secondary ring or zone | Zone or ring on age structure considered to form sub-annually; not counted for age determinations. |
| Circulus | -ridge | Raised, mineralized plate-like structure on the surface of a scale (appears as rings around focus). |

| TERM | SYNONYMS | DEFINITION |
|---|---|---|
| Edge | -margin | Outer periphery of an age structure; represents most recent growth. |
| Opaque | -summer zone or ring<br>-fast-growth zone<br>-optically dense zone | Optically dense zone on age structures formed during periods of active growth. |
| Radius | — | Groove-like depression radiating from the focus to the edge on some scales. |
| Regenerated scale | — | Scale formed to replace one previously lost. |
| Split (annulus) | -double ring | Annulus composed of two or more closely-spaced zones formed within one winter season. |
| Translucent | -winter zone or ring<br>-hyaline zone<br>-slow-growth zone | Zone of low optical density formed during periods of slow growth. |

BOX 16.2: SCALE GROWTH PATTERN RECOGNITION

The following criteria are used to identify a true scale annulus and are readily recognized after experience:

1. Relative spacing of the circuli. (See text.)
2. "Cutting over" or "crossing over" of the circuli across previously deposited circuli, particularly on the lateral edges. On some scales the outer circuli tend to flare outward or end abruptly on the side of the scale. Often associated with crossing over is a thin clear zone with no circulus ridges which extends across the anterior field of the scale. The erosion or absorption of the scale edge during slow growth periods may result in cutting over. (Figures 16.12, 16.13, and 16.14).
3. Bending or waviness of unsegmented circuli (clupeidae). A similar description for sunfish is "bell marks" which often form at the radii. These appear as bell-shaped blank spots located at the junction of the radii and the annulus.
4. Circulus counts. An average number of rows of circulus ridges may be associated with a given annulus. This number decreases with age.
5. Changes in circulus shape. Circulus segments may be thicker, more wavy, or fragmented during active growth. During periods of slow growth these segments are thin, straight, and less fragmented (Figure 16.10). Changing of focus on the microprojector will show the change in circulus thickness which is present in the annuli.
6. Radii may be used. Radii are scale flexion lines extending in an anterior/posterior direction. New radii may form at the outer edge of an annulus or existing radii may bend or branch (Figure 16.13).

This criteria is not generally reliable.

The following criteria are used to identify accessory rings or checks:

1. A ring with closely spaced rows of circuli, or "cutting over" that is discontinuous around the edge of the scale.
2. A ring with fewer rows of circuli than are present in obvious annuli.
3. Circuli of the wrong type (broad rather than narrow, as found in a true annulus) comprise the ring.

A split annulus may be identified by:

1. Unusual spacing of rings, especially in a paired pattern.
2. Observation of fast growth on the edge during the winter months.

BOX 16.3: AGE VALIDATION

An example from Mayo, Gifford, and Jearld (1981) serves as an illustration of age validation of redfish from the Gulf of Maine-Georges Bank region. Validation techniques used were:

1. Reviewing the literature on aged redfish from the study area.
2. Taking samples from fish from the same general location through several seasons.
3. Processing all samples for age mark recognition in the same way.
4. Examining all otolith sections by two experienced age readers for percent age agreement.
5. Examining otolith sections for the type of edge deposition (hyaline and opaque) found throughout the year.
6. Comparing estimates of mean length at age with observed modes of length frequencies specific to the 1971 year class.
7. Discerning progression of length modes of the dominant 1971 year class from length frequency distributions of catch data 1971-78.

The terminology used to specify edge types include the four categories proposed by Jensen (1965) and four additional categories for intermediate edge types. Jensen's categories are the first four in the following list:

Hn Narrow hyaline

Hw Wide hyaline

On Narrow opaque

Ow Wide opaque

Hnv Very narrow hyaline edge, appearing after a wide opaque zone during the summer or transition period

Hm Medium hyaline edge, appearing after a wide opaque zone during late transition to mid-winter

Onv Very narrow opaque edge, appearing after a wide hyaline zone during the winter or early transition period

Om Medium opaque edge, appearing after a wide hyaline zone during late transition to mid-summer

**(Inserts 1, 2, 3, and 4 will be placed here.)**

Highlights of results.

1. The analysis substantiates the methods used by researchers at the Woods Hole Laboratory to describe redfish growth of relatively young redfish based on otolith age determinations.

2. Seasonal formation of hyaline and opaque edges on redfish otoliths occurs at a frequency of one cycle per year.

BOX 16.4: BACK-CALCULATION USING PROPORTION METHOD

Commonly, a regression back-calculation technique uses a simple linear regression relationship between the age structure and fish length. The equation used is:

$$Y = bX + a \quad \text{(see figure 1 below)}$$

where:

- Y = the length of the fish at the time the annulus in question was formed.
- b = the slope of the fish length/age structure size relationship (the change in fish length per unit change in structure size)
- X = the measurement to the annulus in question
- a = the size of the fish when the structure was formed

Given a fish/age structure size relationship in which b = 0.5 and a = 2:

$$Y = 0.5X + 2$$

(The fish size/structure size data points fitted by least squares methods.)

If the annulus measured is equal to 100 units, then by substitution, the fish length at the time the annulus formed equals:

$$Y = 0.5(100) + 2$$

$$Y = 52 = \text{fish size at annulus formation}$$

An alternative method is to use the intercept value (a) of the above example as a correction factor in the direct proportion method, the formula used would be:

$$Y = \left[\frac{\text{annulus size (x)}}{\text{total structure size}}\right] \times (\text{total fish length}) + a$$

substituting will give the predicted fish length for the annulus in question.

TABLE 16.1 ANATOMICAL STRUCTURE COMMONLY USED FOR AGE DETERMINATION

| Family | Common Name | Structure |
| --- | --- | --- |
| Squalidae | Dogfish sharks | Dorsal spine |
| Rajidae | Skates | Vertebral centrum |
| Acipenseridae | Sturgeons | Fin ray |
| Elopidae | Tarpons | Scale |
| Anguillidae | Freshwater eels | Otolith |
| Clupeidae | Herrings | Otolith |
| Engraulidae | Anchovies | Otolith |
| Salmonidae | Trouts | Scale |
| Esocidae | Pikes | Scales-cleithra |
| Cyprinidae | Minnows and carps | Scales |
| Catostomidae | Suckers | Scales-fin ray |
| Ictaluridae | Freshwater catfish | Pectoral spine |
| Batrachoididae | Toadfishes | Otolith |
| Gadidae | Codfishes | Otolith |
| Cyprinodontidae | Killifishes | Scale |
| Atheriinidae | Silversides | Otolith |
| Percichthyidae | Temperate bass | Scale |
| Serranidae | Sea basses | Scale |
| Centrarchidae | Sunfishes | Scale |
| Branchiostegidae | Tilefishes | Otolith |
| Pomada syidae | Grunts | Otolith |
| Sparidae | Porgies | Scale |
| Sciaenidae | Drums | Scale |
| Cichlidae | Cichlids | Scale |
| Scombridae | Mackerels and tunas | Otolith, fin ray |
| Bothidae | Lefteye flounders | Otolith, scale |
| Pleuronectidae | Righteye flounders | Otolith, scale |
| Muglidae | Mullets | Scale |
| Lophiidae | Goosefish | Vertebrae-ray |
| Carangidae | Jacks and pompones | Otolith |

TABLE 16.2 GRAPHICAL METHODS FOR SEPARATING POLYMODAL SIZE FREQUENCY DISTRIBUTIONS INTO COHORTS

| Methods | Limitations, Advantages | Reference |
|---|---|---|
| "Petersen method":<br>simple inspection of modes | - quick-and-dirty estimation<br>- often unreliable (e.g., a small year class may be missed entirely) | Petersen, 1891 |
| Probability-paper plotting plus trial-and-error estimation of parameters to fit plot | - most often used in fisheries research<br>- assumes normal distribution of size-at-age | Buchanan-Wollaston and Hodgson, 1929<br>Harding, 1949<br>Cassie, 1950, 1954 |
| Semi-logarithmic paper plotting plus fitting of parabolas to fit plot | - assumes normal distribution of size-at-age | Tanaka, 1962 |
| Natural logs of size frequencies plotted, straight lines fitted to first significant differences | - assumes normal distribution of size-at-age | Bhattacharya, 1967 |
| "Method of successive maxima":<br>modal classes split from left to right side of size distribution plot | - assumes only symmetrical distribution of size-at-age<br>- could introduce bias in mean size-at-age<br>- provides no statistical data for abundance of cohorts | Gheno and LeGuen, 1968 |

TABLE 16.3 STATISTICAL METHODS (COMPUTER-ASSISTED) FOR SEPARATING POLYMODAL SIZE FREQUENCY DISTRIBUTIONS INTO COHORTS

| Methods | Limitations, Advantages | Reference |
| --- | --- | --- |
| "Maximum likelihood" using NORMSEP (FORTRAN program): provides calculated mean size-at-age and standard deviations for each mode | - requires initial estimate of number of size groups, points of overlap (cutoff points)<br>- assumes normal distribution of size-at-age<br>- most often used in fisheries research | Hasselblad, 1966<br>Tomlinson, 1971<br>McNew and Summerfelt, 1978 |
| Method similar to above using ENORMSEP (to be used with NORMSEP) | - does not require input of initial estimates of number of size groups or cutoff points | Yong et al., 1975 |
| Maximum likelihood in an interactive program (from PDMM; FORTRAN program) | - requires initial estimate of number of size groups, other parameters<br>- can constrain parameters (e.g., to conform to biologically-plausible patterns such as Von Bertalanffy growth curve) | MacDonald and Pitcher, 1979<br>Schnute and Fournier, 1980 |
| Method tracing age group through sequential data using ELEFAN I (BASIC program) | - does not require input of initial estimates of number of size groups or other parameters (therefore assumes objectivity)<br>- can be used on minicomputer | Pauly, 1980 |

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Insert # 1

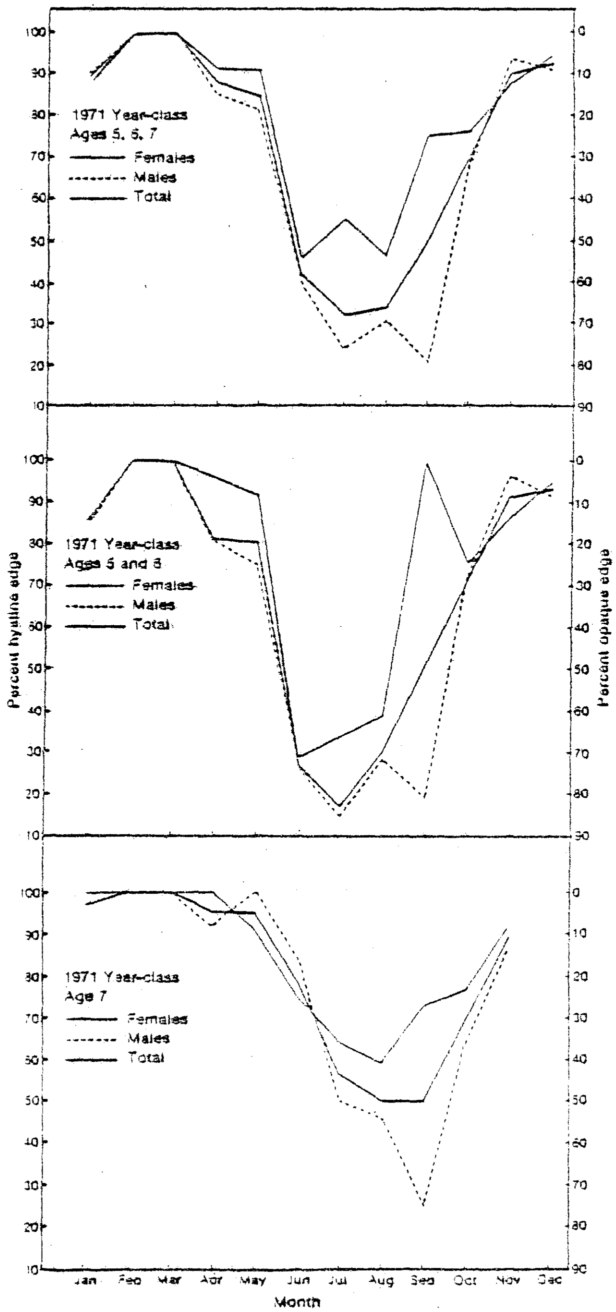
Figure to be included in Box 16.3

TABLE 1. Comparison of otolith edge type determinations by two readers for redfish from the Gulf of Maine-Georges Bank region.

| | Edge type[a] | Reader 1 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Hnv | Hn | Hm | Hw | Onv | On | Om | Ow | Total |
| Reader 2 | Hnv | **81** | 19 | 1 | 2 | 1 | 0 | 0 | 0 | 104 |
| | Hn | 6 | **94** | 16 | 1 | 0 | 0 | 0 | 0 | 117 |
| | Hm | 0 | 15 | **78** | 26 | 0 | 0 | 0 | 0 | 119 |
| | Hw | 2 | 6 | 28 | **193** | 2 | 1 | 1 | 0 | 233 |
| | Onv | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| | On | 1 | 2 | 0 | 1 | 0 | **43** | 4 | 2 | 53 |
| | Om | 5 | 1 | 0 | 0 | 0 | 5 | **40** | 13 | 64 |
| | Ow | 15 | 4 | 0 | 0 | 0 | 0 | 8 | **64** | 91 |
| | Total | 110 | 141 | 123 | 223 | 4 | 49 | 53 | 79 | 782 |

[a]See ~~Materials and methods for definitions~~. validation text p.

