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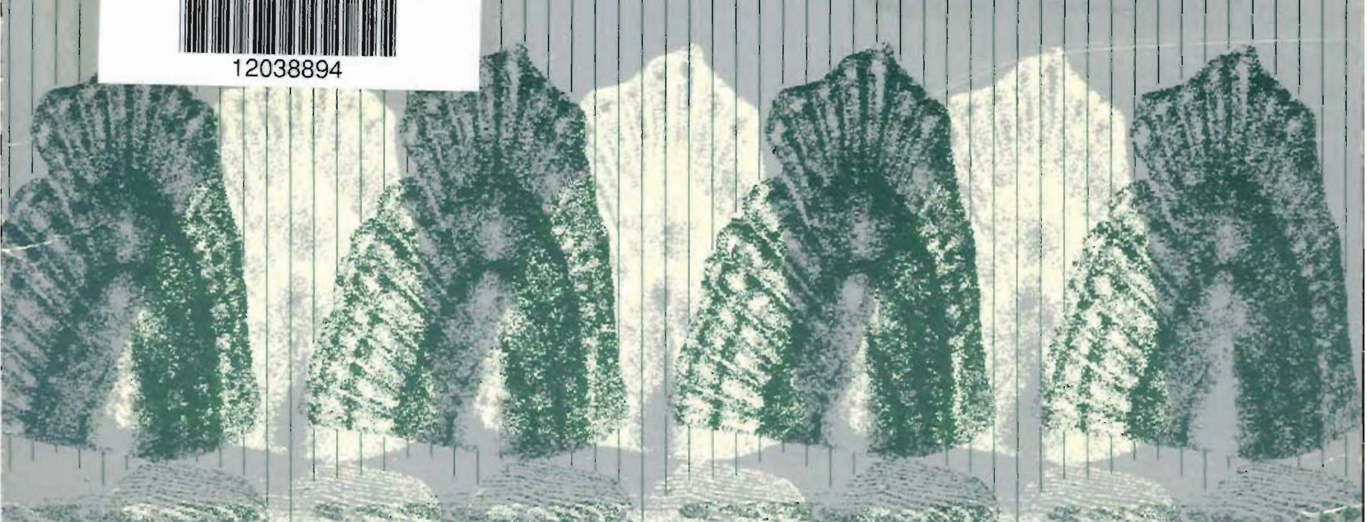

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Age Determination Methods for Fishes Studied by the Groundfish Program at the Pacific Biological Station

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Contents

Abstract

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This manual describes methods and equipment used to estimate the ages of groundfish species studied by the Pacific Biological Station. Procedures for routine identification of annuli on scales, otoliths, otolith sections, fin-ray sections, and spines are described and illustrated. Although the techniques are applied to species found off Canada's west coast, they should be applicable to other marine and freshwater fishes from temperate and arctic waters. Because some of the procedures are experimental and many have not been validated, it is expected that this manual will be revised several times.

Key words: aging techniques, otoliths, scales, fin rays, annuli, spines, checks

Résumé

CHILTON D. E., AND R. J. BEAMISH. 1982. Age determination methods for fishes studied by the Groundfish Program at the Pacific Biological Station. Can. Spec. Publ. Fish. Aquat. Sci. 60: 102 p.

Le présent manuel contient une description des méthodes et des appareils utilisés dans la détermination de l'âge des espèces de poissons de fond étudiées à la station de biologie du Pacifique. On y décrit et figure les méthodes d'identification routinière des annuli sur les écailles, otolithes, sections d'otolithes, de rayons des nageoires et d'épines. Ces méthodes, bien qu'appliquées à des espèces du large de la côte ouest canadienne, pourraient également l'être à d'autres poissons marins et dulçaquicoles habitant des environnements identiques. Ce manuel devra probablement être révisé plusieurs fois car certaines methodes sont expérimentales et plusieurs n'ont pas encore été validées.

Introduction

The ability to age fish accurately is essential to the total understanding of the dynamics of fish populations. Recognition of annual patterns requires the understanding of how these patterns relate to the annual growth cycles in fishes. The manual attempts to describe methods used to read and interpret growth patterns found on a variety of aging structures.

The manual will be available to any individual or laboratory interested in the aging of fish. Anyone using the manual is encouraged to send comments to either of the authors. Some of the procedures are experimental and many have not been validated for all age-classes in a particular stock. Thus, it is anticipated that methods will be revised and descriptions of the procedures will be updated prior to the final publication of this manual. The reader is encouraged to read the key references that are listed for each species as all the documentations upon which our methods have been developed have not been repeated.

The terms used throughout the text are defined in the Glossary.

General Procedures

(Beamish 1973, 1979a, b, 1981; Beamish et al. 1976; Beamish and Chilton 1977, 1982; Beamish and Harvey 1969; Bilton and Jenkinson 1969; Blacker 1974; Chilton 1970; Christensen 1964; Chugunova 1959; Power 1978; Williams and Bedford 1974)

Fish from each stock or population should be treated as if they were being aged for the first time. In this laboratory, several different structures are examined and if ages determined from these structures are similar, the structure that is most easily aged or more readily accessible is used for routine work. Structures currently being used are scales, otolith surfaces, otolith sections, broken and burnt otoliths, and fin-ray sections. When rapidly growing fish are being examined, it may be possible to use length frequencies to age the younger fish. Frequently, several methods are needed to age all fish in any particular stock and it is advisable to become proficient in the preparation, understanding, and interpretation of all methods.

When commencing to age a new species or a new stock it is important to examine structures from juvenile fish to determine the location of the first annulus. The position of the first annulus is often the most difficult interpretation problem and its appearance can vary among stocks, possibly as a consequence of exploitation.

Validation

One of the most serious mistakes made by fisheries biologists is the failure to validate age determination procedures (Beamish and McFarlane unpublished results). Without exception, age determination techniques must be validated for all age-classes in the population each time they are applied to a new species. In some cases the technique should be validated each time it is applied to a different population or stock. If a technique has been validated for one species there is no reason to believe it can be applied to other species, other populations, or other stocks. If a technique is valid for younger fish, it is incorrect to consider that it is applicable for older fish.

Traditionally, age determination methods have been validated by mark–recapture studies, identification of strong year-classes, length frequency analysis, and examination of the edge of a particular structure throughout the year to show that only one annulus is formed. As part of our validation procedure, fish tagged at sea are injected with oxytetracycline (OTC) before they are released. The dosage and most appropriate method of applying the OTC is presently being investigated. Upon recovery, otoliths and other structures are removed and stored in the same manner as noninjected fish structures except that they are kept in the dark. At present we have no special techniques for examining the fluorescent yellow mark that appears on the structure other than the conventional UV lamp and a research microscope. The marked structure should not be exposed to the UV light, or other light, for any length of time as the OTC mark will lose its fluorescence. (It is recommended that eye protectors be worn while examining structures under the UV light).

If there are a number of age-groups present in the population beyond the age of maturity, it is probable that only the mark–recapture method can validate the annuli for all age-classes in a population because the growth pattern of the structure being used often will change drastically soon after the fish reaches maturity. In the absence of a tag and recapture study, we recommend that a variety of structures be examined and if the resulting differences in age determination are substantial, then ages should be considered as rough approximations until a method of validation can be developed. Confirmation of older ages may be possible by examining the content of natural radionuclide concentrations in aging structures. Failure to assess the accuracy of age determination will usually lead to a wasting of resources and can result in the mismanagement of a fishery.

Structures

(Beamish 1979a, b, 1981; Beamish and Chilton 1977, 1982; Bilton and Jenkinson 1969; Blacker 1974; Boiko 1951; Deedler and Willemse 1973; Gulland 1958; Kennedy 1970; Mills and Beamish 1980)

Scales, otoliths, and fin rays used in this laboratory are collected from commercially captured fish or from fish taken on scientific cruises. In the majority of cases, the structures must be prepared by standard or specialized methods before a reader is able to determine an age from a structure.

When selecting a structure it is important to try to determine how it forms in relation to fish growth as growth zones may not form in the same manner throughout the life of the fish.

SCALES

Ctenoid and cycloid are the two types of scales most commonly used for age determinations. The cycloid scales, which are produced by fish with soft-rayed fins, are usually circular and the center or focus is surrounded by an equal amount of growth whereas ctenoid scales, which grow on spiny-rayed fish, have unequal growth around the center and produce ctenii, small spines, on the posterior of the scale. Some fish, like the flatfishes, have both types of scales.

Scales form a protective outer covering for fish and increase in size as the fish grows. The major increases in scale size occur prior to maturity. After maturity, the growth of most fish, and especially males, is reduced. This reduced body growth rate can affect scale growth and change the pattern of circuli formation. This means that the appearance of the annulus for younger fish may not be the same for older fish. If fish growth is very slow or nonexistent, then scale growth may be minimal or nonexistent. Annuli can become very crowded on the edge of the scale and often cannot be identified. In addition, during the later years, calcium may be resorbed from the scales resulting in some reworking or breaking of the circuli. In some species, scales may become thin (transparent) and the annuli are difficult to observe. Regenerated scales (Fig. 1A) are a problem as fish age and it is sometimes difficult to find a whole scale from a preferred area (Fig. 1B).

Scales should always be used with the clear understanding that beyond the age of maturity the age of fish may be underestimated. Failure to consider the consequences of error may result in an incorrect assumption that growth remains relatively rapid and the fish are not long-lived. Hence, ages determined from scales should be validated for all age-groups in the population or a second method of age determination should be used for the larger (and older) fish in the population. In some species, scales are the best structure used for aging young fish.

Scale samples are usually taken from above the lateral line and below the second dorsal fin and stored in small coin envelopes. They can be cleaned and lightly stained in an aqueous solution of gentian violet and then mounted on microscope slides in De Faures solution (Chilton 1970). A temporary mounting of dry scales between two microscope slides is satisfactory if the scales are clean. Acetate impressions also provide a convenient and permanent record of scales, although this laboratory does not use this method on a routine basis.

A scale annulus can be described or identified as a crowding or narrowing of the circuli (Fig. 1B) that can usually, but not always, be followed around the anterior and lateral portion of the scale. Sometimes there is a "crossing or cutting" over or a series of broken circuli within the crowded or annual zone in the vicinity of the posterior quadrant. Immediately following, there often is a zone of widely spaced circuli, indicating the resumption of new growth.

OTOLITHS

There are three pairs of otoliths in the inner ear of a fish. The largest pair, the sagittae, are routinely used for age determination and the general term otolith is used synonymously for the sagitta. It is essential that one realize that otolith formation is not isometric throughout the life of the fish. As the fish ages, more material is deposited on the internal surface than on the external surface and it is possible that no material is deposited on the external surface in any particular year. Thus, it is important that the number of annuli is estimated from transverse sections through the nucleus or breaking the otolith

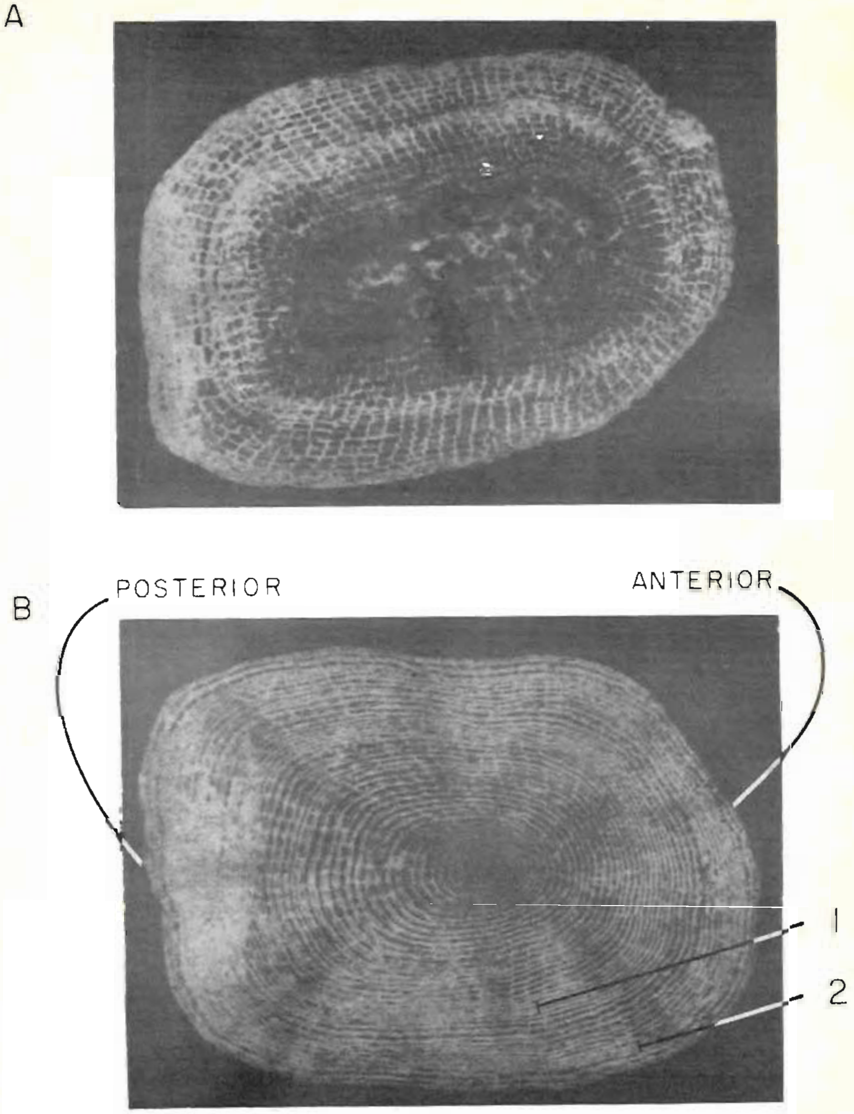


FIG. 1. (A) Regenerated or replaced Pacific cod scale. (B) Annuli on a Pacific cod scale.

through the nucleus, in addition to the normal method of viewing the external surface.

Otoliths probably contain the best permanent pattern of fish growth as bone resorption is not known to occur from the otoliths and some deposition appears to occur each year. Other aging structures may be required for routine work if the fish must be kept alive.

Both of the saggital otoliths are taken so that the surface, sections, and burnt halves of the otoliths can be examined. After both otoliths have been removed from the head of the fish they can be stored in several ways. The preferred method in this laboratory is to use plastric trays containing separate compartments for each pair (Fig. 2A, 2B). These trays come in several sizes but the advantage of heavier and larger trays is that they provide a litte more stability when sampling at sea. After being filled with a storage medium, the trays can be sealed off with a pressure-sensitive film or parafilm before replacing the plastic "snap-down" lid. Individual glass or plastic vials filled with liquid medium can be used to store otoliths. These are quite satisfactory, but they are expensive and often difficult to handle and store.

There are various types of storage mediums. Glycerin and water (about a 50/50 mixture) plus a little thymol is most commonly used in this laboratory. At present we are testing a number of storage mediums. Otoliths from several species are being held in ethanol, glycerin, water and thymol, seawater and thymol, fresh water and thymol, and with no medium. It is anticipated that this experiment will be monitored for about 5 yr to determine if there is any change of clarity in the growth rings on the otoliths among the various mediums. Results of a preliminary experiment showed that growth zones on

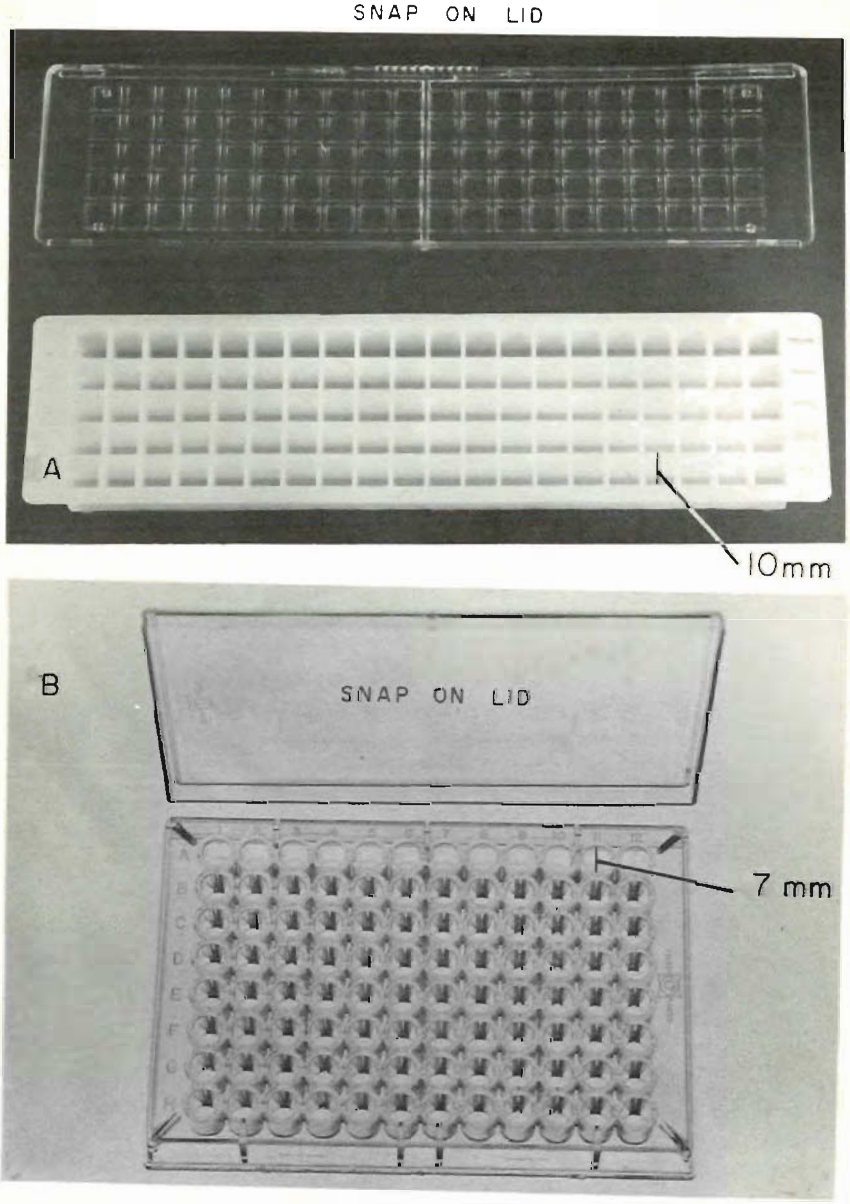


FIG. 2. Plastic storage trays. (A) Trays used for large otoliths, such as rockfish and hake. (B) Trays used for small otoliths, such as sablefish.

Pacific hake otoliths stored in seawater for 5 yr showed no loss of clarity. It is important that when using water, sufficient thymol be used to reduce the growth of natural bacteria, fungae, and algae. Care must be taken to remove all of the membranes and surrounding tissue from the otoliths.

Ages determined from the external surface on the whole otolith appear satisfactory for young fast-growing fish of most species. An otolith surface can be aged by immersing the otolith in water on a black background and examining it with a dissecting microscope using reflected light. Some otoliths have a cloudy or chalky surface inhibiting the identification of the growth zones. These zones may be made slightly more distinct by rapidly dipping the otolith in a weak solution of HCl (usually 20%) before placing it in the water.

Otoliths of older, slow-growing fish become thick and the yearly growth zones do not form equally on all surfaces (Irie 1960). To detect annuli on otoliths from older fish, cross sections must be examined. The otolith can be broken in cross section through the nucleus and one of the broken surfaces carefully burned in an alcohol flame (Chugunova 1959; Christensen 1964). The greater concentration of organic material in the translucent zones produces distinct dark bands that contrast with the light bands of the opaque zones when viewed with reflected light. The burnt otolith is held in Plasticine or a similar substance (Fig. 3) and painted with a little cedar wood oil (or cooking oil which is nontoxic) to enhance the contrast between zones. The burnt half of the otolith should be stored dry until the aging has been completed as storage mediums will blur the burn. The burns also tend to fade with time and must be reburnt or the other half of the otolith should be burnt if there is much delay between readings. The internal edge of the broken otolith occasionally burns too dark and it is then difficult to observe new growth when examining small growth zones. The edge of the sulcus acusticus will be referred to in the text as the sulcus edge.

Thin sections can be cut from the center of the otolith by using the following procedure. All bony structures such as otoliths being prepared for sectioning are first dipped in toluene, to ensure the epoxy will adhere to the structure. The otolith is embedded in epoxy on a parafilm-covered board that has been marked off into squares and suitably labeled or identified (Fig. 4A). Enough epoxy should be applied to form a grip to fit in the machine clamp (Fig. 4B). The epoxy, which comes in an Epoxi-patch kit, can be purchased in bulk quantities from the Hysol Division of the Dexter Corporation and is recommended because it dries to a clear hard substance which can be easily handled.

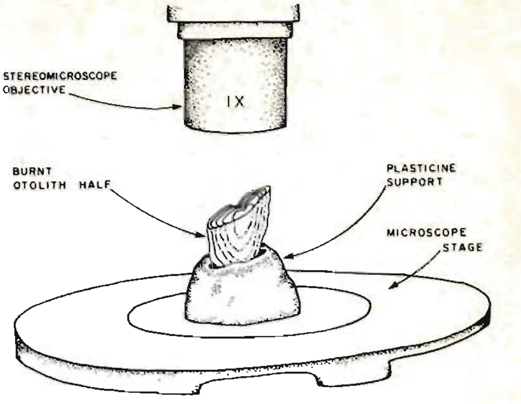


Fig. 3. Drawing of a broken and burnt otolith positioned in Plasticine under a microscope.

Most sections are ~ 0.5 mm thick; however, this may vary depending on the appearance of the growth zones within the structures. A trial run is recommended when starting a new sample and at least three sections of different widths should be examined. A periodic check of the thickness and the angle of the cut should be made. When the correct thickness has been determined, several sections from one structure are placed on a microscope slide and covered with a fast-drying liquid cover slip such as Flo-texx. The growth zones on sections appear a little more distinct if a coating of oil is applied and allowed to "soak in" for a few hours before adding the liquid cover slip. The clarity and contrast of the growth zones, especially in fin rays, improves if the Flo-texxed section is allowed to dry for about 24 h.

Thin sections should be made and examined under a high-powered compound microscope when sufficient magnification is not possible with a stereomicroscope to magnify the small growth zones on the broken and burnt otolith section. If this method also fails to distinguish the annuli then the otolith is declared unreadable.

Rapidly growing otoliths sometimes produce wide growth zones which often contain checks. However, by examining an area of slower growth it is usually possible to eliminate the problem of having to identify checks (Fig. 5). The zone of faster growth is often useful to determine if new growth is present on the otolith's edge and to resolve crowded annuli along the edge of an older otolith.

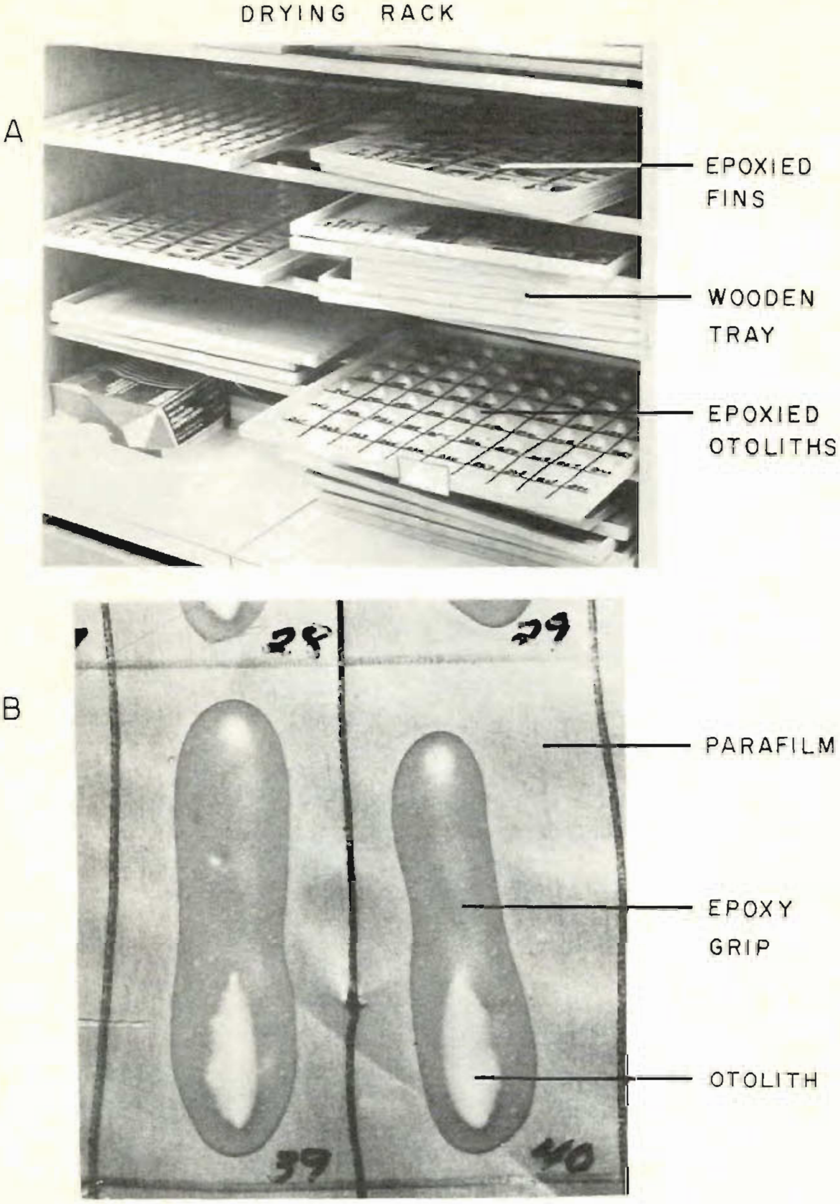


FIG. 4. (A) Epoxied fin rays and otoliths drying on parafilm-covered boards. (B) Enlargement of embedded otoliths.

FIN-RAY SECTIONS

Fin-ray sections should always be considered as an alternative technique to the more conventional methods of using scales and otoliths. In theory, ray or spine sections from any fin could be utilized but in practice the dorsal, pectoral, and pelvic fins are most commonly used. Each fin ray has two elements and usually the larger element is examined. Each time a new species or in some cases a new population or stock is studied, sections from all spines and rays should be considered. Previous experience has shown that the most suitable rays or spines are the ones that have an intermediate rate of growth. There is a preferred location on the fin ray for aging that can only be determined by experimentation. The thickness of the section will vary among species in order that optimal contrast between opaque and trans-

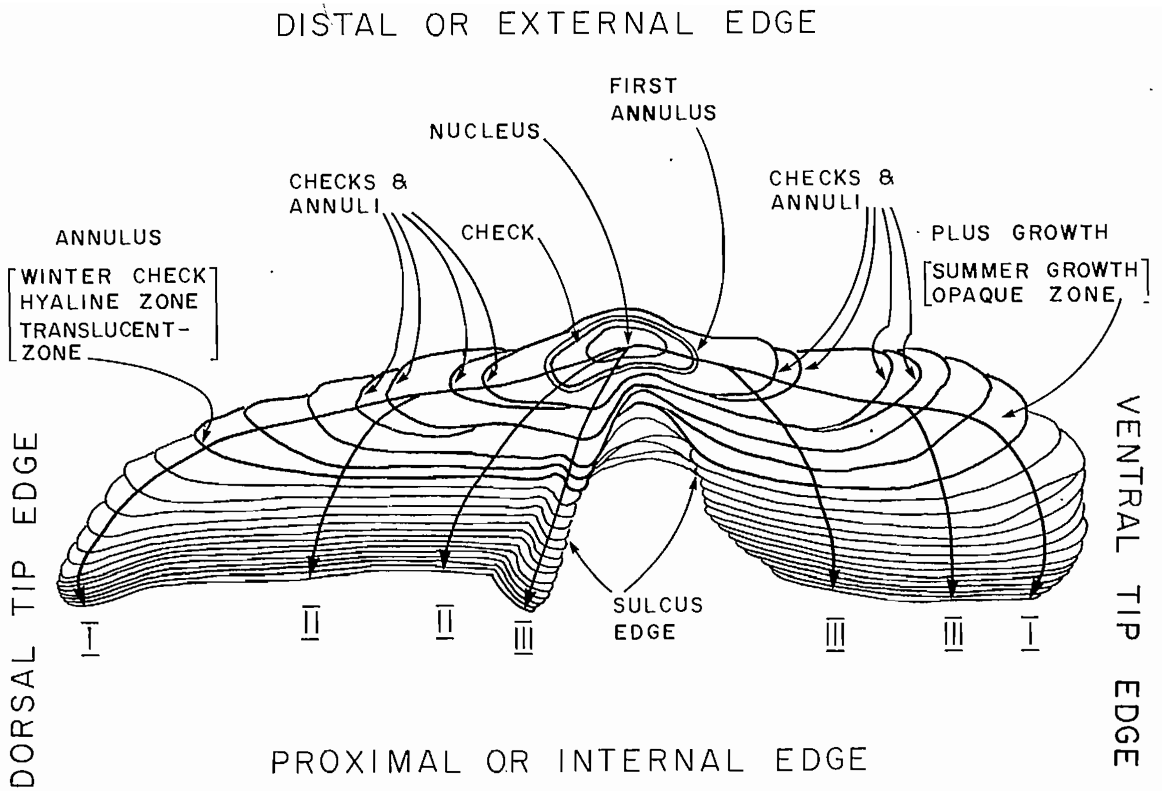


FIG. 5. Drawing of an otolith cross section showing areas used for counting and pertinent aging characteristics. Arrows pointing to I indicate the fast-growing area and those pointing to II and III are slower growing areas. II is the preferred counting area for most rockfish. However, the clearest pattern may not always be on the preferred area and therefore other areas should be examined. More than one area should be counted at all times.

lucent zones is obtained. Sections must be made at right angles to the length of the ray. If a growth pattern is visible but the interpretation is difficult, improving the quality of the section will often clarify the pattern. Although it is only necessary to examine one fin ray, time is often saved by sectioning four to six rays at once and making three sections of the combined four to six rays.

The advantages of this technique are that it is often possible to age older fish more accurately and the fish does not have to be sacrificed. The disadvantages are that the interpretation of the first annulus can be difficult and fins can be more difficult to handle, store, and prepare than scales or otoliths. The correct application and interpretation does require some experience.

Fins should be placed in 14.5-kg kraft ungummed envelopes to be frozen or air-dried immediately (2–5 d). Air-drying appears to be better than forced drying. A thinner envelope tends to dry and stick to the fin. Particular attention must be taken to place the cut edge of the fin at the open end of the envelope while ensuring that the cut surface is perpendicular to the longitudinal axis of the fin rays. This ensures that the rays are flat and dried so that the cut surface is at right angles to the fin rays. They should never be stored in a crumpled or twisted condition as sections perpendicular to all rays couldn't be taken from the same area without separating and remounting all rays.

Fin rays are prepared in a similar manner as discussed under the otolith section using a parafilm-covered board and embedding the structure in epoxy. The epoxy is usually applied smoothly up to about 3.5 cm from the basal area of a fin (Fig. 6) to provide rigid support when clamped in the sectioning

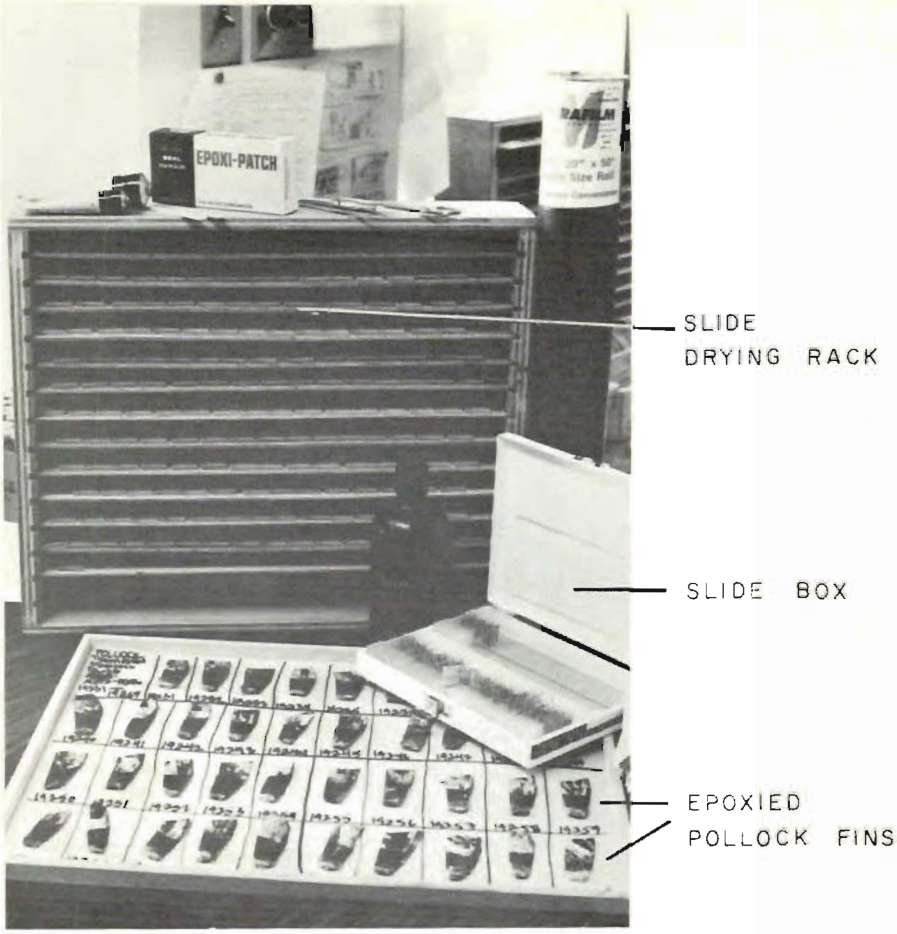


FIG 6. Epoxied fin rays drying on a parafilm-covered board. Supplies used for the preparation and storage of fin-ray sections are also shown.

machine (Fig. 7, 8). For easier handling small fins less than 2.5 cm long are completely embedded in the epoxy and if the fin is very wide it should be cut between the rays to sizes of about 2.5 cm in width. Do not try to section while the epoxy is soft, as structures vibrate during sectioning, causing the rays to shatter. The sections are placed on glass slides and covered with a liquid mounting medium such as Flo-texx.

OTHER STRUCTURES

At present, structures such as cleithra, opercular, or interopercular bones, etc., are not used in this laboratory. We have not found it necessary to use structures other than scales, otoliths, and fin rays and consider that these structures are the simplest to sample and process.

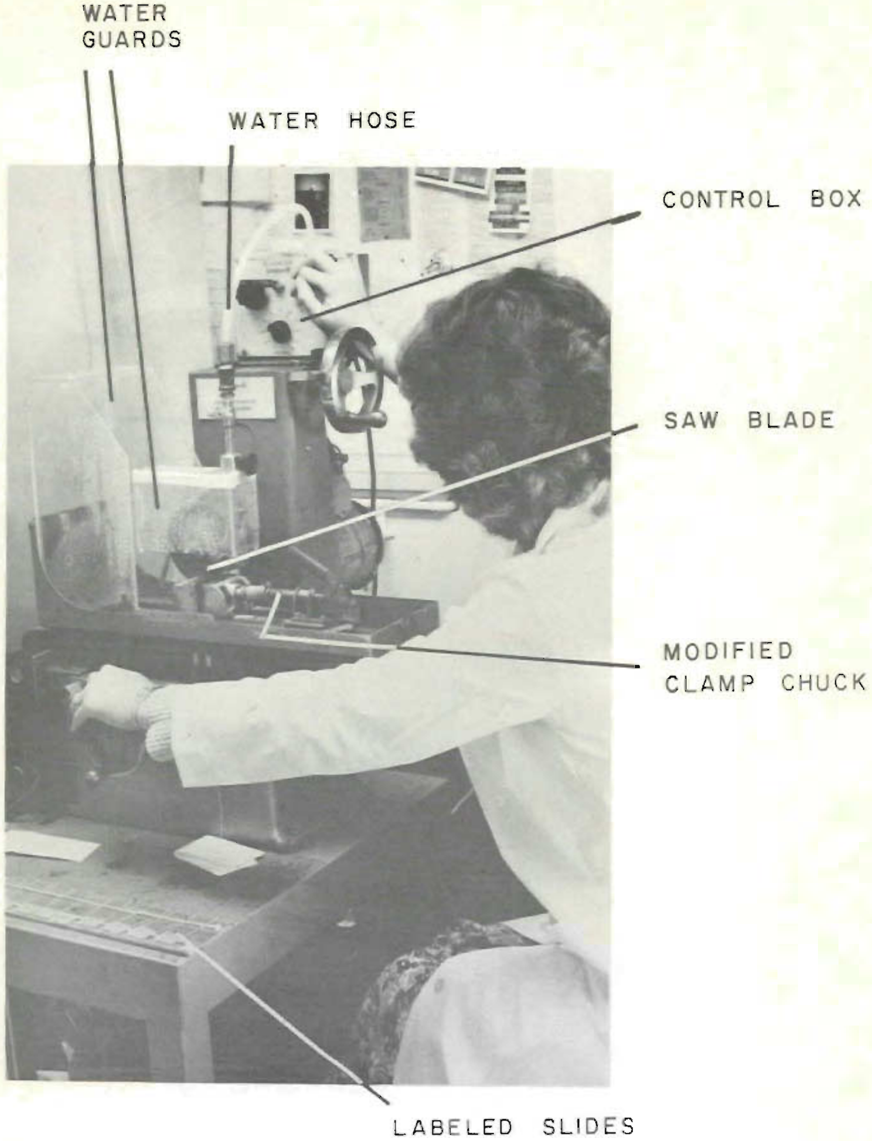


FIG. 7. Bronwill sectioning machine.

LENGTH FREQUENCY ANALYSIS (Schnute and Fournier 1980)

In general a length frequency analysis can only be used to identify the first few age-groups in a population. Often ages beyond 3 or 4 will be unreliable. However, the method may be applicable to rapidly growing and short-lived species such as Pacific cod.

Interpretation

Fish age estimates require that annuli be identified and counted. In this laboratory we identify the annulus as the translucent or hyaline zone for otoliths and fin-ray sections and the area of crowded circuli for scales. For older fish (age 10 yr or more) the appearance of the annulus on otoliths or fin-ray sections usually changes as the fish grow older and

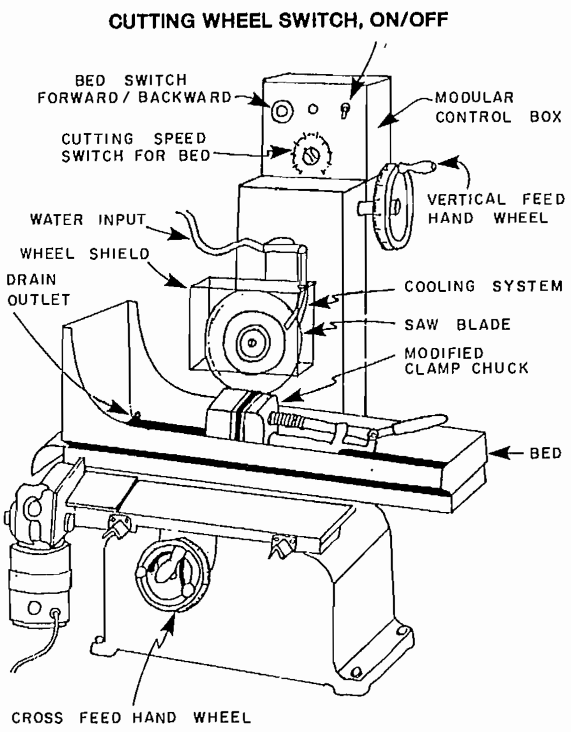


FIG. 8. Drawing of a Bronwill sectioning machine showing the various parts in more detail.

the scale annulus may not be identifiable. In general, we recommend that scales not be used to age fishes that are suspected to be long-lived.

It is recommended and internationally accepted that January 1 be used as a birthdate. Use of a January 1 birthdate will ensure that fish aged correctly will be assigned to the proper year-class. It is, therefore, necessary for the reader to know when the fish was caught. In this lab the presence of opaque material is noted with a + notation when applicable. If the fish is caught late in the year and an annulus has been formed on the edge, the reader records this as 3(4), meaning the age is 3 but the 4th-yr's growth is complete. If a fish is aged during the spring when new growth has not started and an annulus is not visible on the edge from the previous year, but it is past January 1, the reader writes 3(2+). This means the fish is 3 yr old but the 3rd annulus has not formed. If there is an annulus on the edge during the early months of the year, then the reader simply writes 3. In all cases, the number preceding the parenthesis is the best estimate of age and the number in parentheses refers to the number of annuli identified. This system is necessary because the reader is most qualified to judge if opaque growth (or wide spacing of circuli) is new or old growth. If fish have been sampled at the time of the year that new opaque growth has just started to form, such as June or July in our area, it can be extremely difficult to interpret the edge of the structure. This may not be a problem for young fast-growing fish as the opaque zones are usually large. However, it is a problem for older, slow-growing fish because it cannot be determined easily if the narrow band of opaque material has just formed or was formed the previous year. Usually an educated guess must be made by comparing the widths of previous opaque zones, but in many cases interpretation is difficult.

Many mature fishes do most of their growing during the period of June to September; therefore, it can be expected that new opaque growth is not evident until mid-June or July. Because a small amount of new growth can be confused with last year's growth, it is better, if possible, to select fish for age determination from periods other than when the new opaque growth is beginning to form.

The annual growth pattern seen in both fins and otoliths is comprised of an opaque zone and a translucent or hyaline zone, the latter called the annulus, representing a time period of reduced growth. The former represents a zone of active growth. With transmitted light, the annulus appears light and the opaque growth zone is dark. When using reflected light the "opposite" is observed, the annulus is dark and the growth zone is light (Fig. 9A, 9B). Because both types of lights are used, the reader must be aware of the difference.

The initial few years as seen on an aging structure section are represented by large wide growth zones. As fish grow older and growth slows down, the annual growth zone laid down is much smaller and the opaque and translucent zones may be equal in width (Fig. 10).

Precision and Accuracy

It is important to distinguish between accuracy and precision. Precision refers to the degree of reproducibility and thus relates to the variability between readers or between readings. Accuracy relates to the degree of closeness to the true value and thus relates to the departure from the true age. Investigations that involve determining the age of fishes usually deal adequately with the problem of precision but relatively little effort has been expended on the problems of accuracy.

Standard statistical techniques can be used to estimate the precision of age determination. A

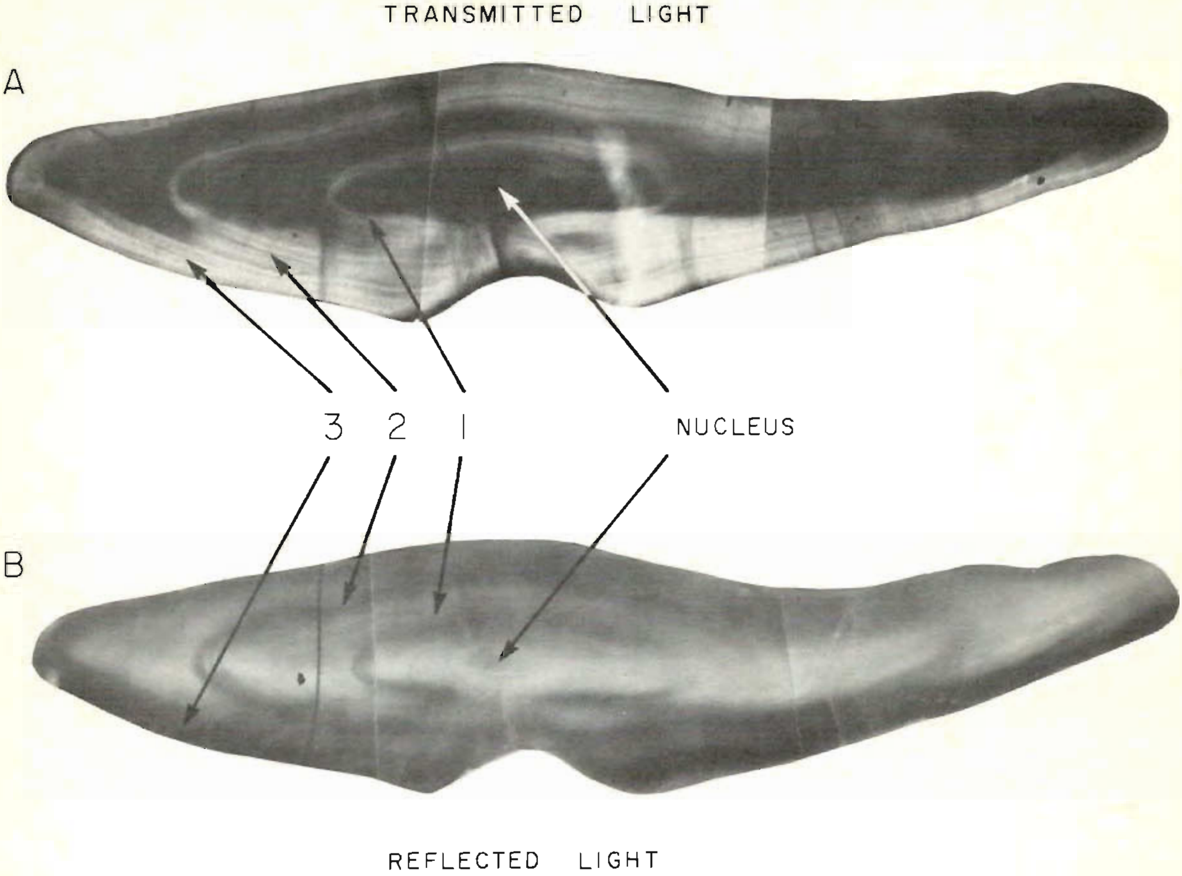


Fig. 9. (A, B) Sections of Pacific hake otoliths showing the appearance of annuli under transmitted and reflected light. Arrows indicate position of the annuli.

number of tests such as the chi-square test have been used to compare readings or readers. More common is the use of a "percent agreement" comparison, i.e. one set of readings is in agreement with another by a certain percent and ±1 yr by another percent, and so on. But this method does not compare the relative importance of an aging error and can't compare sets of determinations easily.

In this laboratory an index of "average percent error" has been developed to provide a more meaningful comparison of repetitive age estimates (Beamish and Fournier 1981). The conventional percent agreement technique does not evaluate the degree of precision equally for all species. For example, if 95% of age determinations between two readers agree within ±1 yr for Pacific cod, this would represent low precision as most commercial samples contain only a few year-classes. However, if 95% of spiny dogfish age determinations agree within ±5 yr, this can be interpreted as high precision as dogfish may be as old as 60 yr with ~ 30 age-groups in a fishery.

The index of average error or average percent error can also be used to compare the precision of sets of determinations of either the same species or different species, determinations from different structures, or determinations from different laboratories.

The index is

$$\frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij}-X_j|}{X_j}\right]$$

where N fish are aged, R is the number of times each is aged, Xij is the ith age determination of the jth

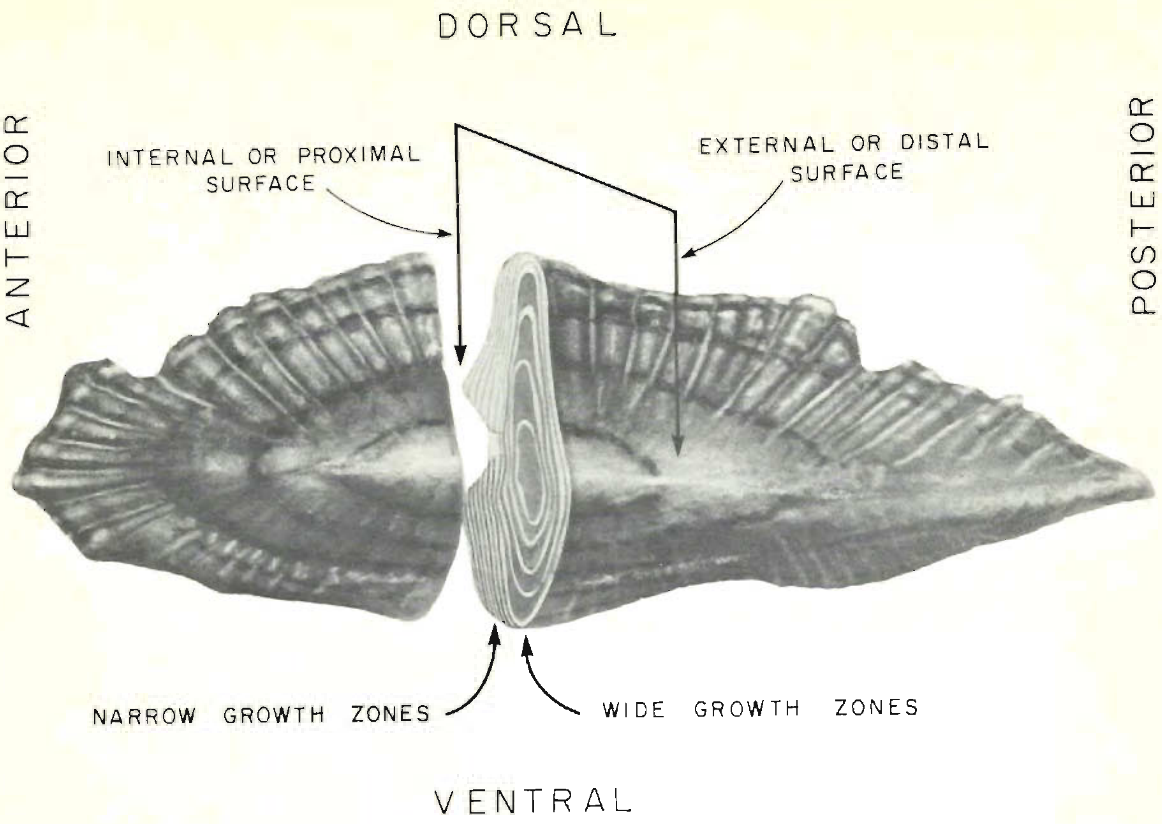


FIG. 10. Drawing of a Pacific hake otolith showing the relation between annuli visible on the surface and from sections. Note the smaller growth zones as the fish grows older.

fish, Xj is the average age calculated for the jth fish. Multiplied by 100 the index becomes the index of average percent error.

The problem of estimating accuracy is more difficult and errors in accuracy can be important particularly when the relative abundance of a cohort is being determined. For example, if a strong year-class appears in a population and it is followed or preceded by weak year-classes and if aging errors increase with the age of the cohort, there will be a significant overestimate of the original size of the weaker year-classes. Also, if it is not recognized that a particular species can be very old, there will be an erroneous accumulation of fish in the age-groups around the age at which the age determination technique breaks down. This kind of an error can have serious consequences when an exploitation strategy is being developed.

Accuracy can be estimated only when a method is validated for all age-groups in the stock or population. If a method is validated for all age-groups and it often is difficult, rarely will the validation be repeated for more than one stock. In practice, the accuracy of age determinations is almost never estimated.

One method of approximating an estimate of accuracy is to compare determinations by several techniques, such as surface otolith readings, otolith sections, or breaking and burning otoliths and sections of fin rays. If all readings are similar then there is an added degree of confidence in the determination. However, if the ages are not similar then it is essential that the procedure be validated. This usually requires a tagging and recapture study and possibly the injection of a substance such as OTC to mark the bone.

Equipment

A modified Bronwill sectioning machine (Fig. 7, 8) is used in this laboratory to section otoliths and fin rays. Some experience in operating the machine is required for a smooth, continuous operation. Cutting speed, water flow, and height of the saw blades (controlled by the vertical feed handwheel) should be determined before commencing to section. To prevent burning delicate, brittle structures such as otoliths, we use a slower cutting speed than for coarser fin-ray sections which can withstand a higher speed. The thickness of the sections is controlled by a cross feed handwheel (Fig. 11).

A Buehler isomet low-speed saw (Fig. 12) is used occasionally to section embedded structures, but it has the disadvantage of being too slow for most routine work. It is useful for experimental purposes because the slow speed provides needed control. It can be used to section otoliths too thick to snap in half for burning, by embedding them in Plasticine on the chuck. The saw can also be very useful for polishing the broken surface of an otolith by gently holding the broken edge against the diamond grit on the cutting wheel as some readers prefer to view the cut and polished edge of an otolith rather than the broken edge.

Examination of scales for aging is carried out on the Eberbach microprojector (Fig. 13) with a 40X magnification or a microfiche reader–printer (Fig. 14). When using the latter, aging structures such as scales and sections which are mounted on glass slides can be placed in the self-opening glass flats of the microfiche handler. This device moves easily and smoothly in any direction and an image is projected onto a nonglare screen up to a magnification of 48X. The use of the reader–printer has the advantage of being able to produce a picture of the structure in seconds that can be used for discussion or as a permanent record. Microfiche readers should not be

FIG. 11. Bronwill high-speed sectioning machine isolating the cutting action of the diamond blade saw making a third section into a fin ray. Sections of varying thickness are being cut.

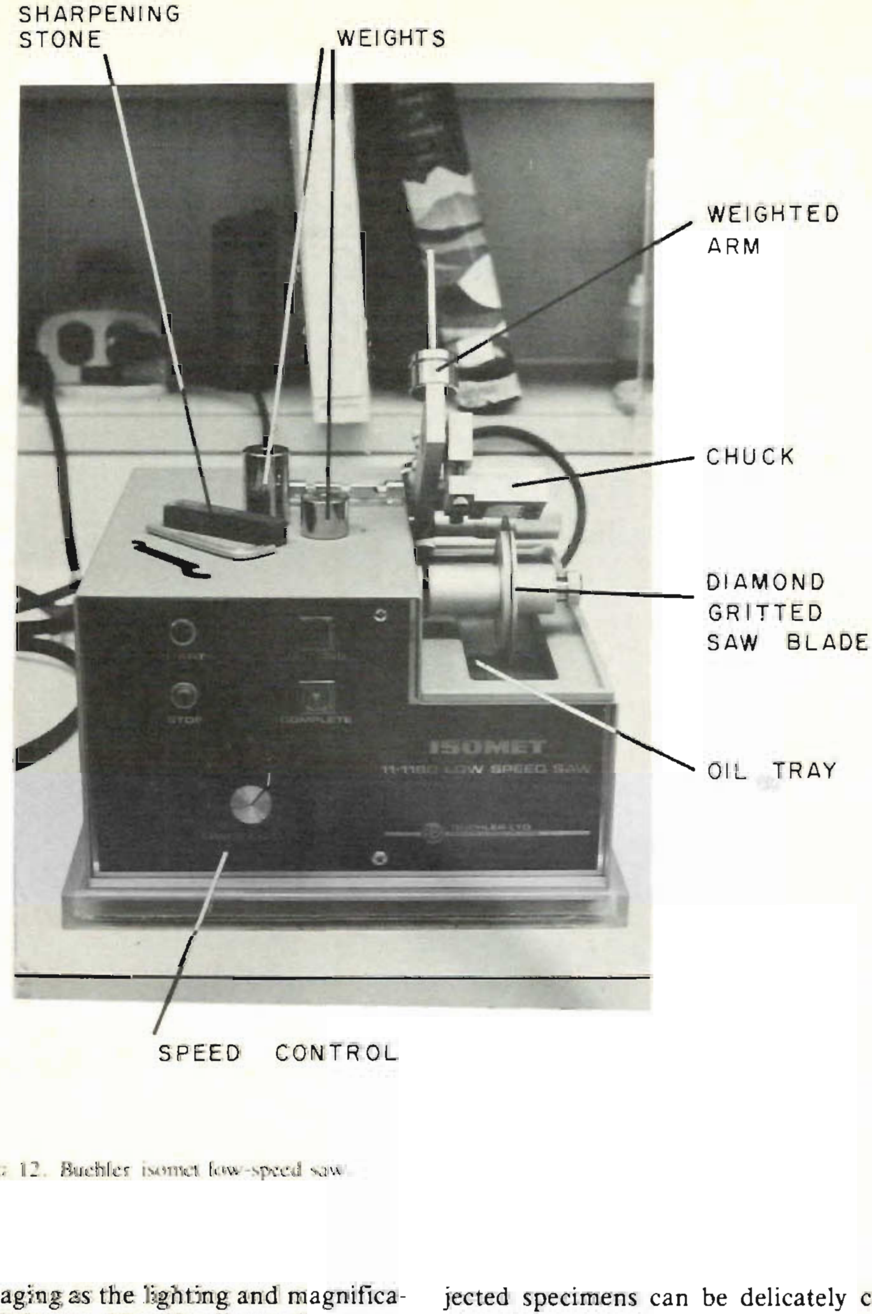


FIG. 12. Buehler isomet low-speed saw.

used for routine aging as the lighting and magnification are not sufficient to identify all annuli.

Television microscopy is being used to advantage in this fish aging unit (Fig. 15). Controversial aging structures are projected through a CCTV Hitachi camera system onto a video monitoring screen using a Wild M20 research compound microscope or a Wild M8 stereomicroscope. Because of the electronic focus and beam controls, the sharpness of the projected specimens can be delicately controlled with very little distortion.

Other equipment used in this laboratory includes the Wild M5, M8, and M5A stereomicroscopes which give a three-dimensional image of the specimens. The M5A is equipped with apochromatic optics which are fully color corrective and have a higher numerical aperature for greater resolution. Compound microscopes such as the Olympus BHA

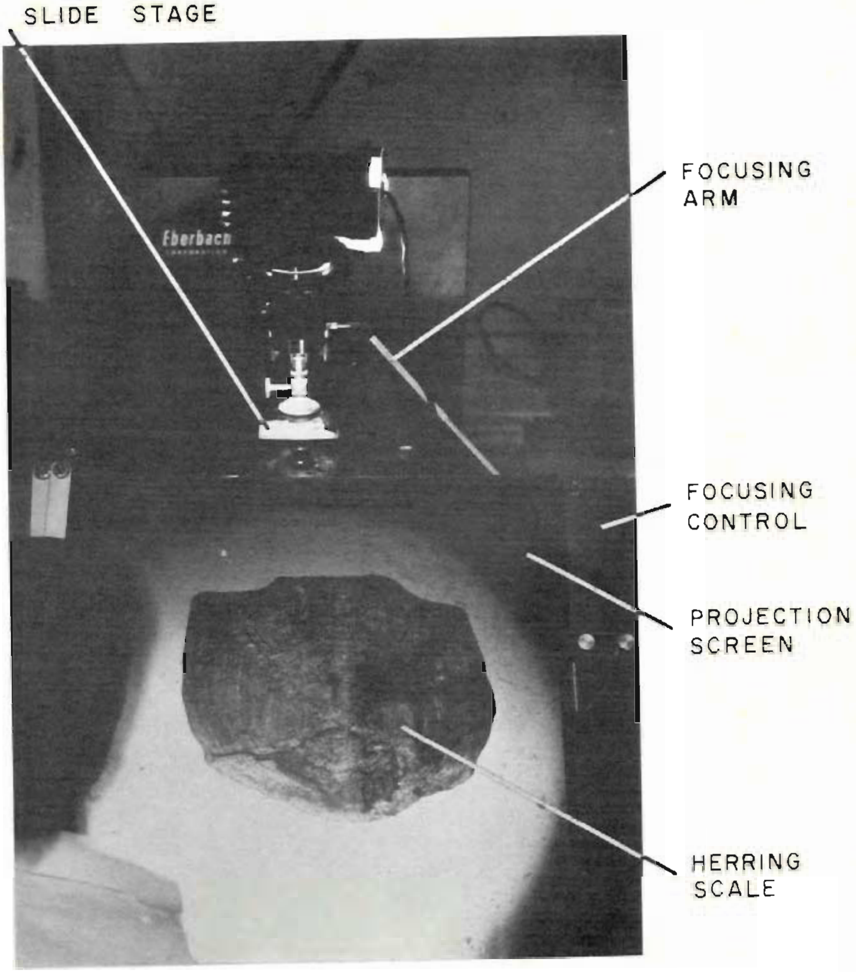


FIG. 13. Eberbach microprojector showing a screen image of a herring scale.

models and the Wild research model M20 have been set up with phase-contrast condensors, green filters, and special high quality phase objectives. We often view cross sections with 4X objective (which is not a phase objective) in association with a phase-contrast condensor resulting in better contrast. Special apochromatic objectives 10X and higher greatly enhance the resolution power of these microscopes.

Lighting methods are very critical (Blacker 1964). Both reflected and transmitted light can be used together when examining slides. Often one will enhance some feature which is unclear under the alternative type of light. We have found the ring, four-point, gooseneck fiber optic illuminators and free-standing lamps to be the most satisfactory as reflected light sources (Fig. 16).

All of our compound microscopes and stereomicroscopes have an attached or attachable third

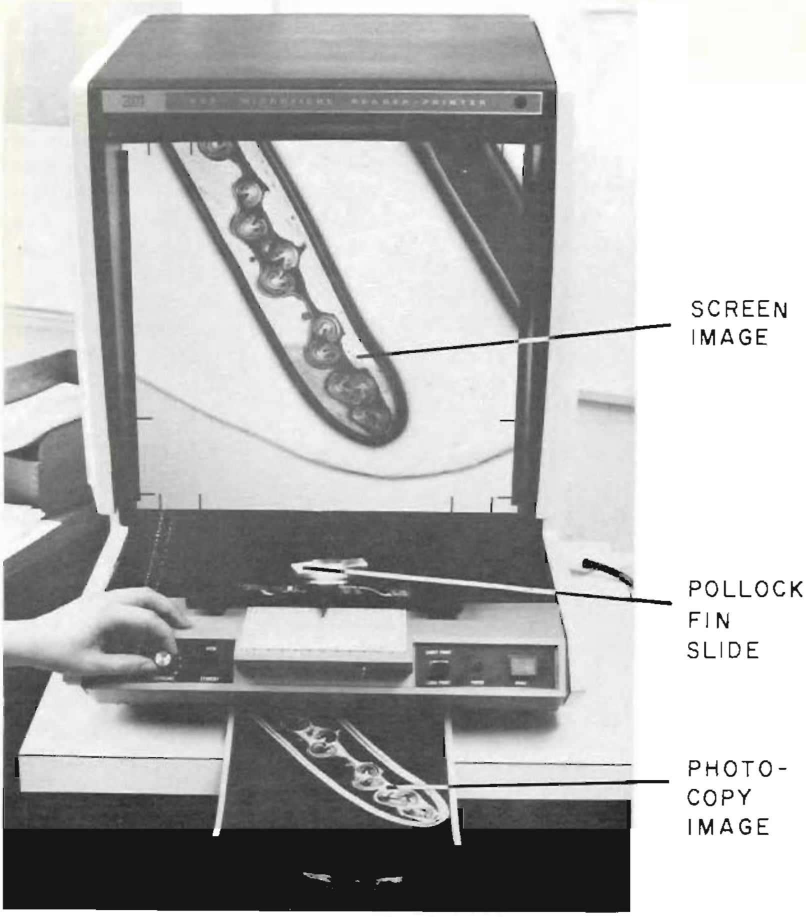


FIG 14. Microfiche reader-printer showing a screen image and a photocopy image of pollock fin-ray section.

occular which can be hooked up via adapters to the TV, 35-mm, or large-format Polaroid cameras. The latter produces instant 4 X 5 in. (1 in. = 25.4 mm) photos of fairly good quality, and film packets of different types (color, black and white, recoverable negative, etc.) can be ordered. Our program routinely photographs specimens which can be used for interlaboratory comparisons and we also have the use of a darkroom, where we develop film and prints for demonstration and publication.

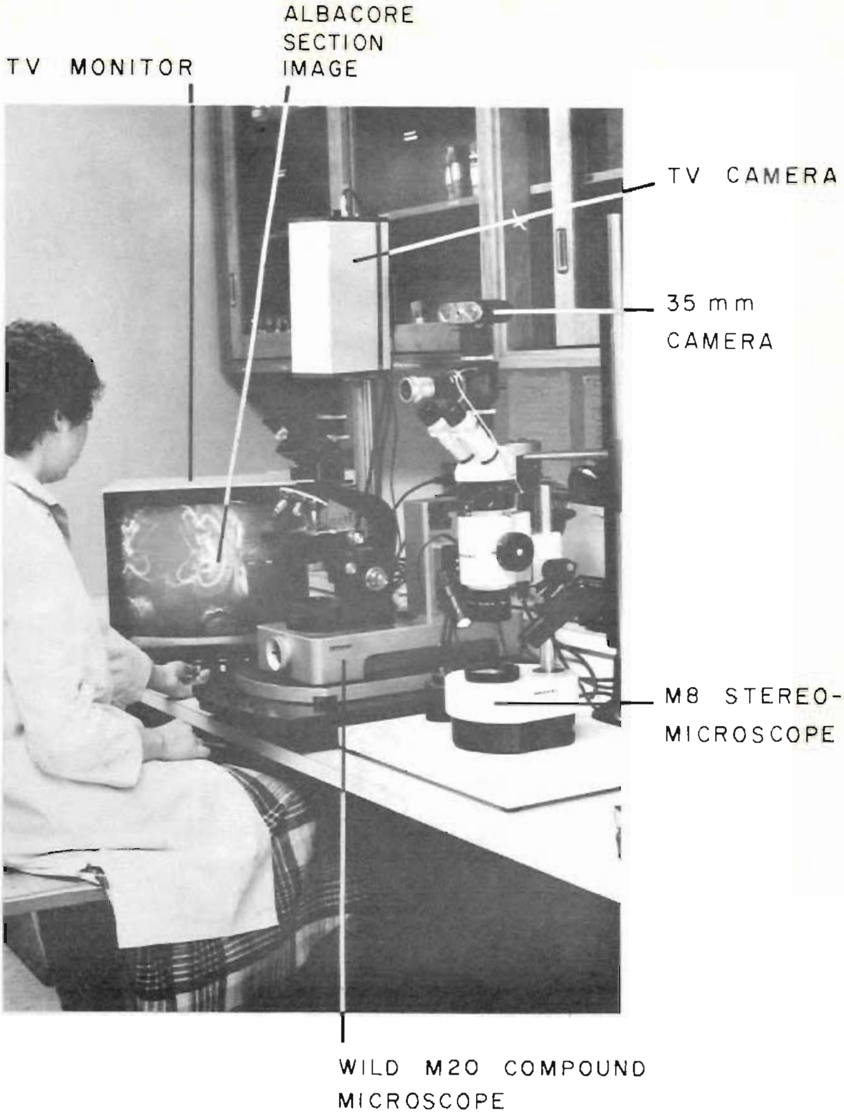


FIG. 15. Hitachi TV monitoring screen using a Wild M20 research microscope showing an albacore fin section. Also, a Wild M8 stereomicroscope set up for photomicroscopy.

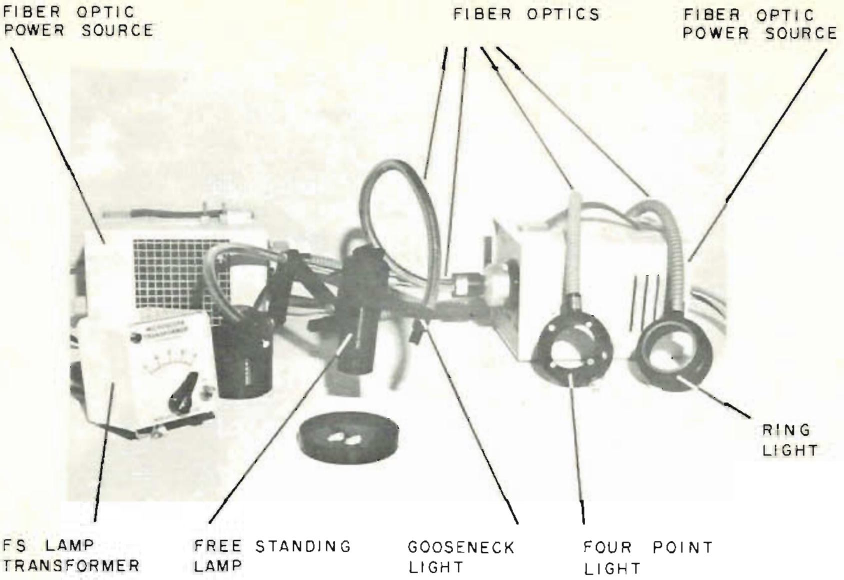


FIG. 16. Various light sources.

Aging Methods by Species

ANOPLOPOMATIDAE

SABLEFISH (BLACKCOD) *(Anoplopoma fimbria)*
(Beamish and Chilton 1982)

Sablefish or blackcod have been a commercial species since the turn of the century but a satisfactory aging method wasn't obtained until recently. The few previous reports of age and growth of sablefish have tended to overestimate annual growth and underestimate age because these studies used scales and otolith surfaces for the identification of annuli (Pruter 1954; Heyamoto 1962; Shubnikov 1963; Kodolov 1967, 1976; Kennedy and Pletcher 1968; Webb and Lockner 1973). The interpretation of annuli on these structures probably is satisfactory for the juvenile fish (Fig. 17A), but recent results of tagging studies have confirmed that sablefish can be considerably older than previously estimated.

An examination of thin sections and broken and burnt otolith sections indicated that a pattern of alternating growth zones was present on the internal surface (Fig. 17B–E, 17CC). This pattern was most easily identified on freshly broken and burnt otoliths and suggested that larger sablefish could be as old as 40–50 yr.

A tagging study, which included injecting fish with OTC to mark the otoliths, validated that the zones identified as annuli did form once a year and that otolith growth was greatly reduced for older, larger fish (Beamish and Chilton 1982). Although the interpretation of annuli appears valid, it must be emphasized that the identification of annuli can be difficult and the growth pattern of the otolith will vary among stocks.

The first years of growth are usually difficult to identify on the broken and burnt section because of the relatively rapid otolith growth, the indistinct annuli, and the numerous checks. Also the width of the annual growth zones during the first few years is quite large compared with widths of zones laid down in subsequent years. Width measurements of the first three annuli will aid in their identification. The first annulus forms at some distance from the nucleus and the position of this annulus should be confirmed by examining age 0+ or 1+ fish as determined from length and time of sampling. (Sablefish spawn in February and grow to a length of 20–30 cm in 1 yr.) The second and third annuli can be difficult to identify; however, the fourth annulus usually is distinct. It is sometimes possible to tilt the burnt section under the microscope to expose the first few annuli on the external surface. Thus, the more easily recognized surface annuli can be followed to the broken surface. As sablefish grow older, the pattern of alternating growth zones becomes more distinct and concentrated on the internal surface of the otolith. High magnification is necessary to examine otoliths from very slow-growing individuals as the annual zones are very narrow and difficult to distinguish. Occasionally the zones are so dense it is impossible to separate them with conventional techniques. On some of the more difficult to age burnt otolith sections there may be an array of fine "hairlike" checks on the ventral edge which converge to form one annual growth zone at the sulcus edge of the section; therefore it is recommended that ages be estimated from zones that intersect this edge when this occurs.

A study of daily growth rings was initiated to establish the location of the first annulus and to examine the early life history of sablefish. Several methods were tried to delineate the daily increments, but the most successful was the acetate impression method (Pannella 1971, 1973).

An otolith was fastened to a flat surface such as a SEM pin or microscope slide with crystalband heat-sensitive glue. The internal or concave side was ground slightly and the otolith was removed by gently heating the glue or epoxy and turned onto the flat internal side. The external side of the otolith was used for replication as the sulcus or auditory nerve groove on the internal side obstructs viewing on the nucleus. The otolith was ground with coarse microtome knife sharpening paper until the nucleus was just visible on the flat surface. Following this the surface was polished with a fine microtome paper and finally a polishing paste. The otolith was then etched with 2% HC1. The etching time varies between 1 and 10 min depending on the thickness and fragility of the otolith. Some increments on the edge may be lost during the grinding and etching process. A thin strip of acetate, which had been soaked in acetone for 3–4 s, was applied to the clean dry otolith surface making certain all air bubbles were removed. When dry, the acetate was peeled away from the otolith. It can then be placed between two microscope slides and examined and photographed with a compound microscope mounted with a 35-mm camera.

The daily increments appear to be deposited in distinct concentric layers for the first 50 d. Because daily increments have been shown to appear after yolk sac resorption (Brothers et al. 1976), it is possible that the first ring observed was formed at the onset of feeding. As the pattern is constant we

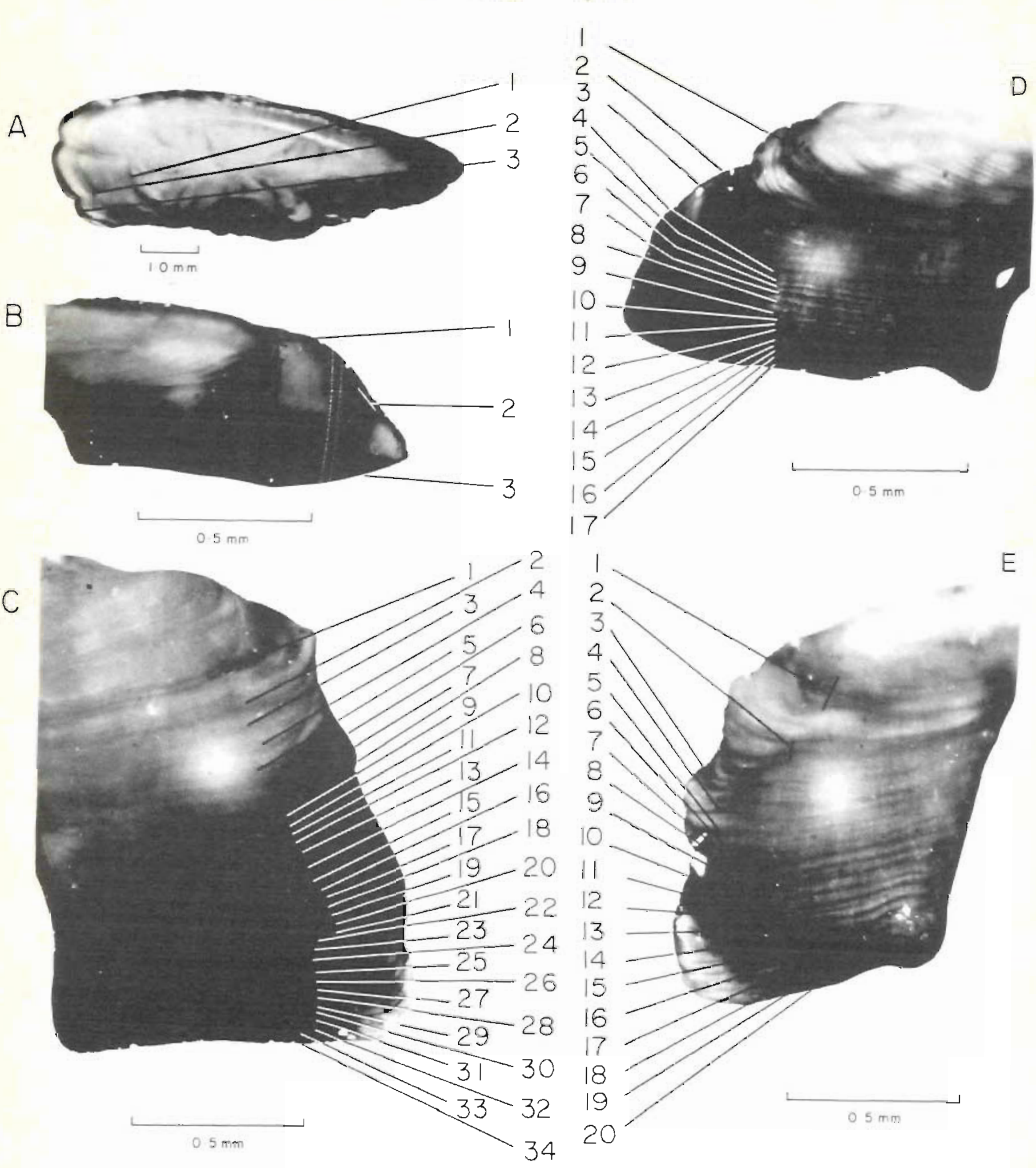


FIG. 17. (A) Sablefish otolith surface. (B) Burnt cross section of above. (C–E) Burnt cross sections of older sablefish otoliths; (C) average section of a very old sablefish; (D) a slow-growing fish; and (E) easily aged fast-growing sablefish. (CC) A partial enlargement of Fig. 17C indicating annuli on a burnt otolith section from an older sablefish.

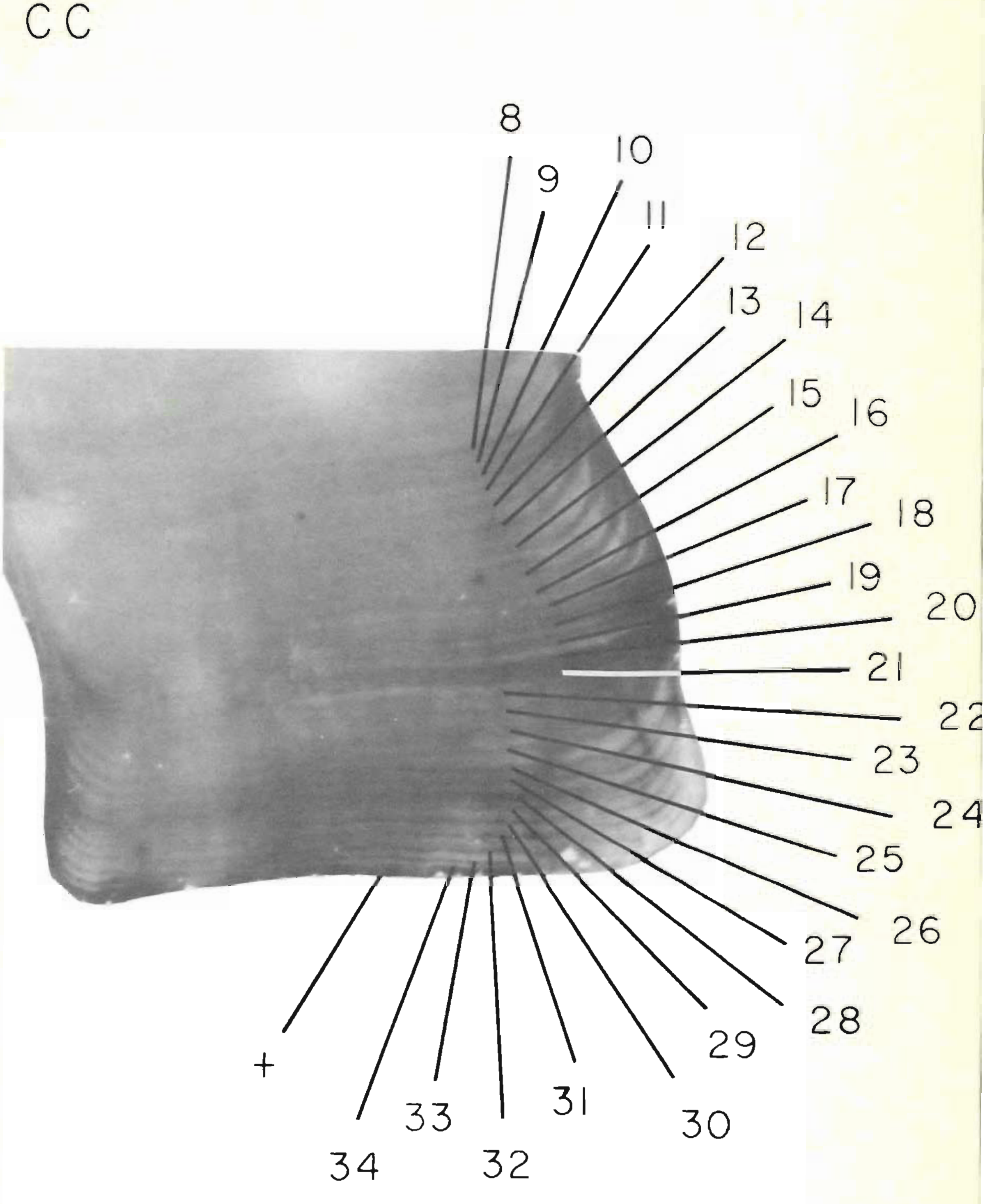


FIG 17. Continued.

interpret this to mean that larval sablefish remain at hatching depths of ~500 m during this period. For the following 100 d there appears to be an area of incomplete and grouped increments which might be interpreted as the time of larval ascent to the surface waters.

From 100 to 300 d the clarity of the rings improves which could suggest the gradual establishment of young sablefish in the surface waters. Juvenile sablefish (5–15 cm) have been seen in surface waters in Queen Charlotte Sound in August (Beamish and Chilton 1982).

Annual rings on the acetate impressions are seen as one dark or a series of quite distinct dark bands. The position of the first annulus occurs after about 275–300 daily growth increments have formed and because sablefish hatch in early spring, the zone identified as a first annulus (Fig. 17A, 17B) appears to be the true first annulus.

GADIDAE

PACIFIC COD (GREY COD) *(Gadus macrocephalus)*

(Kennedy 1970; Beamish 1981; Foucher and Fournier 1982)

Initially, age determinations and growth rates of Pacific cod were determined from length frequencies and data from tagging studies (Ketchen 1961, 1964). Otoliths of Pacific cod found in Canadian waters have been determined to be of little use (Ketchen 1970). This laboratory has found that although some burnt sections of otoliths did exhibit an apparent annual pattern of growth zones, the otoliths were more difficult to interpret than sections of fin rays or scales. When a criterion for distinguishing annuli on Pacific cod scales was described by Kennedy (1970), routine processing of scales and aging of this species began. However, in 1978 the use of the scale method was suspended until the results of this method could be validated or until a more rapid method could be developed. The scale method was suspected of producing incorrect ages for older fish as checks occur more frequently on scales of faster growing fish. The circuli are more widely spaced and any interruptions in growth appear as a check that is difficult to separate from an annulus.

The annulus or winter check of older Pacific cod scales from some areas is not completed until late spring and the new growth does not begin to show until well into the growing season. New growth on scales of younger fish is visible as much as 6 wk earlier (unpublished personal observation). Occasionally there is difficulty in determining whether new growth or the previous year's growth is showing on the edge of aging structures sampled in the spring and early summer. In the younger, faster growing fish, it probably would be safe to assume that it is new growth. But as the fish grows older it becomes harder to establish the year to which the slight amount of growth belongs.