Figure to be included in Box 16.3




Seasonal changes in the proportion of redfish otoliths displaying hyaline and opaque edges.

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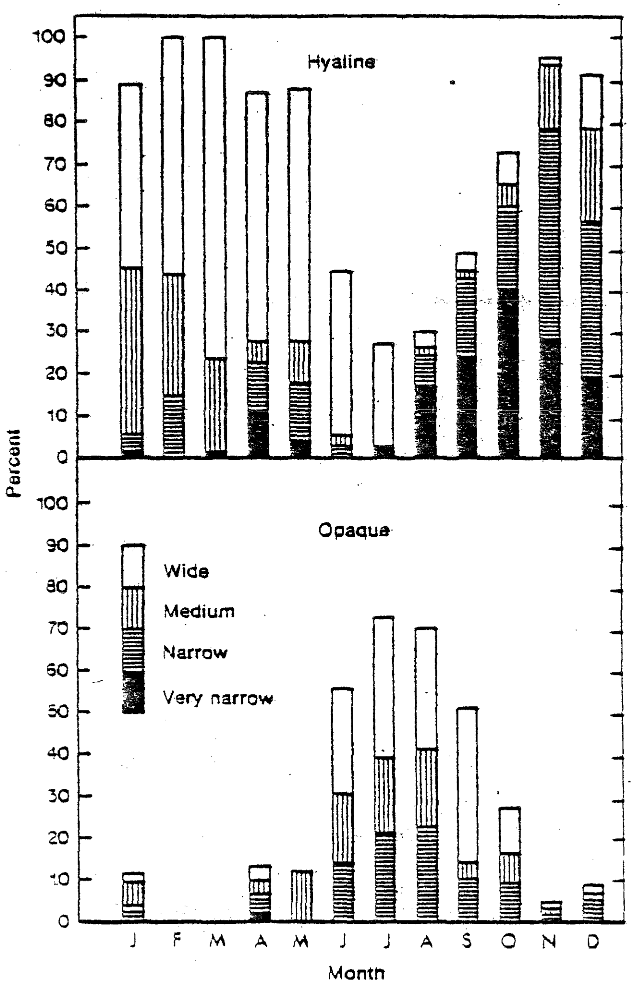
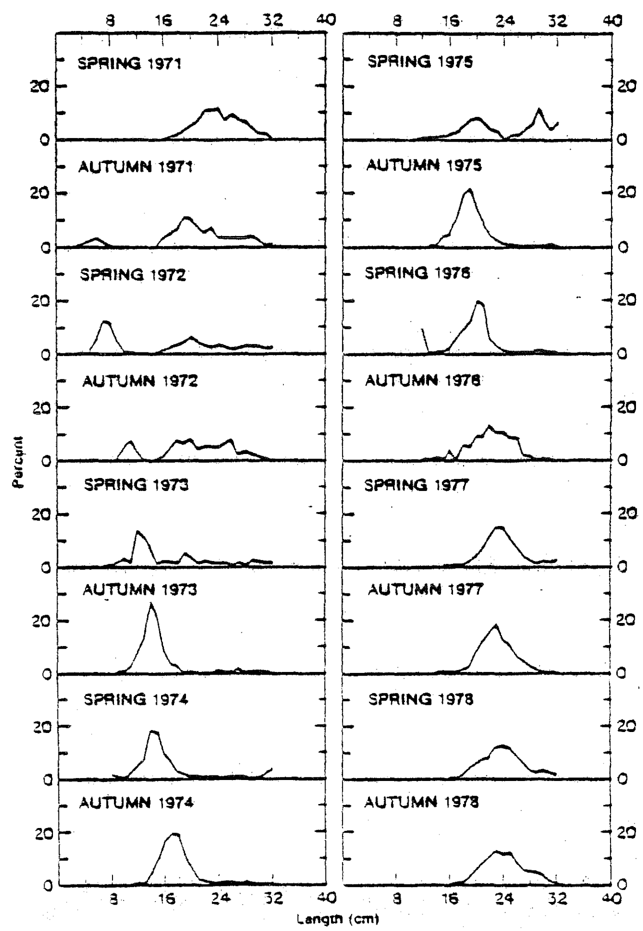



[redacted] Seasonal changes in the width of hyaline and opaque edges of redfish otoliths.




[redacted] Length frequencies of redfish, displaying the progression of modal length groups representing the 1971 year-class, from trawl surveys of inshore areas (<111 m) in the Gulf of Maine during 1971–78.

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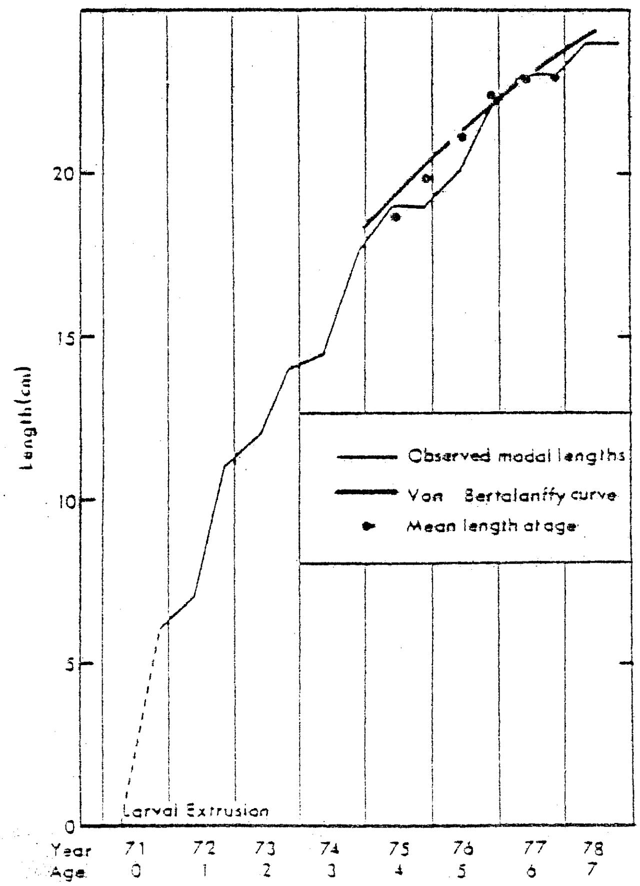
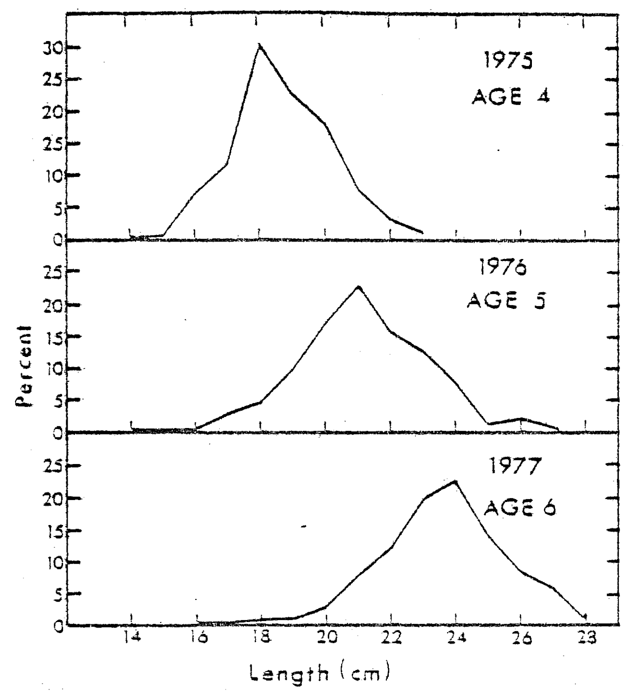
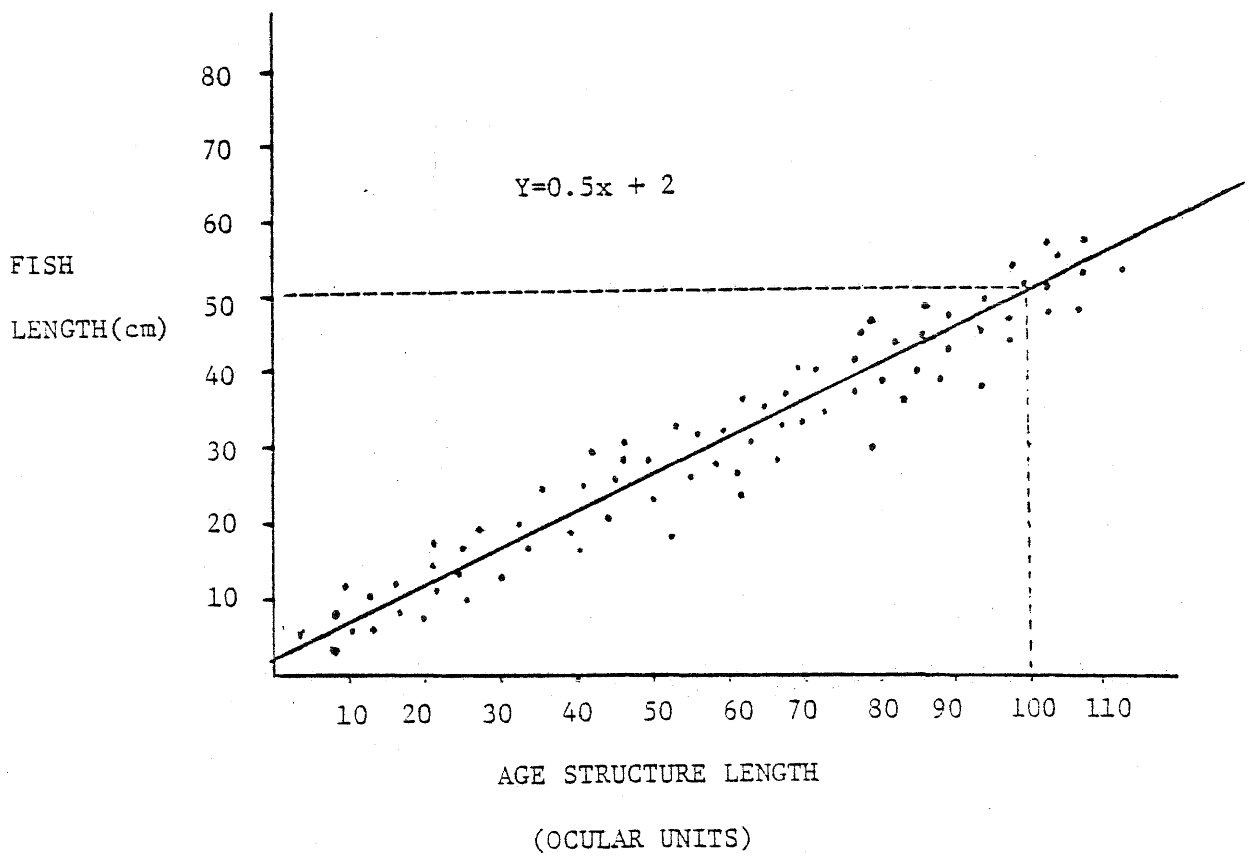



Growth of redfish in the Gulf of Maine as indicated by observed modal lengths of the 1971 year-class from spring and autumn surveys during 1971–78 and by mean lengths of age-groups 4–6 based on otolith ageing, together with a segment of the von Bertalanffy growth cruve from Mayo (1980).




Length distribution of ages 4–6 redfish of the 1971 year-class from spring surveys in the Gulf of Maine, 1975–77.