Because of the difficulty of applying the scale method, a study of the value of fin-ray sections was undertaken. The second dorsal and pectoral fin rays were found to be suitable for aging Pacific cod. Some technical problems were experienced with the second dorsal fin becoming twisted and not drying flat in the envelope making mounting and sectioning of the fin rays difficult. This results in inferior sections and it is then difficult to separate annuli from checks. Comparisons of ages using fin-ray sections and scales indicated that similar ages were obtained for the first few years (Fig. 18A–D) and the fin-ray method appears to be more reliable for older fish.

At present we are experimenting with a combination of techniques. Because Pacific cod grow very quickly it appears that length frequencies may provide a good method of estimating age of younger fish. A judicious mixture of scale and fin-ray ages and a computer analysis of length frequencies may ultimately prove to be the most efficient age determination method for Pacific cod in the Canadian zone.

PACIFIC HAKE *(Merluccius productus)*

(Dark 1975; Beamish 1979a)

In the past, the surface ages of whole otoliths were considered satisfactory for Pacific hake. Recent studies by Beamish (1979a) have provided evidence that older, thicker otoliths and otoliths from some stocks are underaged when viewed from the external surface (Fig. 19A–H). In many older fish it is difficult and sometimes impossible to detect all growth zones when viewing the whole otolith, as distinct growth patterns form only on the internal surface (Fig. 20A–F). Surface aging of the hake otolith is useful for the younger fish and the method described by Dark (1975) appears reliable for the bulk of the fish found in the "offshore" fishery but it is not suitable for the slower growing fish from the Strait of Georgia or some of the "offshore" hake in the Canadian zone. Because the age at which surface ages become unreliable may change among stocks, comparisons should always be made between the section and surface ages. We routinely section or break and burn all hake otoliths as the population in the Strait of Georgia consists of older, slow-growing individuals, and the hake off the west coast of Vancouver Island are larger and older; thus both can be difficult to age using the surface of otoliths.

Sections should be prepared and mounted according to the instructions given previously. They are aged with a compound microscope using 10 power eyepieces and 4 power objectives. Higher power objectives (10–20X) are used to resolve any difficulties in interpreting the presence/absence of new growth on the edge or in separating individual annuli which may be crowded on the otolith's edge. A higher power can be useful also when attempting to resolve difficult sections which appear blurred and have many zones (Fig. 21A–C, 22A, 22B). Phase-contrast objectives and tipping the slide slightly on the microscope stage may provide greater contrast.

Routine aging of hake otolith sections should begin by ensuring that the reader can properly identify the first three annuli and the presence or absence of new opaque growth on the otolith's edge. The first three annual growth zones are much wider than subsequent growth zones and the annuli within these zones can be obscured because of the numerous checks. The best approach is to become thoroughly familiar with the growth pattern of annuli on otoliths from younger (immature) hake. The reader must become familiar with decreases in the widths of the annual growth zones and the change in the relative width of the opaque and translucent zones as the fish ages. This problem is illustrated in Fig. 23 (and Fig. 9A, from which Fig. 23 was drawn). When examining the otolith sections it is useful to compare the relative positions of the nucleus and annuli with the surface of otoliths from younger fish.

Identification of presence or absence of new growth on the section edge could be a problem if new growth is not visible on all surfaces of the otolith. New growth is often detected first on the ventral edge of a section. If the reader suspects plus growth may be present (because of the time of year sampled), a careful examination of the outside edge of a section should be made using higher magnification. It is also useful to examine the surface edge of the whole otolith.

In summary, the assignment of an age for Pacific hake is often the result of several observations involving the surface, section, and broken and burnt age. An age derived from a section is likely to involve counting in one or more locations as well as aging more than one section of the same otolith. Recent studies suggest that the technique of breaking and burning otoliths reveals growth zones of similar or even better clarity than the sectioning method and has the advantage of requiring less time and effort. The section method is still necessary for otoliths difficult to age and otoliths from very old fish.

WALLEYE POLLOCK *(Theragra chalcogramma)*
(La Lanne 1975; Beamish 1981)

Scales (Ogata 1956) and otoliths (La Lanne 1975) have been used for many years to age walleye pollock. However in this laboratory, after examining the rays of various fins, sections from the pectoral fin were found to be the most useful structure because of the clarity of the annuli (Fig. 24A–D).

Scales and otoliths were examined, but the former from older fish were difficult to interpret because of closely spaced circuli, indistinct annuli, and checking. Otoliths were not suitable for most pollock because annuli were obscured. Kasahara (1961) and Ishida (1954) noted similar problems for otoliths from pollock larger than 35 cm.

Preparing and sectioning of pollock fins is similar to the procedures for other species. When the dried fin is removed from the envelope, excess skin and rays are cut away with scissors, leaving only the first 7–10 rays. After mounting, several sections are cut about 0.6–1.0 mm from the base. The distance that the section is cut from the fin base is important. Those made at the base have greatly distorted growth patterns, whereas sections made too far from the base may not contain the first annulus. Therefore, it is important to cut at least three sections (Fig. 25A, 25B). The first should be made through the basal area to orientate the reader, who will know that the next two have been taken as close to the base as possible and these are usually the easiest to age. The sections are air-dried overnight and then glued to the microscope slides with Flo-texx. Sections mounted in this way have been clear enough to age at least 4 yr later.

As with other species, preparation problems often cause difficulties in interpreting fin rays. If the sections are too thick or too thin, optimum contrast between growth zones is not obtained. Oblique cuts exaggerate the spacing between annuli in some portions of the ray while concentrating it in others, and often decrease contrast (Fig. 26).

Using transmitted light, the wide summer growth or opaque zones are dark and the narrow winter growth or translucent zones are light. The opaque zone diminishes in width after the first few years. The center of the ray is identified as a small translucent oblong or crescent-shaped area (Fig. 24C). A problem with the application of the fin-ray method for pollock age determination is distinguishing the first annulus (Fig. 27). It is

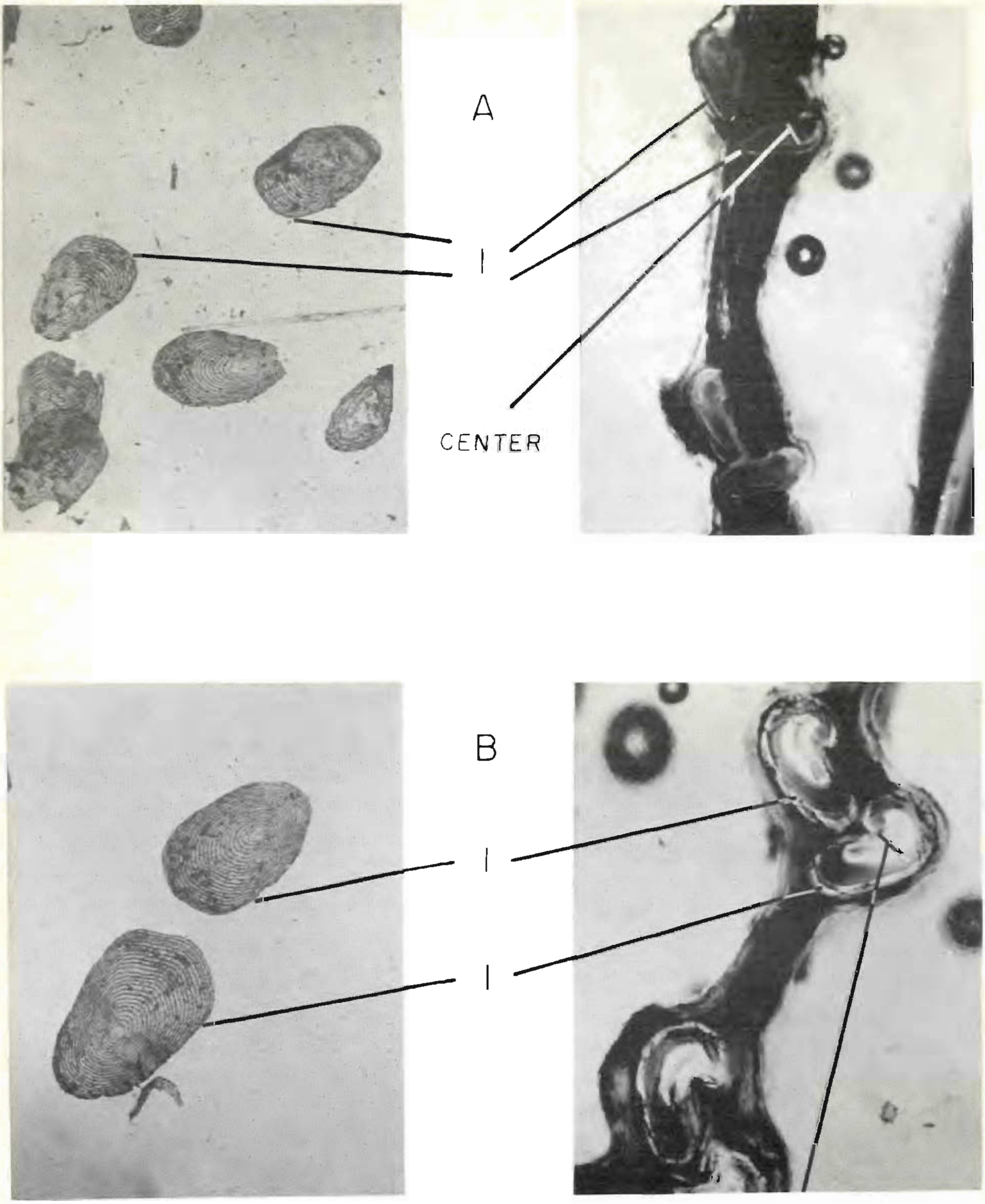


FIG. 18. Comparison of Pacific cod scales and sections of fin rays. Ages from sections of fin rays and scales are similar for the first few years. (A) 12-cm fish caught in February; age 1 yr. (B) 22-cm fish caught in February; age 1 yr. (C) 45-cm fish caught in October; age 2(2+) yr. (D) 52-cm fish caught in October; age 3(3+) yr.

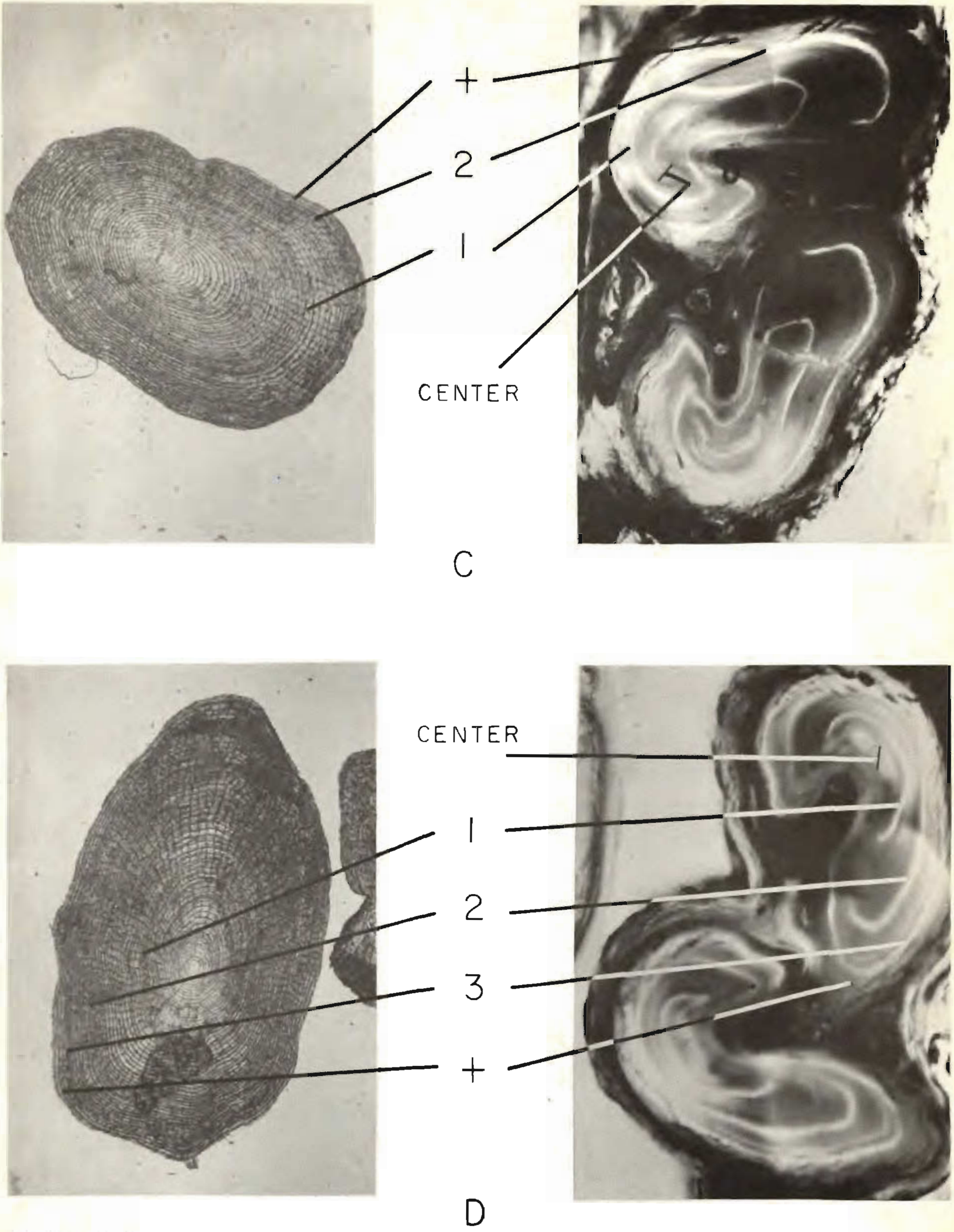


Fig 18. Continued.

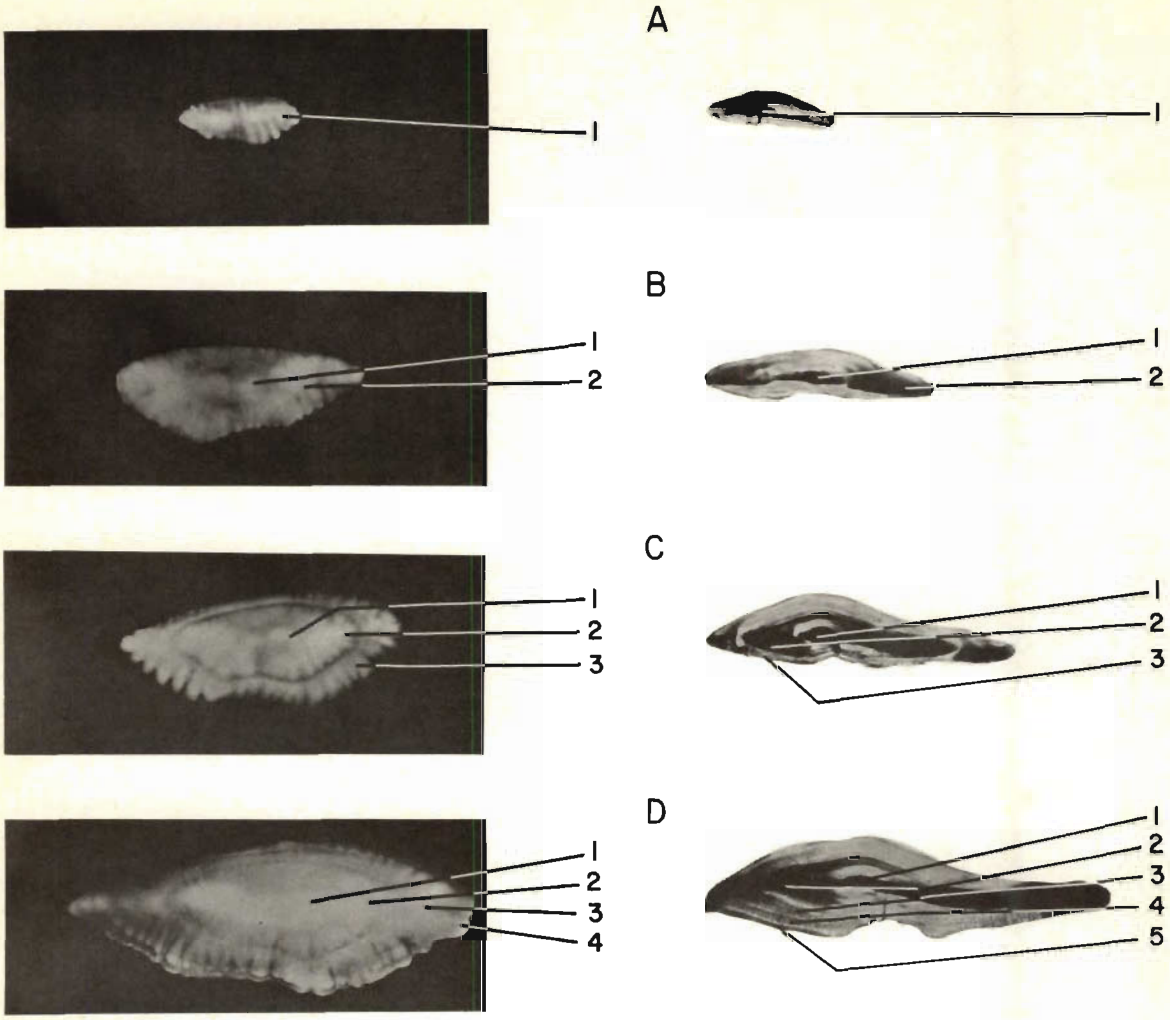


FIG. 19. (A–D) A comparison of hake otolith surfaces and sections of otoliths showing identical age for the first few years and older ages using the section method for older fish. (E–H) Older, thicker hake otoliths underaged on the surface.

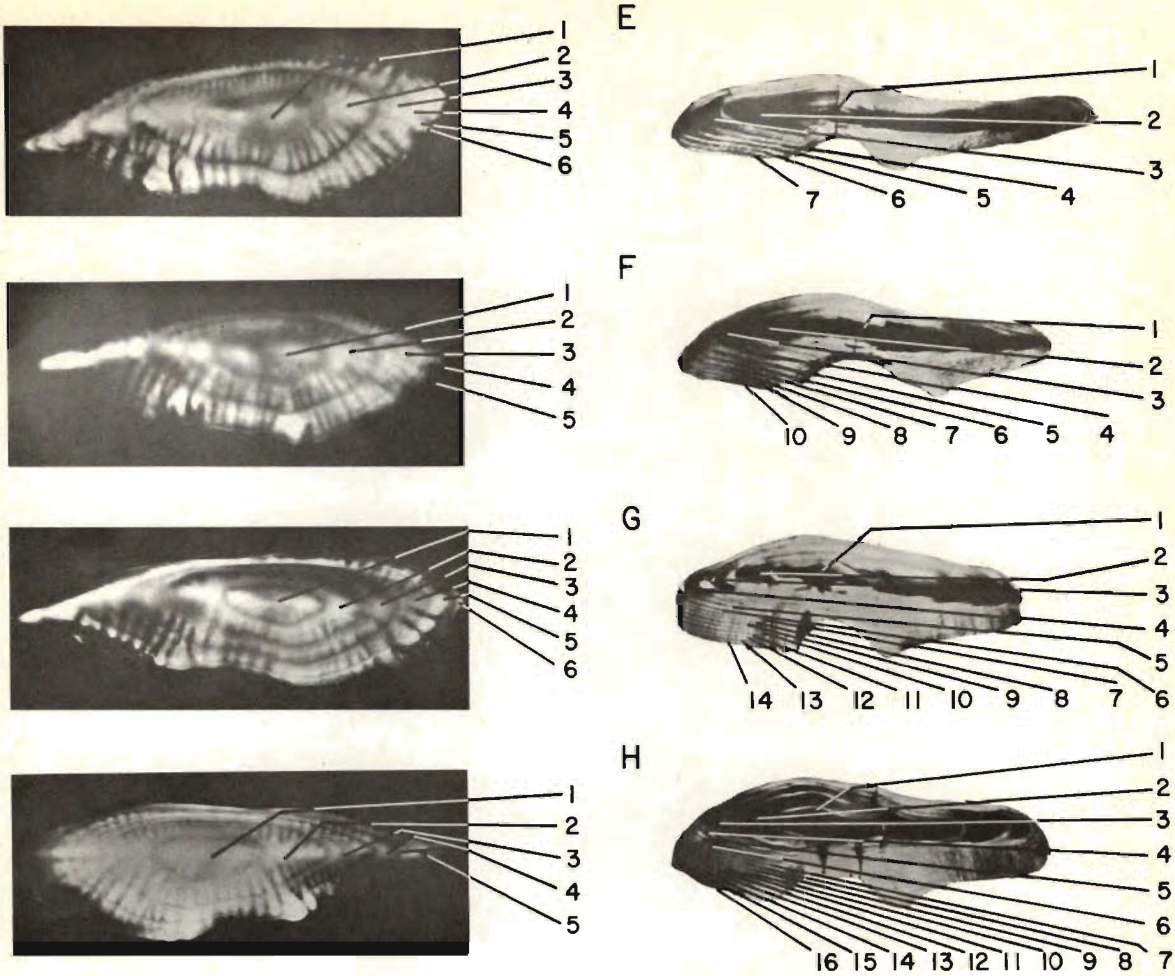


FIG. 19. Continued.

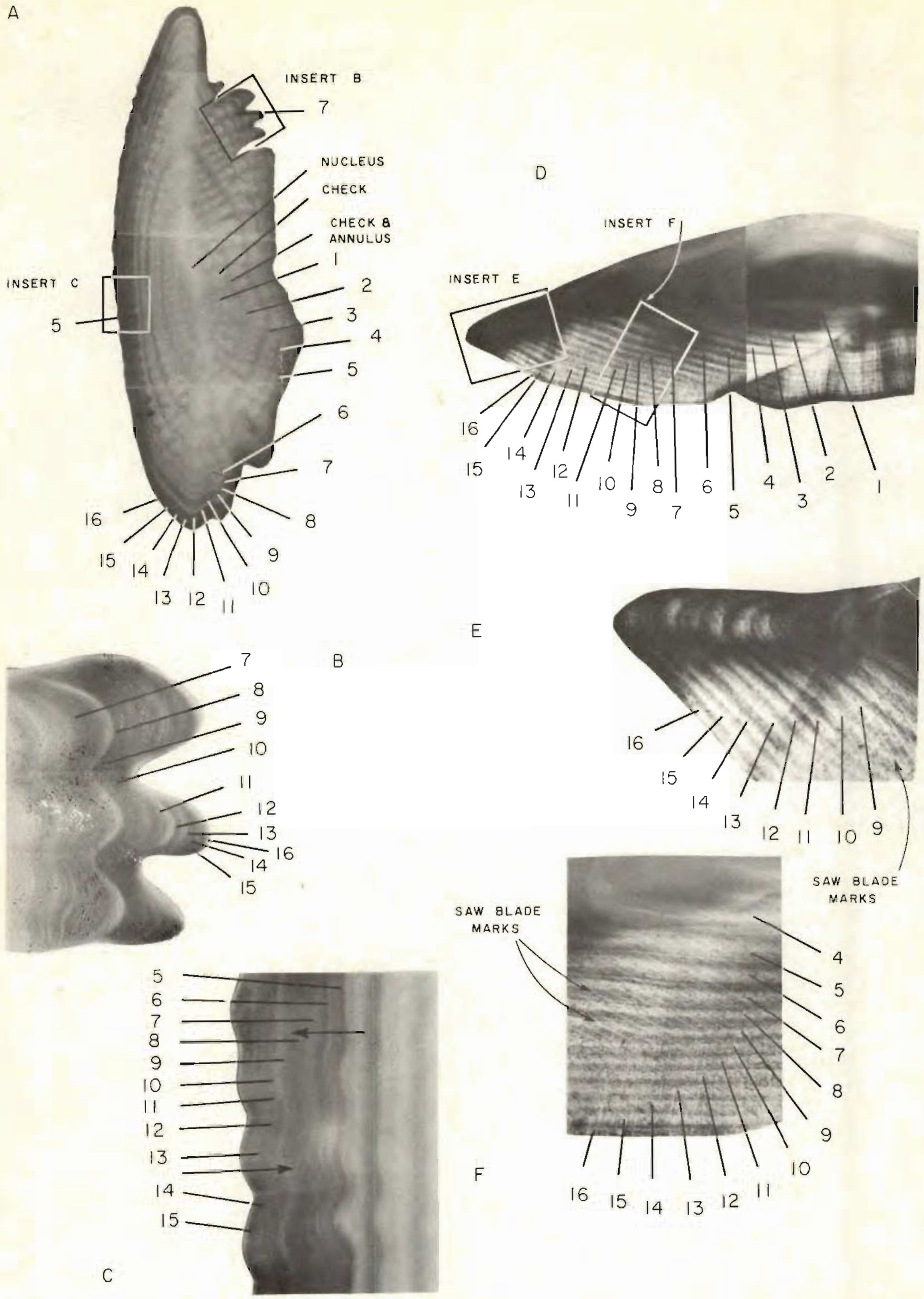


FIG. 20. (A–C) A surface of a 16-yr-old hake otolith with enlargements of edges. In (C), if the dark zone between the seventh and eighth annuli is counted, an age of 16 yr would be obtained on the counting area indicated, see arrows. (D–F) A thin section of the same 16-yr-old hake otolith with enlargements of counting area. (F) shows the preferred counting area.

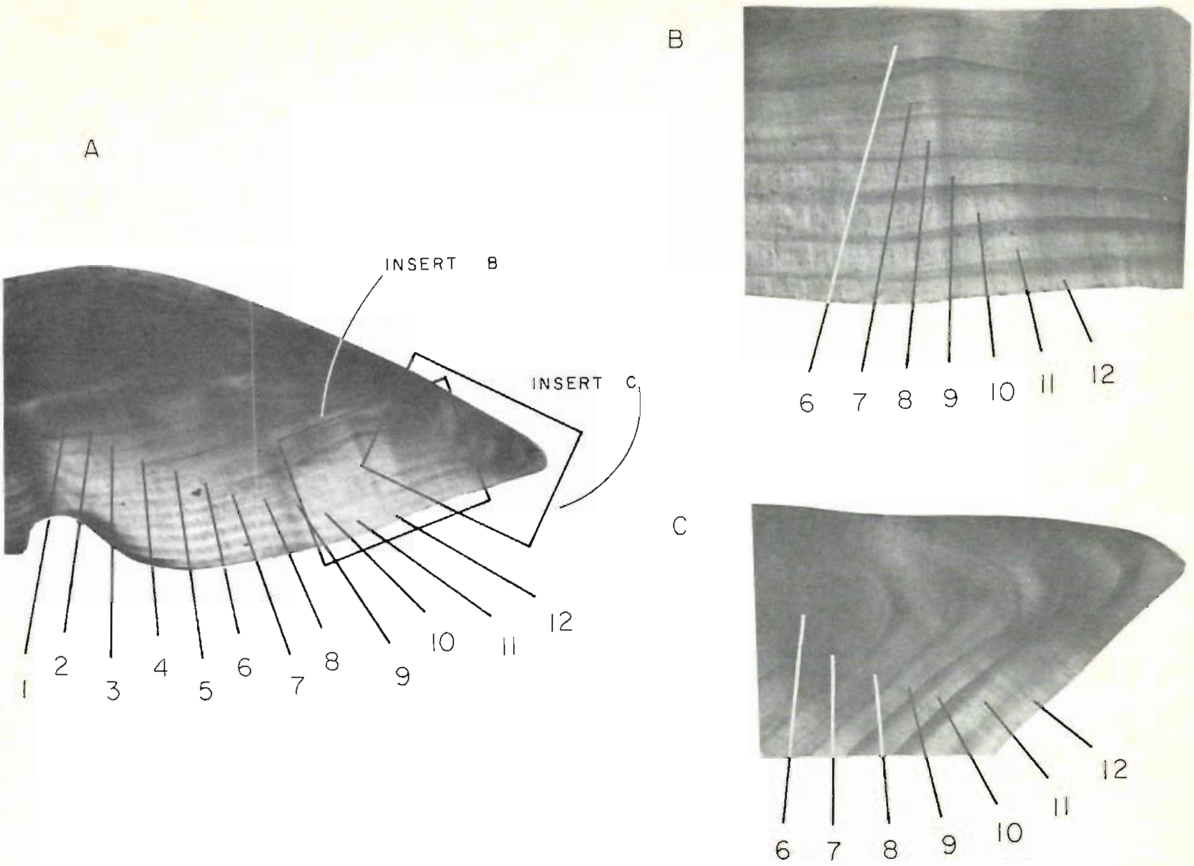


FIG. 21. (A–C) A high power magnification is used to aid interpretation of the edge of a hake section. It is difficult to identify the presence of new growth on the edge. Because the fish was caught in July, the reader must suspect there should be new growth present and should therefore inspect the edge of the whole otolith. In the case of this sample, there was a slight amount of new summer growth; a good example of using two aging methods to assign a fish age.

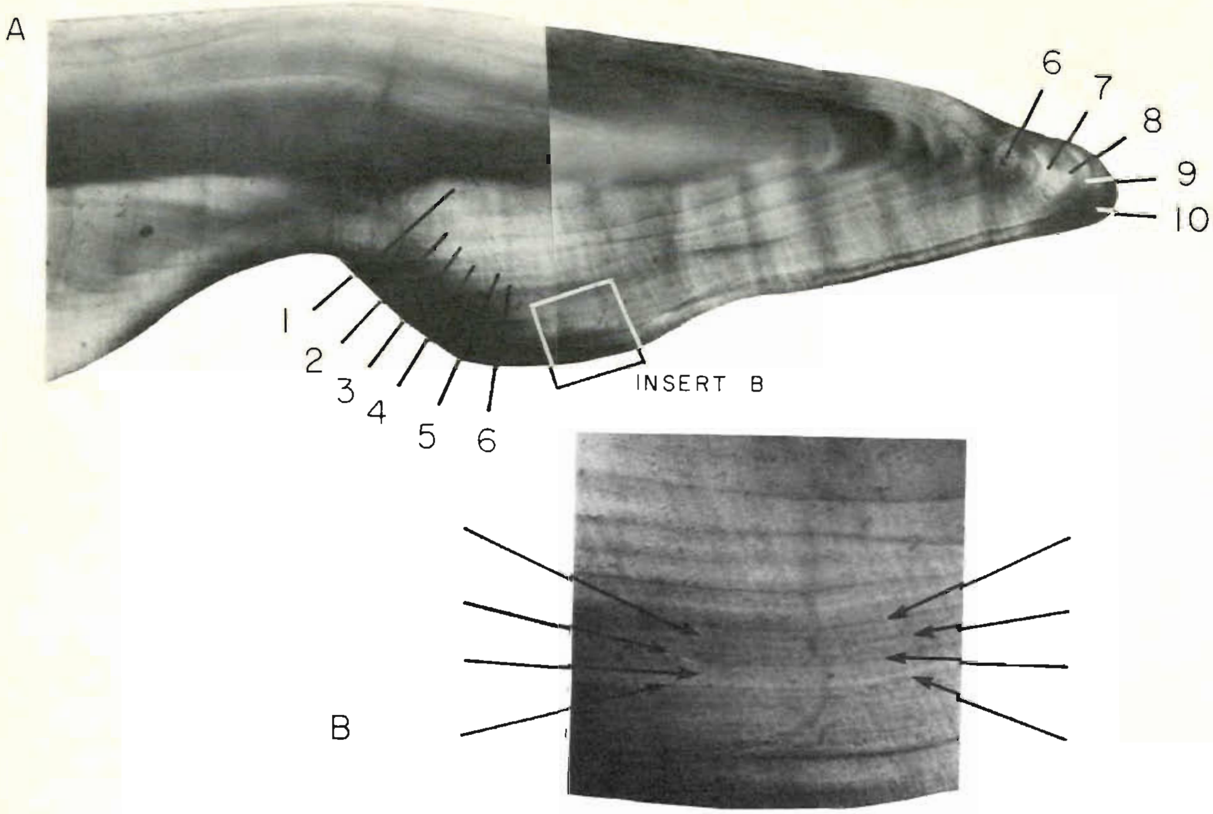


FIG. 22. (A) A hake section which is difficult to interpret. The years assigned after 6 are tentative as the remaining growth pattern is unclear. (B) Arrows indicate the many small zones and checks which make it difficult to establish an annual zone.

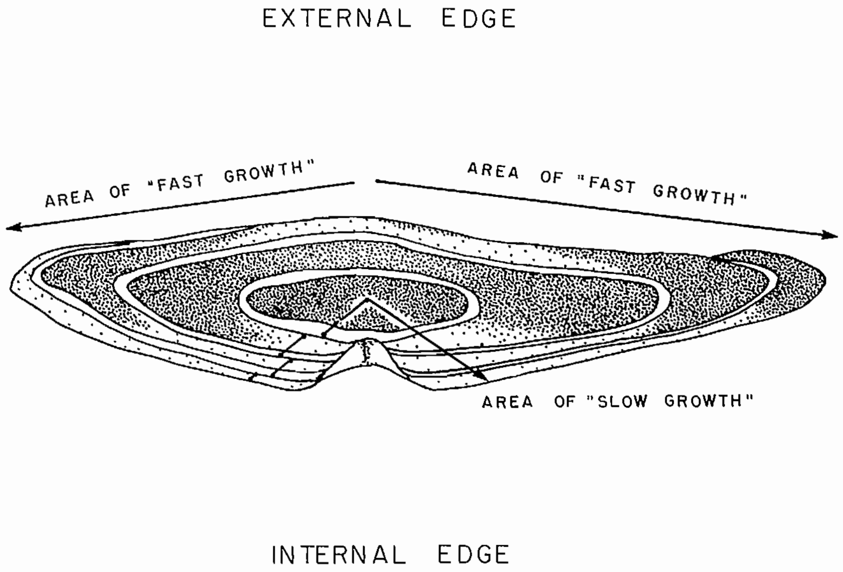


FIG. 23. Drawing of the first four annual growth zones of a hake otolith section. The area of "slow growth" is the preferred area for aging. The dots connected by a bar on this area show 1 yr growth. NOTE: After the 2nd to 5th yr the contrast between the winter and summer growth is reduced and therefore care must be taken when identifying an annual zone; see Fig. 9A from which the figure was drawn.

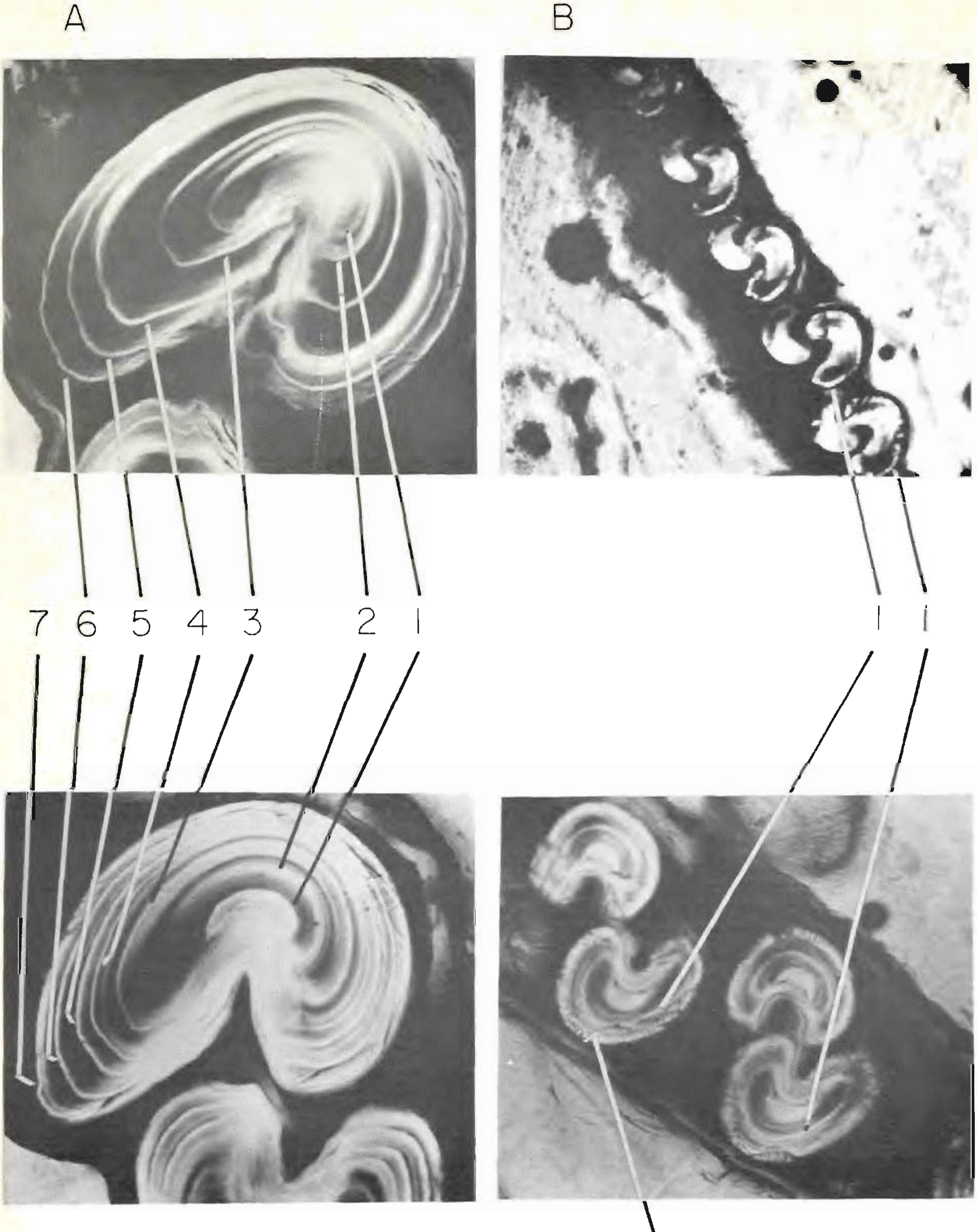


FIG. 24. (A–D) Examples of pectoral fin sections from walleye pollock indicating the annuli; (D) shows a zone which is difficult to interpret.

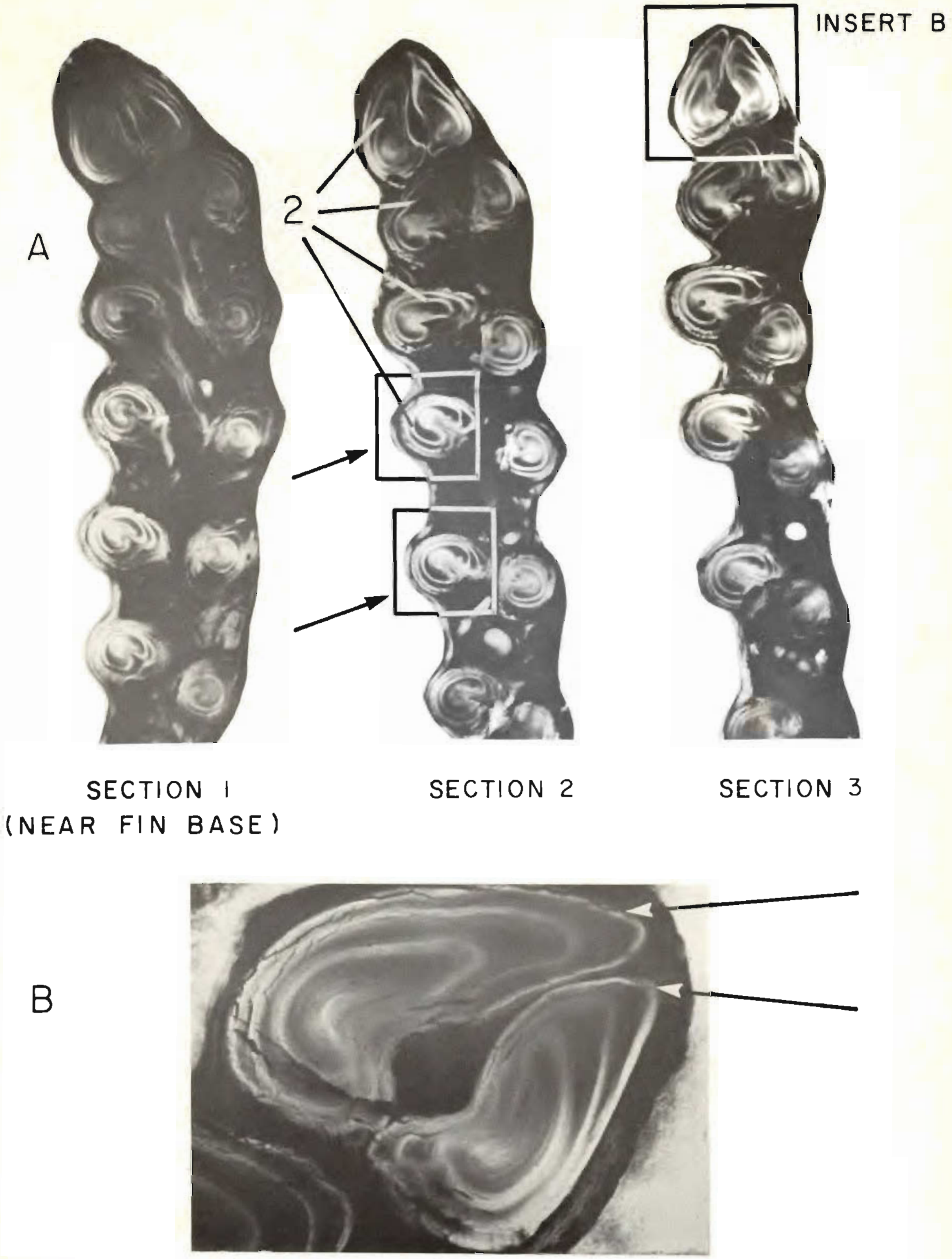


FIG. 25. Three successive sections from a pollock fin ray emphasizing the different growth patterns of each ray. (A) arrows are pointing to rays which should be measured to identify annuli if they are obscure. Note the difference in size and shape of the 2nd annulus when comparing the first four rays. (B) The condition of the edge is easier to determine on the first ray, see arrows. Color is an important factor when differentiating the boney growth from the dried soft tissue around the ray.

FIG. 26. Example of an oblique fin-ray cut distorting the spacing of the annuli. The bars indicated show blurred translucent zones which are not definite enough to identify as annuli.

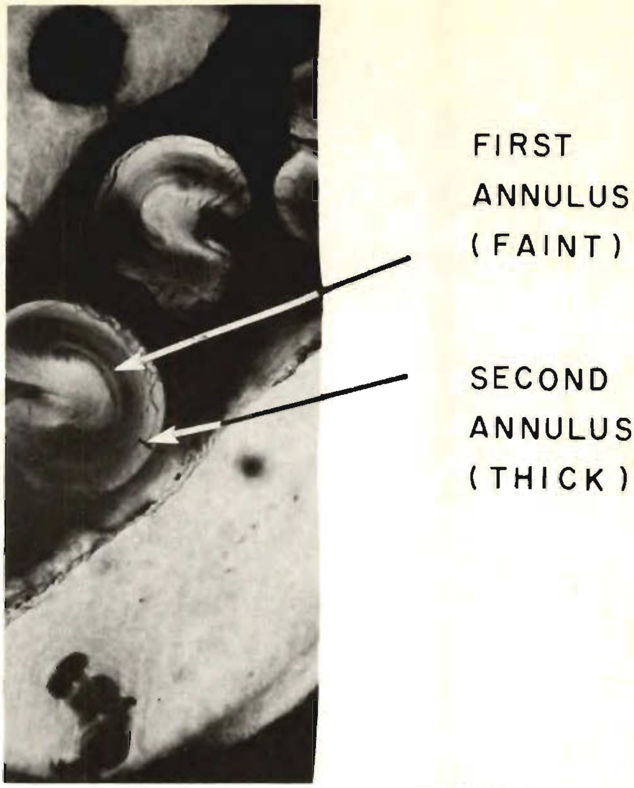


FIG. 27. A difficult first annulus for a novice reader.

recommended that juveniles be collected from the stock being aged to obtain measurements of the center width and the first and second annuli. Measurements are useful if the first annulus is indistinct and the next prominent translucent zone appears to be the second annulus. In this case, the distance to the apparent second annulus will usually be well outside the range observed for the first annulus. Taking measurements of the diameters of the annuli is important for the novice reader (Fig. 28A). Measurements should be taken from the 3rd-5th ray on the 2nd section from the base (Fig. 25A). Note that each fin ray is composed of two bony structures (Fig. 28B), and the larger of the two should be measured because the growth pattern is more easily identified on this bone.

The presence of checks may create interpretation difficulties. However, they are usually less prominent than annuli, do not form completely around the ray, often are close to the annuli, and are not present in all sections. Examination of sections of slower growing rays or the smaller fin-ray element will often not contain a check that is present in faster growing rays.

Determining the condition of the edge of a section is most easily accomplished by examining the first two rays where the growth zones are larger (Fig. 25B).

One of the advantages of the fin-ray method is the facility with which annuli can be identified in older fish. Even when annuli form close together, it is usually possible to observe that the fish is older and accumulating annuli on the edge of the ray. This is particularly important when slow-growing fish are being studied.

HEXAGRAMMIDAE

LINGCOD *(Ophiodon elongatus)*
(Beamish and Chilton 1977)

Although lingcod has been an important sports and commercial species for over 50 yr, a practical method for aging this species wasn't available until 1977. Scales and otoliths were found to be unsatis-

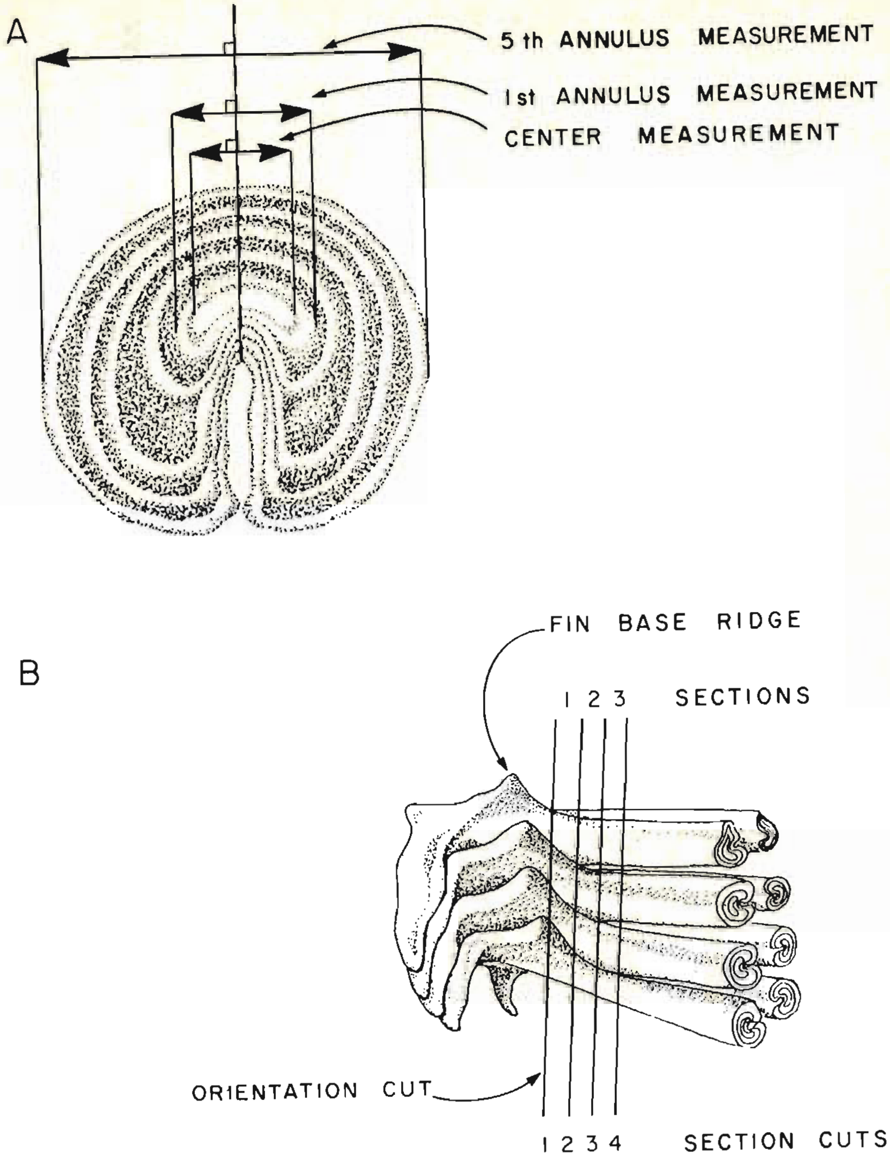


FIG. 28. (A) Drawing showing a representative section of a fin indicating the method for estimating annulus width. (B) Drawing of a fin ray from a pollock. The distance that the section is cut from the fin base is important. Those made at the base (1) are greatly distorted, and are made solely to orientate the reader, who will know that the next two (2 and 3) have been taken as close to the base as possible.

factory (Chatwin 1954, 1956, 1958; Beamish and Chilton 1977) and although vertebrae appeared useful for young fish, they were not practical to use routinely for commercial catches and there was considerable doubt if vertebrae could accurately age older fish. In 1977, we published an article that showed that cross sections of the 4th–8th fin rays from the second dorsal fin provided a method for estimating age (Fig. 29A).

Preliminary results from a tagging study in which lingcod received an injection of OTC appear to validate ages in the age range of the few fish recovered (Cass and Beamish 1982).

The application of the fin-ray method requires

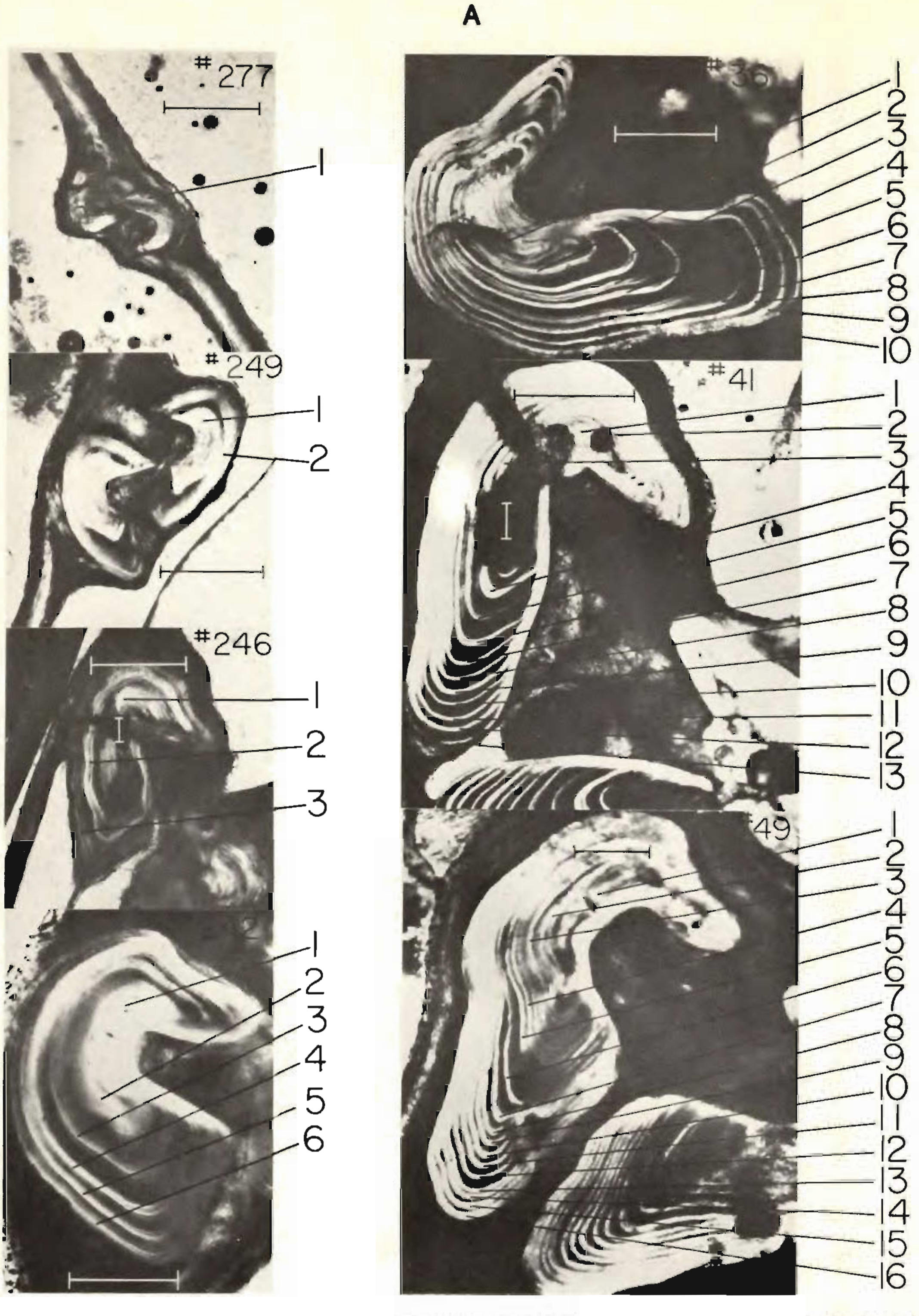


FIG. 29. (A, B) Scales and fin rays from lingcod to show that scales are not reliable for older fish. Fish No. 277, 249, 246, and 242 were captured in late October and measured 23, 33, 50, and 69 cm, respectively. Fish No. 36, 41, and 49 were captured in June and measured 97, 103, and 115 cm, respectively.

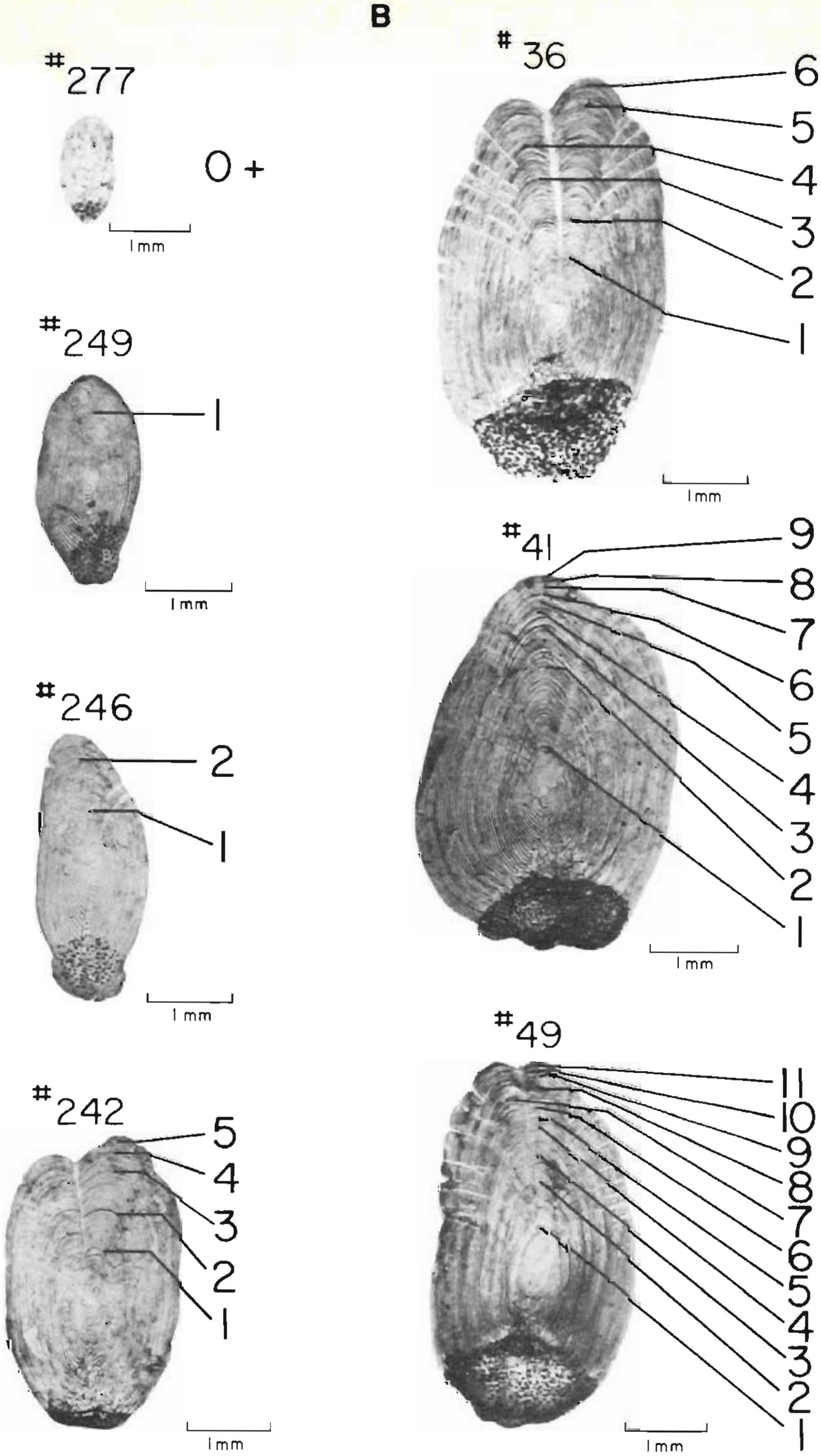


FIG 29. Continued.

some patience and experience. The position on the ray where sections are taken must be carefully selected and sections of varying thickness should be used. Adequate transmitted lighting along with a quality microscope is essential. Other fins can be used but cross sections of the 4th–8th dorsal fin rays provide the clearest annuli. For juvenile fish we suggest that length is the best indicator of age. Scale ages are acceptable to about age 5 or 6 (Fig. 29B), but after this they can underestimate the true age by as much as 8 yr. Sections of dorsal fin rays should always be examined when accurate ages for older fish are required.

The first two annuli may be difficult to identify for young fish because of a lack of prominence or the presence of checks and for older fish because of the resorption of the fin-ray center. In both cases an estimate of the position of the third annulus can be made by measuring the width of the first and second annuli from juvenile fish or fish where these annuli are visible. Often it is possible simply to examine more sections, particularly from those rays that grow relatively slowly. As fish age, the first few annual growth zones may become translucent (Fig. 29: No. 242, No. 36). Again it is nearly always possible to identify the third annulus.

The problem of identifying checks can be partially solved by comparing sections of slow- and fast-growing rays. The checks that are present in fast-growing rays seldom appear in the slower growing rays. In general, a check is much narrower than an annulus and is rarely continuous around the ray. In this laboratory, if a translucent zone forms in close association with another translucent zone of about equal width and they both are prominent in all sections and both are continuous around the ray, then they are each considered to be annuli. This rule does not apply to fish younger than age 3.

PLEURONECTIDAE (FLATFISH)

For the commercially important species of flatfish, the external surface of the otolith has been used for age determination. Recently, it has been demonstrated in this laboratory that additional procedures must be followed to obtain accurate ages. Some of the species will be discussed individually and photographs provided to illustrate the growth zones on the external surface and on cross sections of the otoliths.

Surface otolith readings are usually satisfactory for ages up to 7 yr. Beyond this age, the growth zones on the surface tend to be less discernible, becoming narrow and crowded near the edge. The older otoliths, particularly from males, grow thicker and all annuli cannot be identified from the external surface. Bläckner (1974) and this laboratory have observed a thickening of older flatfish otoliths primarily through deposition on the internal surface, as growth zones accumulate in this area. Broken and burnt otoliths or thin sections indicate that flatfish in our area are older than previously throught. Therefore, the breaking and burning technique is recommended, particularly when the otoliths are thick and difficult to age. If annuli are difficult to distinguish using the breaking and burning method then thin sections must be made. Mouting sections of otoliths on slides and examining these with transmitted light under a compound microscope should, under optimum conditions, yield similar results to the breaking and burning technique. However, sections are technically more difficult to prepare.

ARROWTOOTH FLOUNDER (TURBOT) *(Atheresthes stomias)* (Hay 1966)

An examination of the external surface of the turbot otoliths appears to provide a satisfactory aging method for the first few years (Fig. 30A). The older otoliths are often cloudy and difficult to age on the surface because of the many checks (Fig. 31). Dipping them in a solution of 20% HC1 occasionally improves the clarity. The use of thin or broken and burnt sections should be employed along with the surfaces to obtain accurate ages. Hence, the benefit of collecting two otoliths from each fish. Figures 30B, 32A, 32B, and 33A–C are photographs of thin sections from turbot otoliths. The annuli are fairly clear on the younger sections (Fig. 30B), but some problems occur on the older otoliths with crowded annuli and checks that form near the edge. The use of a higher magnification to observe the crowded zones on the cross section usually eliminates the density problem and enables the separation of annuli from checks.

PACIFIC HALIBUT *(Hippoglossus stenolepis)*

As part of a cooperative study with the International Pacific Halibut Commission (IPHC) to compare age determination estimates, this laboratory used the break and burn technique for otoliths and IPHC staff examined the surface of the otoliths. Preliminary results from our laboratory indicate that ages of very old halibut may be underestimated if only the otolith surface is examined. The study is

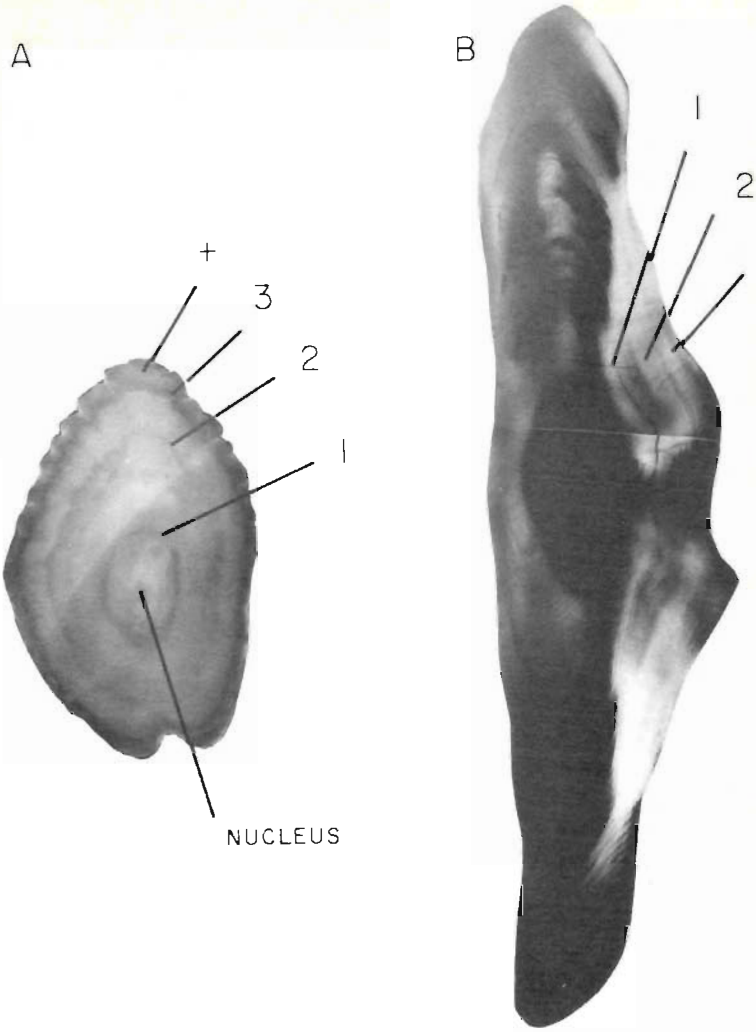


FIG. 30. (A) A surface of young turbot (arrowtooth flounder) otolith. (B) Thin section of a young turbot otolith.

continuing and results will be published when the study is complete.

A Buehler sectioning machine is used to cut otoliths for the burning procedure that are too thick to break through the center by hand. On the burnt section, the first and second annuli, which vary in prominence, are usually associated with several checks and can be difficult to locate. The remaining growth zones form a clear pattern of alternating translucent and opaque bands especially along the sulcus edge (Fig. 34A, 34B, 35A, 35B). Occasionally, there is difficulty with a convolution of growth zones or with a blurred area on the burnt section (Fig. 36A, 36B) but upon examination of an alternative area an age can usually be determined.

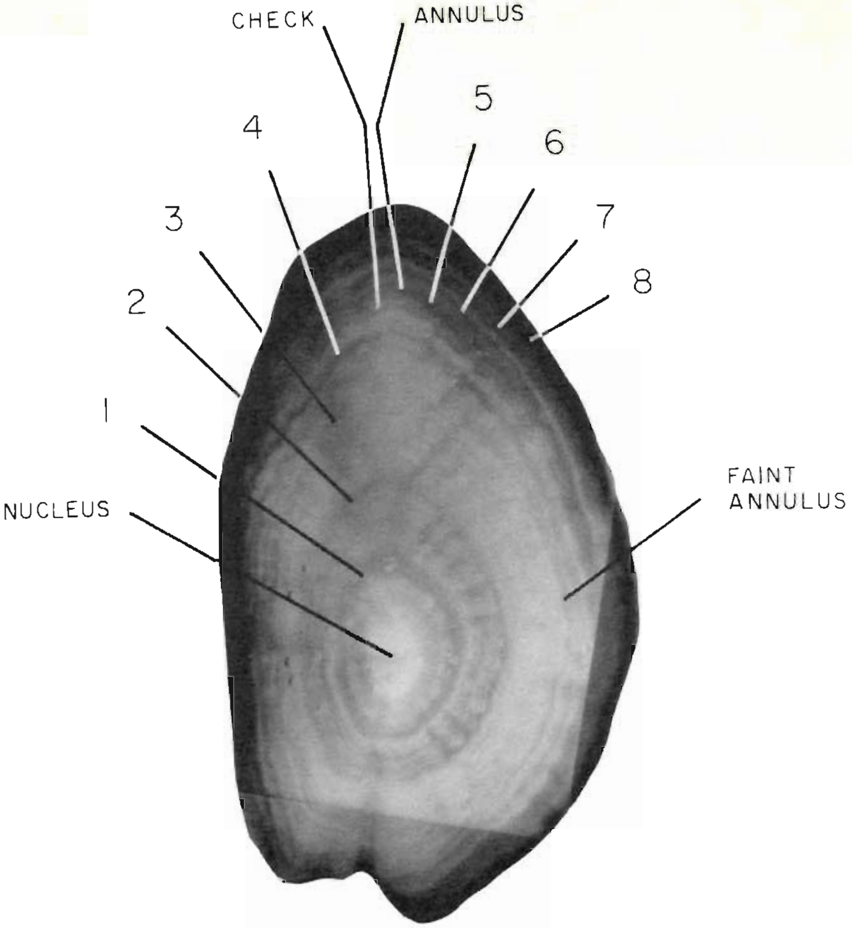


FIG. 31 Surface of an older turbot otolith. The third annulus is indistinct and the fifth annulus appears with a check close by. There are many checks for the first few years. Annuli beyond the 8th yr are not indicated because of the dark edge.

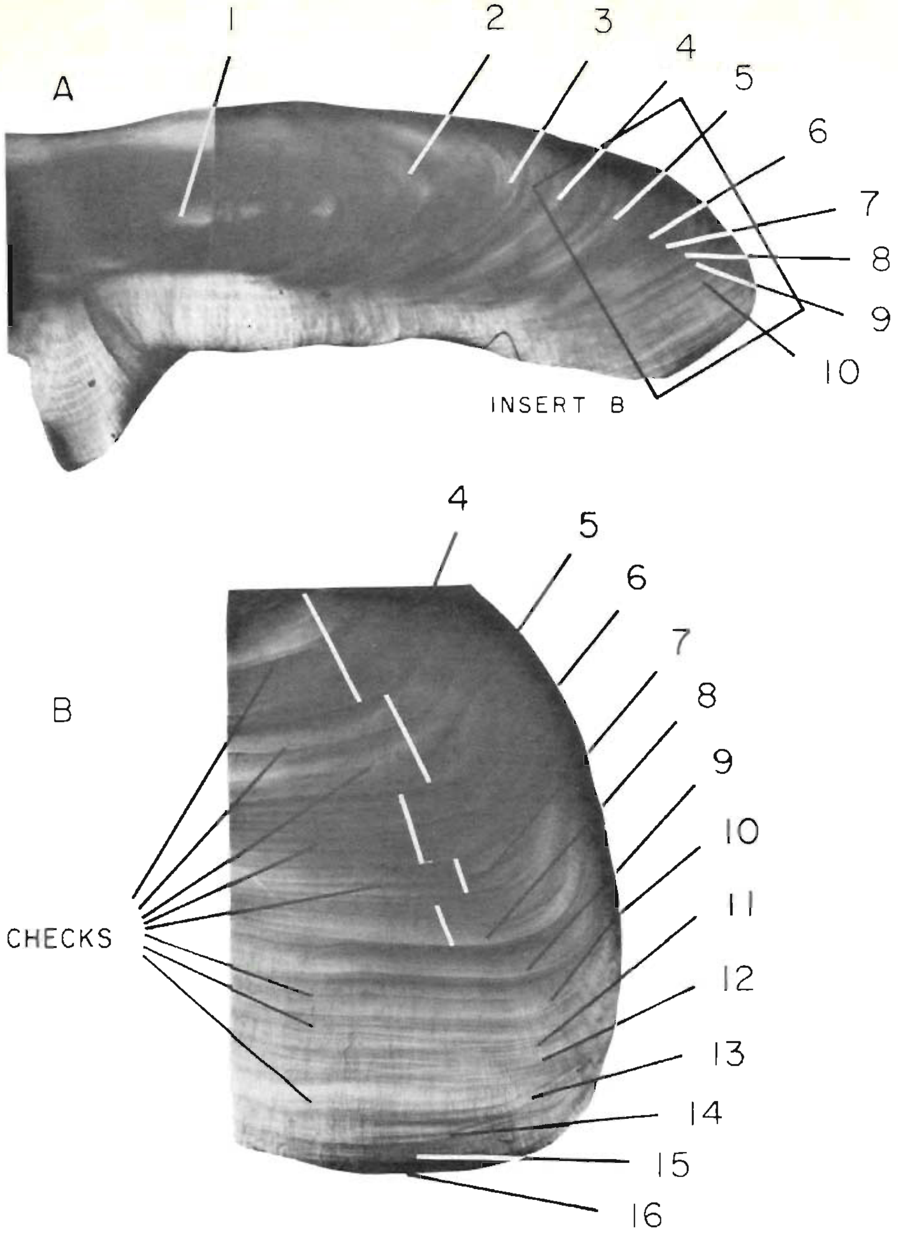


Fig 32. (A, B) Thin cross section of a turbot otolith showing an enlargement of a difficult counting area.

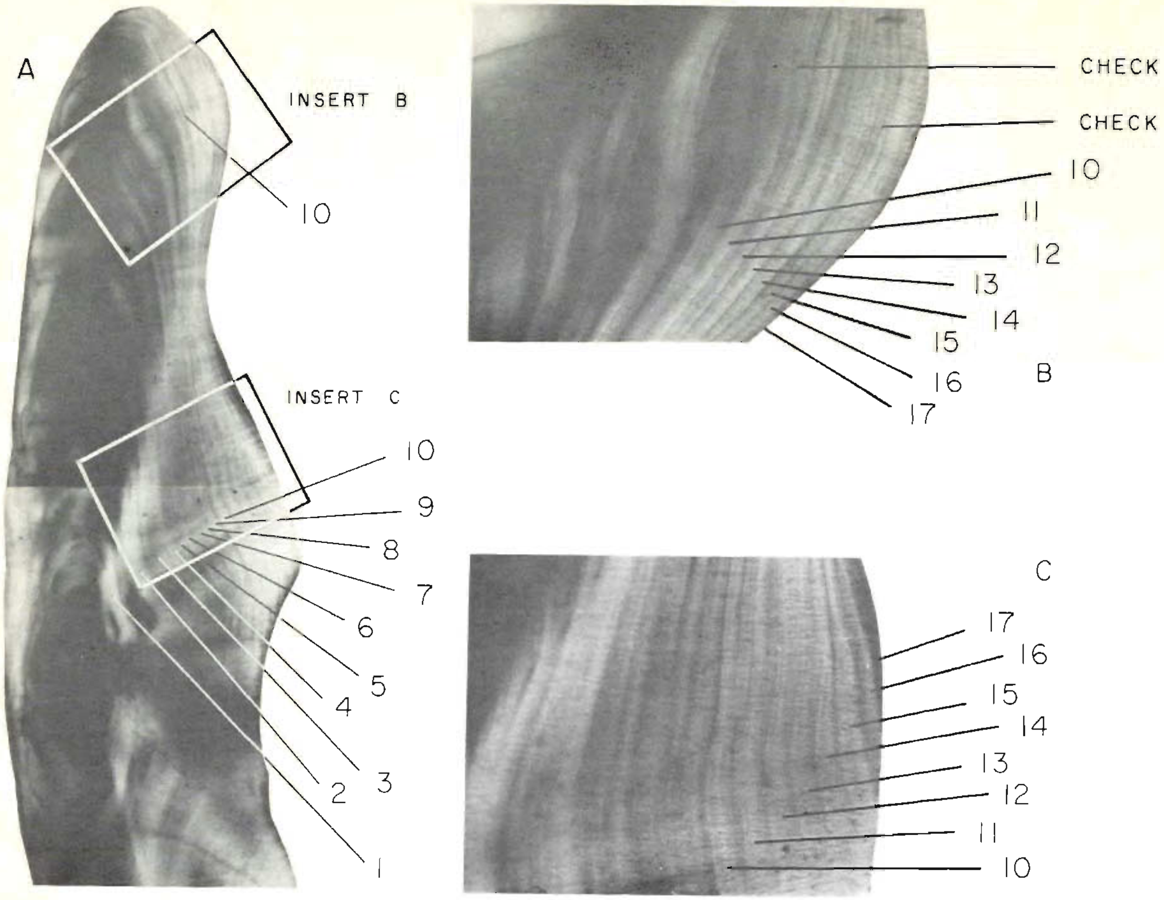


FIG. 33. (A–C) Thin cross section and enlargement of an older turbot otolith showing crowded zones and numerous checks

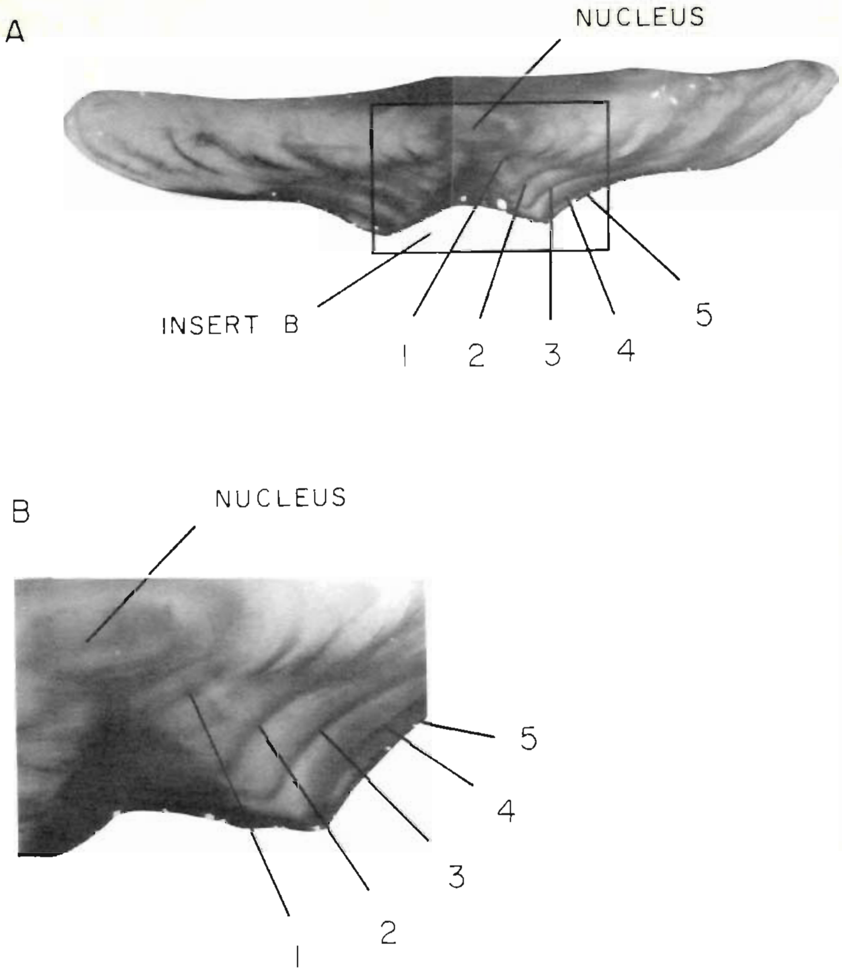


FIG. 34. (A) Broken and burnt section of a young halibut otolith showing growth zones on the internal surface. (B) Enlargement of the counting area along the sulcus edge.

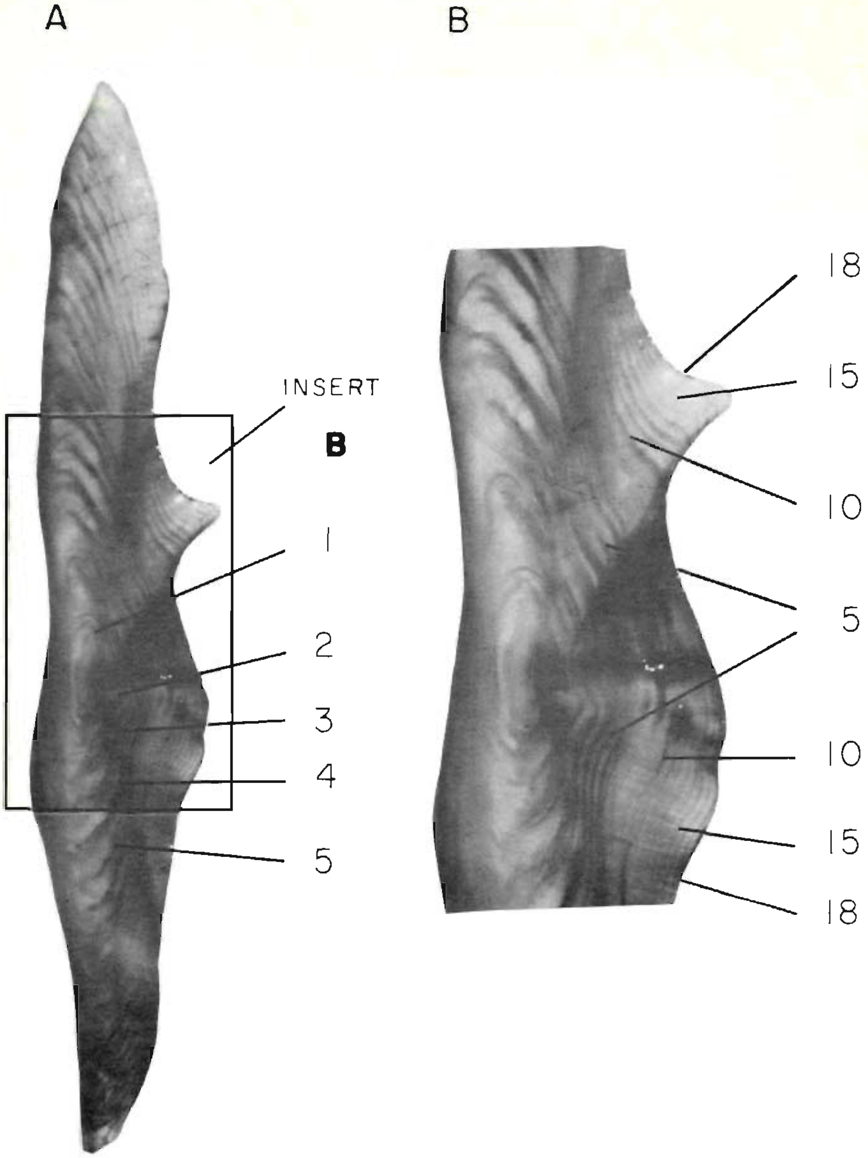


FIG. 35. (A) Broken and burnt section of an older halibut otolith showing growth zones on the internal surface. (B) Enlargement of the counting area along the sulcus edge.

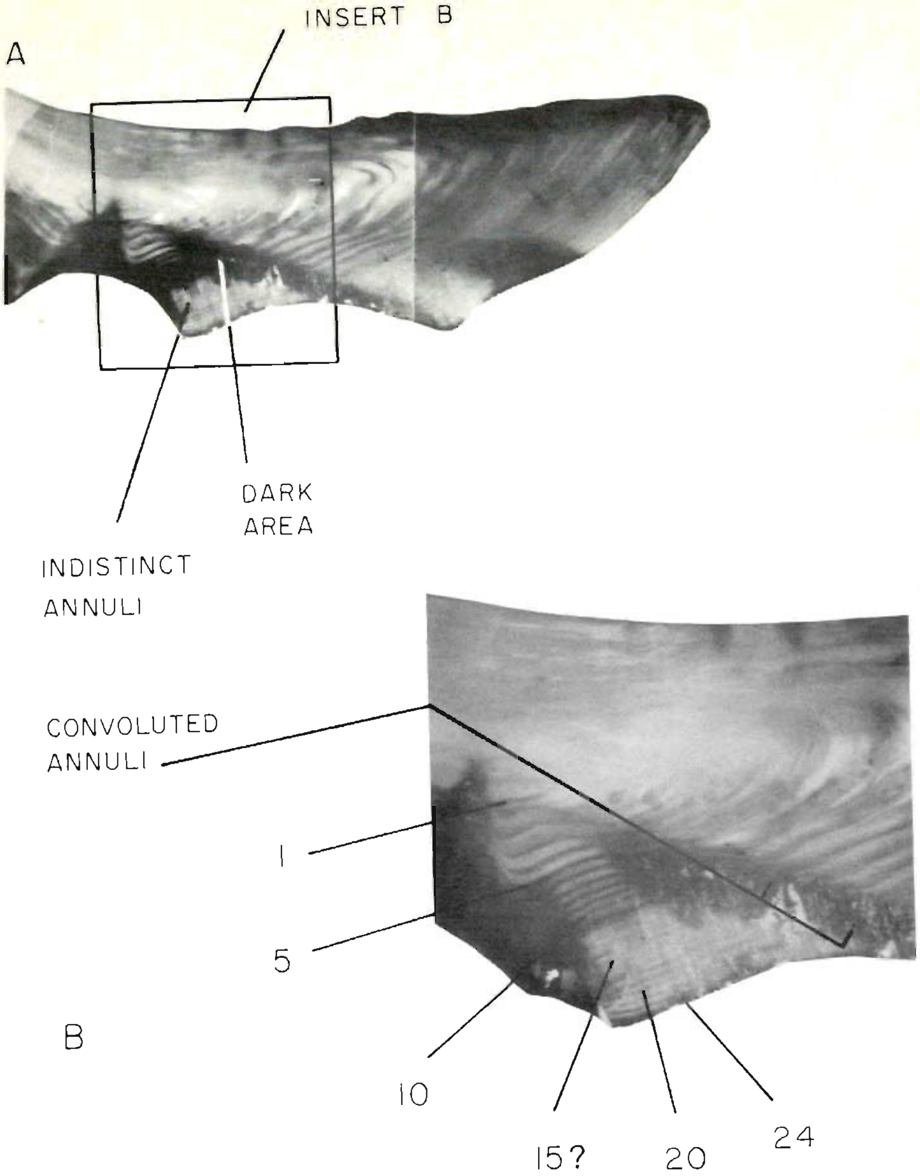


FIG. 36. (A, B) Broken and burnt otolith section of an older halibut otolith and enlargement of one counting area showing problems such as indistinct zones, convolution of zones, and dark areas.