Y=0.5x + 2
FISH
LENGTH(cm)
80
70
60
50
40
30
20
10
10 20 30 40 50 60 70 80 90 100 110
AGE STRUCTURE LENGTH
(OCULAR UNITS)

CHAPTER 16

FIGURE HEADINGS

Figure 16.1: diagram indicating general areas on a fish where scales of various species may be removed

Figure 16.2: diagram describing a typical method of cutting head for otolith removal

Figure 16.3: diagram of a typical flounder showing where head may be opened to remove otoliths

Figure 16.4: (left)diagram of a redfish otolith. (right)transverse section from a redfish otolith

Figure 16.5: (left)diagram of a channel catfish spine showing location of cut for section. (right)cross-section from a channel catfish spine, under brightfield transmitted light

Figure 16.6: (left)diagram of a fin ray (biserial) showing the location of cut for section, after filaments are separated. (right) thin section of fin ray filament from a summer flounder after treatment with clove oil, viewed under darkfield transmitted light

Figure 16.7: thin transverse section from the marginal fin ray of pectoral fin of shortnose sturgeon (after Jack Buckley (unpublished), with permission)

Figure 16.8: (above)inner surface of the valve of the surf clam indicating location of cut for chondrophore and valve sections. (below) chondrophore section

Figure 16.9: cycloid scale of a haddock

Figure 16.10: ctenoid scale of a yellowtail flounder

Figure 16.11: scale of a fallfish (after Michael Ross (unpublished), with permission)

Figure 16.12: scale of a summer flounder, showing thin clear rings in anterior field representing annuli

Figure 16.13: (left)scale of a bluefish showing "cutting over" of circuli (above right)"cutting over" on outer edge of second annulus

Figure 16.14: (left)scale of a white sucker, showing extreme crowding of annuli near edge; only 3 annuli evident. (right)fin ray section from the same fish, showing 6 annuli (after Stephen Quinn (unpublished) with permission)

Figure 16.15: regenerated scale of a haddock

Figure 16.16: whole otoliths, cleared in glycerin, of a silver hake

Figure 16.17: thin transverse section from a redfish otolith

CHAPTER 16, FIGURE HEADINGS, continued:

Figure 16.18: diagram of a cleithrum

Figure 16.19: thin section from the centrum of a little skate

Figure 16.20: diagram of a typical scale (A), whole otolith (B), and fin ray section (C) showing radius of measurements for backcalculations

Figure 16.21: the length frequency distribution of a catch of haddock, showing the different size groups of fish caught and corresponding year classes (after Lux (1971), with permission)

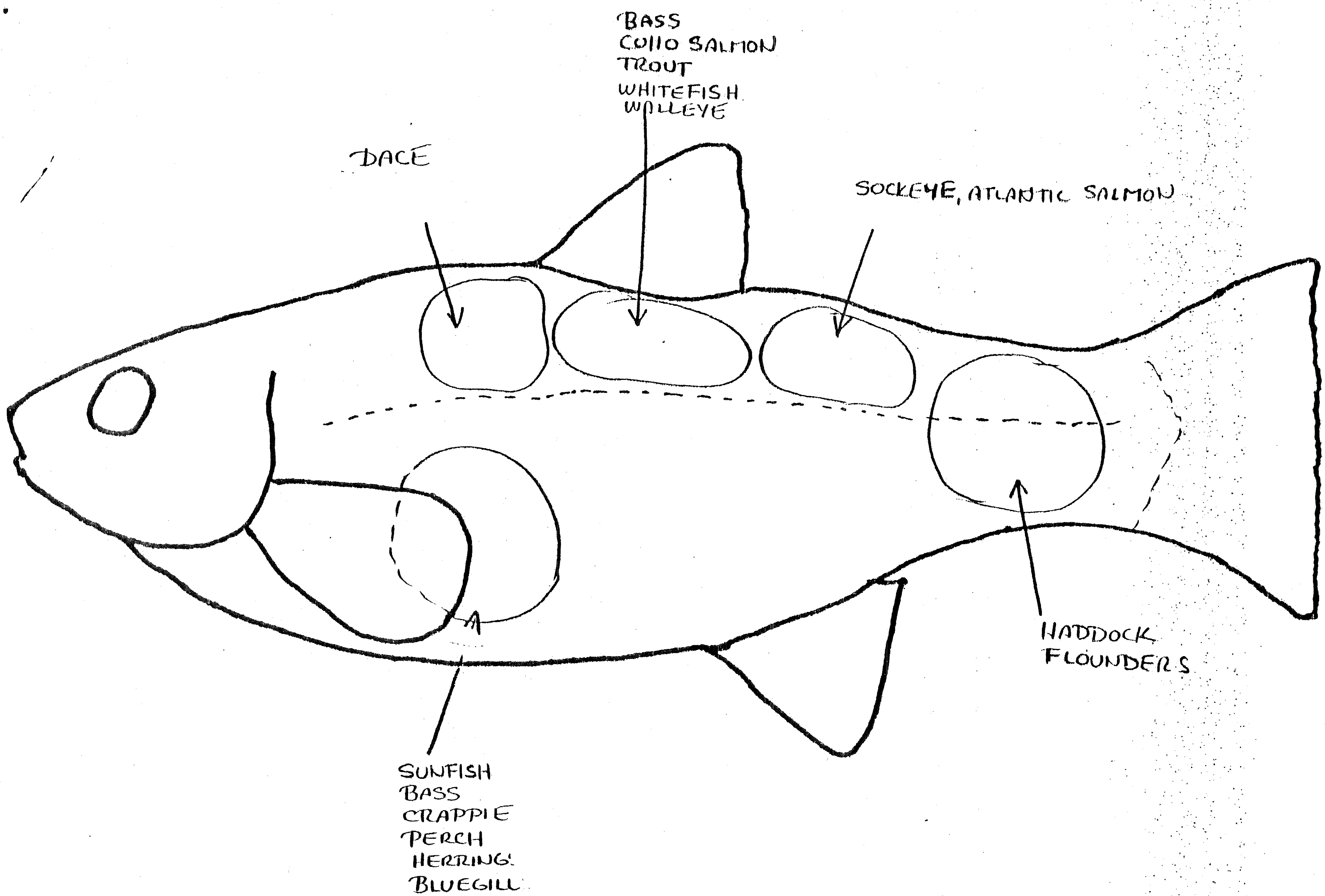



Figure 1. Diagram indicating general areas on a fish where the scales of various species may be removed.

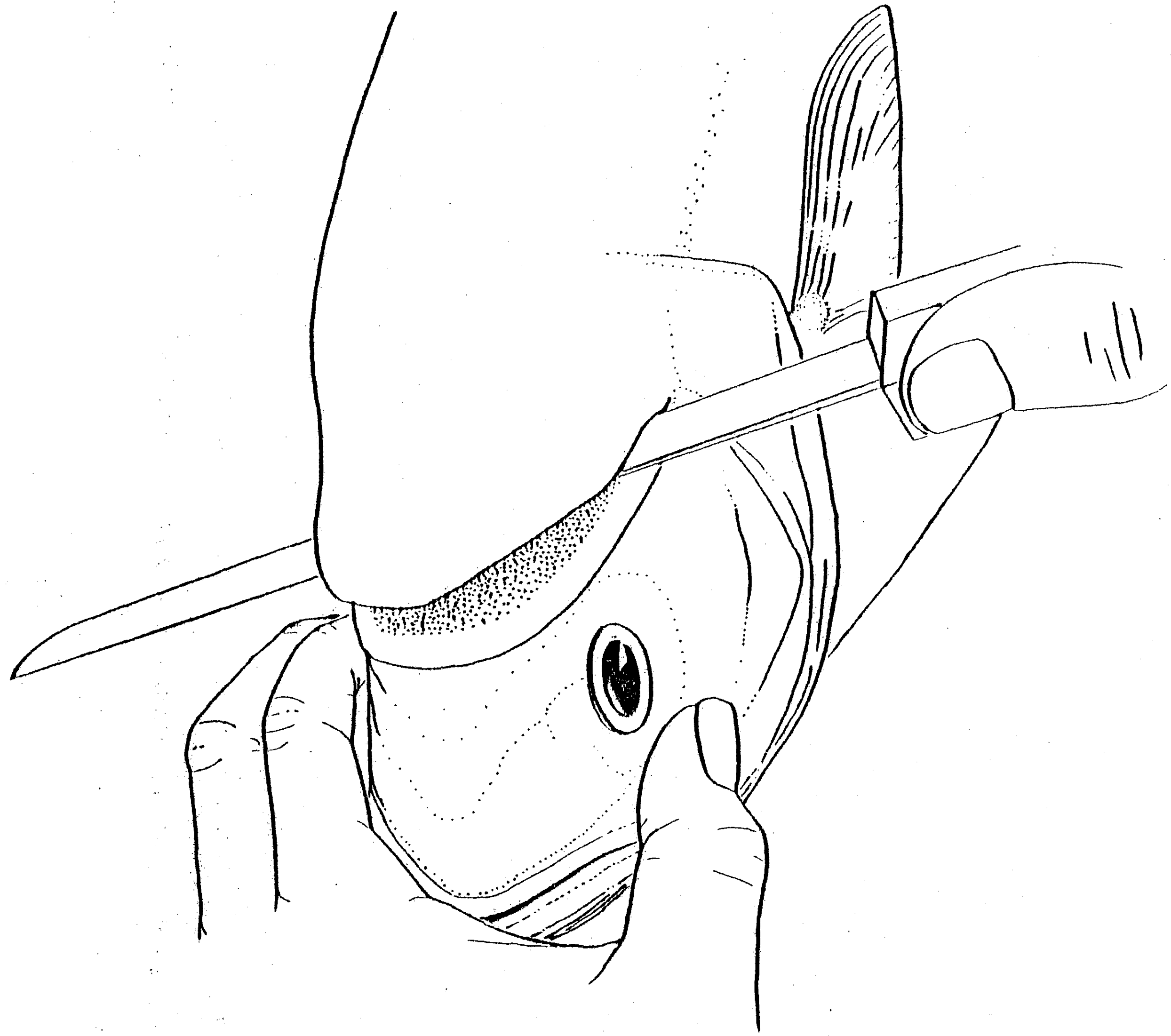


Figure 2. Diagram describing a typical method of cut for otolith removal.

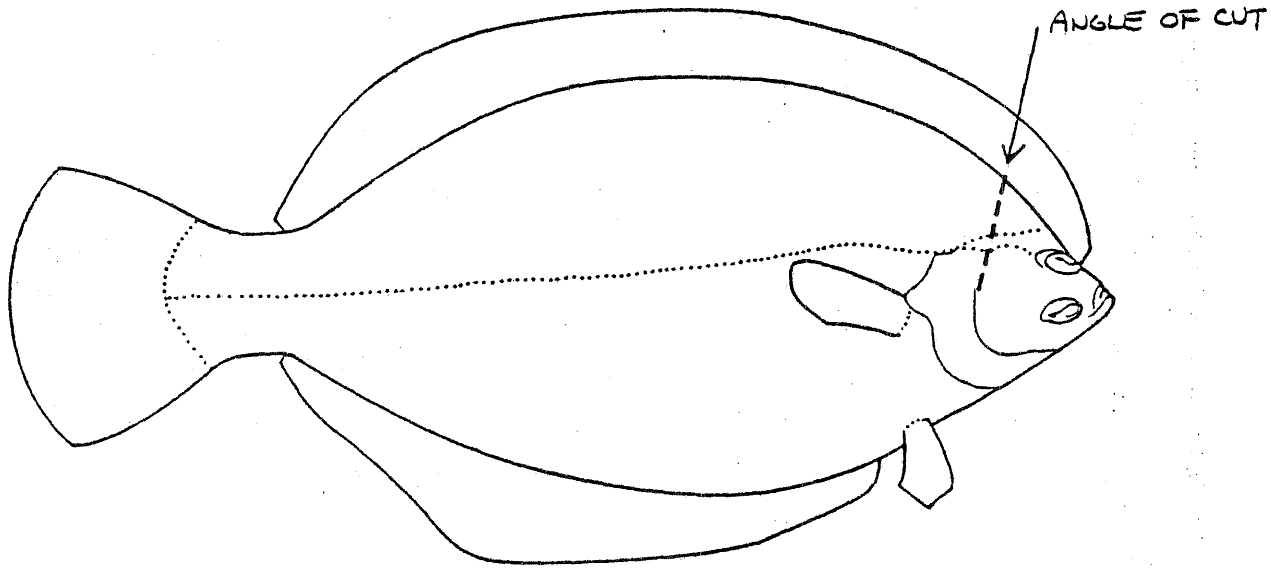



Figure 3. Diagram of a typical flounder showing where the skull may be opened to remove otoliths.

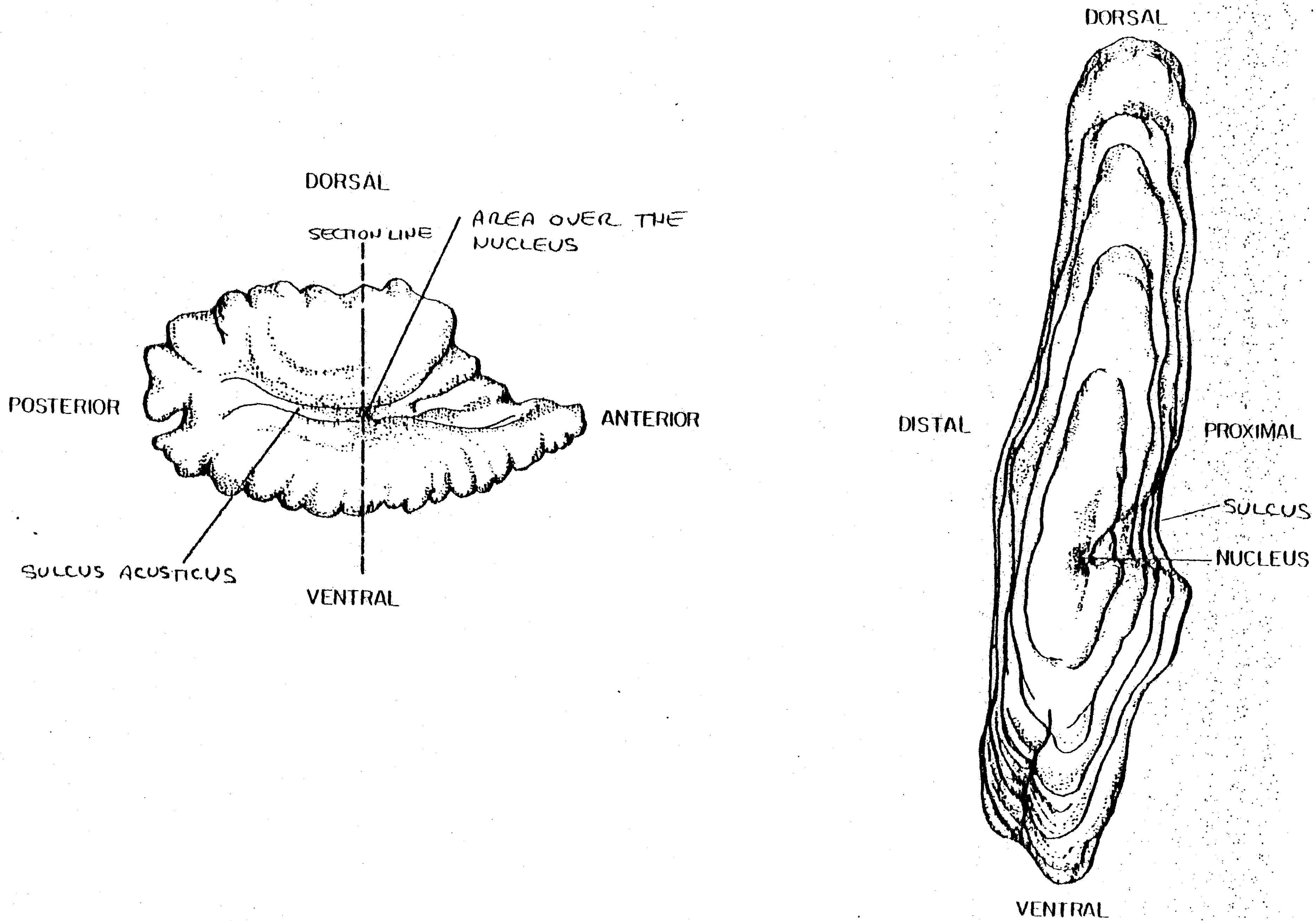



Figure 4. (left) Diagram of a redfish otolith. (right) Transverse section from a redfish otolith.