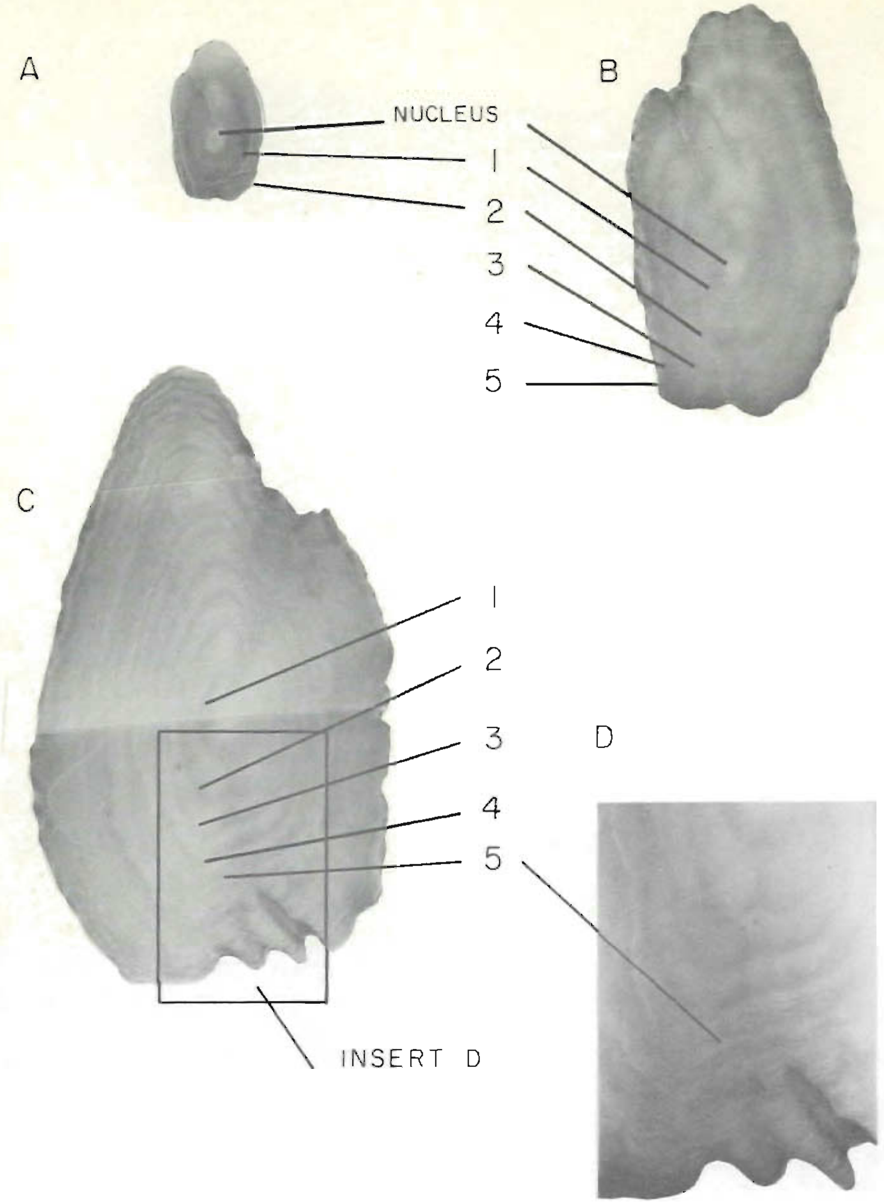


FIG. 37. (A, B) External surfaces of young halibut otoliths showing clear, widely spaced growth zones. (C, D) External surface of an older halibut otolith showing crowded growth zones in later years.

On the external surface, the first few annuli appear to be easily located (Fig. 37A, 37B). The amount of deposition between the nucleus and the first annulus appears to vary, probably between sexes and geographical areas. The remaining annuli, although not always distinct because of numerous checks, seem to form a fairly regular pattern (Fig. 37C, 37D). Several examinations along various regions of the otolith surface should be made to obtain a confident age.

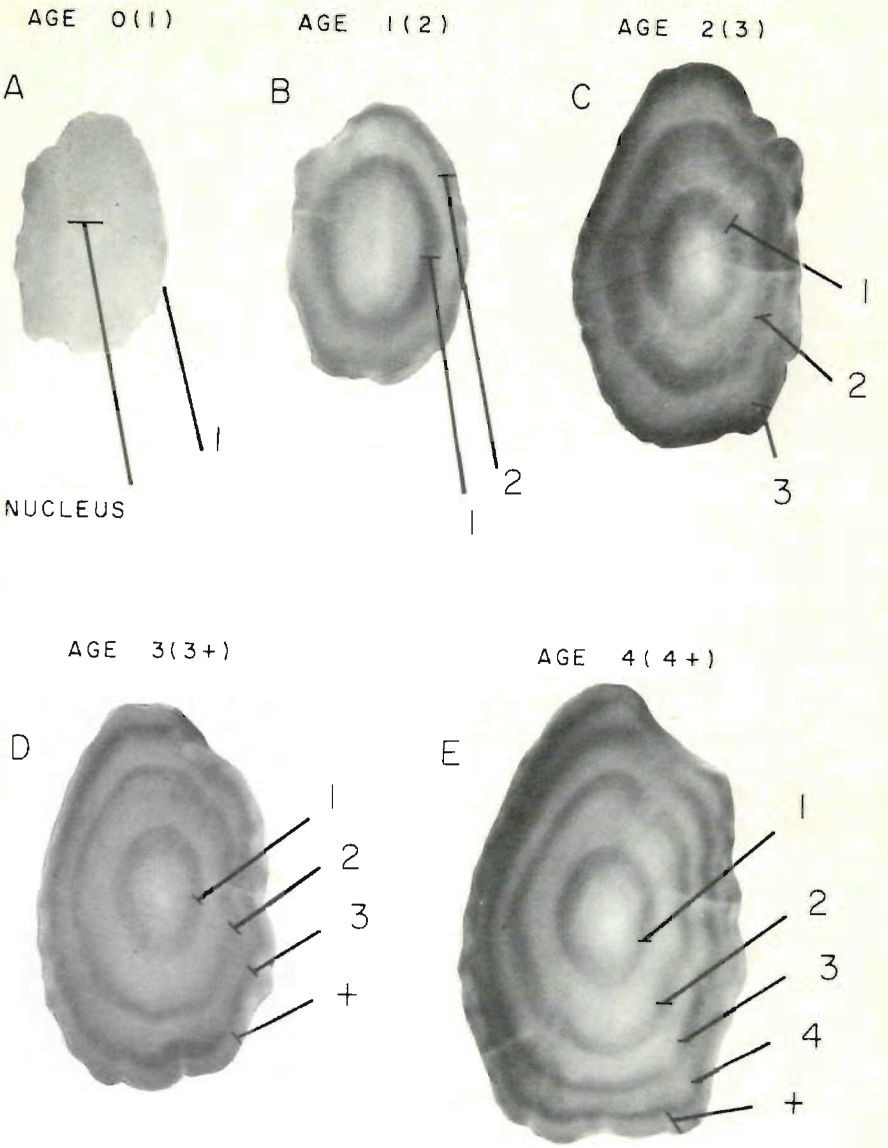


Fig. 38. (A–E) Surfaces of young rock sole otoliths. Otoliths (A), (B), and (C) were taken from fish caught late in the year and each has an annulus on the edge.

ROCK SOLE *(Lepidopsetta bilineata)*

The ages for young rock sole can be determined using either the external otolith surface (Fig. 38A–E) or the broken and burnt section. To obtain accurate ages for older fish, a thin cross section or a broken and burnt section of the otolith must be examined (Fig. 39A, 39B, 40A, 40B). Growth zones on the cross sections are usually clear and evenly spaced and gradually decrease in width after about 6 yr. The nucleus appears as a distinct small dark area and is easily identified.

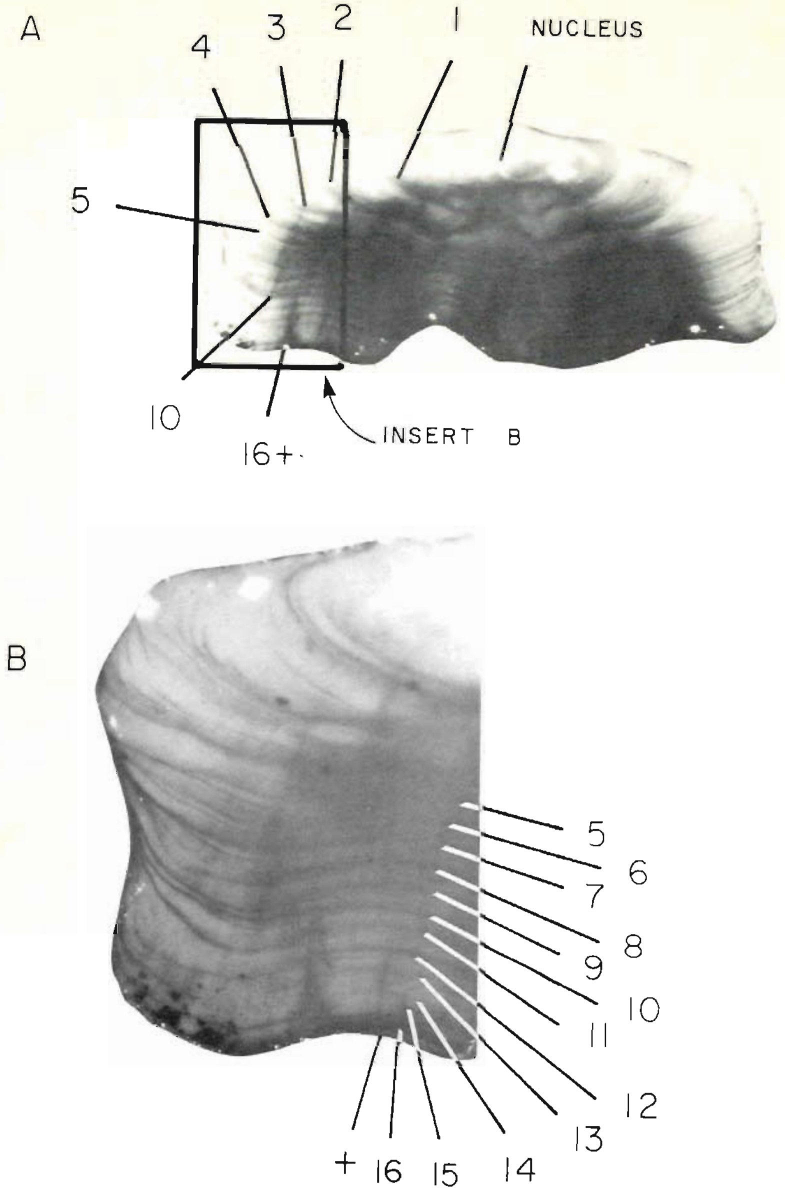


FIG. 39. (A, B) Burnt cross section and enlargement of a counting area on an older rock sole otolith.

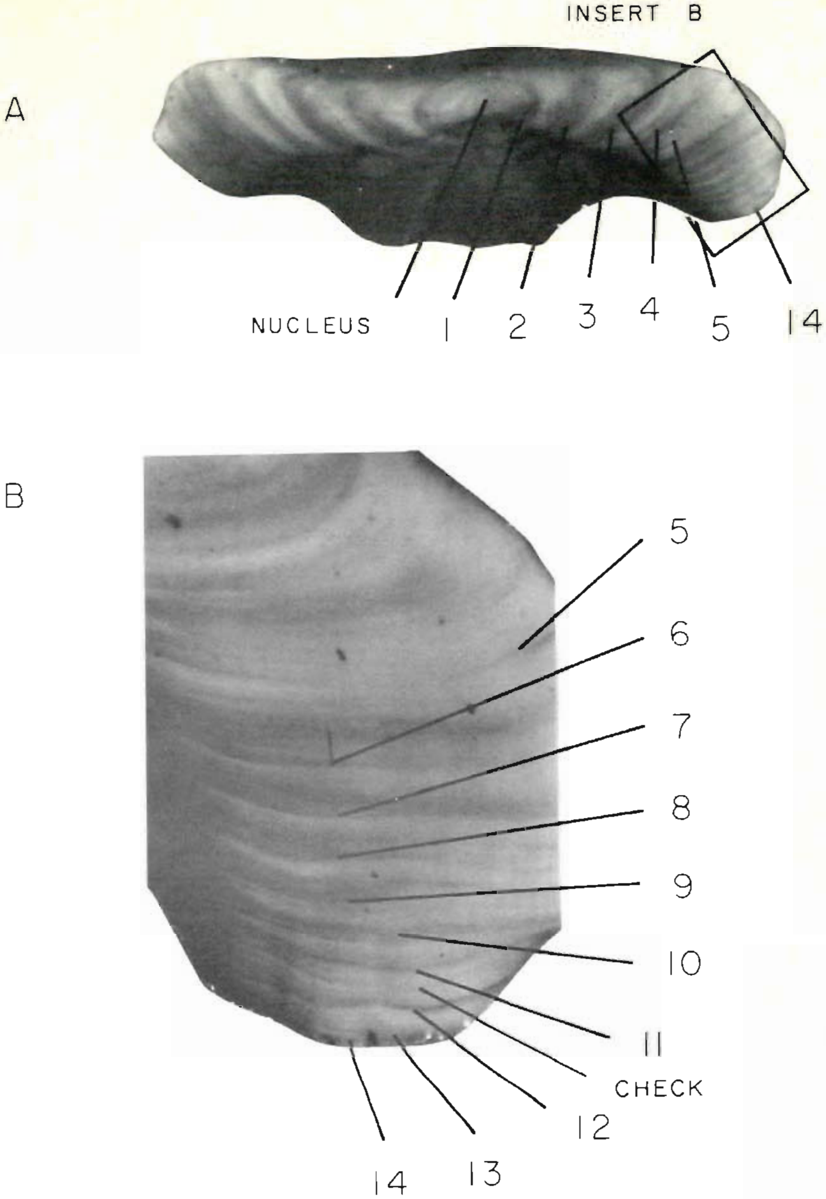


FIG. 40. (A, B) Burnt cross section and enlargement of a counting area on an older rock sole otolith

DOVER SOLE *(Microstomus pacificus)*
(Demory 1972)

According to Demory (1972), scales appear to be satisfactory for aging most Dover sole, except the large males. As mentioned, we feel that scales are not always the most reliable structure for aging older fish and should only be used if there is an independent method of confirming the interpretation (see Beamish et al. 1976; Mills and Beamish 1980; Power 1978). Dover sole scales also are difficult to sample. The scales are deeply embedded in the dermis of the skin and sampling must be done with care to ensure that the scales do not break leaving part of the scale in the fish skin. Some samplers prefer to take a flap of skin from the fish when

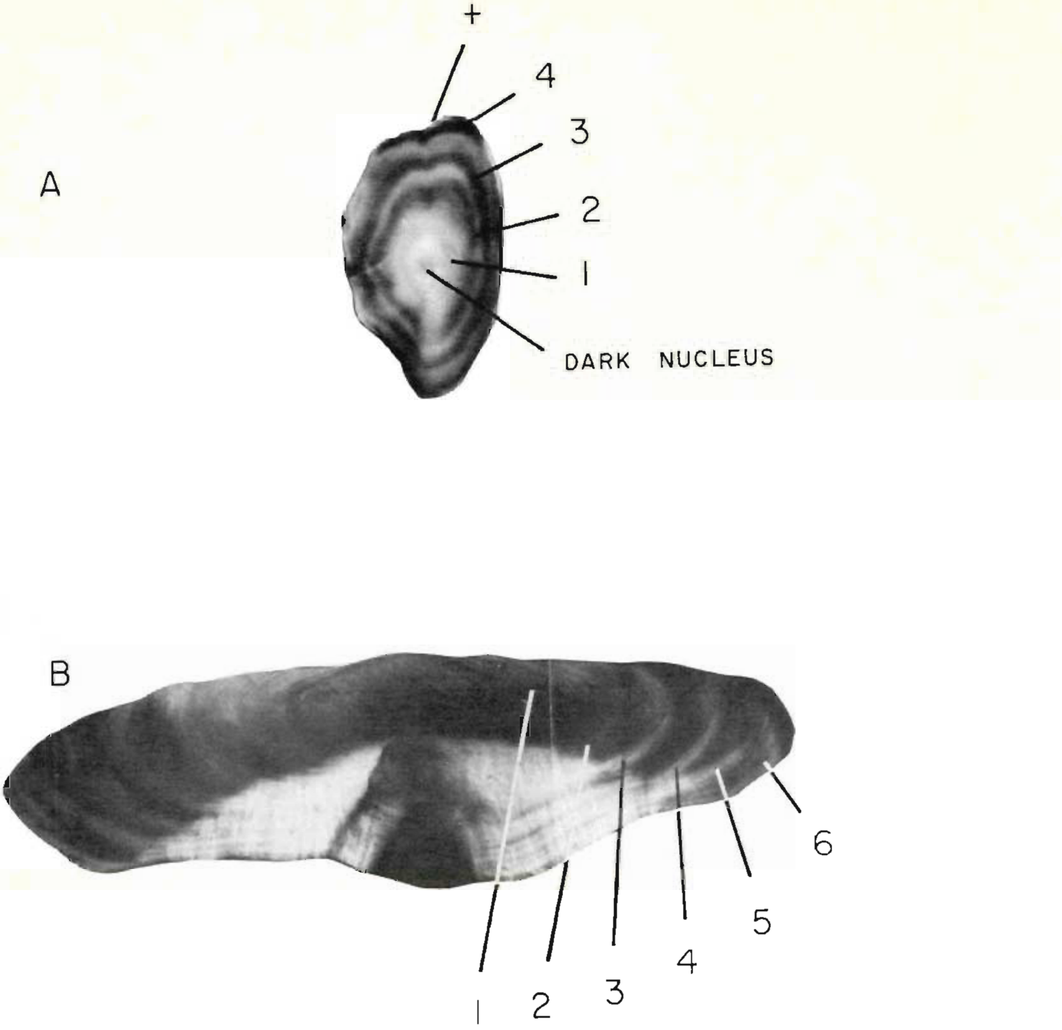


Fig. 41. (A) Surface of a young Dover sole otolith exhibiting a faint first annulus after the black center or nucleus. Positioning of the first annulus should be confirmed by obtaining "known-age" (by length) juvenile fish (see text). (B) Thin cross section of a young Dover sole otolith.

sampling at sea or at the dockside and remove the scales in the laboratory. Regenerated scales are extremely common.

A comparison of ages using scales, otolith surfaces, cross sections of otoliths, and broken and burnt sections indicated that ages obtained using cross sections of otoliths were much older than ages obtained from the otolith surface. Scales produced lower ages than any of the structures; therefore we recommend that they should only be used for young Dover sole.

Dover sole otoliths are small when compared with those of other flatfish and they can become very thick. In this laboratory, the first annulus was counted as the first translucent zone outside of the dark nucleus (Fig. 41A). The position of the first annulus should be confirmed by examining "known-age" (by length) juvenile fish which were not avail-

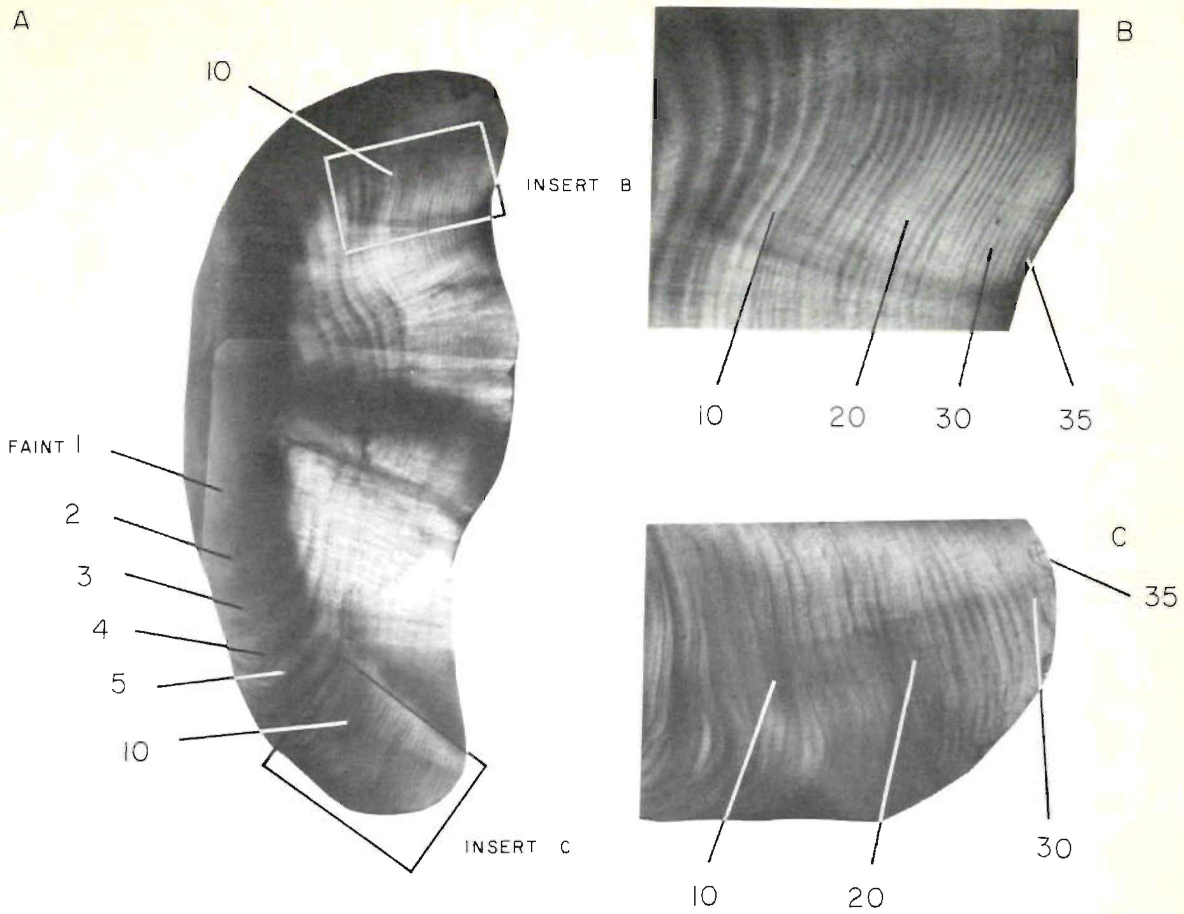


Fig. 42. (A–C) Thin cross section and enlargements of counting areas of older Dover sole showing the very dense and compacted annuli.

able. We speculate that the first annulus is poorly defined and otolith growth is relatively slow during the 1st yr, possibly as a consequence of the Dover sole's pelagic existence during its 1st yr of life (Carey et al. 1981). Surface ages appear satisfactory for the first 7–8 yr but beyond this age a cross section of the otolith should be examined as the internal surface provides a clearer view of the annuli.

Thin sections should be made when the otoliths have become too thick to break for the burning method. Annuli for the first few years can be readily identified on the sections but a higher magnification is often necessary to separate and follow the crowded growth zones that form towards the edge of the otolith. Figure 41B shows the clarity of annuli from a section of an otolith from young Dover sole; Fig. 42A–C, 43A, 43B are examples of thin otolith sections from much older Dover sole.

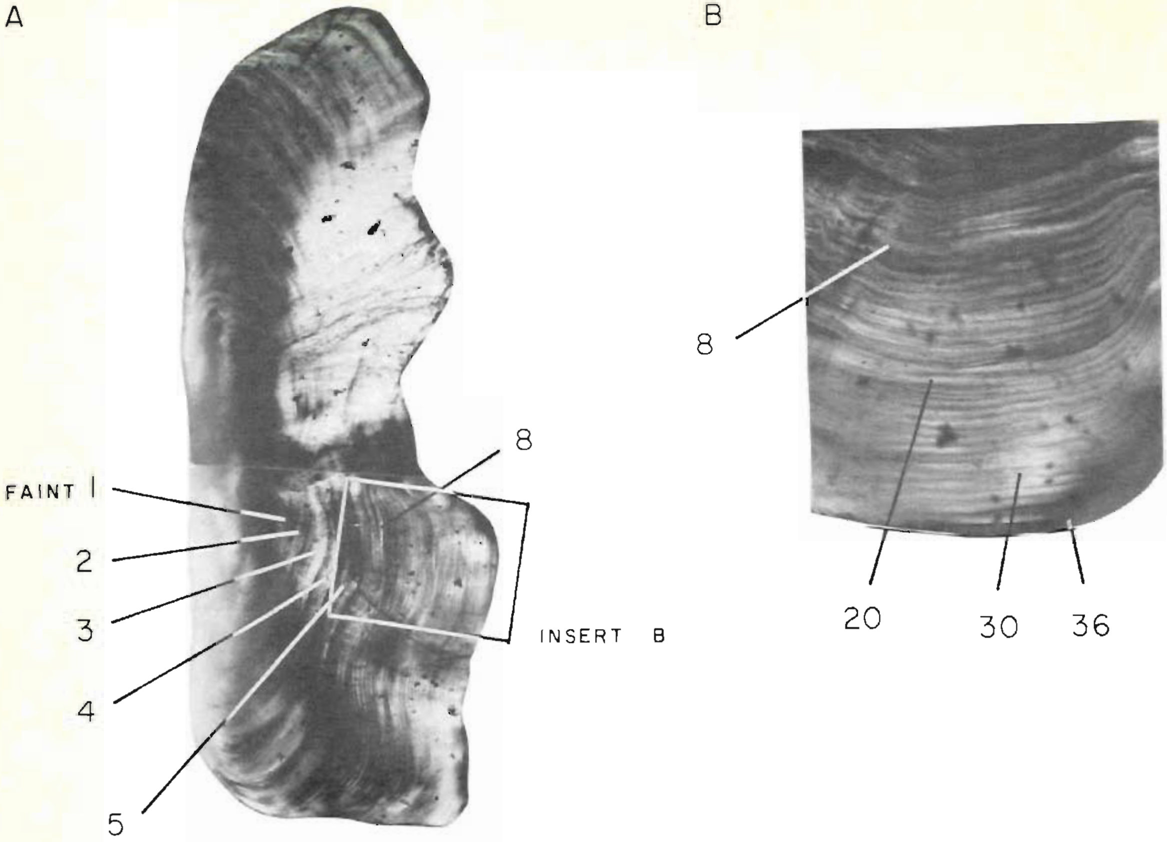


FIG. 43. (A, B) Thin cross section and enlargement of counting area of older Dover sole showing the very dense and compacted annuli.

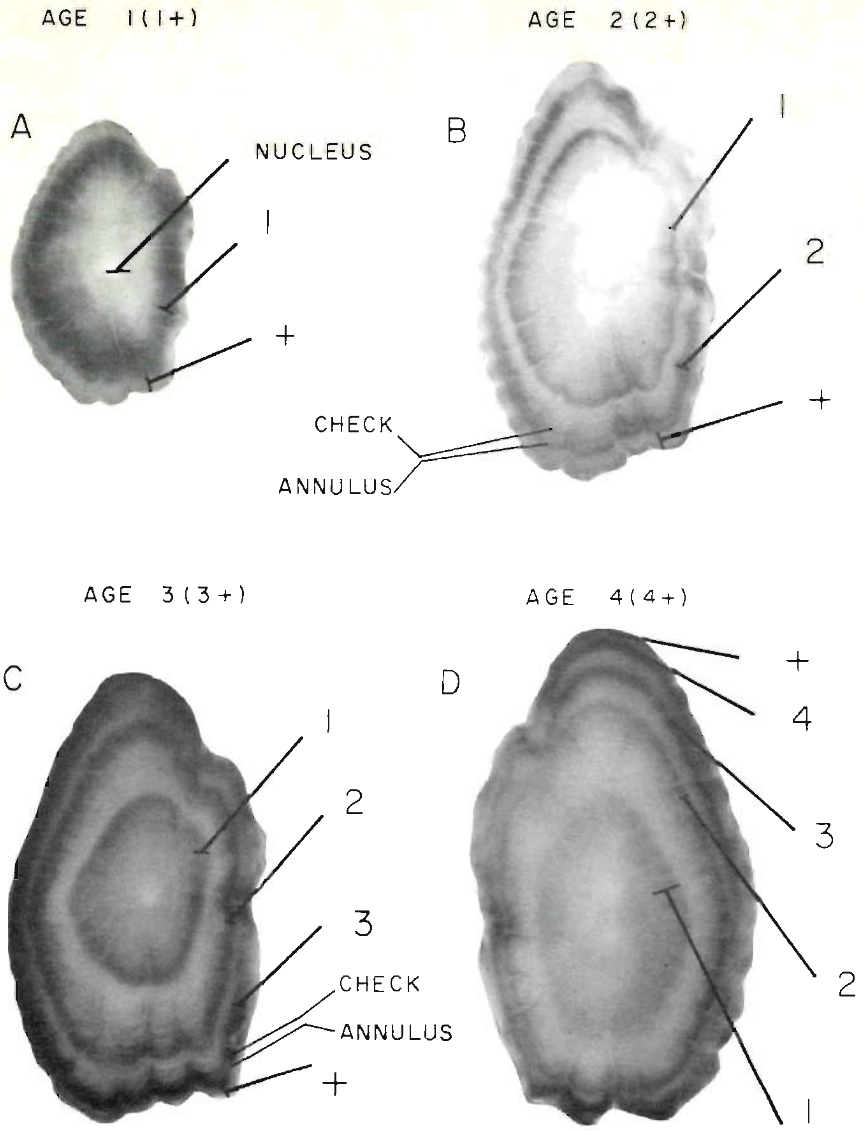


FIG 44. (A–D) Surface of young English (lemon) sole otoliths. Note the relatively large amount of growth from the nucleus to the first annulus, as compared with rock sole and Dover sole.

ENGLISH (LEMON) SOLE *(Parophrys vetulus)*

The external surface of English sole otoliths has clearly marked annuli for the first few years (Fig. 44A–D). However, our unpublished studies indicate that after an age of about 8 yr, the surface ages may underestimate the true age of the fish. Therefore, thin cross sections or broken and burnt sections which expose the inner surface of the otolith must be examined to obtain accurate ages for older individuals. A distinguishing feature of these otoliths is the fairly large amount of growth which is laid down before the formation of the first annulus. The annuli on the burnt sections are usually prominent and evenly spaced with the growth zones gradually decreasing in width as the fish grows older. Sections of some of the thicker otoliths can be aged up to 21 yr (Fig. 45A, 45B).

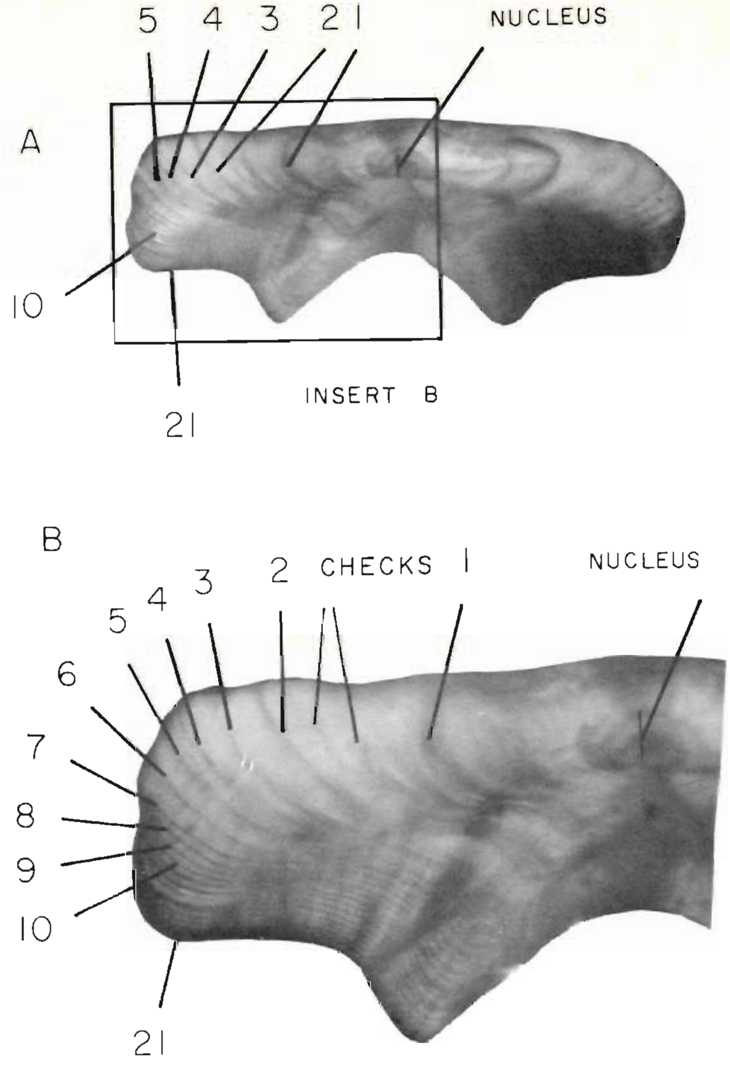


FIG 45. (A, B) Burnt cross section and enlargement of an older English sole otolith.

SCOMBRIDAE

ALBACORE *(Thunnus alalunga)* Beamish 1981)

Developing a successful aging method for albacore has been a difficult problem for fisheries biologists and many methods have been tried and rejected. The fin-ray method appears to provide a rapid and convenient method of assessing age for albacore and possibly other tuna; however the method has not been validated.

A study of the suitability of the dorsal, pectoral, pelvic, and anal fins indicated that the dorsal and anal fins were the most satisfactory for albacore age determination (Fig. 46A–H). The procedure for preparing and sectioning these rays differs slightly from other species because of the thick fin rays.

Two methods are used to mount tuna fins depending on their condition. Some amount of force must be used when removing the tough fins. This

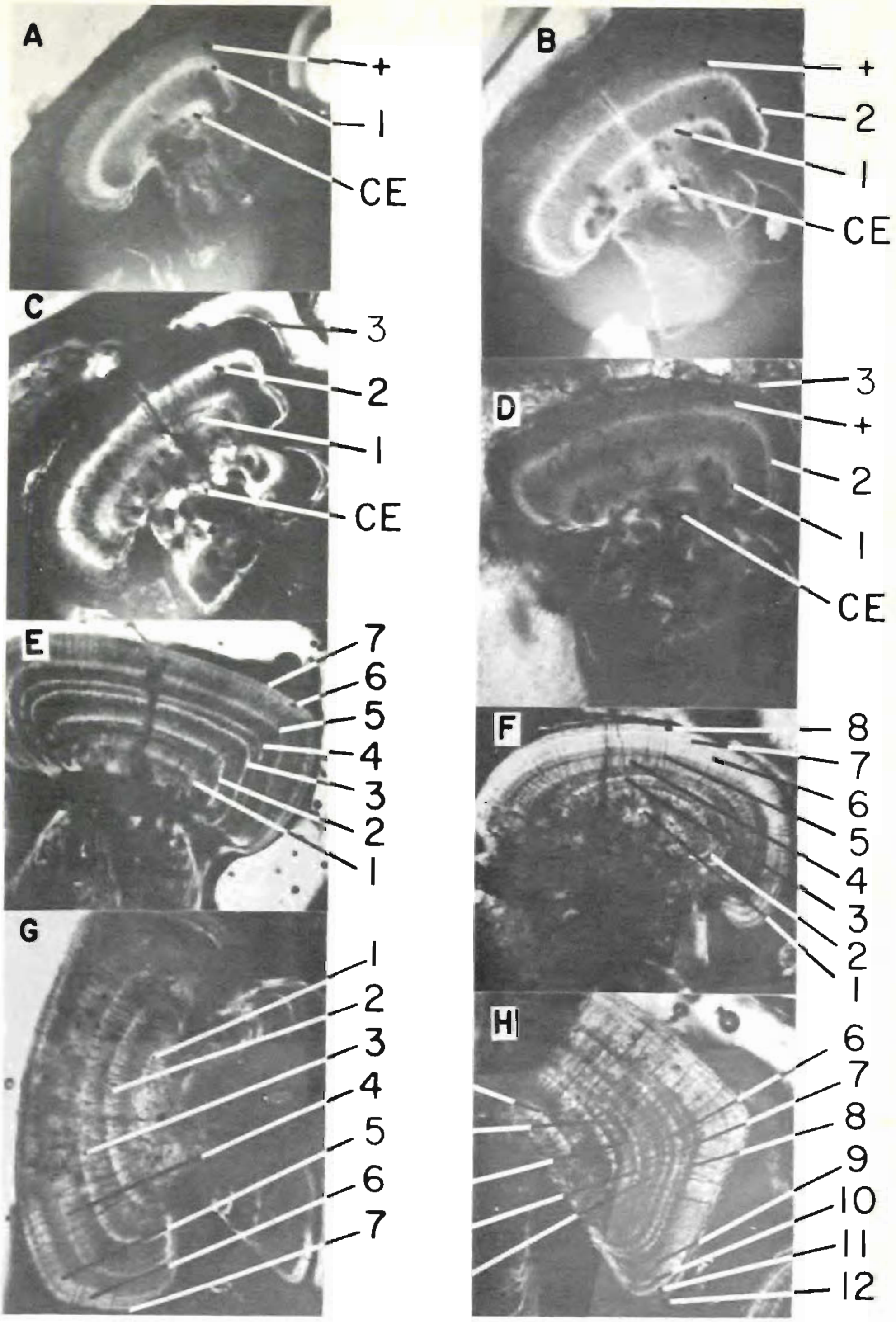


FIG. 46. (A-H) Examples of fin sections from albacore tuna. CE indiates the center of the fin ray. (A) Section from anal fin of a 1-yr-old fish, 63 cm. (B) Section from anal fin of a 2-yr-old fish, 71 cm. (C) Section from the second dorsal fin of a 3-yr-old fish, 76 cm. (D) Section from anal fin of a 3-yr-old fish, 76 cm. (E) Section from second dorsal fin of a 7-yr-old fish, 117 cm. (F) Section from the anal fin of a 8-yr-old fish which was difficult to interpret because of the diffuse nature of some translucent zones. (G) Section from the anal fin of a 7-yr-old fish, 99 cm. Three annuli are close to the edge of the ray. (H) Section of an anal fin was not aged, 110-cm fish. Twelve translucent zones are evident but zones could not be separated around the whole circumference.

may break or shatter some of the fin rays and in such cases Method 1 is used. If the fin is whole, Method 2 is used.

Method 1 — Dip the dried fins in warm water and peel off the skin with a scalpel. Separate each ray by cutting through connecting tissue with a scalpel. If possible, use the first 6 rays; however the first 10–12 rays are usable. Shape a 2.5 X 2.5 cm square of epoxy on labeled parafilm. Dip each ray into toluene and place each one so that the rays are parallel and bases are at right angles to the length of the ray. Spread epoxy over all the rays, ensuring that they stay in position. The epoxy should reach about 3.5 cm up from the fin-ray base on all sides (Fig. 47A).

Method 2 — When the fin is unbroken and dried so that it is flat, cut through the skin and connecting tissue with a scalpel after about the fifth to sixth ray (depending on size of the fin) to reduce the width of the fin for sectioning. Mount the rays while still connected by the skin. Dip in toluene and spread epoxy over the rays to 3.5 cm above the ray base. Dry fins 1–2 d until the epoxy is hard.

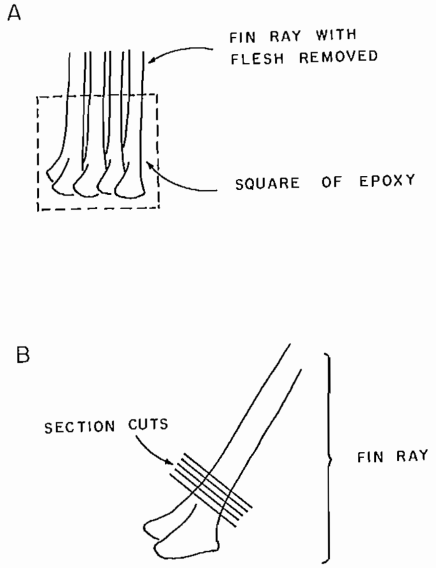


FIG. 47. (A) Method 1 for mounting tuna fin rays. The illustration shows the positioning of the stripped fin rays in the square of epoxy. (B) Drawing showing the area from which to take the best sections from the tuna fin rays, usually 1-1.5 cm above the basal structure of the ray.