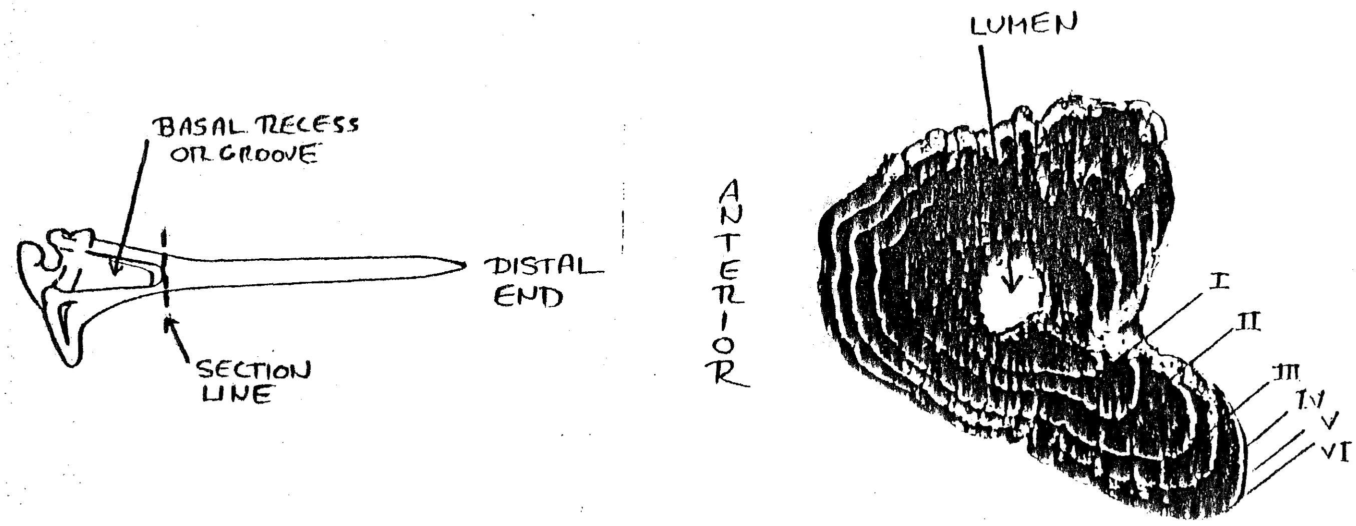



Figure 5. (left) Diagram of a channel catfish spine showing the location where the section is taken.
(right) The cross section from a channel catfish spine under brightfield transmitted light.

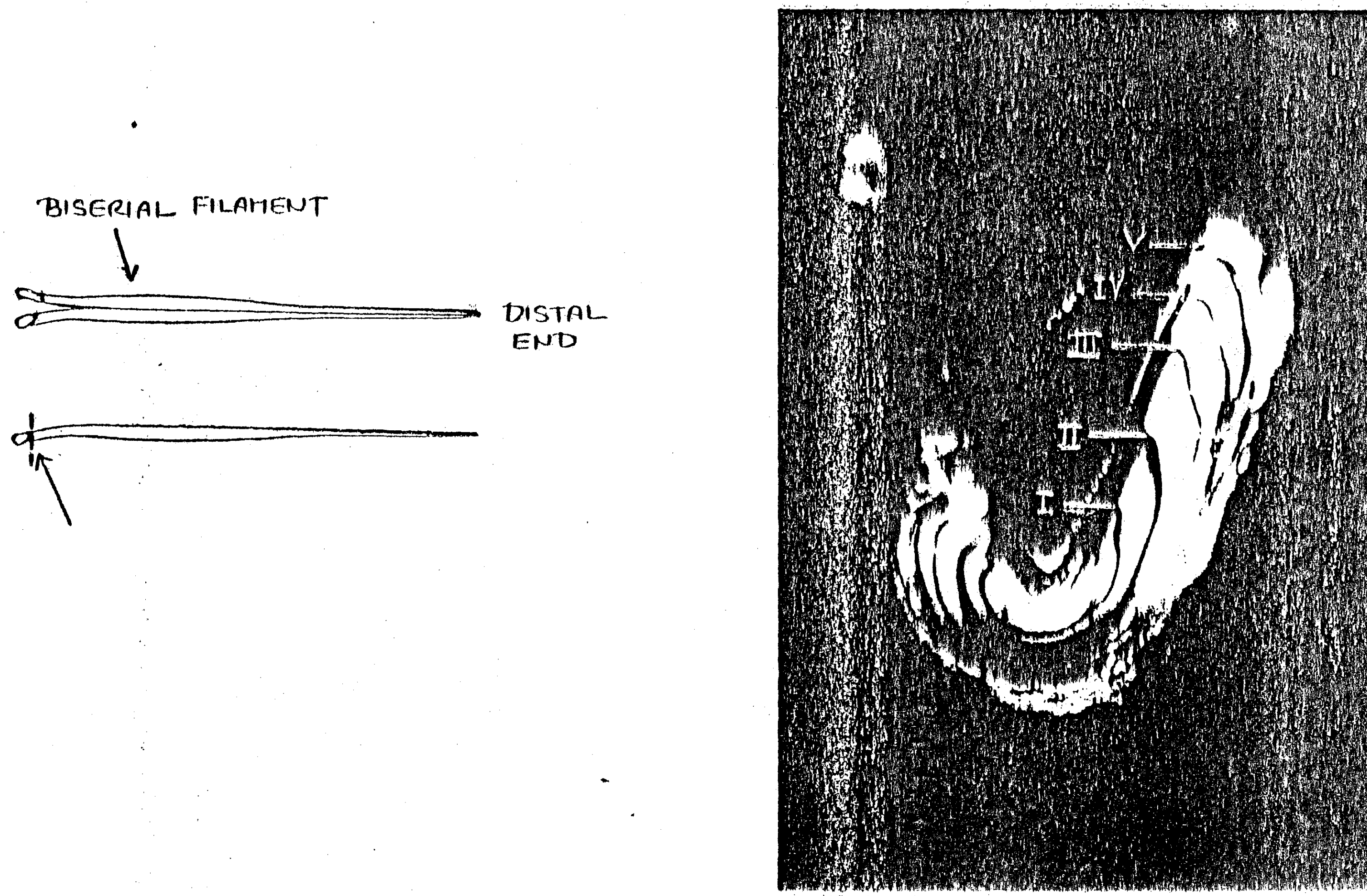



Figure 6. (left) Diagram of a fin ray (biserial) showing the location where the section is taken after the filaments are separated. (right) Thin section of a fin ray filament from a summer flounder after treatment with clove

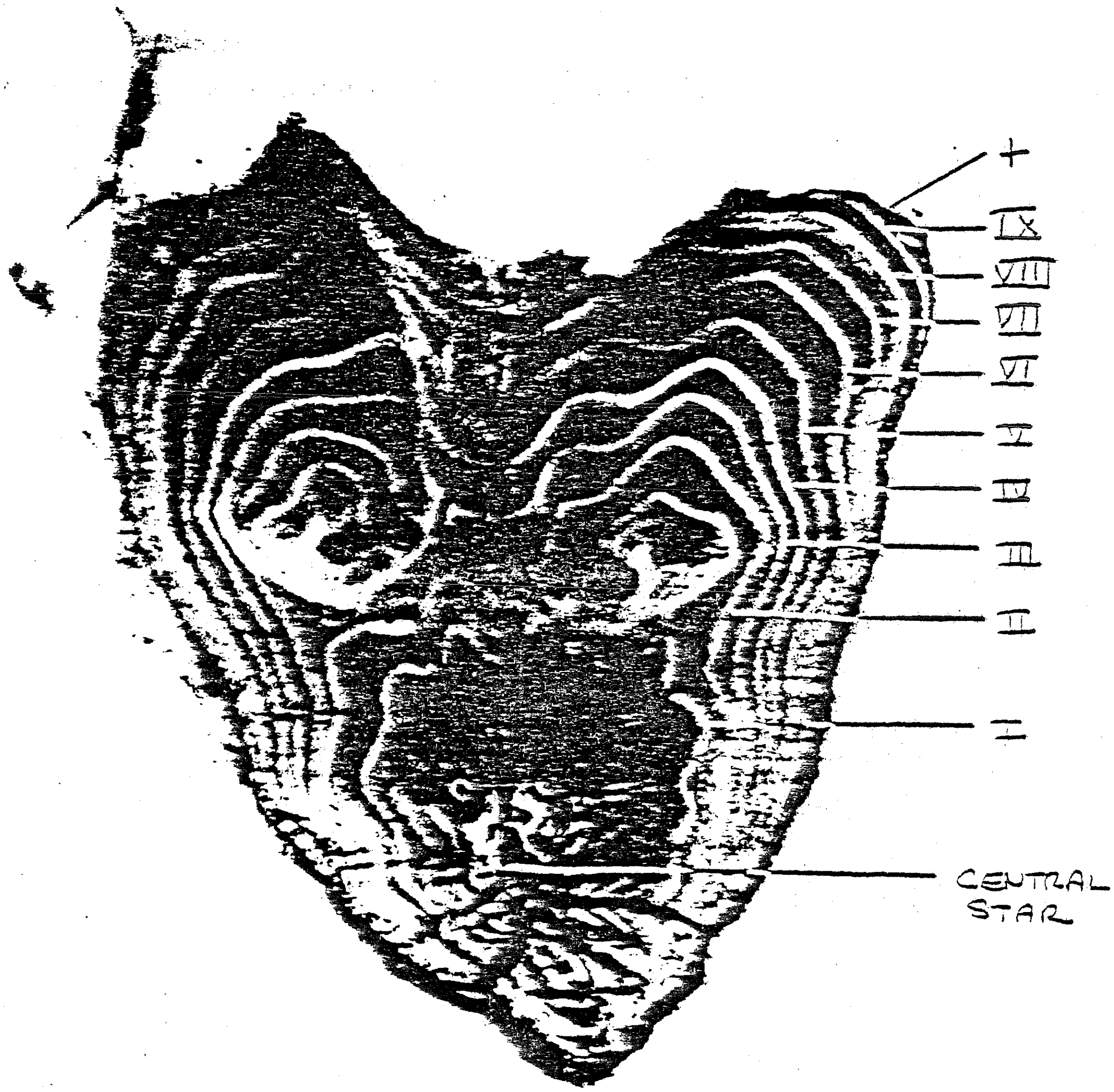



Figure 7. Thin transverse section from the marginal fin ray of the pectoral fin of the shortnose sturgeon .

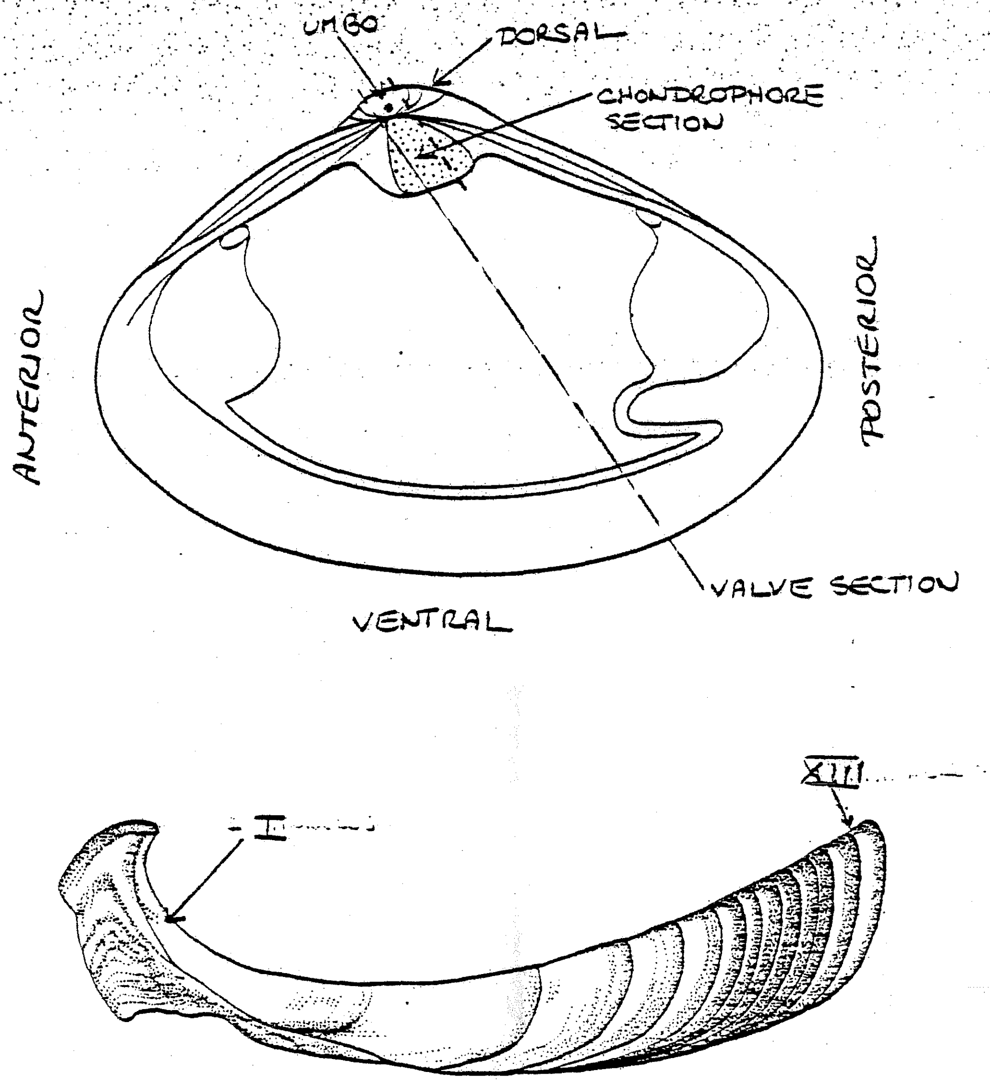



Figure 8. (above) Inner surface of the valve of the surf clam indicating where the chondrophore and valve sections are made. (below) Chondrophore section.

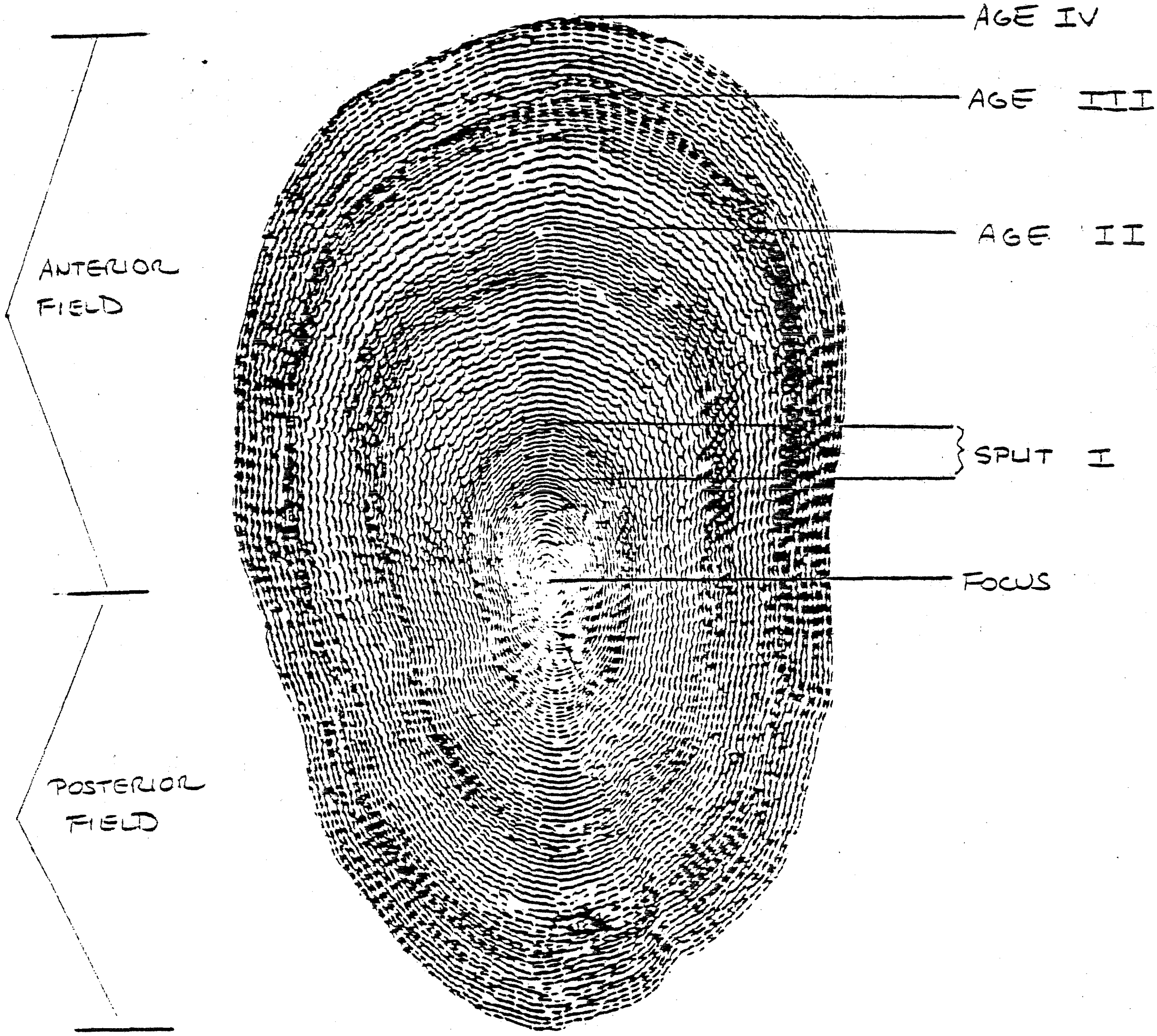



Figure 9. Cycloid scale of a haddock.

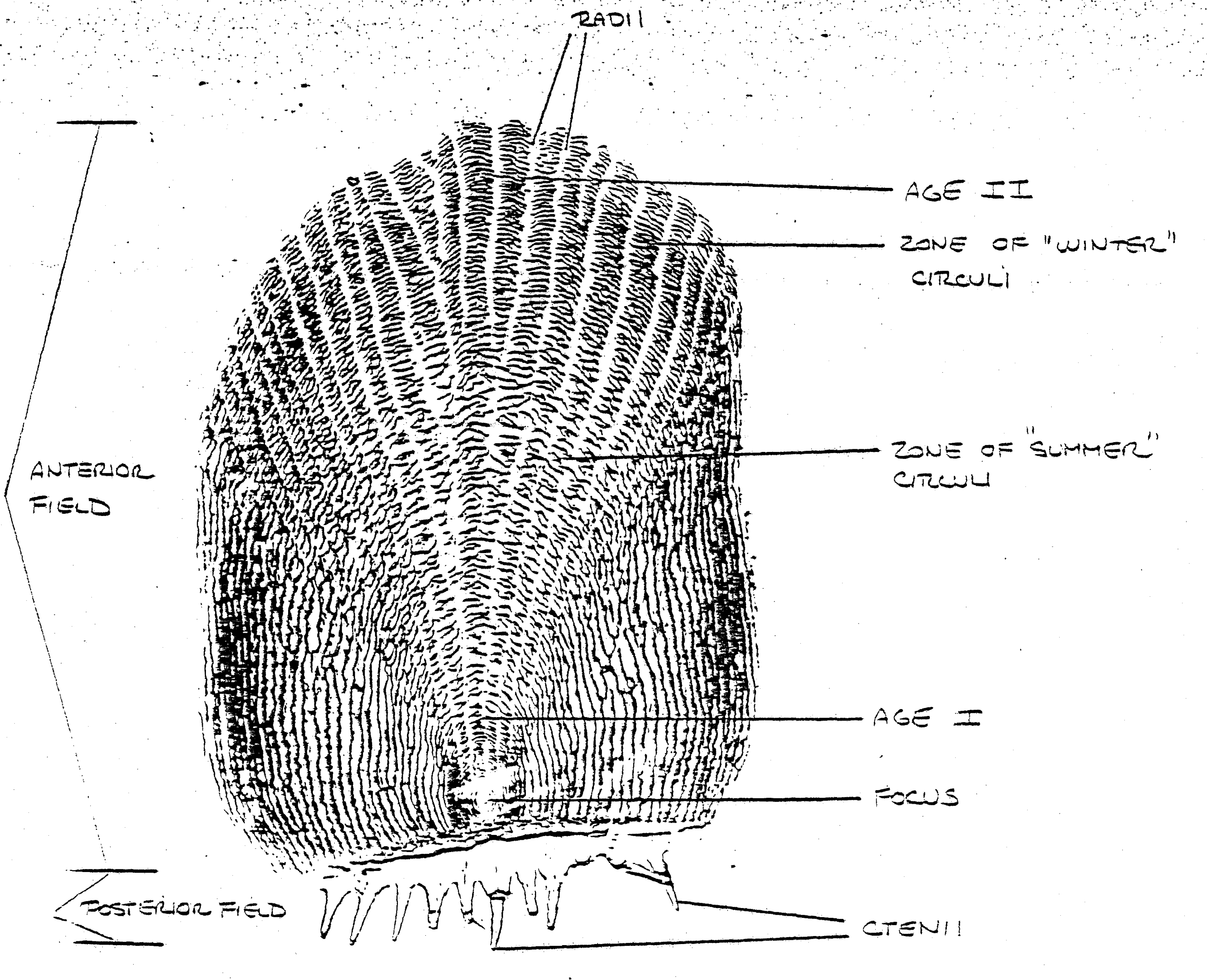
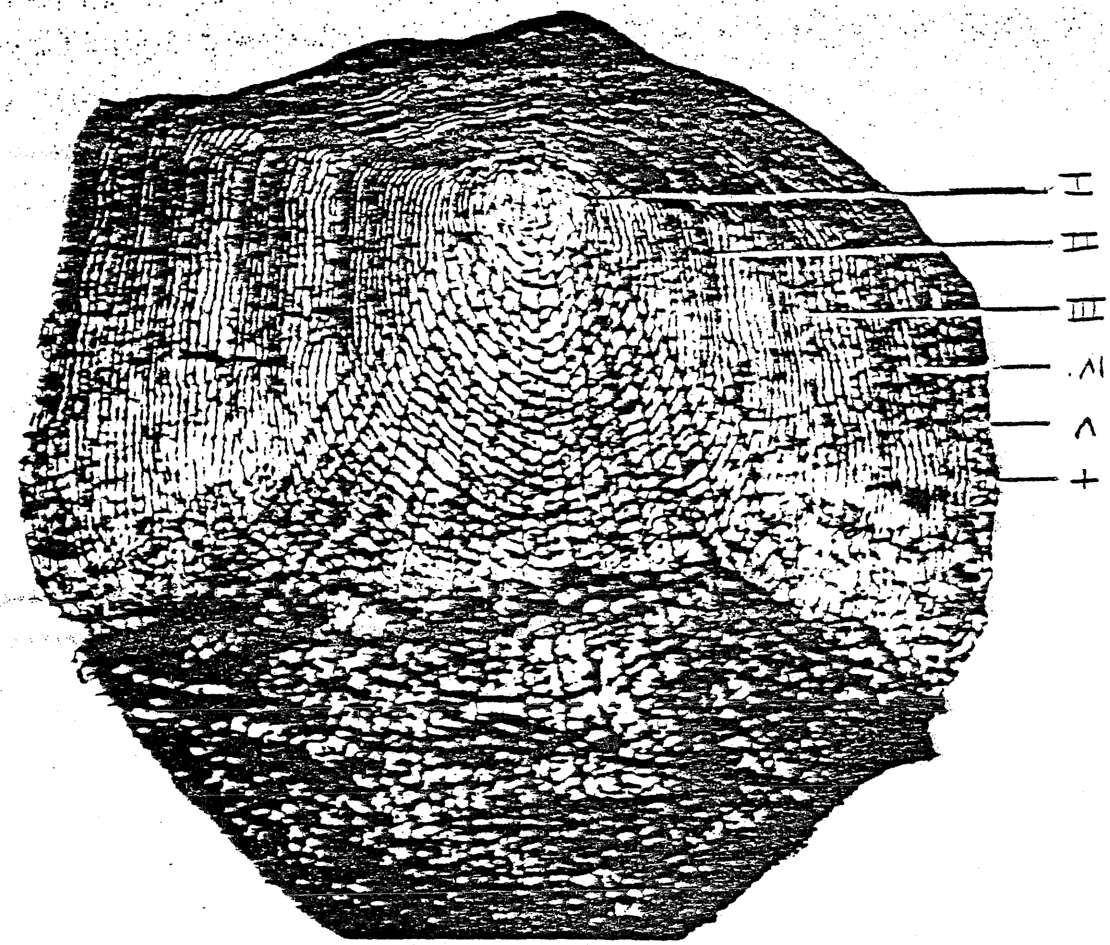



Figure 10. Ctenoid scale of a yellowtail flounder.

(5+)




UBSIDE DOWN
REVERSE

Figure 11. Scale of a fallfish.