The best sections for aging seem to come from sections 1–1.5 cm above the basal structure of the ray (Fig. 47B). Make perpendicular cuts about 1 mm thick through the mounted fin rays using the Bronwill thin sectioning machine. Sturdy tweezers are used to steady the fin as the rays and epoxy provide a lot of resistance when sectioning. Place sections on a labeled slide in sequential order and cover with a liquid cover slip such as Flo-texx.

As previously mentioned, the interpretation of growth zones from fin-ray sections requires experience. The reader must ensure that the best possible sections are obtained and that they are viewed with a suitable research microscope. Difficulties experienced by most novice readers result from poor preparation and inadequate equipment for viewing the material.

There are two major problems with the fin-ray method as applied to albacore. The position of the first annulus has not been confirmed because known age young-of-the-year fish and age 1 fish have not been examined. At present, it is assumed that the first translucent zone that is visible is the first annulus.

The second difficulty is the number of checks that may be present. The problem can be minimized by examining sections from both slow- and fast-growing rays. The sections from fast-growing rays (wider ray sections) often will have checks that are not visible on slower growing rays. However, on some sections it simply is not possible to separate checks and annuli. Although the method shows considerable promise, it must be validated by a mark and recapture procedure before the ages can be considered accurate.

SCORPAENIDAE (ROCKFISH)

The surfaces of whole otoliths have been used for age determinations of rockfish for many years. Recently, a comparison of age determinations from otolith surfaces and from otolith sections showed ages estimated from otolith surfaces may be accurate only to about 15–20 yr (Beamish 1979b). Thin sections and broken and burnt sections have indicated that rockfishes are much older than previously thought and may reach ages of 80–140 yr (Fig. 48A–E, 48CC, 48DD, 48EE). The criteria used to determine the ages of all rockfishes are basically the same but growth patterns and growth rates differ among species so we have attempted to illustrate and describe otoliths from several species.

It is now routine in this laboratory to break and burn all rockfish otoliths within a sample or make a thin section if the broken and burnt section is difficult to interpret. Burning partially eliminates many problems such as checking as this procedure enhances the true annuli. If the burn fails to establish the first few annuli clearly then the position of these annuli should be determined from the external surface of the otolith.

Lighting and the angle at which the broken and burnt otoliths are observed is important; therefore, the reader must be adept in manipulating the otolith to produce the best light angle. To enhance the clarity of the growth zones the broken and burnt surface must be coated with a light oil such as cedar wood or cooking oil.

The annuli of all older rockfish cannot be identified around the surface of the entire otolith because as the fish grows older and the growth rate slows down, otolith growth is concentrated along the internal surface. On burnt otoliths, a zone is usually not designated as an annulus unless it can be traced continuously from the sulcus edge to the dorsal or ventral tip.

In the succeeding pages, photographs are provided and methods are described for aging some of the commercially important species. For those not mentioned, it is recommended that sections or broken and burnt otoliths always be used as a comparison with the surface ages. If surface age determinations are preferred, these estimates should be checked with section estimates. Scales should never be used unless the method has been validated or ages produced are similar to ages obtained using other structures.

PACIFIC OCEAN PERCH *(Sebastes alutus)*
(Westrheim 1973; Beamish 1979b)

Pacific ocean perch otolith formation is uniform and even, with growth zones appearing on the internal surface indicating that Pacific ocean perch can grow very old (Beamish 1979b). The distinction between the nucleus and the first annulus is usually made clearer by breaking and burning the otolith and the first annulus appears as a dark band around a central white nucleus. The remaining annuli show a very even, easy to read growth pattern (Fig. 49A–D). If problems arise with blurred zones and uneven growth on the burnt section (Fig. 50A, 50B) it is recommended that a thin cross section be cut from the unburnt otolith and examined under a higher magnification to ensure accurate counting of the closely spaced annuli (Fig. 51A–D). Figure 52A–C shows a cross section and enlargements of an older otolith. Otolith growth patterns vary greatly among geographic areas and there is some indication that there is also a growth difference between sexes.

The otolith surface contains a nucleus which appears as a group of indistinct rings made up of "lobes." The nucleus is often sufficiently distinct to be confused with the first annulus (Fig. 53A) and the first annulus on the surface of the otolith may be difficult to identify because its radius from the nucleus appears to vary significantly even among otoliths in a single sample. However, with experience and measurement of juvenile specimens this problem can be minimized. Surface ages determined along the posterior and dorsal area of the otolith as described by Westrheim (1973) (Fig. 53A–C) show some merging of annuli occurs along the ventral side after 5 or 10 yr; therefore, aging along this area is not recommended. Figure 54A, 54B shows a surface of an older Pacific ocean perch otolith with a lobe formation on the posterior end.

SILVERGRAY ROCKFISH *(Sebastes brevispinis)*

Breaking and burning silvergray rockfish otoliths provides a clear view of the nucleus and the first few annuli, as well as closely spaced annuli nearing the edge (Fig. 55A–C). Although the otoliths grow quite large and thick, they are easy to break in half through the nucleus and they burn evenly in most cases. On the burnt surface, the first annulus appears as a dark band around a central nucleus. The position of this annulus may vary among stocks. There may be some checks between the nucleus and first annulus but the remaining annuli are usually clear. If problems should occur on the burnt surface with indefinite or crowded zones a thin section should be made and examined under a higher magnification. Annuli are usually identified in the area of the sulcus edge and visible along the interior dorsal surface of the section.

The otolith surfaces consist of broad growth zones that are evenly spaced and these zones may vary among stocks. The relatively small amount of growth to the first annulus is very distinct (Fig. 56A). Because the posterior end of the otolith contains many checks it is usually more accurate to count down the dorsal side (Fig. 56B), and then around the posterior end when growth slows down. After 12–15 yr of growth, the otolith surface starts to form "fingerlike" projections on the posterior end (Fig. 56A, 56C). The fingers grow out flat instead of thickening with age as do other parts of the otolith. Annuli are visible as very fine zones on these fingers.

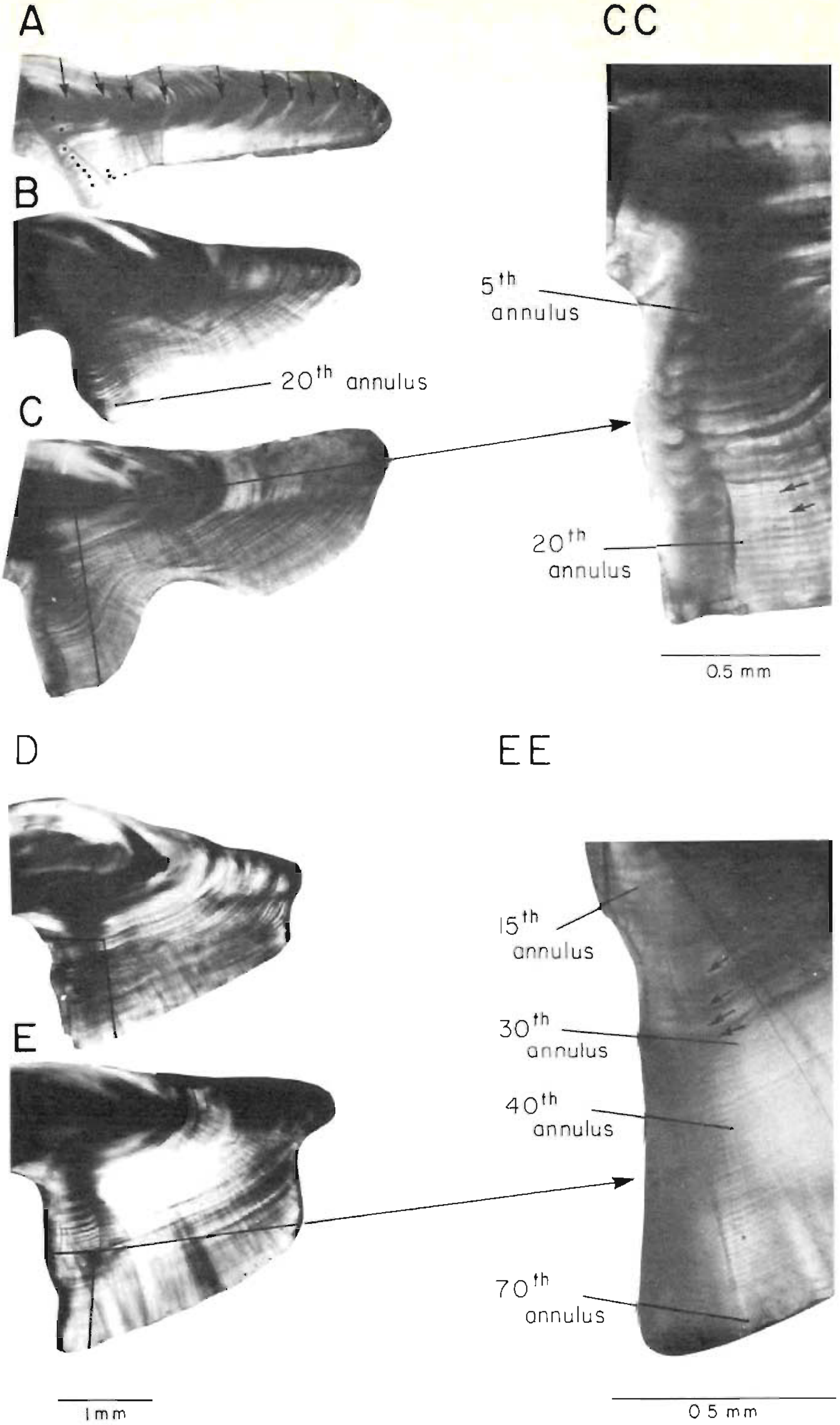


FIG. 48. (A-E, CC, DD, EE) Cross section of Pacific ocean perch otoliths showing the growth zones on the internal side. In most cases these zones are not visible on the external surface of older otoliths. As the fish grow older and body growth slows down or becomes nonexistent it is reflected in the otolith growth and many of the annual growth zones become extremely small. Arrows in (A) indicate position of annuli. Arrows in (CC) and (EE) indicate checks.

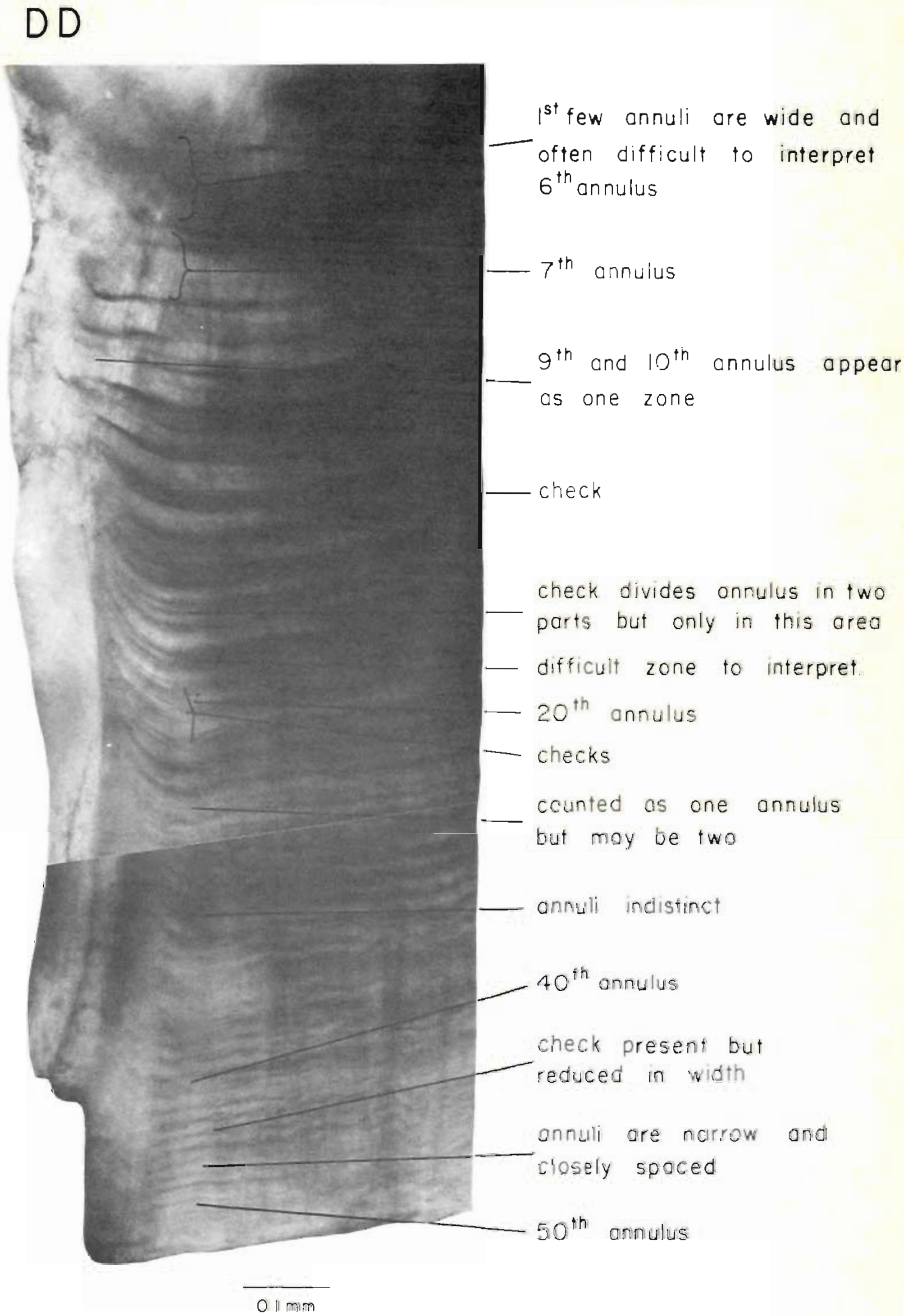


FIG. 48. Continued

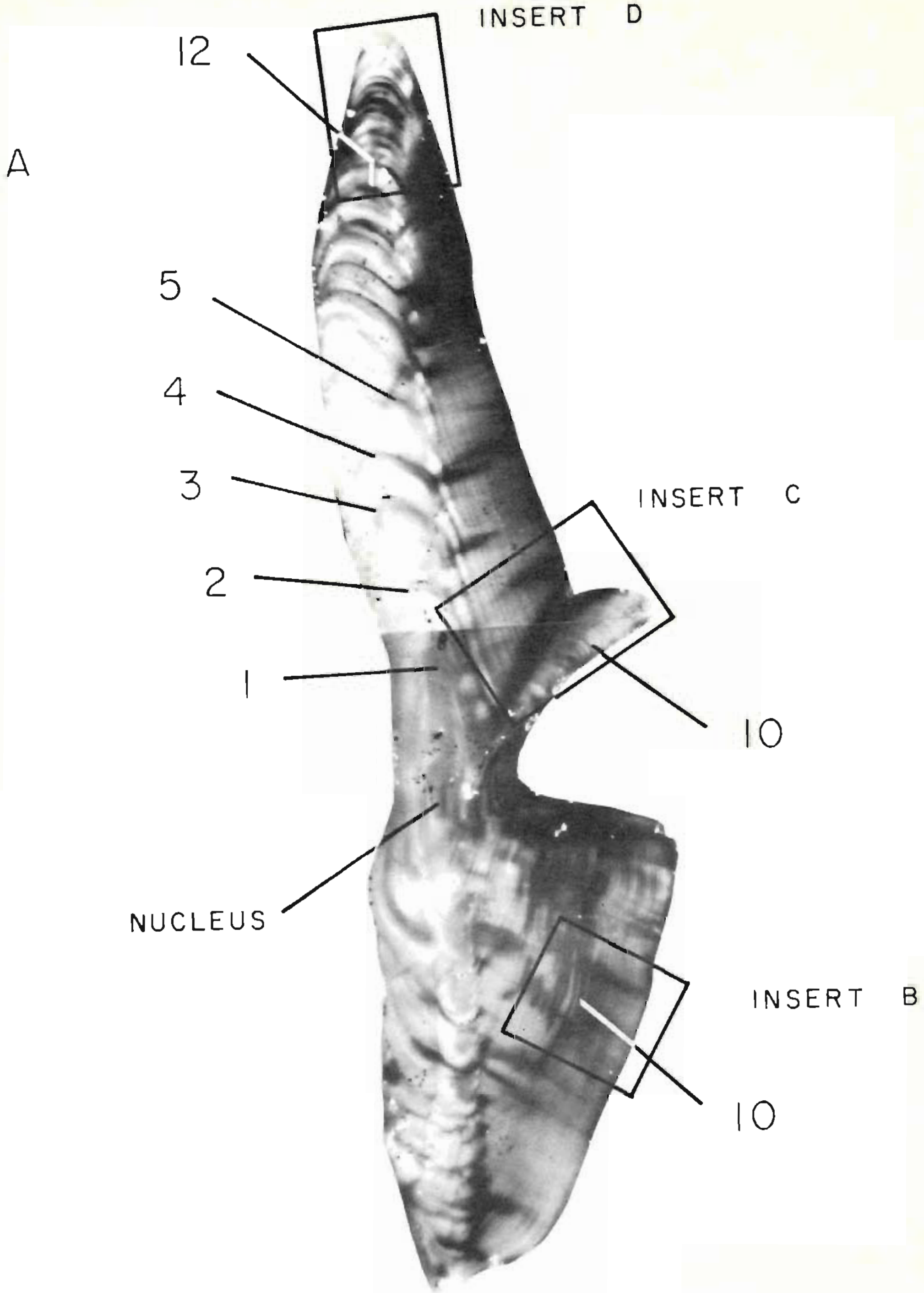


FIG. 49. (A–D) Burnt cross section and enlargements of counting areas of a Pacific ocean perch otolith indicating an even "easy to read" growth pattern.

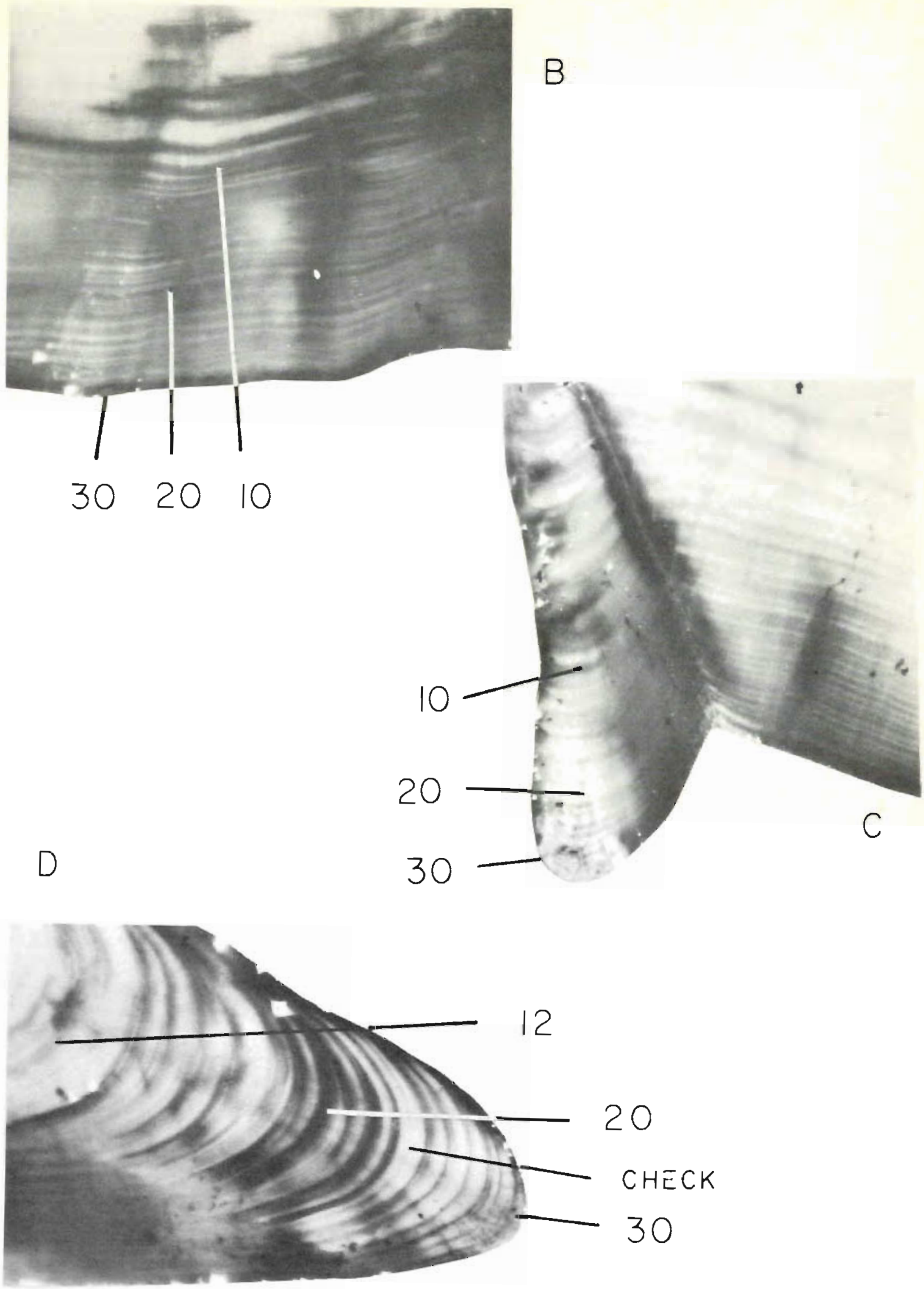


FIG. 49. Continued.

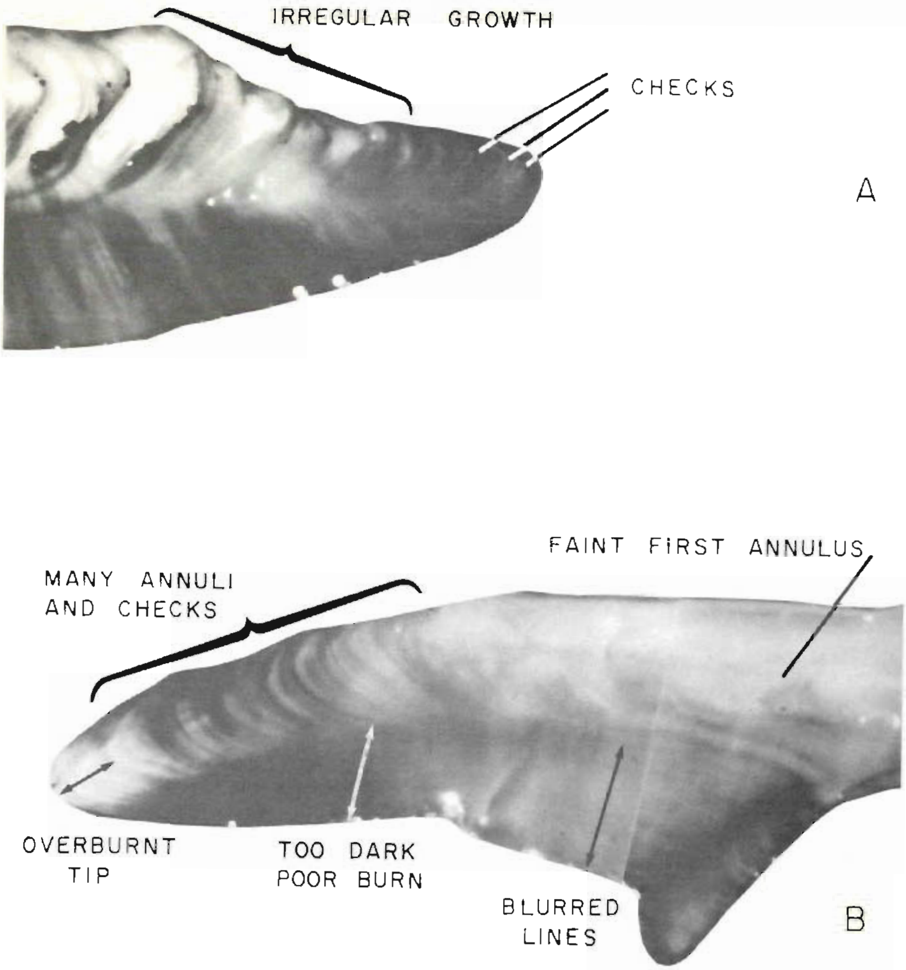


FIG. 50. (A, B) Burnt cross sections of Pacific ocean perch otoliths indicating problems when identifying annuli such as overburnt tip, faint first annulus, and blurred zones.

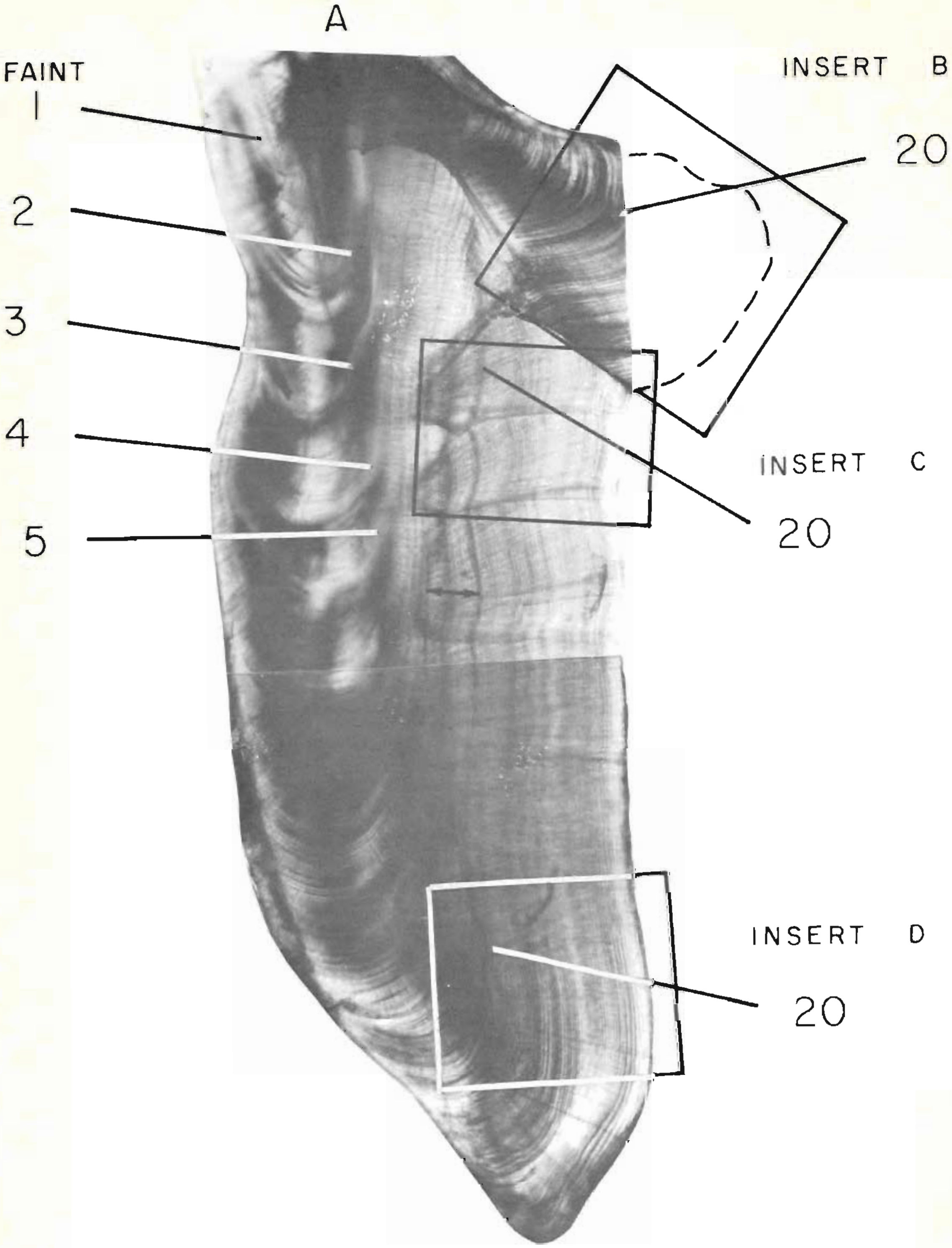


FIG. 51. (A–D) Thin cross section and enlargements of counting area of an older Pacific ocean perch otolith showing closely spaced annual zones. (A) Area indicated by double-headed arrows points out a pattern difficult to interpret as zones are blurred and hard to follow.

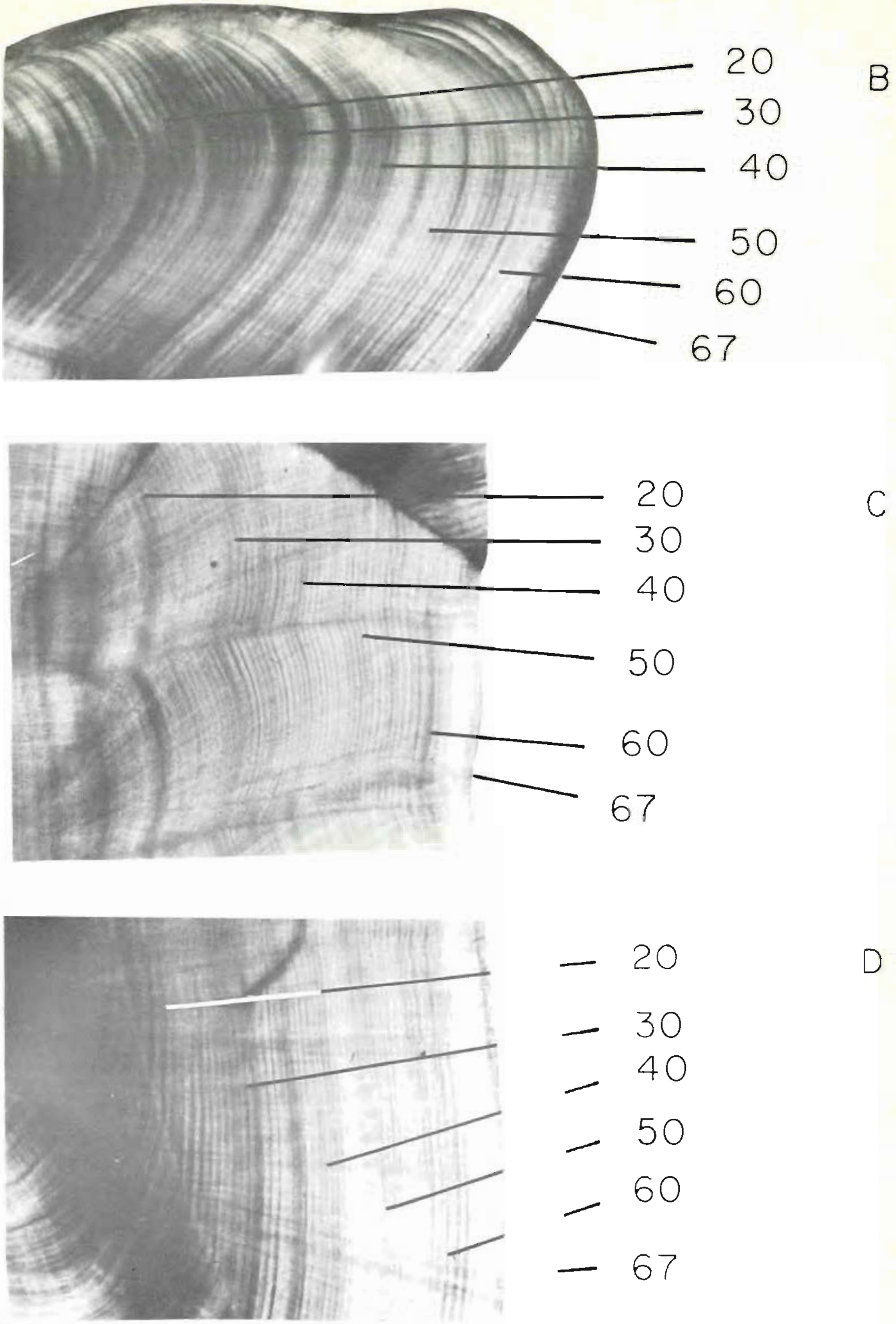


FIG. 51. Continued.

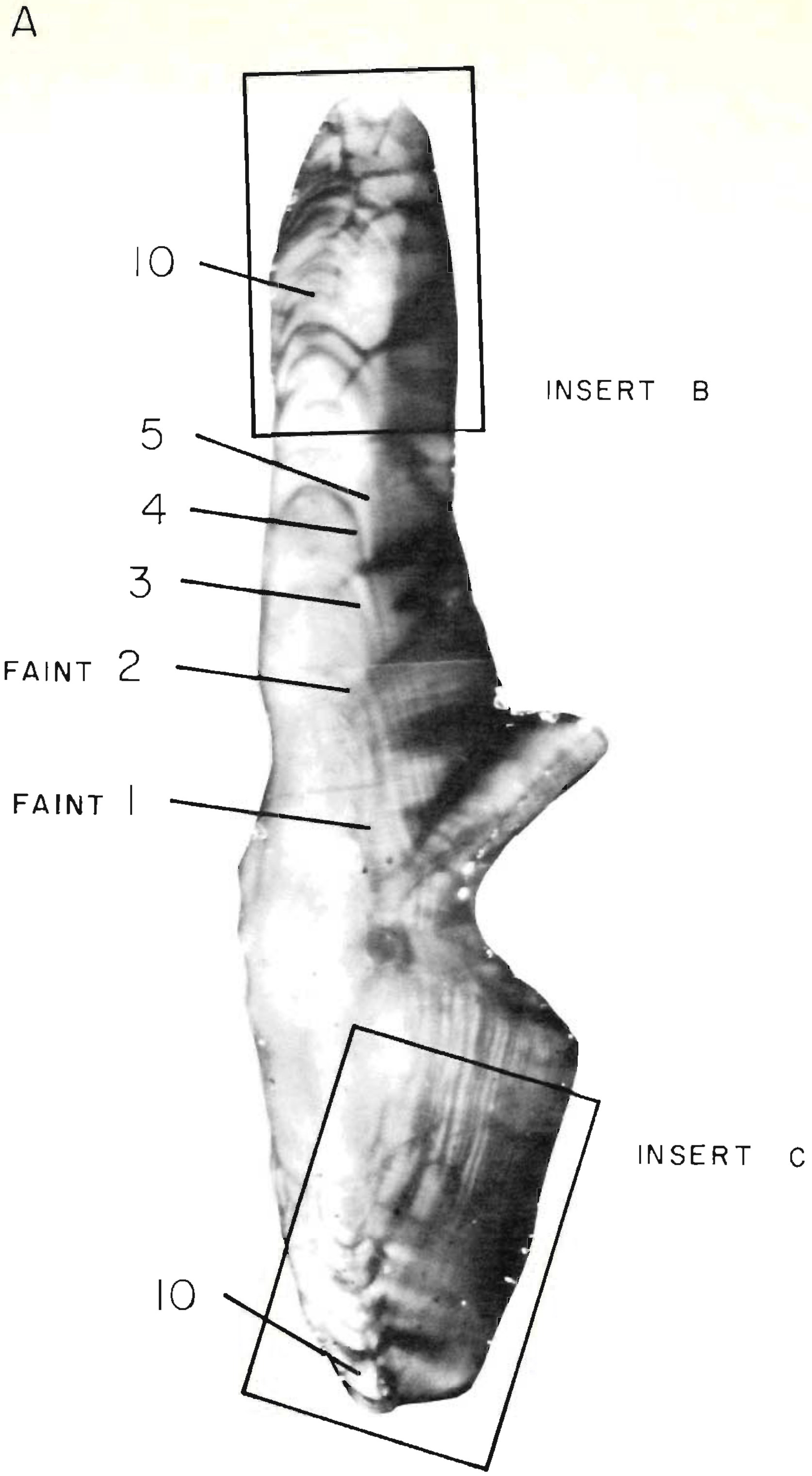


FIG. 52. (A–C) Burnt cross section of a Pacific ocean perch otolith and enlargements of the counting areas showing the even growth of the annuli. In (A), note the faint first and second annulus.

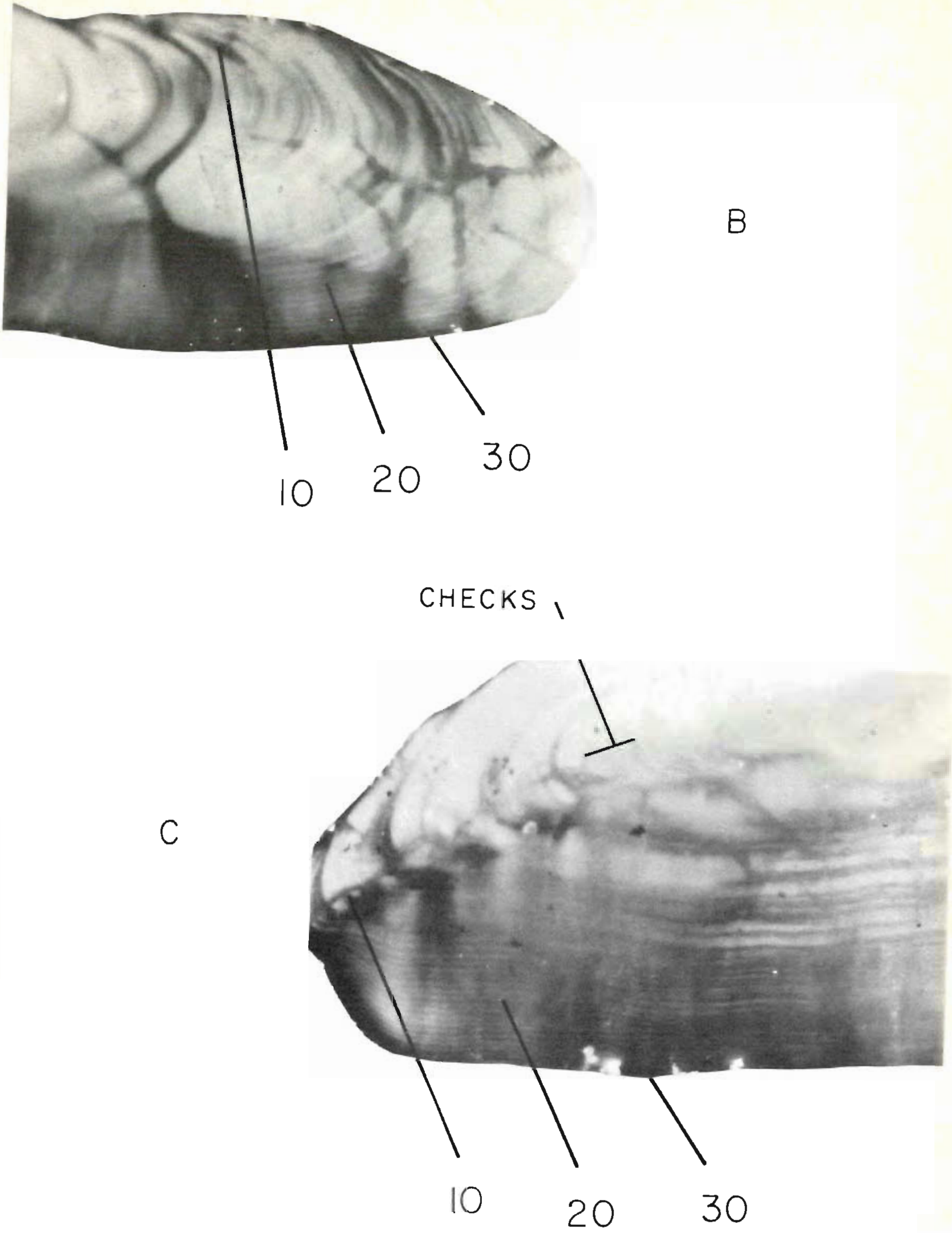


FIG 52. Continued.

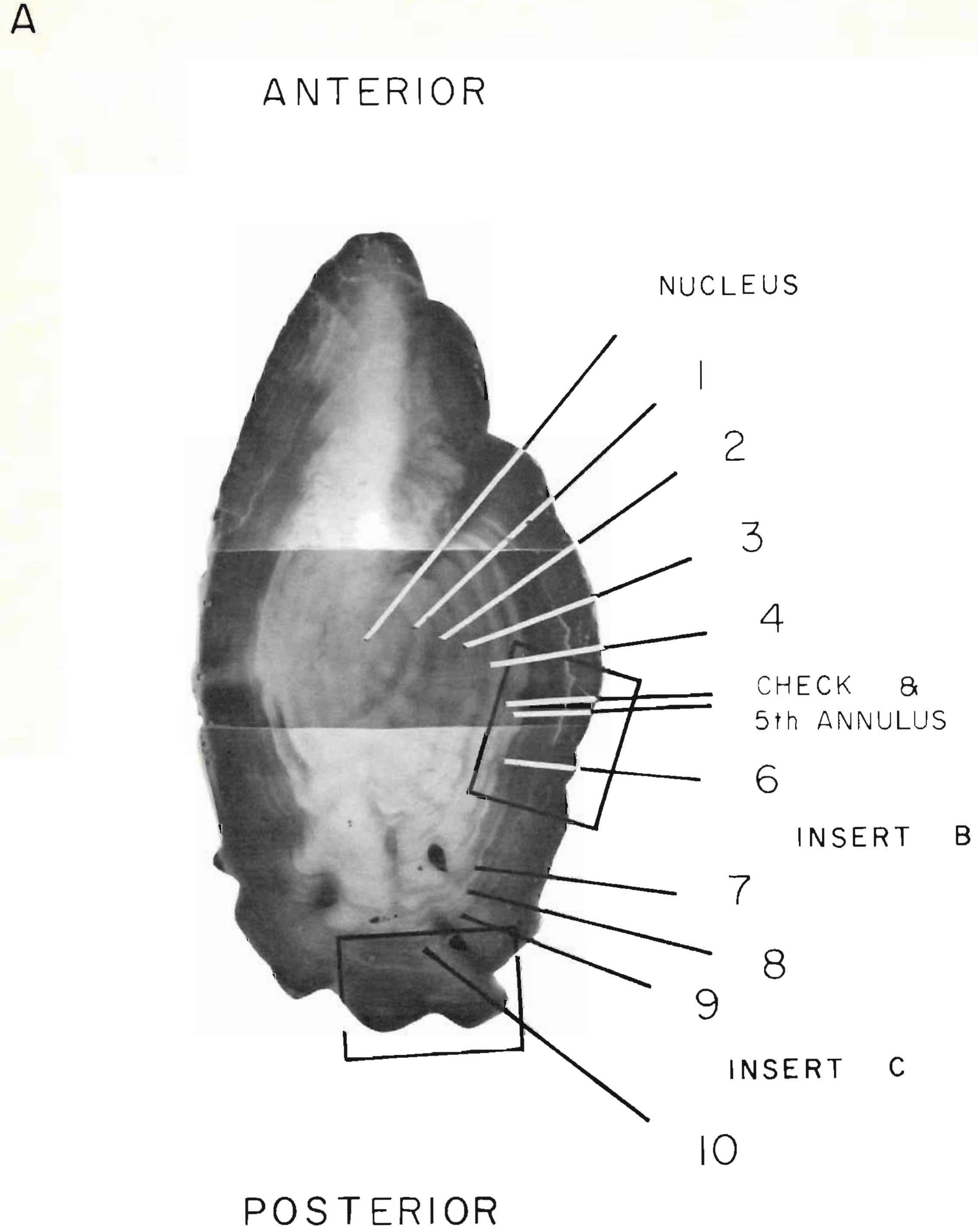


FIG. 53. (A) Pacific ocean perch otolith showing the nucleus and first few annuli. (B) Enlargement of the surface edge showing crowded annuli indicating Pacific ocean perch can grow very old. (C) A more reliable area to count as the annuli continue to grow along the lobe whereas in (B) the annuli are no longer visible on this part of the surface.

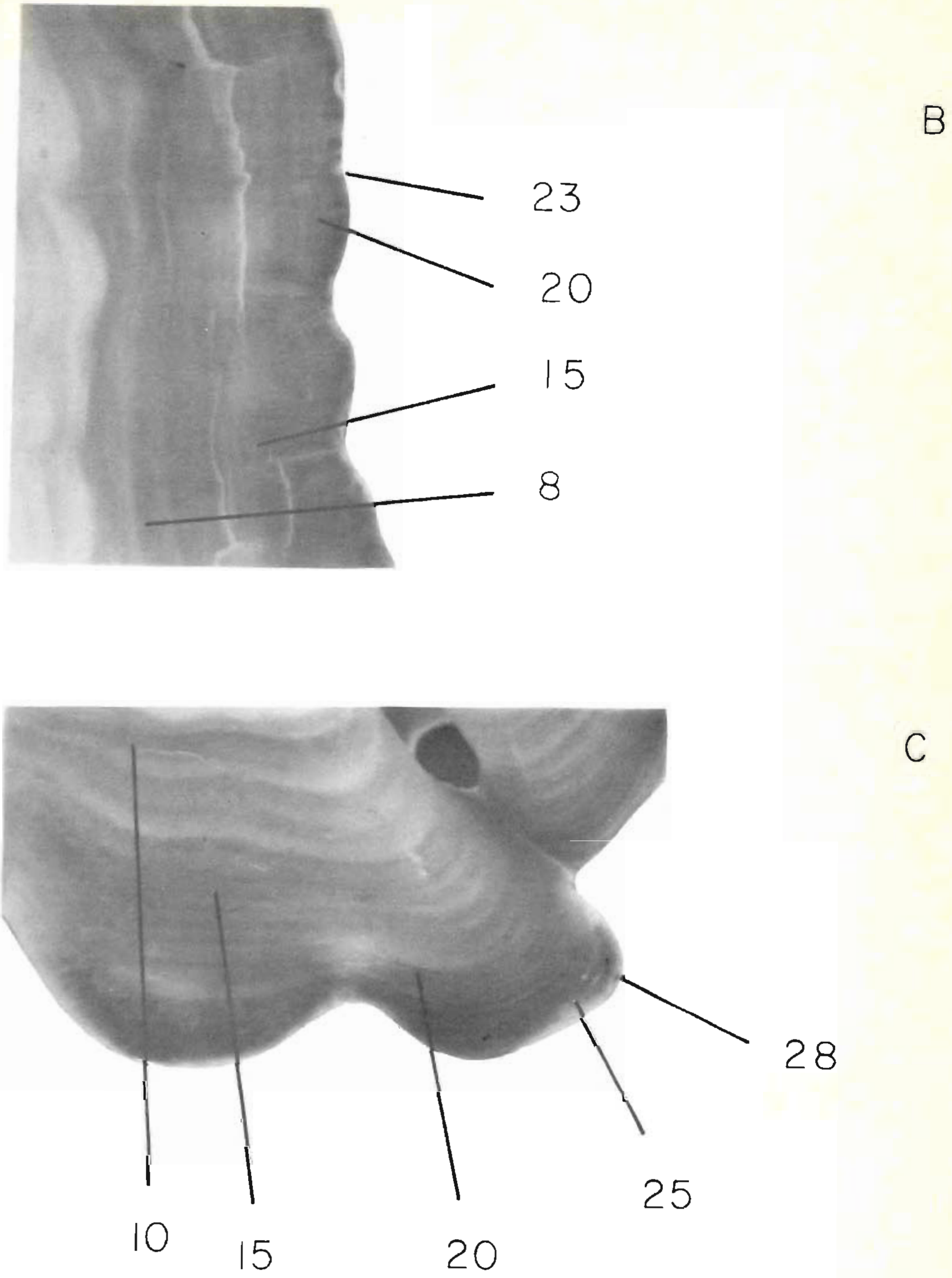


FIG. 53. Continued.

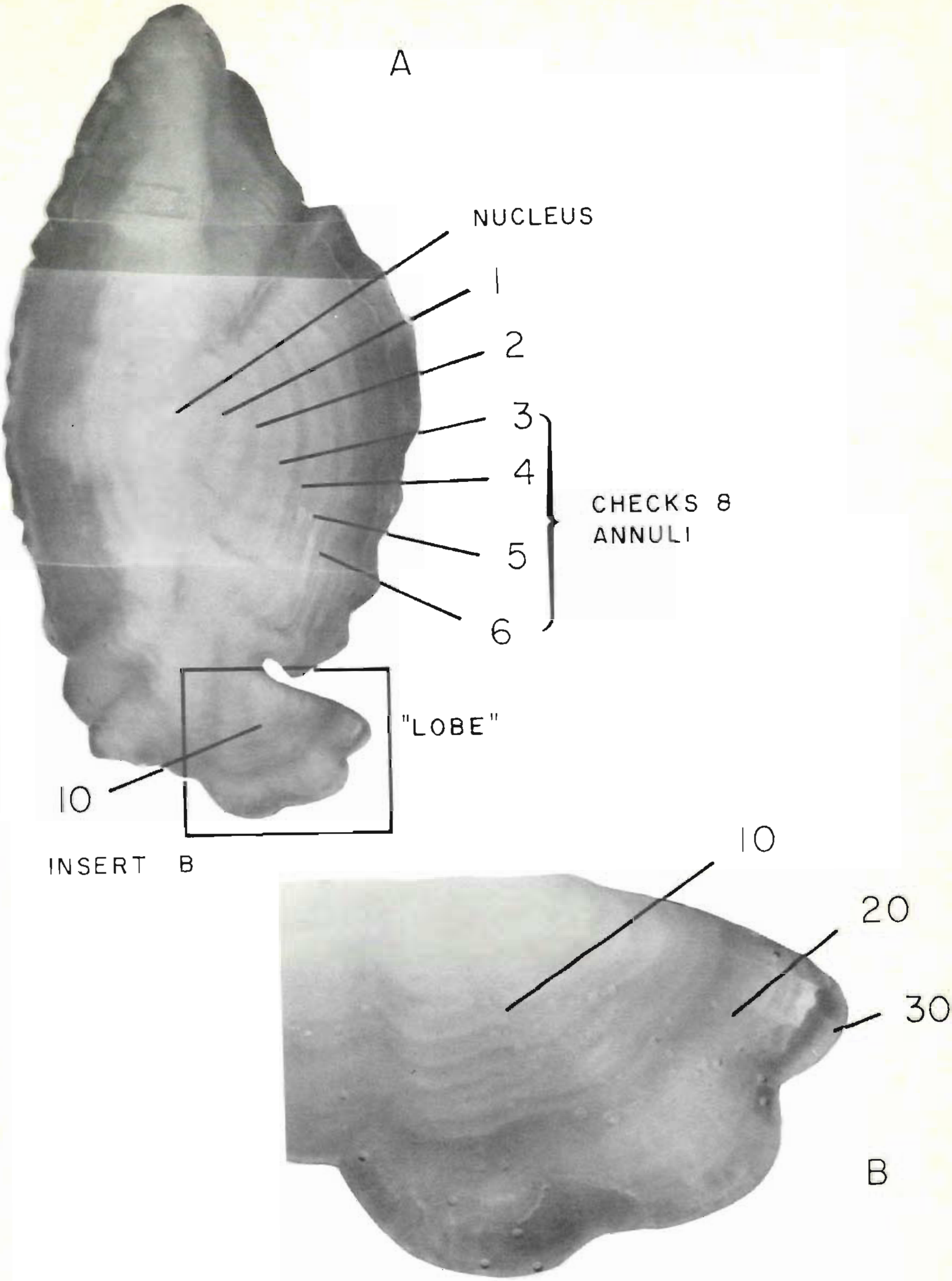


FIG. 54. (A) Surface of an older Pacific ocean perch otolith with "lobe" formation on the posterior end. The third to sixth annuli have checks close by indicating a slowing down in growth. (B) Enlargement of "lobe" showing the many small annual zones.

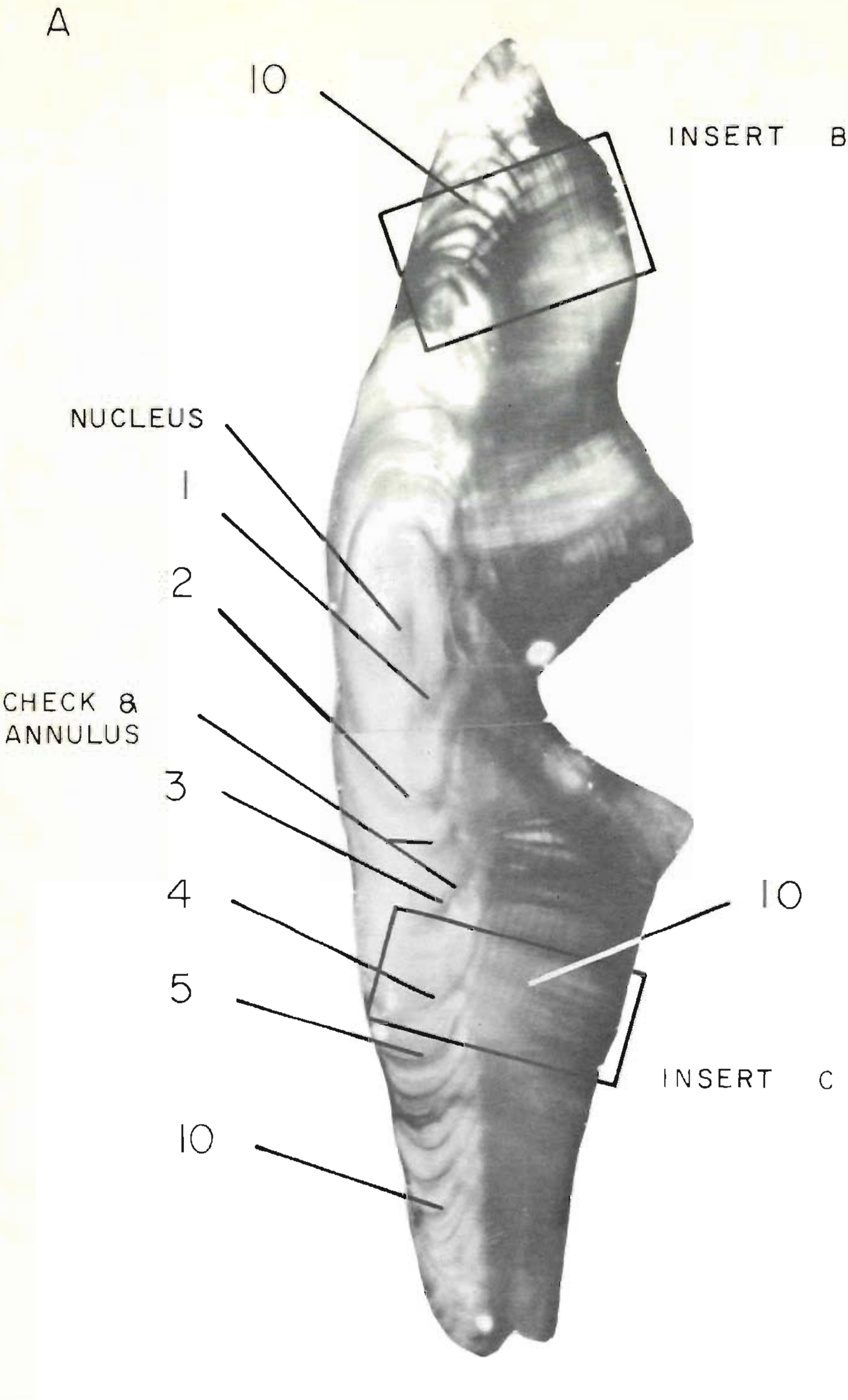


FIG. 55. (A) Burnt cross section of a silvergray otolith showing a clear nucleus and clearly spaced annuli with enlargements of counting areas.

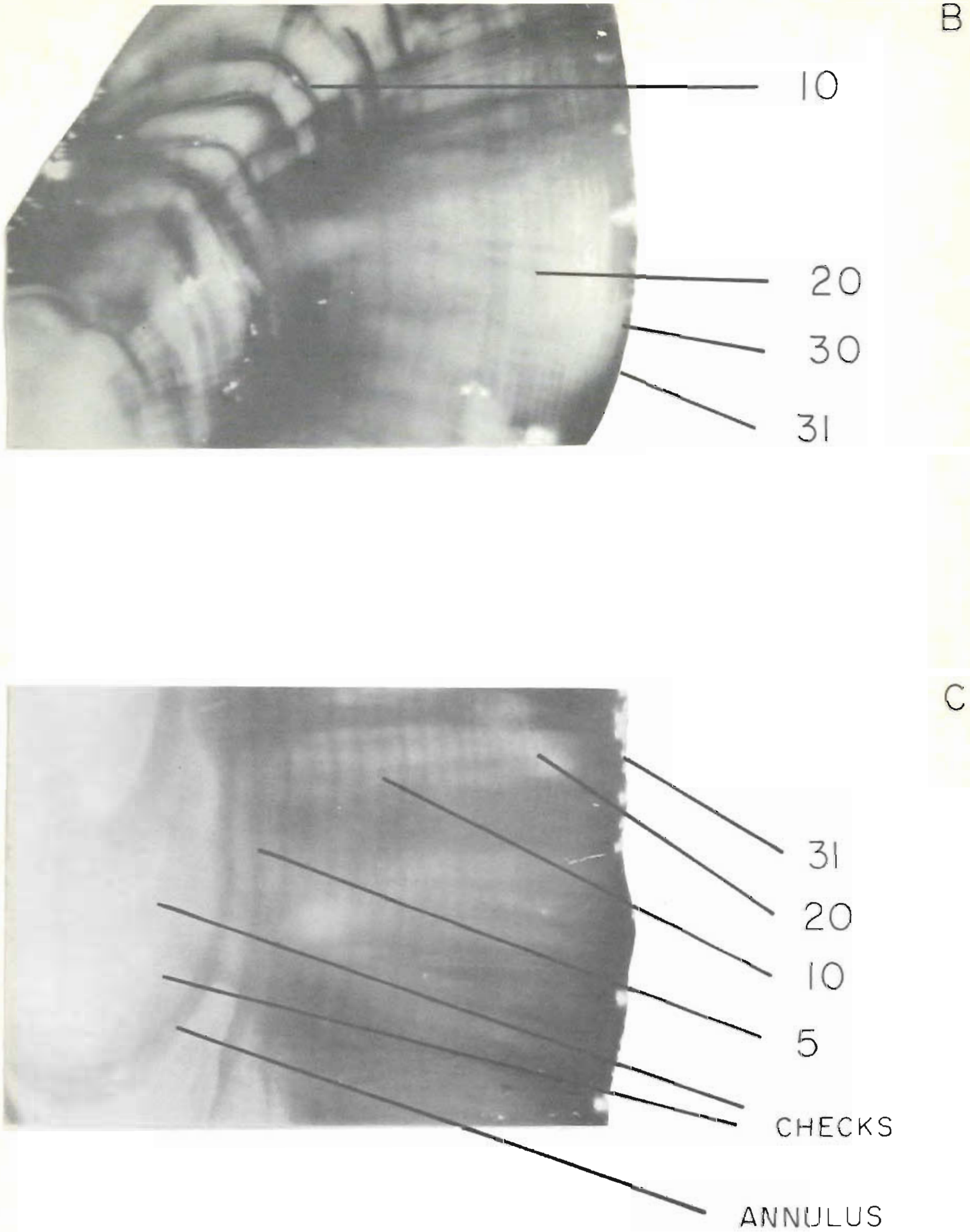


FIG. 55. Continued.

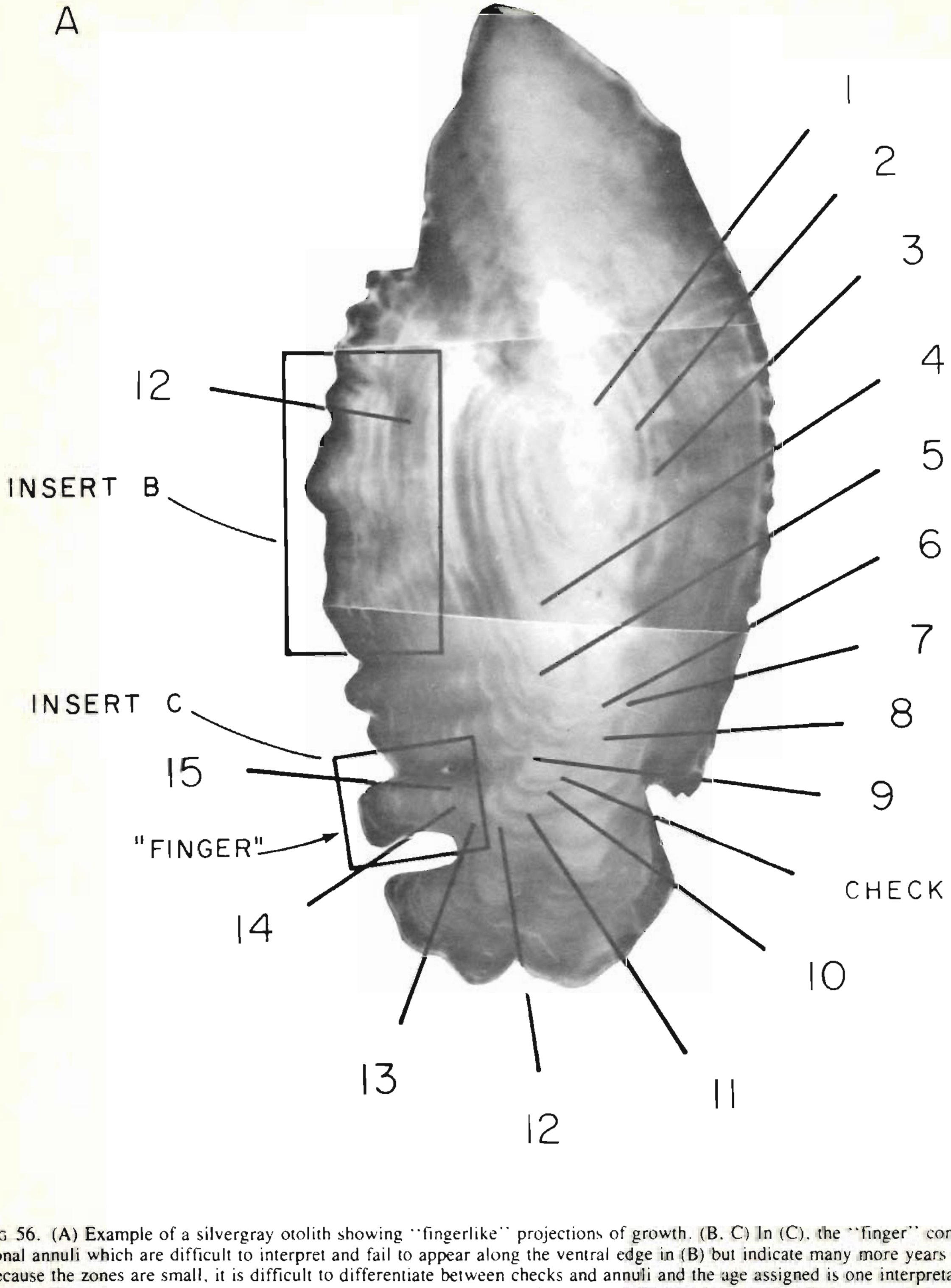
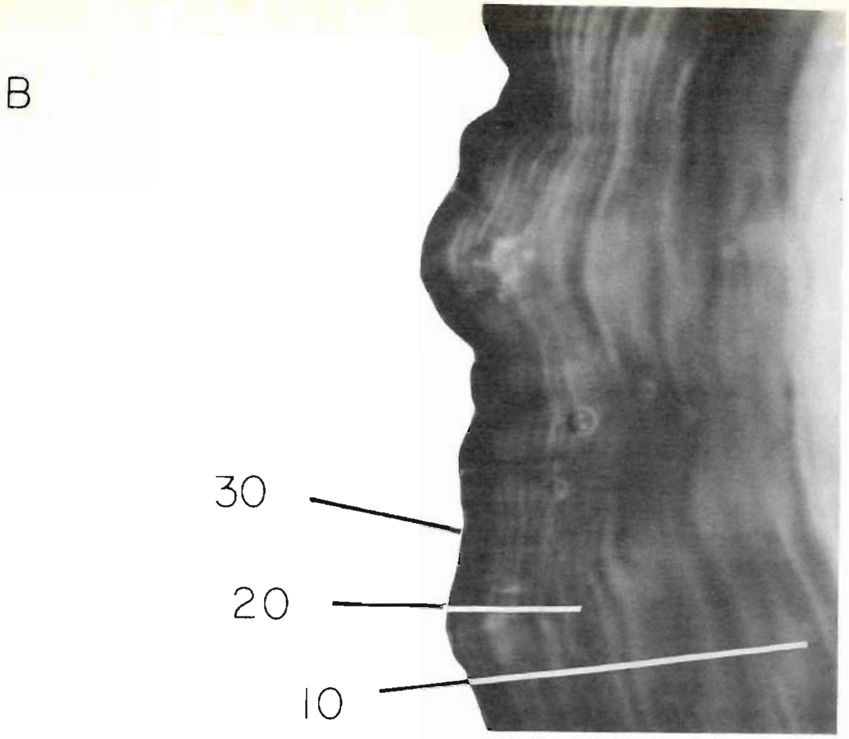


FIG 56. (A) Example of a silvergray otolith showing "fingerlike" projections of growth. (B, C) In (C), the "finger" contains additional annuli which are difficult to interpret and fail to appear along the ventral edge in (B) but indicate many more years of growth. Because the zones are small, it is difficult to differentiate between checks and annuli and the age assigned is one interpretation of the pattern. The arrow pointing to the bar indicates a large clear zone where annuli did not show on the photo.



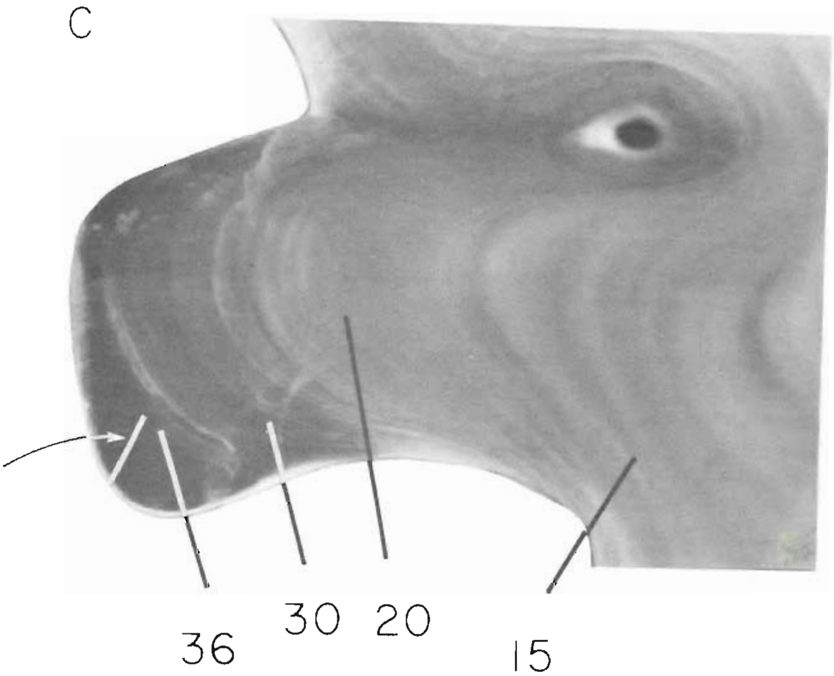


Fig. 56. Continued.

YELLOWTAIL ROCKFISH *(Sebastes flavidus)*

When broken and burnt, yellowtail rockfish otoliths reveal clear annuli which are usually read on the ventral and dorsal sides of the sulcus edge (Fig. 57A–D). Because this rockfish grows rapidly in the 1st yr, the first annulus appears a substantial distance from the nucleus. The next few growth zones are quite large also and many contain several checks, but breaking and burning enhances the annuli and the checks are less noticeable (Fig. 58A, 58B, 59A–C). It is usually possible to follow a distinct band considered to be the annulus along the burnt cross section to the sulcus edge. It is recommended that thin cross sections be made if problems arise interpreting the edge of the burnt surface. The last growth zone may only be visible on the dorsal tip.

The otolith surface is characterized by the relatively large size of the 1st yr of growth as compared with other rockfish. The annuli for the next 4–5 yr tend to remain clear but during the succeeding years the posterior end may produce numerous checks (Fig. 60A–C). Six and Horton (1977) observed deviations between readings due to the presence of these checks after the first few years and the complexity of many crowded growth zones on the otolith edge. Annuli form on the surface edge and on the interior surface. Examination from the surface does not always reveal these annuli. The growth zones on the surface gradually decrease in width and at about 13–15 yr thickening on the internal surface takes place.

CANARY ROCKFISH *(Sebastes pinniger)*

The canary rockfish is relatively fast growing and its otoliths are large. Breaking and burning these otoliths clarifies the true annuli as the checks are less prominent (Fig. 61A–D). The first annulus on the burnt surface is observed outside of a large amount of opaque deposition. It may vary in prominence and distance from the nucleus. After the first few years the annuli form dense zones around the sulcus edge but these zones spread out towards the dorsal and ventral edges. Occasionally interpretation problems can occur on the burnt surface with blurred zones, dark areas, or an overburnt tip. If an age cannot be determined then a thin cross section should be made and examined under a higher magnification.

The otolith surface usually shows a regular growth pattern. It is generally clear, although the older otoliths can be cloudy and have numerous checks (Fig. 62A, 62B). The otoliths of fish under 10 yr old characteristically have wide clear growth zones. The first annulus, which is often faint, surrounds a small nucleus. It may vary among stocks. As the otolith grows the first 10 annuli often have accessory checks, particularly on the posterior portion of the otolith. Therefore, it is advisable to count from the nucleus down the dorsal side and then around to the posterior end of the surface. Older otoliths form "fingerlike" projections on their posterior and dorsal edges (Fig. 62A). These fingers tend to grow out flat instead of thickening as do other parts of the otolith. By the age of 20, canary rockfish otolith formation slows to the point that growth can only be seen as uniformly narrow zones on these "fingers" (Fig. 62B). They may become opaque with age, but dipping the otolith in a mild solution of HCl for a few seconds will sometimes clears the otoliths for viewing using 25 or 50 power on a dissecting microscope. Care must be taken when etching, as the edge is easily dissolved. A general rule for counting the increments on the fingers is to count every zone you can see. To verify counts, compare the surface reading to a broken and burnt reading.

YELLOWMOUTH ROCKFISH *(Sebastes reedi)*

When viewing the broken and burnt surface of the yellowmouth rockfish otoliths, the first few annuli may contain numerous checks (Fig. 63). As previously mentioned burning usually enhances the clarity of the true annuli which can be followed from the sulcus edge to either the dorsal or ventral side. The remaining growth zones are abruptly reduced in size and form alternating translucent and opaque bands that accumulate in densely spaced zones toward the internal section edge of the otolith (Fig. 64A). Several areas of the broken and burnt surface display clear growth patterns that are easily counted (Fig. 64B, 64C). Thin sections should be made and examined under the higher magnification of a compound microscope when growth zones cannot be counted accurately on the broken and burnt surface.

The surfaces of these otoliths are more difficult to age than those of the other rockfishes. For about the first 7 or 8 yr the otoliths contain moderately wide growth zones which can be counted fairly easily on the external surface. The following years produce irregular growth patterns which tend to accumulate on the edges as the growth of the fish slows down. It is difficult then to separate the annuli with any degree of confidence on the otolith surface (Fig. 65A–C).

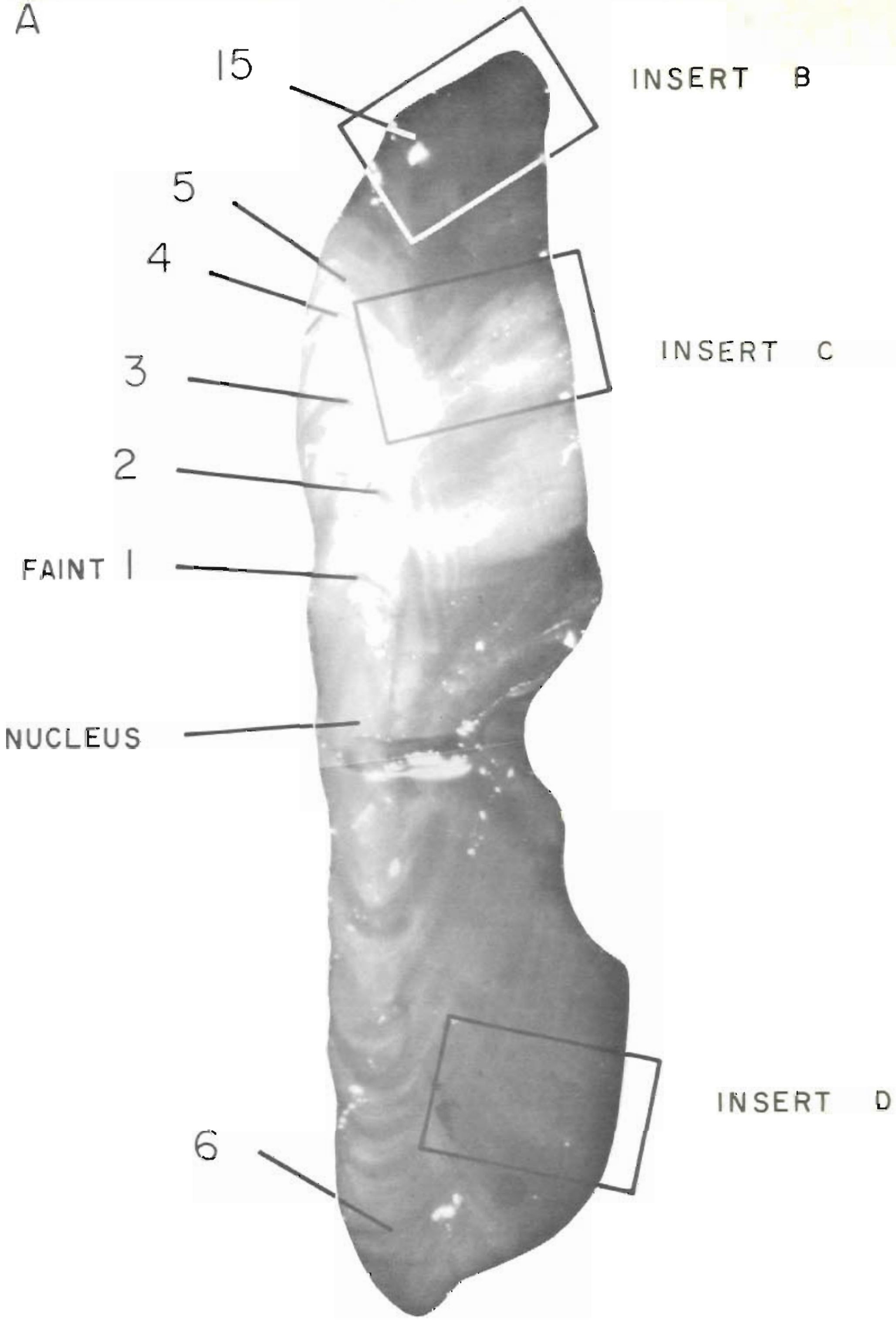


FIG. 57. (A–D) Burnt cross section and enlargement of an older yellowtail otolith with an indistinct first annulus followed by clearly formed growth zones enhanced by burning. (C) is the clearest area for counting.

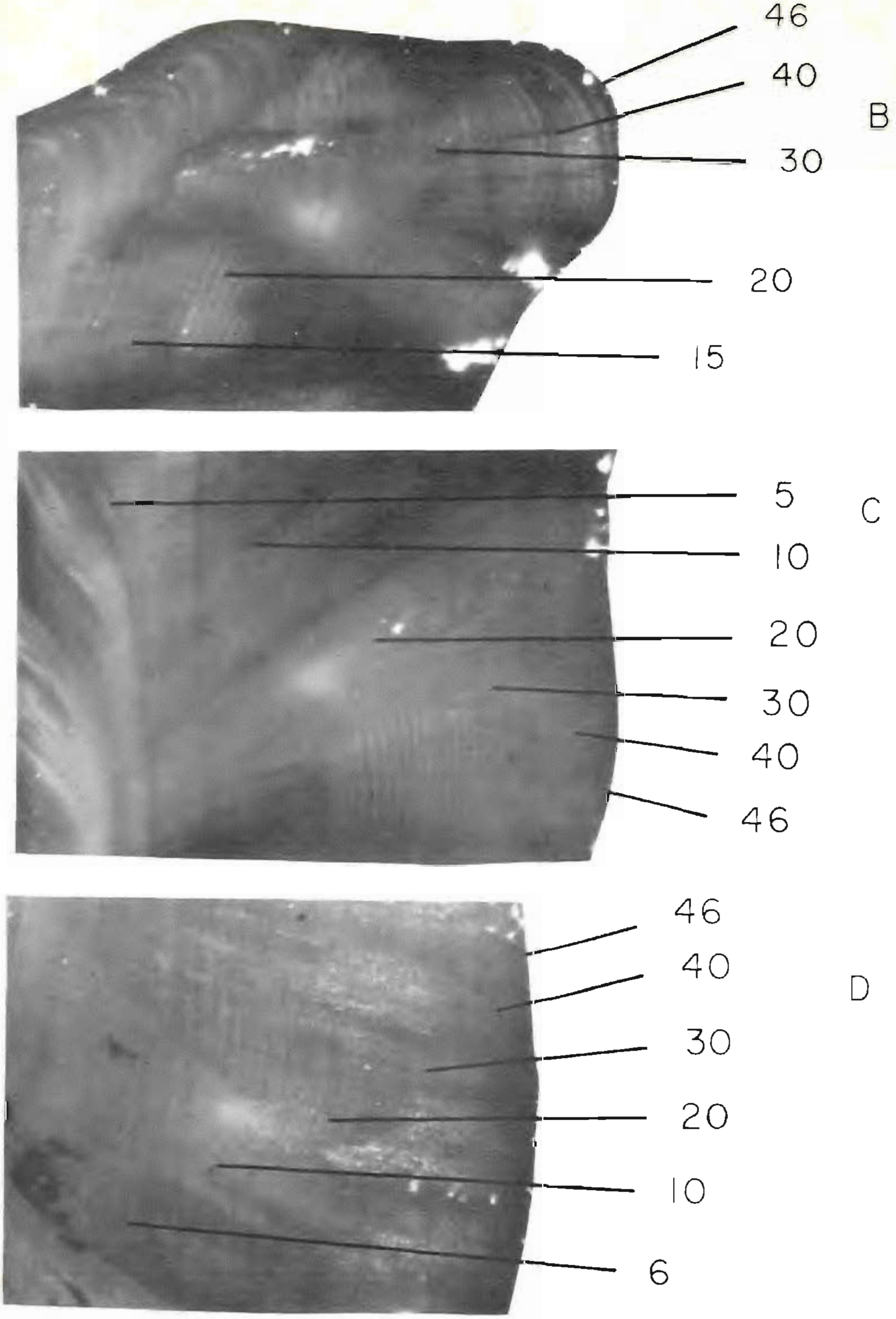


Fig 57. Continued

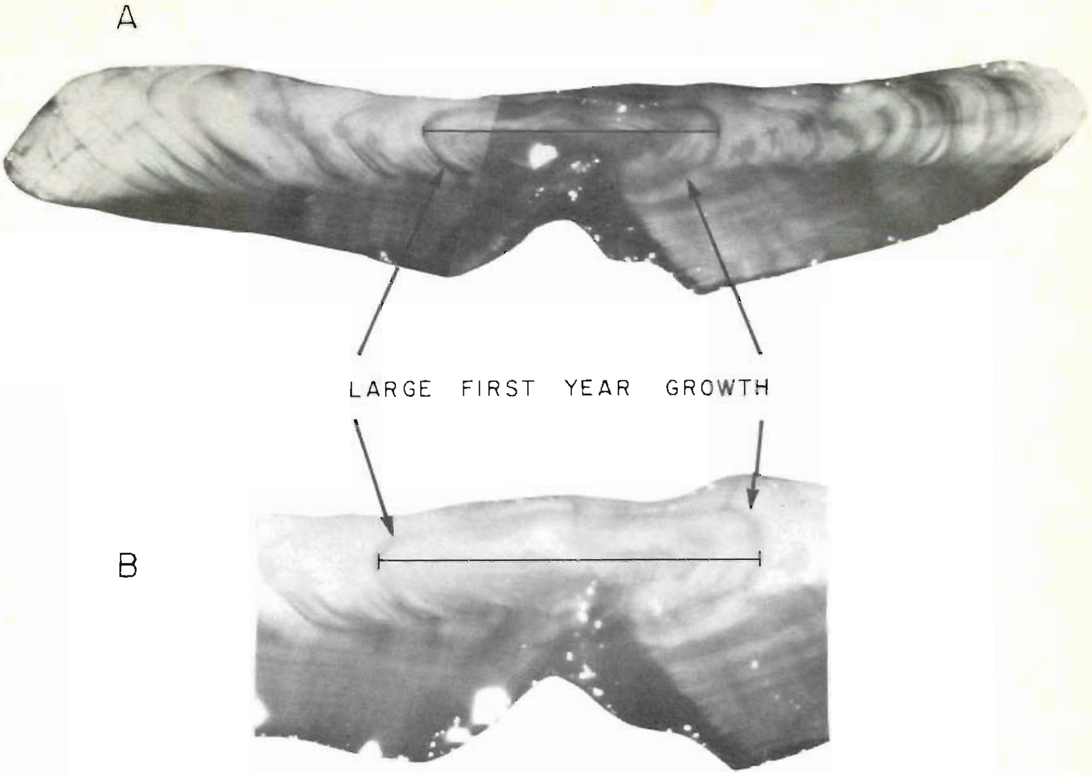


FIG. 58. (A, B) Broken and burnt section of a yellowtail otolith showing a prominent first annulus and subsequent clearly spaced annuli.