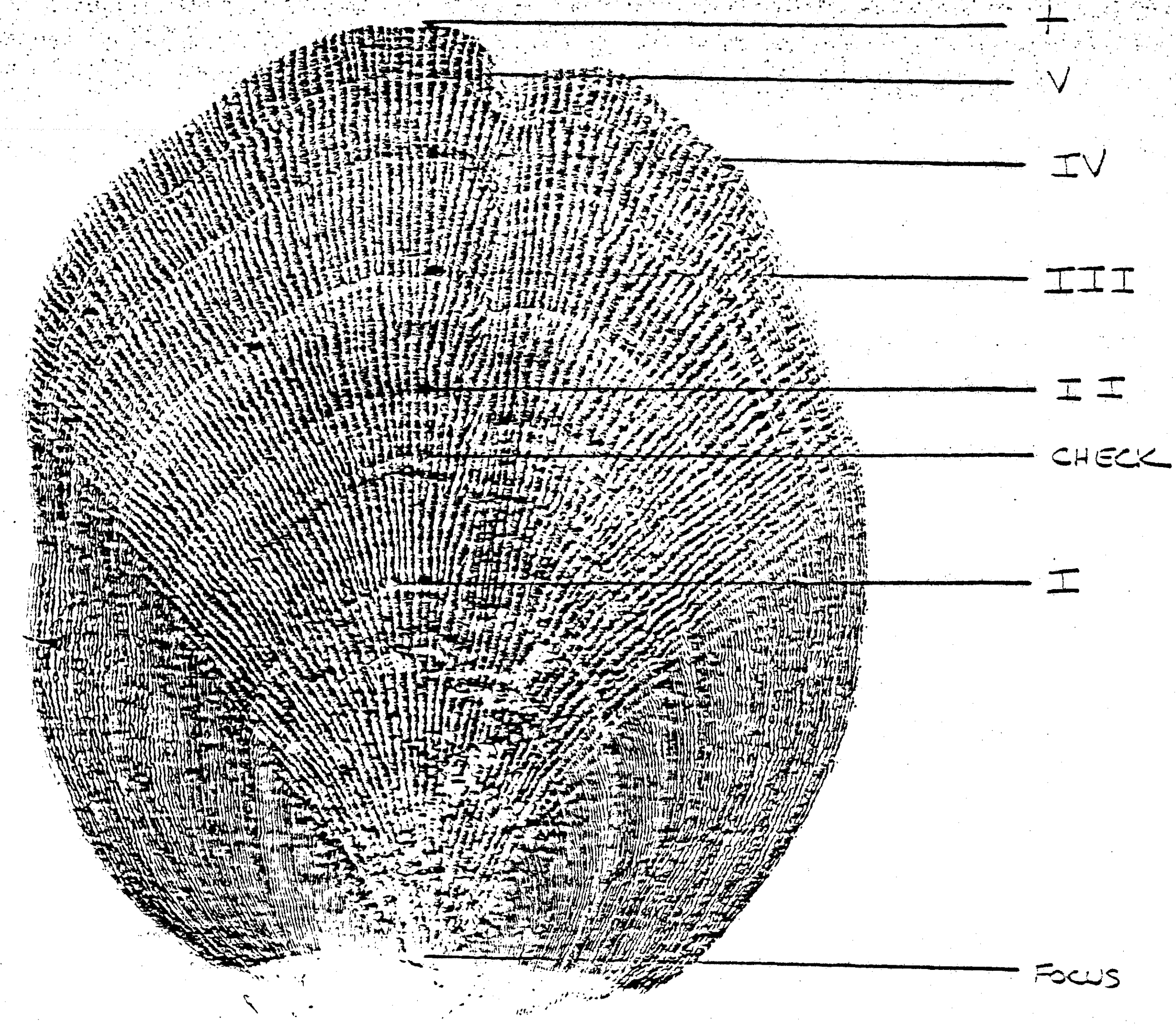



Figure 12. Scale of a summer flounder showing thin clear rings in the anterior field repressing annuli.

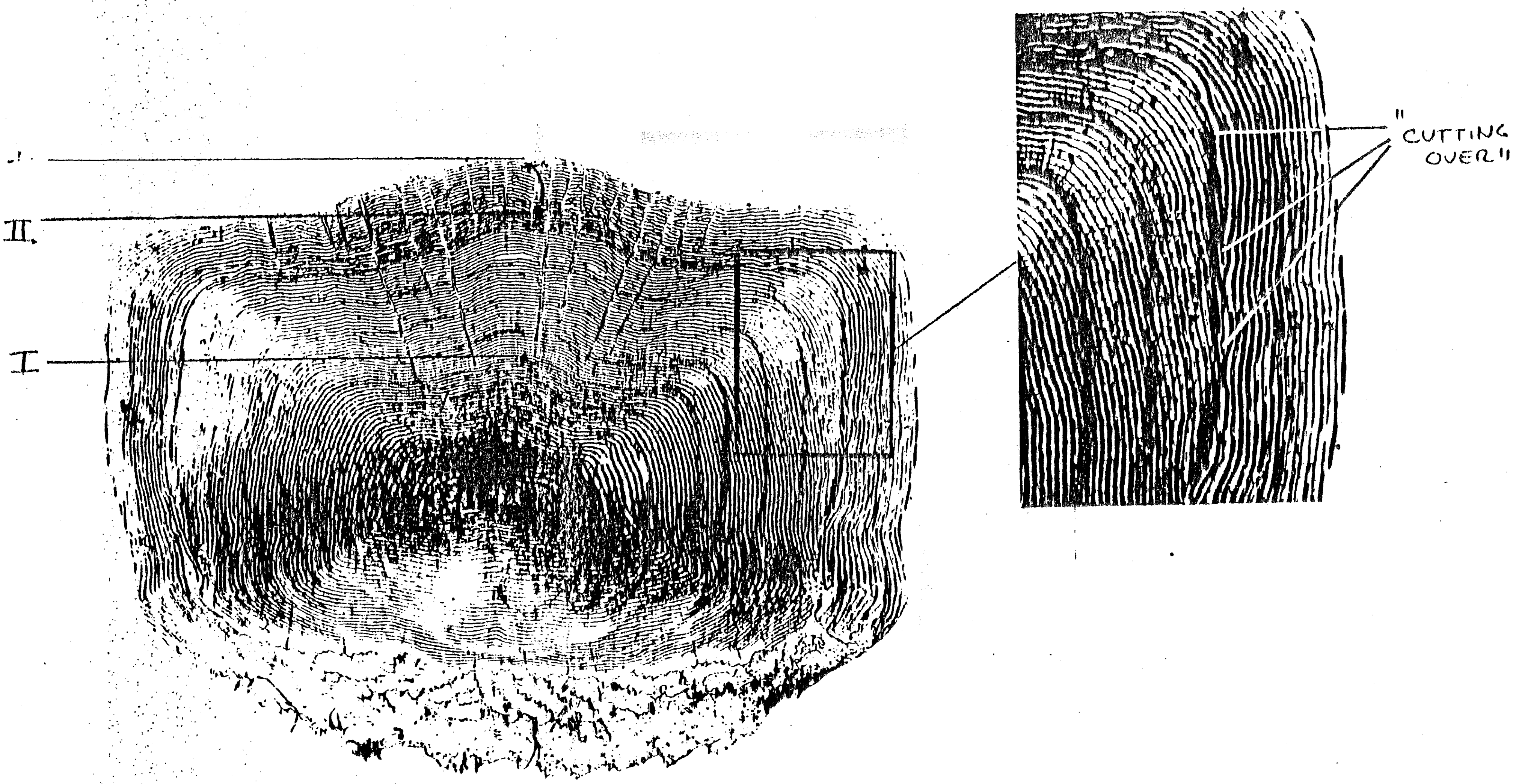
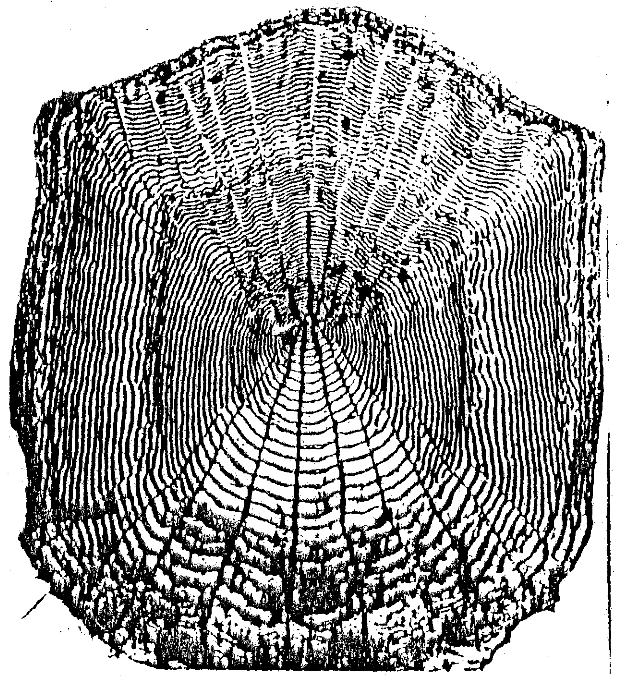



Figure 13. (left) Scale of a bluefish showing the "cutting over" of circuli (right above) on the outer edge of the second annulus.

Figure A. Scale of white sucker showing crowding of annuli near edge



B. Fin ray cross section of (same) white sucker showing 6 annuli.

Figure 14. (left) Scale of a white sucker showing extreme crowding of annuli near the edge, only three annuli evident. (right) Fin ray section from the same fish showing six annuli.

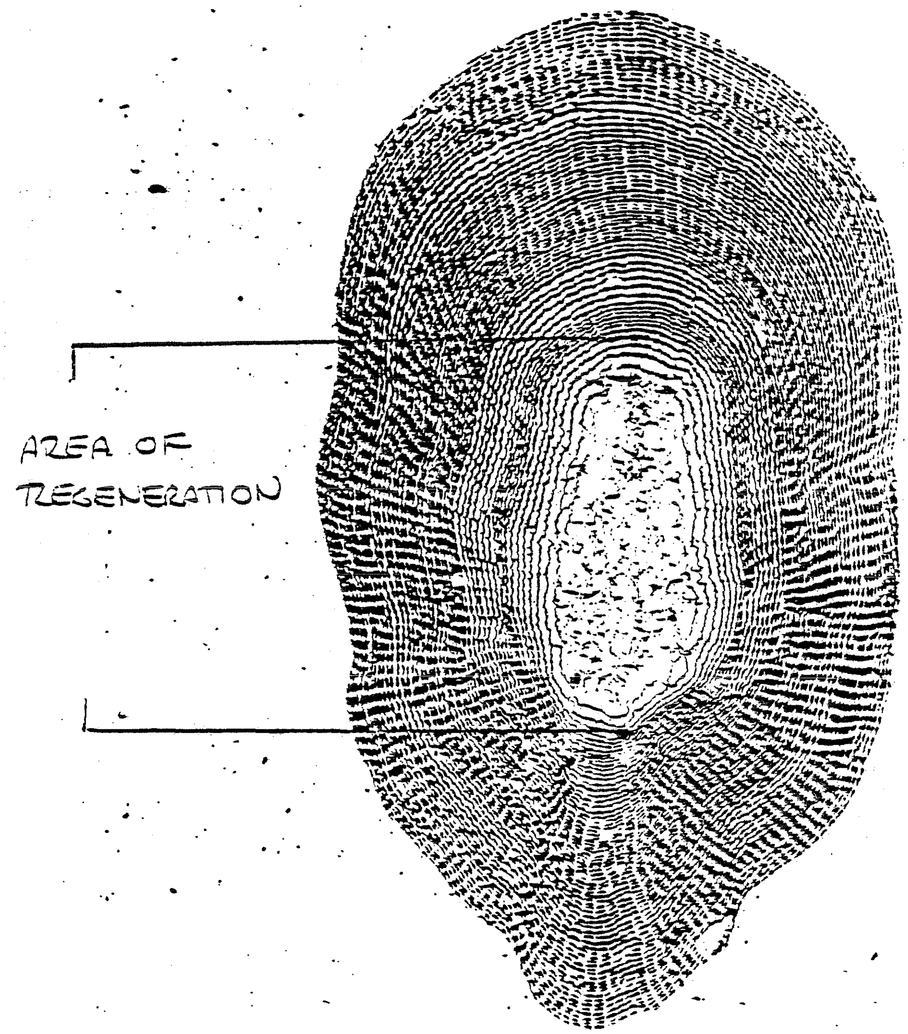



Figure 15. Regenerated scale of a haddock.

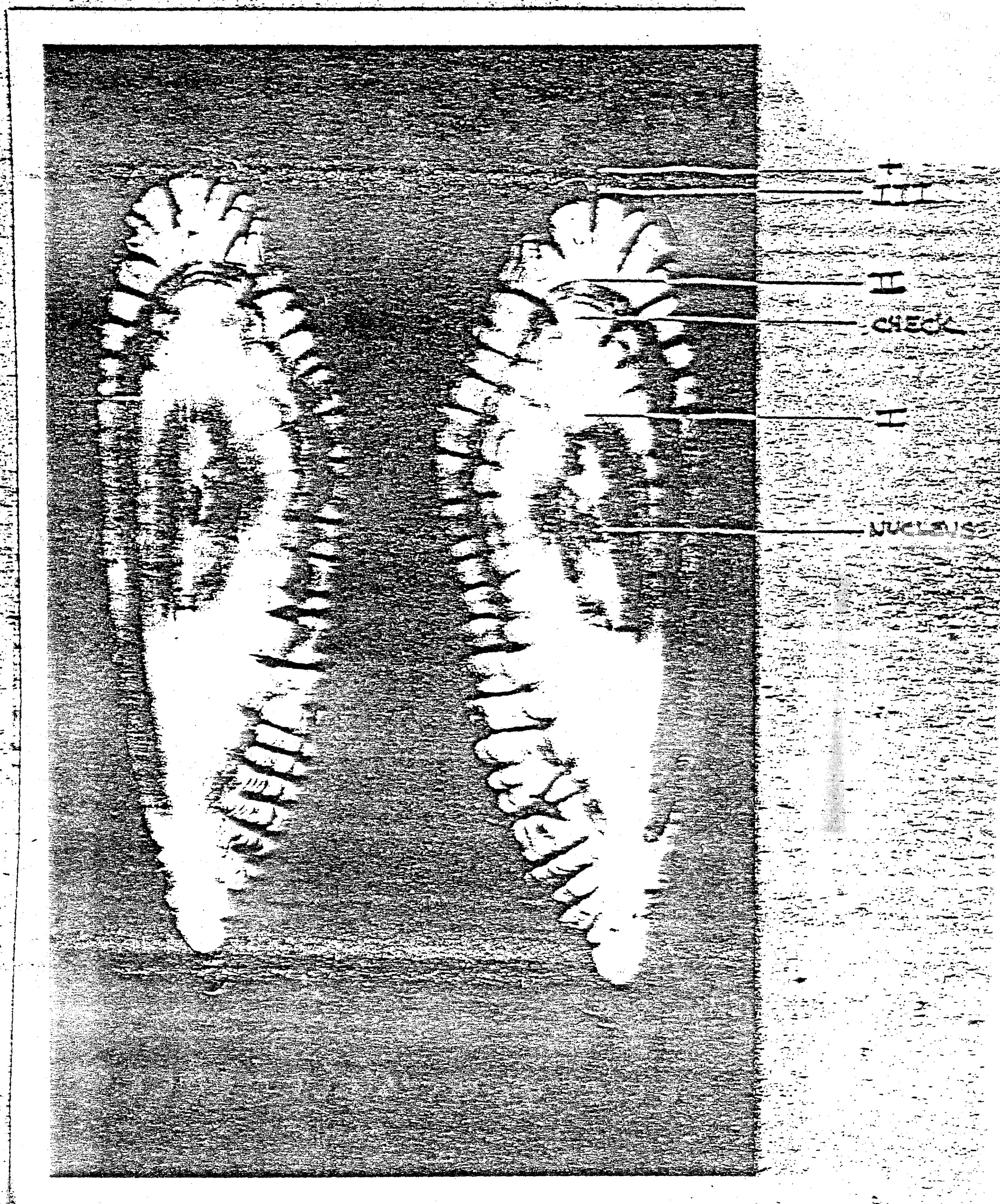



Figure 16. Whole otoliths cleared in glycerin of a silver hake.

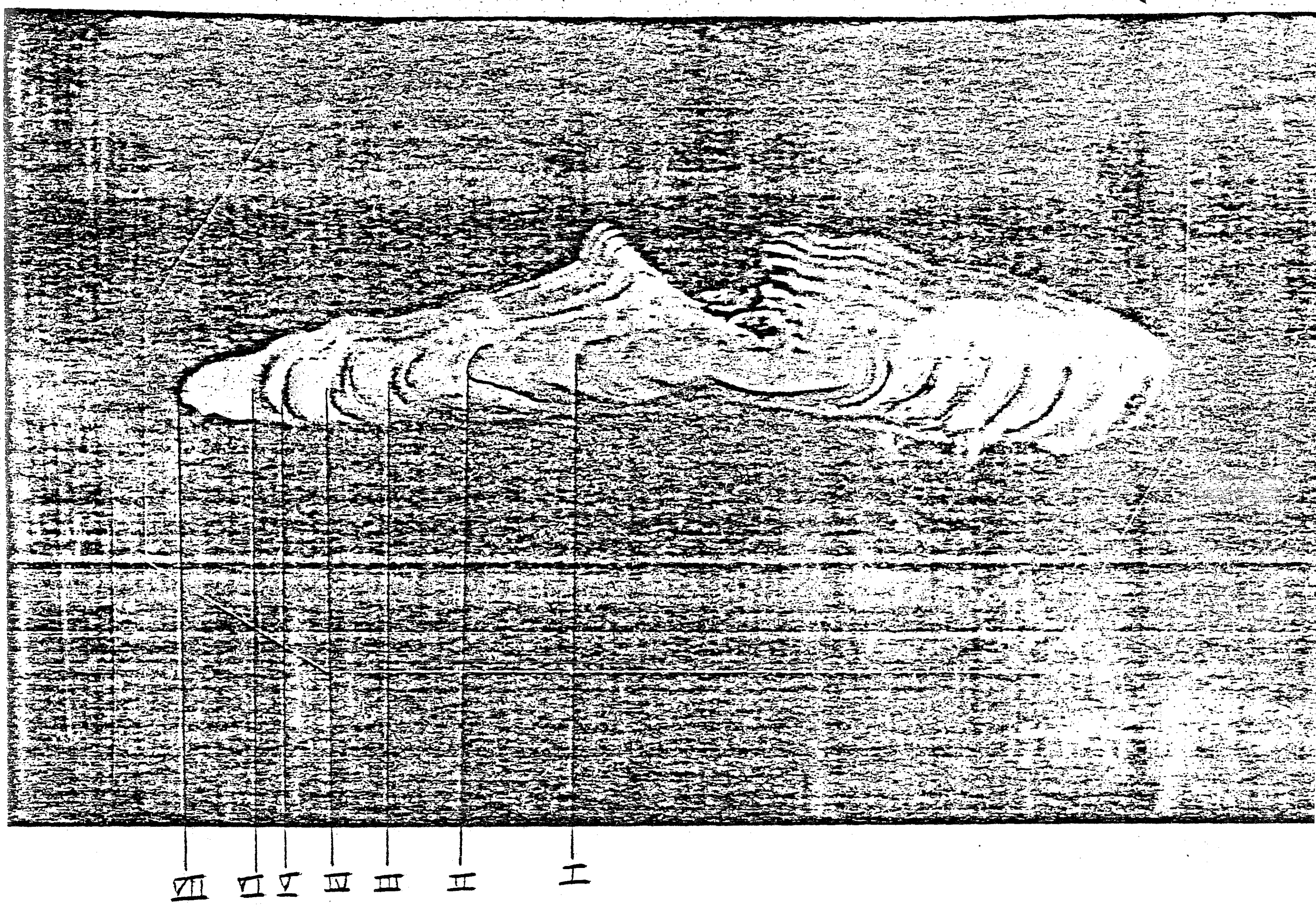



Figure 17. Thin transverse section from the otolith of a redfish.

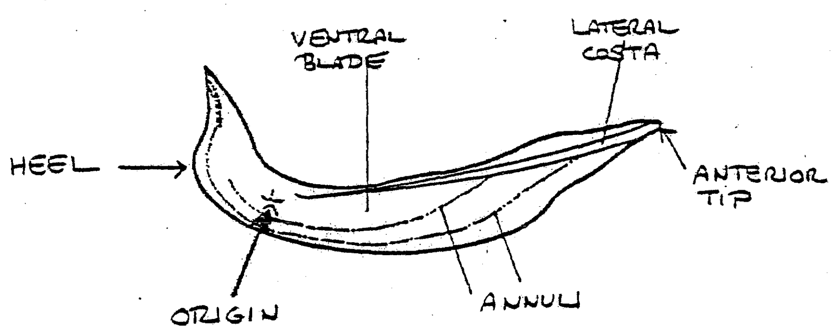



Figure 18. Diagram of a cleithrum.

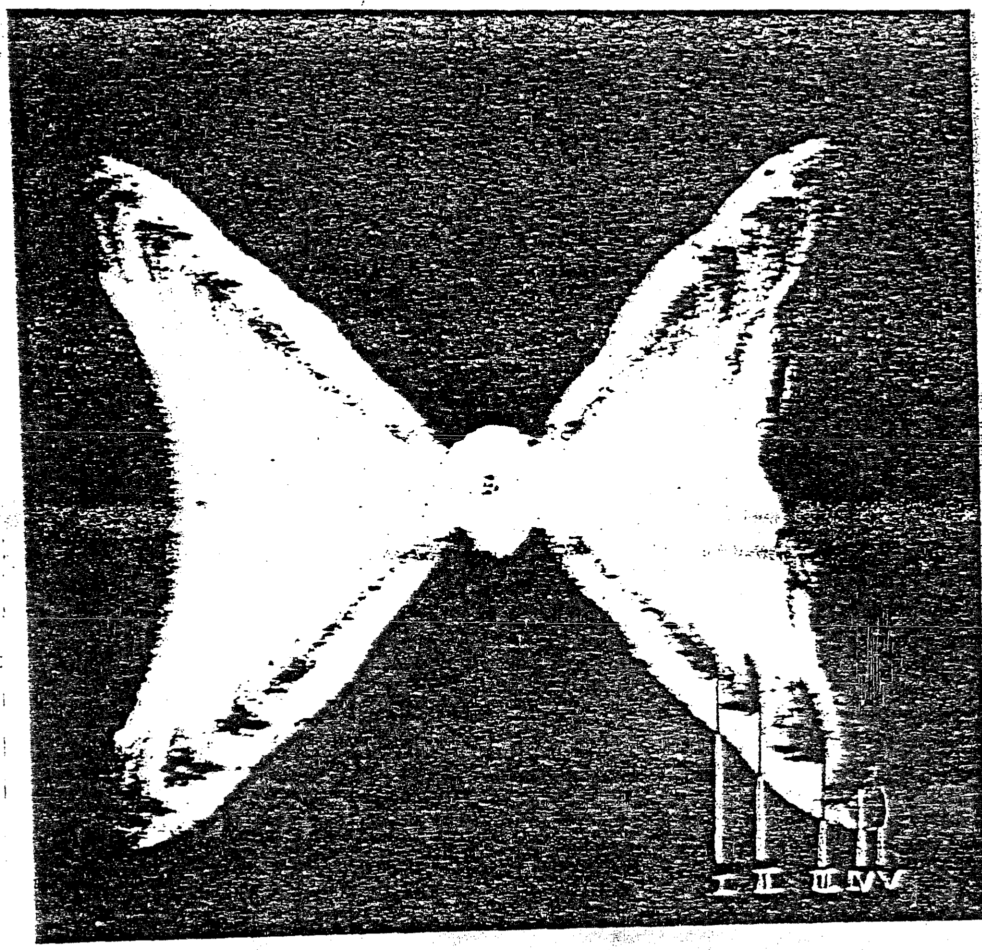


Figure 19. Thin section from the centrum of a little skate.

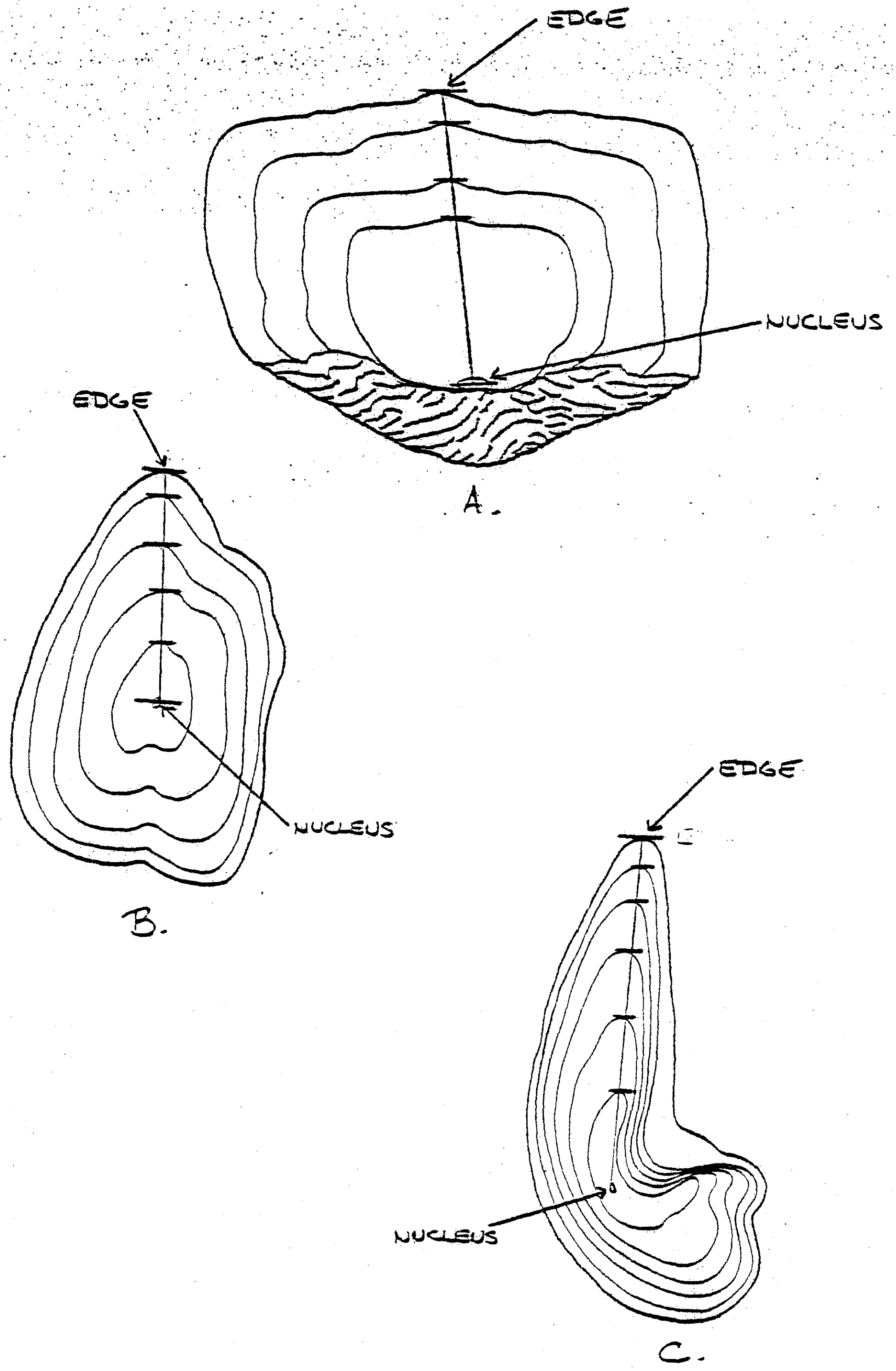



Figure 20. Diagram of a typical scale (A) whole otolith (B) and fin ray section (C) showing radius of measurements for backcalculation.

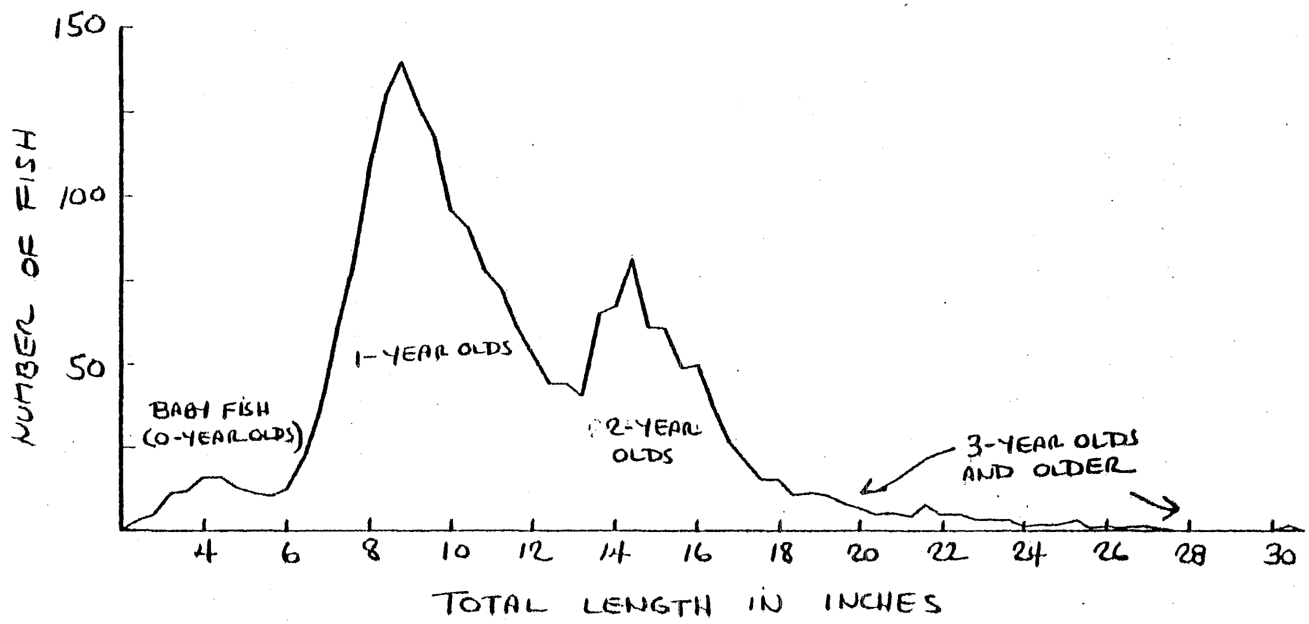



Figure 21. The length frequency distribution of a catch of haddock, showing the different size groups of fish caught (Lux 1971, by permission).

# APPENDIX

## Biostatistical Methods of Age Determination

An alternative method of determining the age structure of a population (as opposed to individuals) has emerged beginning with Petersen's (1891) observations that individual modes might represent age classes, through contemporary statistical analyses of polymodal distributions which can be of length or weight frequency data. In general, such methods assume that (1) length or weight measurements of individuals from the same age group (cohort) collected within a designated period will conform to a normal distribution, and (2) such distributions can be separated by graphical or analytical techniques. Such methods are the only ones available for situations in which individuals cannot be aged directly [such as crustaceans or the first year class (age 1) of juveniles, which is often difficult to determine using hard structures], and also provide a way of validating hard structure ageing techniques and eliminating some difficulty in interpretation. Lange (1980) in an attempt to validate the statolith ageing method utilized a length frequency modal analysis method to estimate age-at-length and establish growth schemes for *Loligo pealei* (LeSueur) and *Illex illecebrosus* (LeSueur). Her technique follows the assumption of normality; modal components of the length frequencies can be separated into adjacent parabolic shaped distributions representing age classes, or cohorts.

The advantages of statistical methods of age determination include circumvention of biological complications of age reading, e.g., the failure of annulus formation which complicates direct measurement of age and growth. They also do not require the slow and tedious preparation necessary in assessing the age of fish from hard structures. However, they are not generally applicable to separating older age groups of long-lived species where there is likely to be considerable overlap between modes. The usefulness of statistical ageing methods is limited (1) for species that have protracted and/or irregular spawnings, (2) for species in which individuals of a given size tend to form aggregates, and (3) in instances where the assumption of fish (cohorts) collected in a restricted period will be normally distributed (Hasselblad, 1966). Also, graphical and statistical methods of age separation are not free of subjectivity, and different techniques for grouping and analyzing the data can produce very different results.

A variety of graphical and statistical methods for age determination of aquatic organisms have been developed based on the above assumptions. Graphical methods for separating polymodal distributions include the probability paper method of Buchanan-Wollaston and Hodgson (1929) or those refined by Harding (1949) and Cassie (1950, 1954), along with estimation by trial and error until the theoretical cumulative distribution closely agrees with the observed distribution. Additional graphical methods for separating mixtures assumed to be normally distributed include the method of Tanaka (1962), which is based on fitting parabolas to the natural logarithms of the size frequencies, and the method of Bhattacharya (1967), which is based on fitting straight lines to the first significant differences of the natural logarithms of the size frequencies. The method of successive maxima (Gheno and LeGuen, 1968) is somewhat simpler because it only assumes that size-at-age is symmetrically distributed. Although such methods are generally straightforward and can be completed without resorting to computers, they often provide conflicting results. In temperate species (NAFO SCS Doc. 811XI/28) as well as with tropical species (Pauly, 1980) widely different interpretations have been obtained for a given set of data by different authors or even by the same authors using different methods.

With the coming of age of the computer, iterative analytical methods have been evolved. Separation by maximum likelihood procedures with the FORTRAN computer program NORMSEP (Normal distribution separator) designed by Hasselblad (1966) and later modified by Tomlinson (1971) has probably been the most common procedure employed in fisheries research in recent years. The method (program) provides calculated mean lengths at age and standard deviations for each mode of length frequency distributions. A weakness of NORMSEP is that the number of size groups and their points of overlap (Hasselblad, 1966; cut off points) must be entered into the program. ENORMSEP (Yong et al., 1975) is based on similar procedures but avoids the necessity for preliminary estimates of the number of size groups and their points of overlap, i.e., ENORMSEP determines such estimates and enters them into the NORMSEP program to complete the analysis of age group relative abundance.

MacDonald and Pitcher (1979) developed a more elaborate program for computing maximum likelihood estimates for the parameters of a mixture of normal distributions. However, like NORMSEP, the program requires the number of components of the distribution and initial estimates for all of their parameters. The advantages of this program are its availability in an interactive version and its ability to allow the user to constrain the parameters (such as forcing all of the component standard deviations to be equal). An extension of the MacDonald and Pitcher program is given by Schnute and Fournier (1980).

Yet another procedure has been devised by Pauly (1980). ELEFAN I (Electronic Length Frequency Analysis) "traces" an age group through sequential length-frequency sample data. The method (which is basically a modal class program analysis) assumes objectivity, i.e., the solution is based exclusively on the length frequency data themselves, and requires no subjective inputs such as the assumed number of age groups. The program is written in BASIC and can be run on most microcomputers.

## Uses of Age Data

### A. Age-length keys

Age determination by use of fish hard structures is valuable in stock studies where length samples of individual fish are more readily collected (within a defined time and space) than the fish structures necessary to age each sample. Hence, there is much greater logistic capability for measuring fish than for ageing them, and there would be relatively smaller variation of ages within lengths than to the overall length distributions.

Essentially, one must then extrapolate or prorate the aged subsample to the total length distribution sampled. This is accomplished by constructing an age-length key, which is assumed to be a representative sample of the stock under study. In a simple form, one constructs an age-length key (table) by indicating the numbers at age in columns that correspond to each length interval within the sample. Total numbers of aged fish for a given length may be indicated in an extreme right column and total numbers of fish at a given age class can be summed across the bottom. This simple table than becomes the foundation for the construction of a similar table indicating the percentage (or ratios) at each age class for a corresponding length. This latter table may then be utilized to convert to age-length frequency observations of fish sampled within the defined time and space.

"In using an age-length key, one must remember that the fish used for age determination must be taken from the sam stock, during the same season, and using gear haveing the same selective properties as that used to take the length-length frequency. Above all, an age-length key cannot be applied to length samples of any year except to one from which it was derived, unless the year-classes represented almost have the same initial abundance, growth and subjected to the same fishing experience--a condition seldom encountered" (Ricker, 1975).

For the most part, it should be kept in mind that age-length keys are subject to errors caused by inconsistent (fluctuations in) length-frequencies and incorrect ageing. What one may find where there is a series of such keys available for a given stock is that estimates based on them may, in fact, show that the most dominant ages provide reasonable estimates of size at age and frequency at age. While similar estimates for the older ages are more suspect because of the usually smaller sample sizes at this length-frequency coupled with greater ageing errors usually encountered with older fish. In general, one finds for young fish at the lower end of the age range that the keys indicate incomplete recruitment when the shape of their length-frequency is truncated to the left hand or smaller sizes. If this distribution in the catch sample is ignored there is a tendency to produce an upward bias in mean size at age (i.e., a large mean length at age) for the younger age groups. This bias in turn will then influence the size at $t_0$ when constructing growth parameters for the Von Bertalanffy growth curves.

## The Use of Scale Shape for Population Differentiation

The use of ageing structures to distinguish between fish stocks dates back to 1913 (Gilbert). The same principle still applies perhaps with the exception of recent sophisticated analytical methods. The basic premise of racial investigations is that fish spawning in separate locations are subjected to different influences. The differences, whether they are genetic or environmentally induces, are reflected in the growth patters of the scales and otoliths. By analyzing the patterns of age structures from fish of known stocks, the stocks of mixed races can be separated as to their origin.

Population delineation based on scale patterns have been commonly used for species of salmon. Measurements are made from a magnified scale image along a transect consistent for all scales. Several characteristics of scales can be quantified for analysis, among them are (1) distinguishable zones made up of a number of circuli representing the fish's life in freshwater, (2) number of circuli laid down while in marine waters, and (3) size of each growth zone. Also taken into consideration should be the age at smolt (the last year in freshwater) and any dominant age group which may exist in the parent stock. Recent work has also involved stock delineation by measurement of the total area of specific scales and growth bands within the scale.

Similar growth characteristics may be determined from otoliths. Several criteria may be measured such as the diameter of the nucleus, the distance from nucleus to each growth band, total length and width of the otolith for each age group, and measurements of any otolith feature peculiar to a given species (such as the rostrum found on clupeid otoliths). Any pattern which may be unique to certain habitats should also be included, such as transition zones between fresh and saltwater, or spawning and metamorphic checks. The differences in fish size/otolith or scale size equations between areas may also be useful in separating stocks (Rojo, 1980).

The separation of the stocks based on these characteristics usually involves discriminate function analyses, a statistical method for determining which characteristics are most useful in differentiating between groups. The best discrimination between groups may be explained by only a few of the measured characteristics. But measurements and testing of many characteristics increases the likelihood of finding the most useful one(s). Once useful criteria are established, characteristics of the scale or otolith can be measured form the mixed stocks and the percentages of the stock from different origins can be deduced.

The use of discriminate function analyses does not yield conclusive results. The researcher should also consider the non-quantitative age characteristics to determine the racial origins, such as age structure of the population, any known migration or spawning differences between the stocks, etc. Other considerations when sampling the age structures under investigation would be determining any clinal trend in the characteristics which may not be the result of separate populations, and the inclusion of hatchery reared fish among the wild stocks. The favorable conditions within the hatchery can affect the otolith or scale patterns creating unique growth patterns; these may be misleading in determinations of the racial origin of hatchery reared fish captured in the wild (McKern, Horton and Koski, 1974). Analysis of age structures for stock discrimination should be carried out with other methods such as tagging or electrophoretic methods whenever possible.