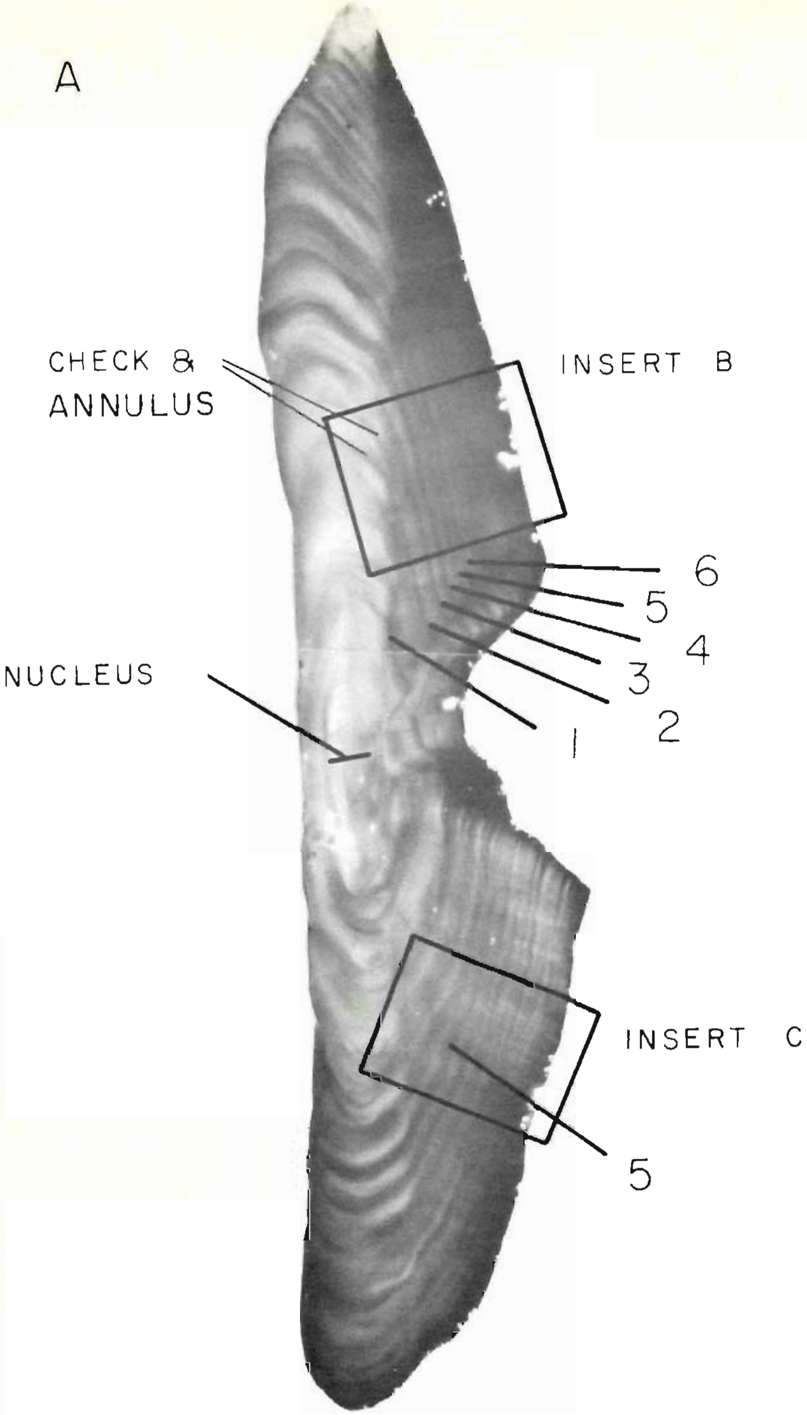


FIG 59. (A-C) Burnt cross section of a yellowtail otolith showing the first annulus consisting of several zones followed by a clear pattern of annuli with the growth zones diminishing as the fish grows older. (B) is a more reliable counting area compared with that shown in (C).

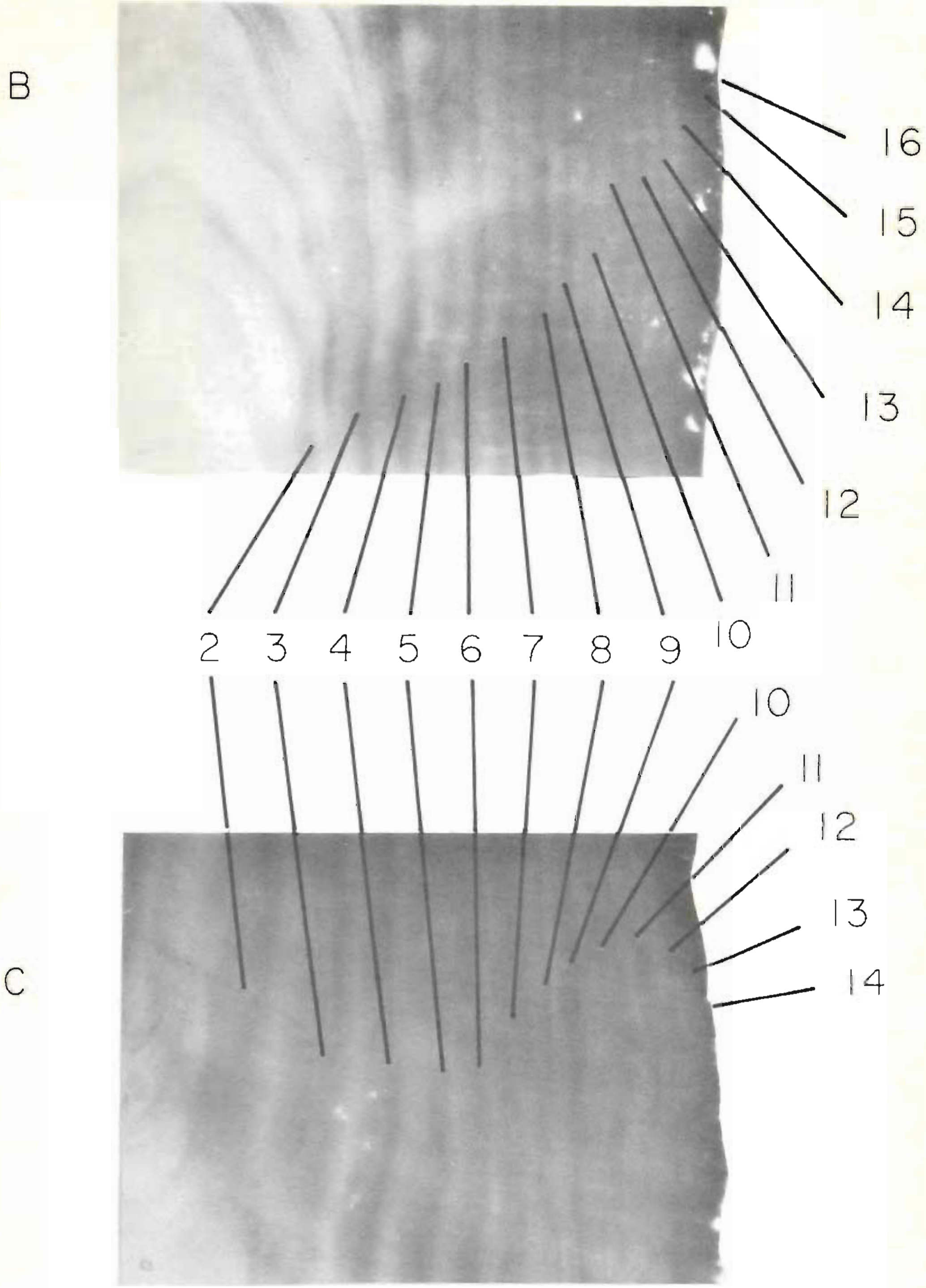


FIG. 59. Continued.

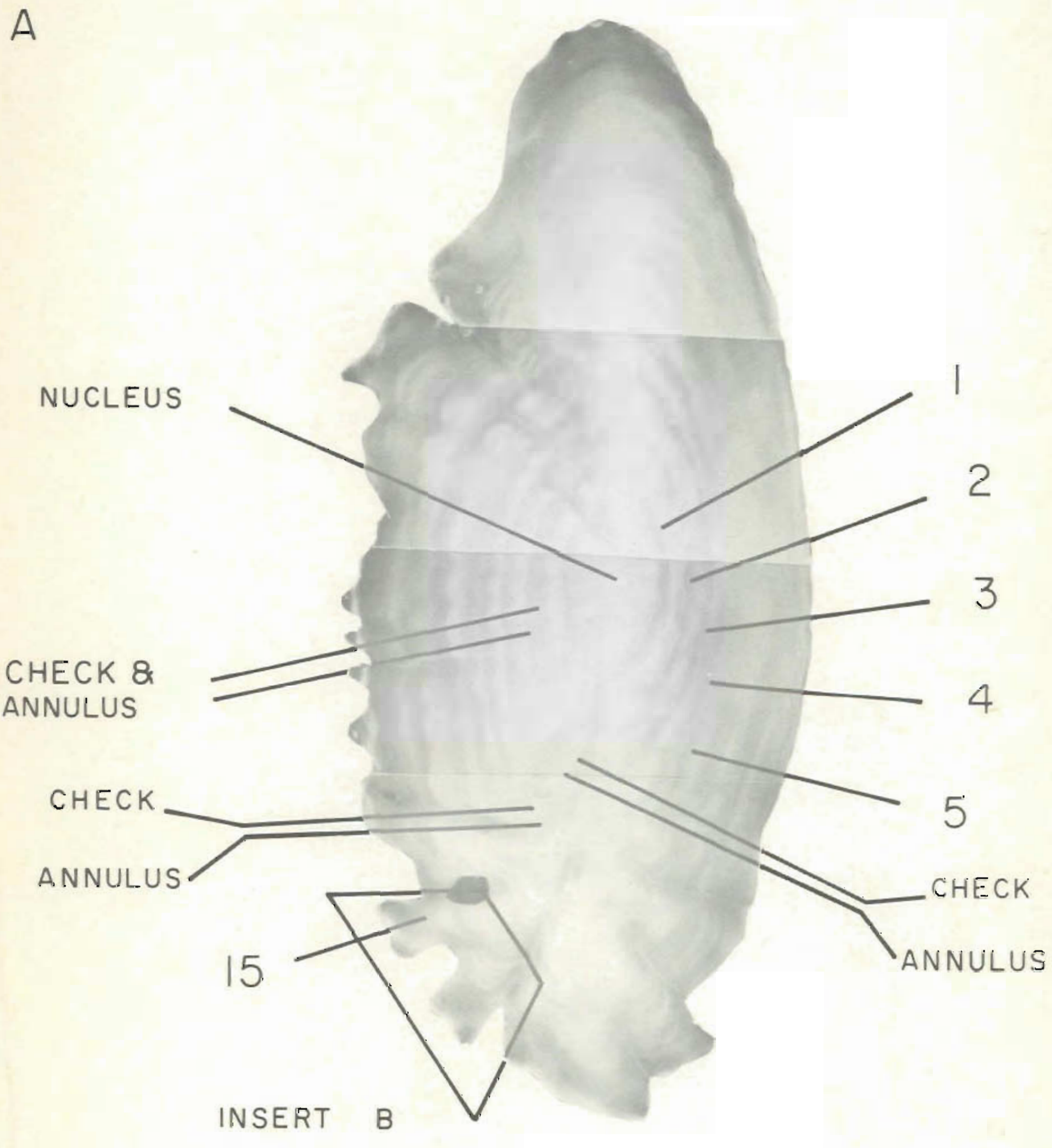


FIG. 60. (A–C) Yellowtail otolith showing the large 1st yr of growth and many checks on the posterior area. (B, C) "Fingerlike" projections on which many small growth zones are apparent.

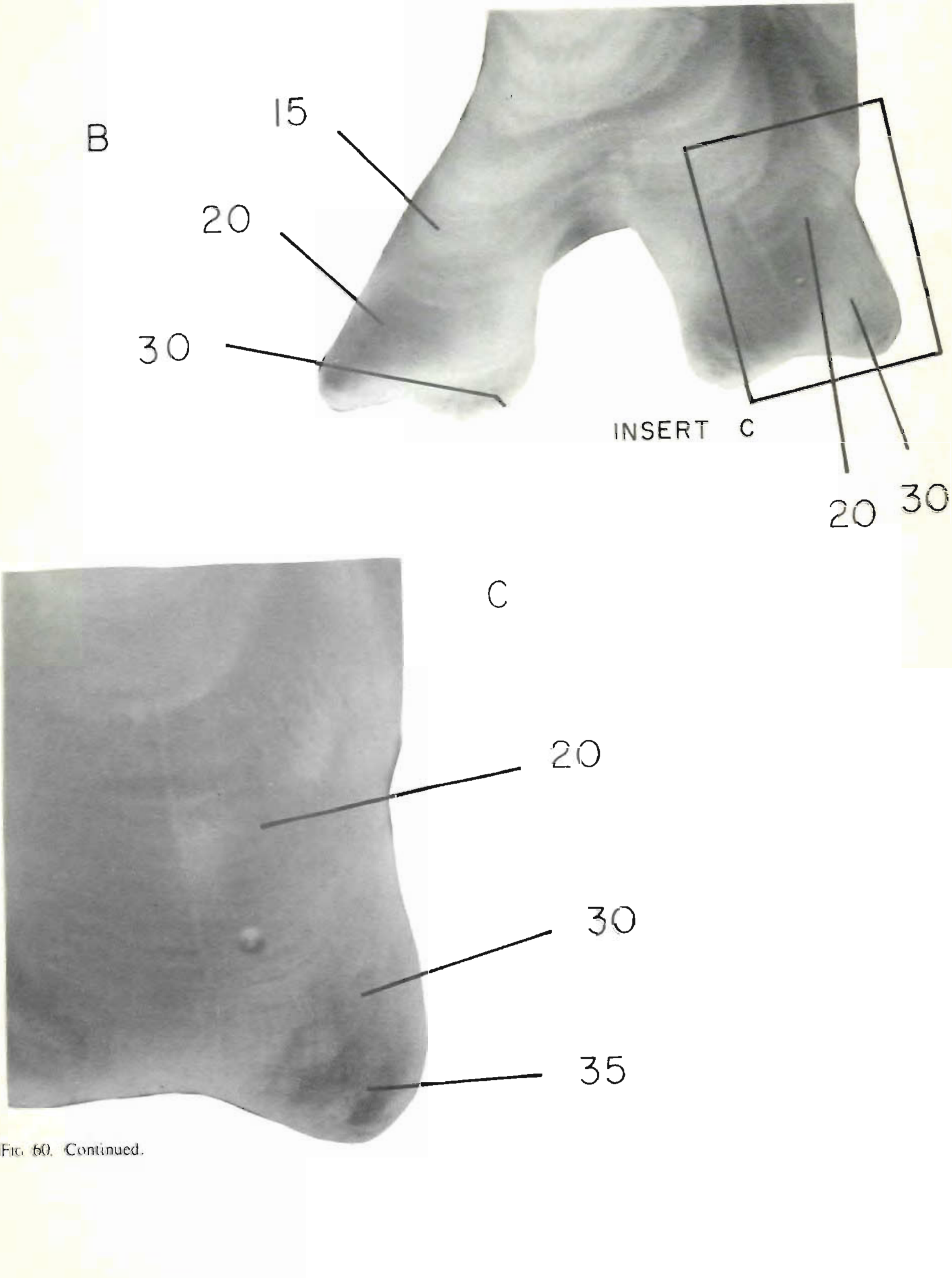


FIG. 60. Continued.

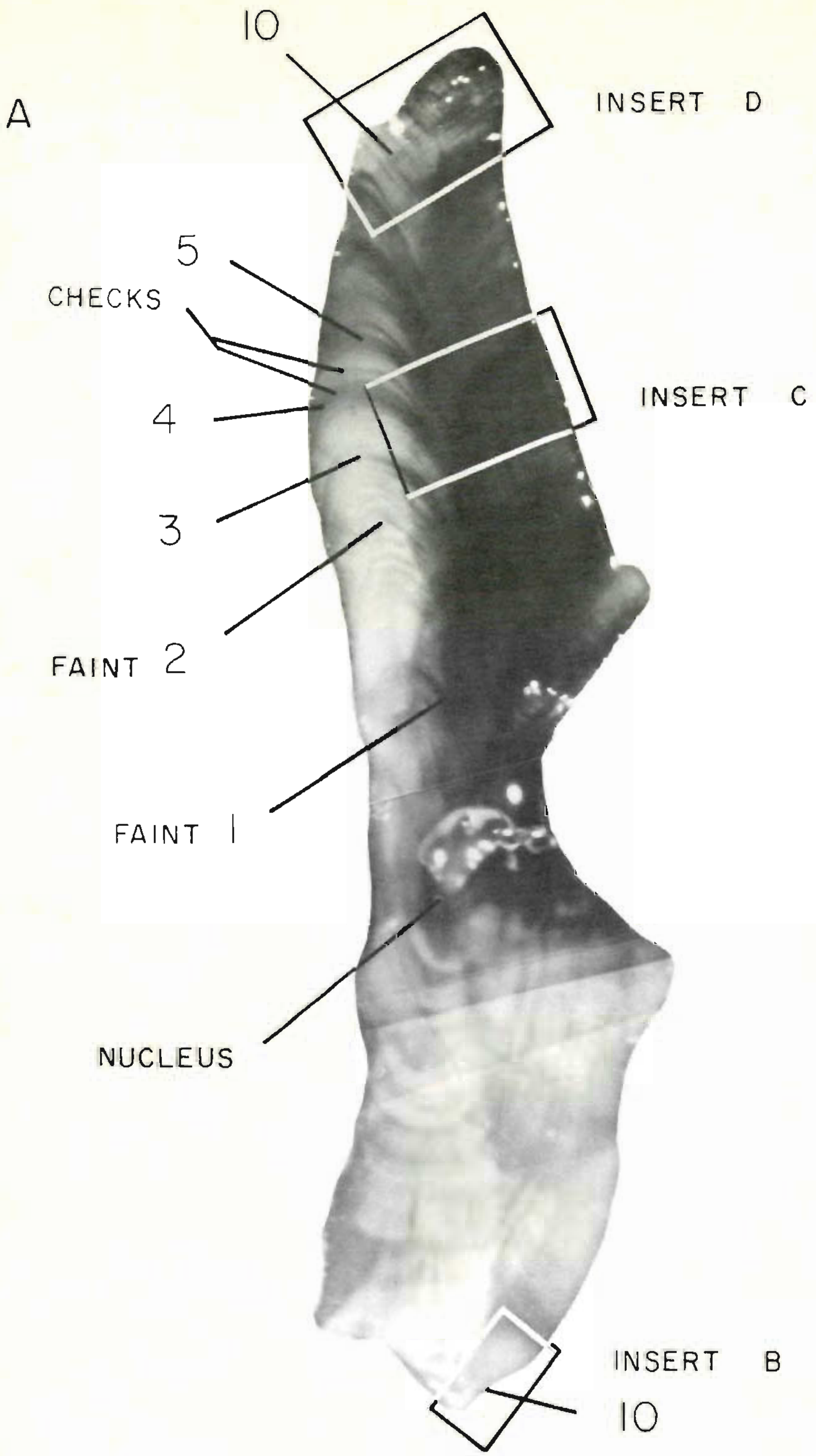


FIG. 61. (A–D) Cross section and enlargements of counting areas of canary otolith showing indistinct first and second annuli followed by growth zones enhanced by burning.

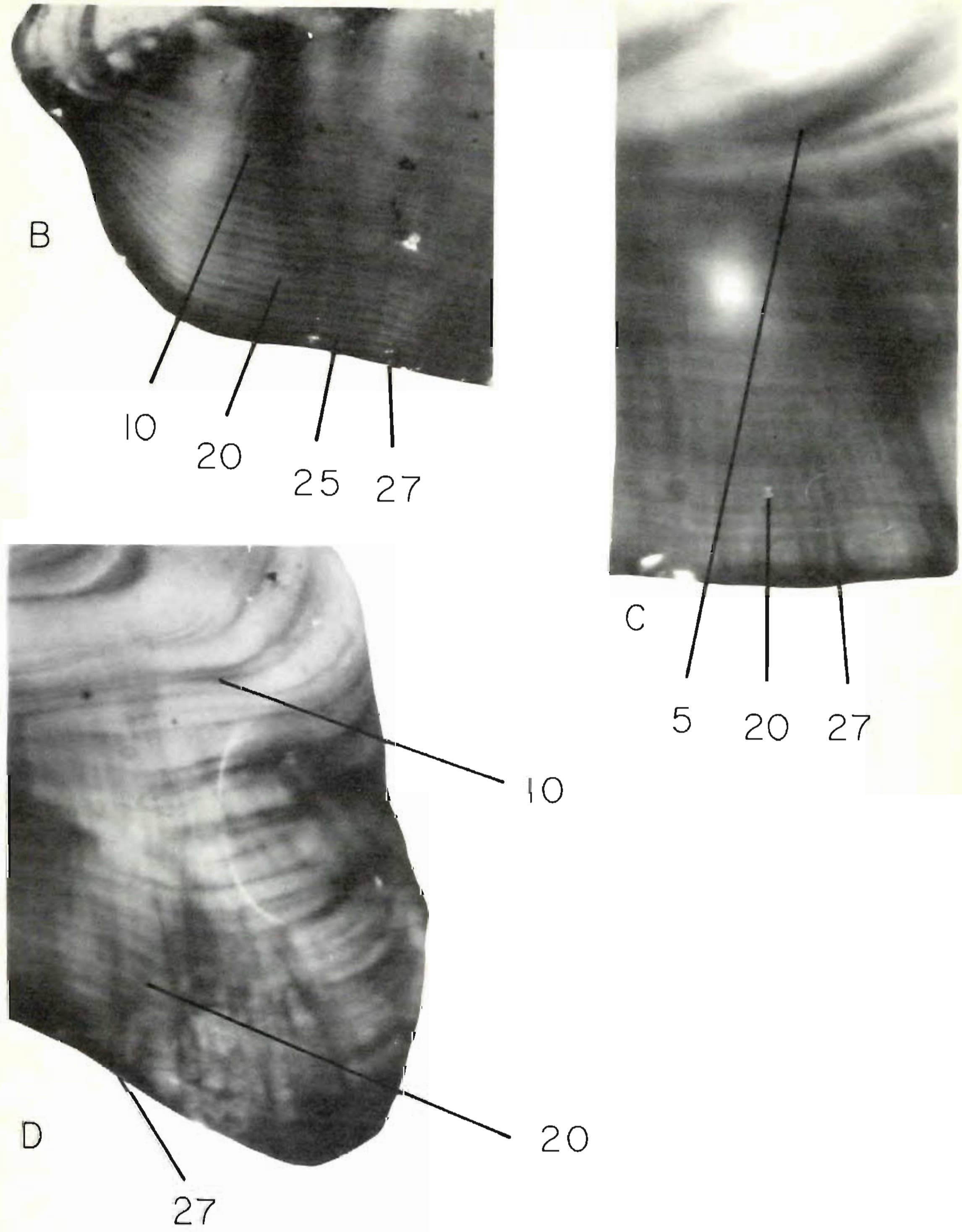


Fig. 61 Continued.

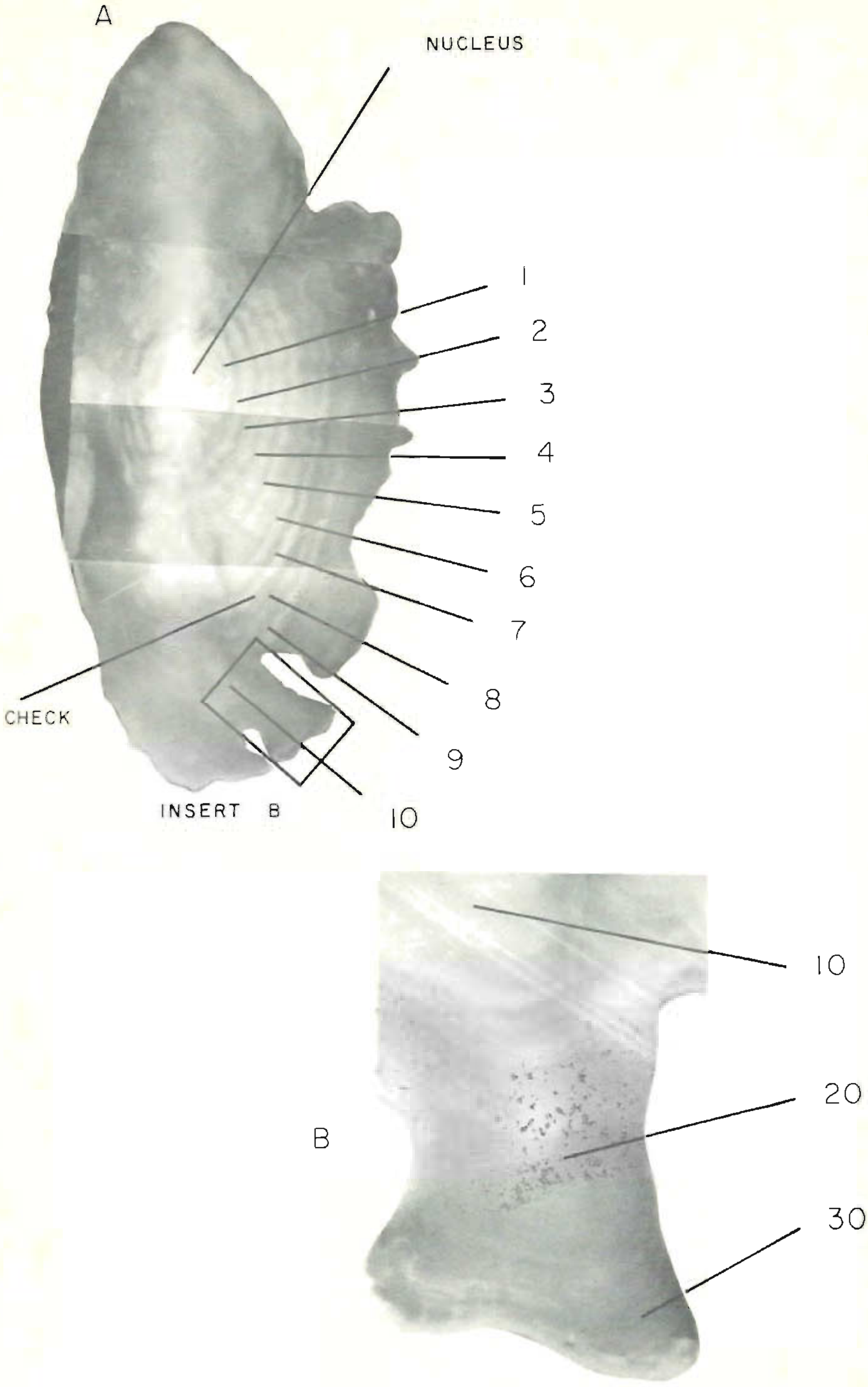


FIG. 62. (A, B) Canary otolith showing regular growth patterns for the first few years with "finger-like" projections on the posterior end. (B) illustrates one of the projections. The otolith is agedat >30 yr as the last few annuli are vague.

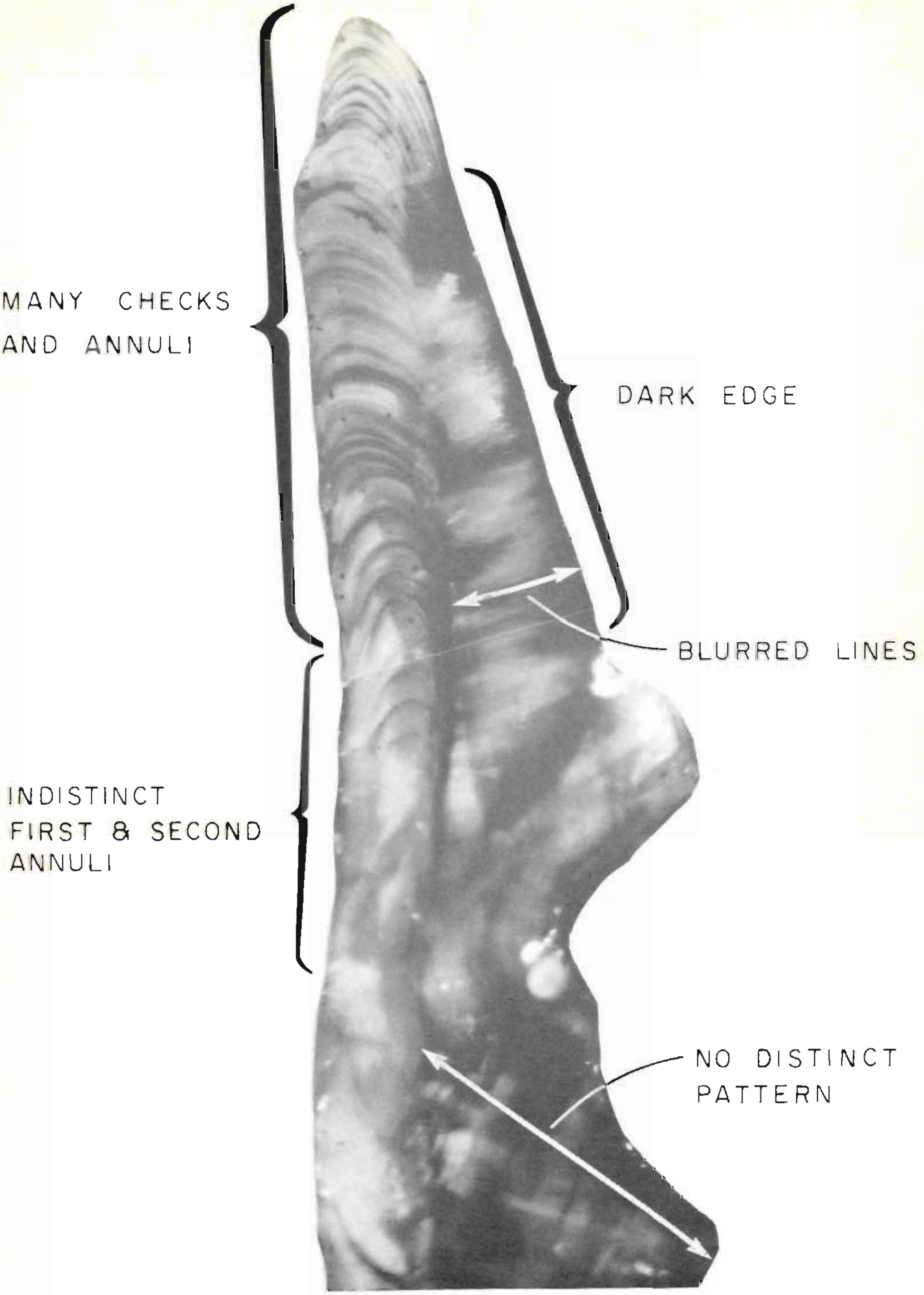


FIG. 63. Burnt cross section of yellowmouth otolith explaining some difficulties that can occur when assigning an age.

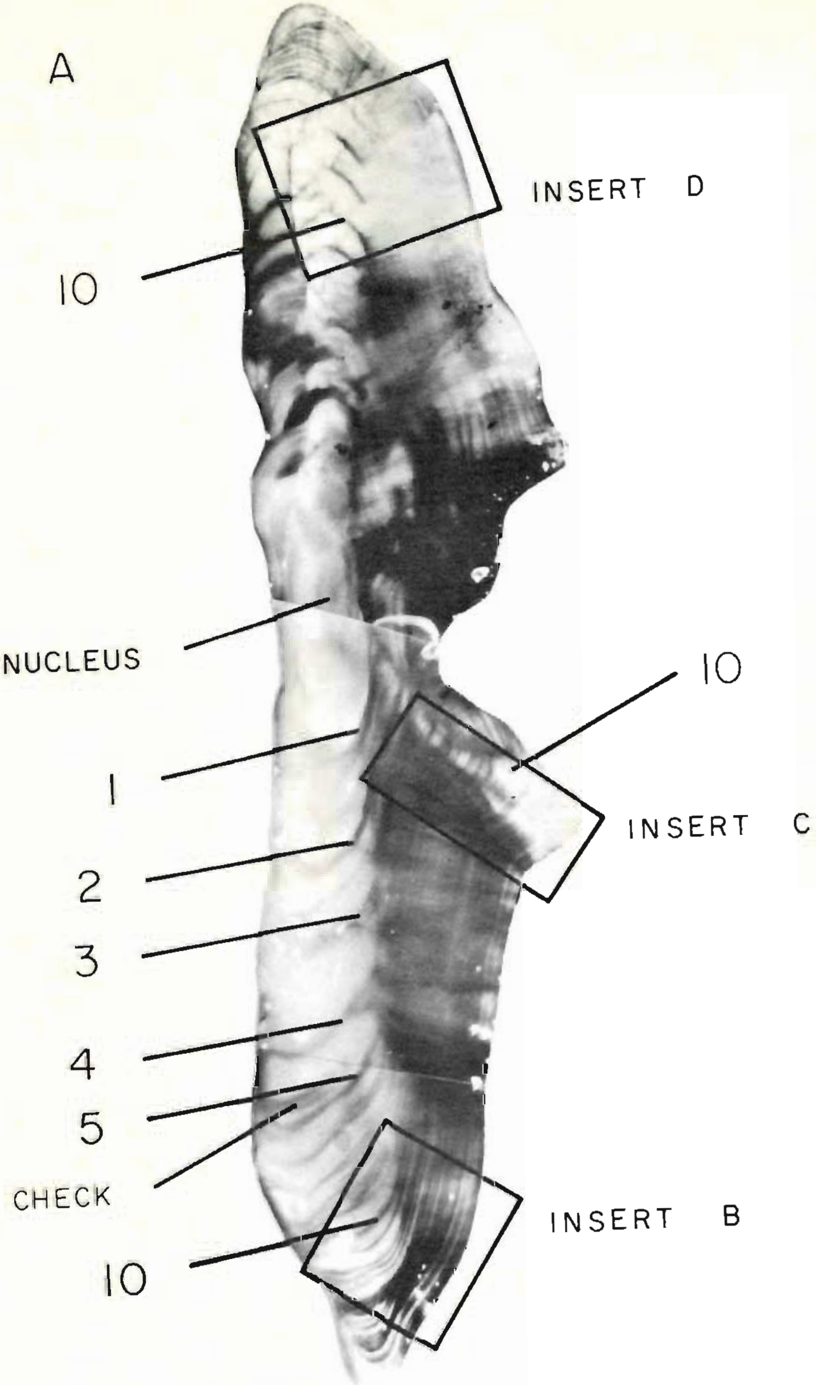


FIG. 64. (A–D) Burnt cross section of a yellowmouth otolith showing growth patterns becoming compacted on the internal edge. Several areas may be examined for aging including along the sulcus edge, see (C).

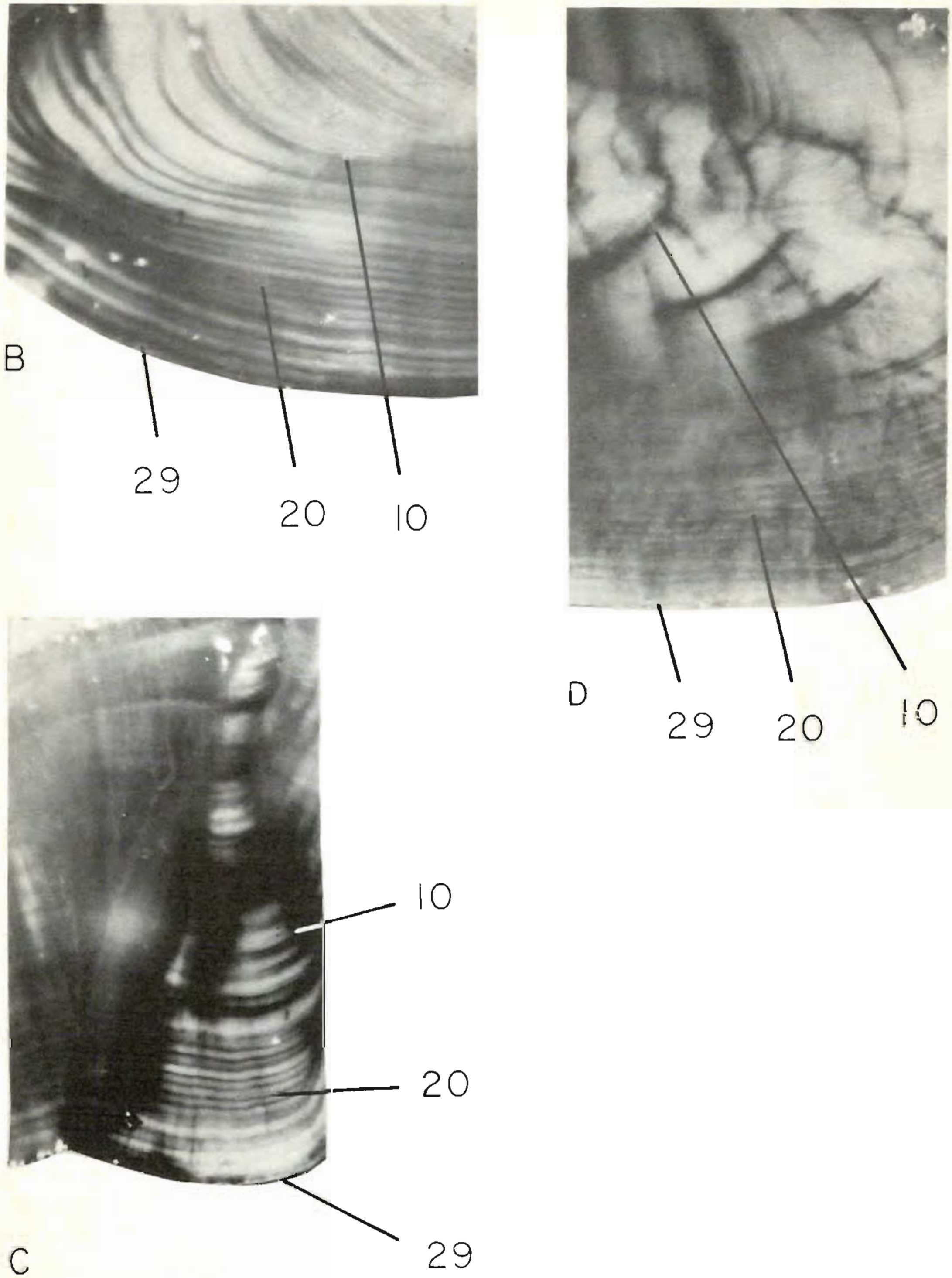


FIG. 64. Continued.

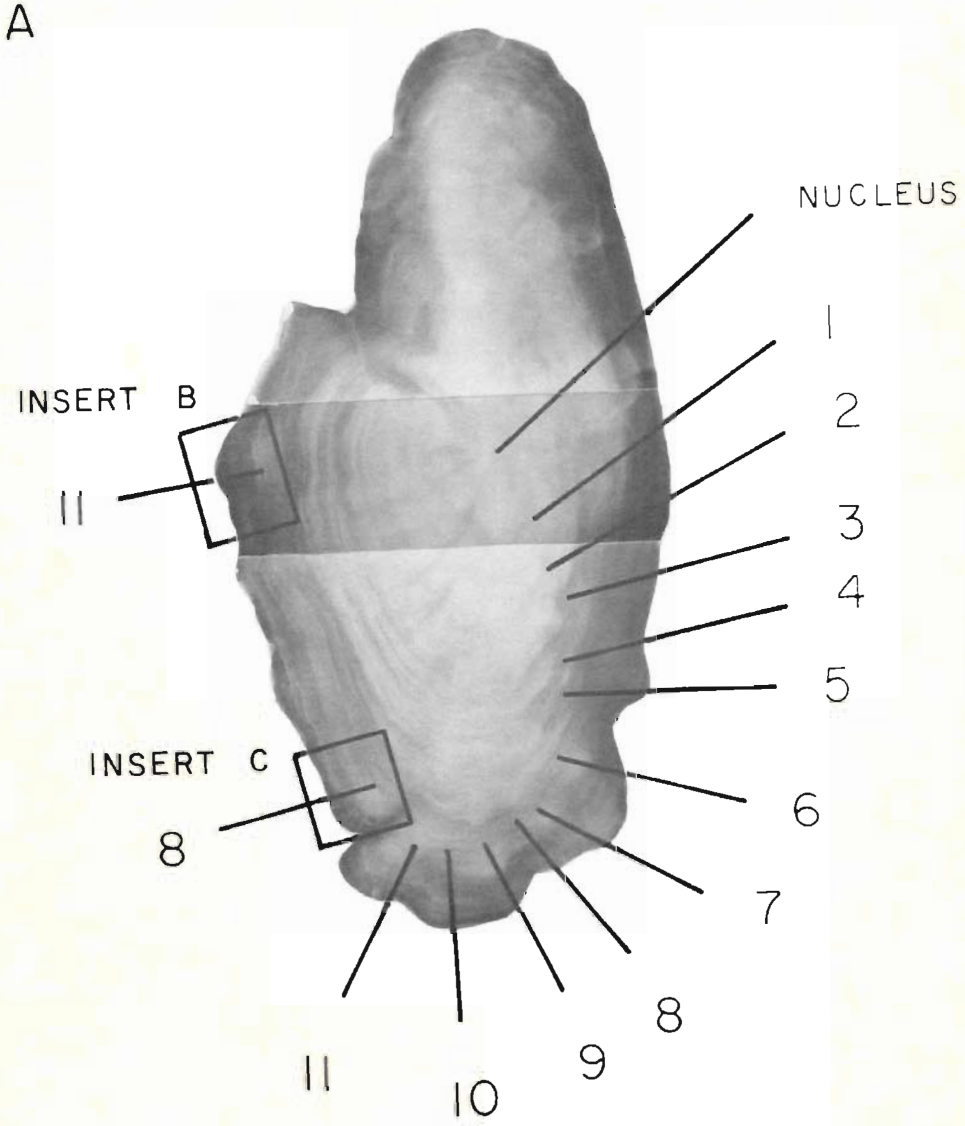


FIG. 65. (A–C) Yellowmouth otolith and enlargement of edges showing difficulties with irregular pattern on the external surface. (A) Note the second and third annuli are composed of many zones and also many checks are evident along the dorsal area. (B) The annuli are only evident to ~25 yr. (C) The annuli are evident to 30 yr.

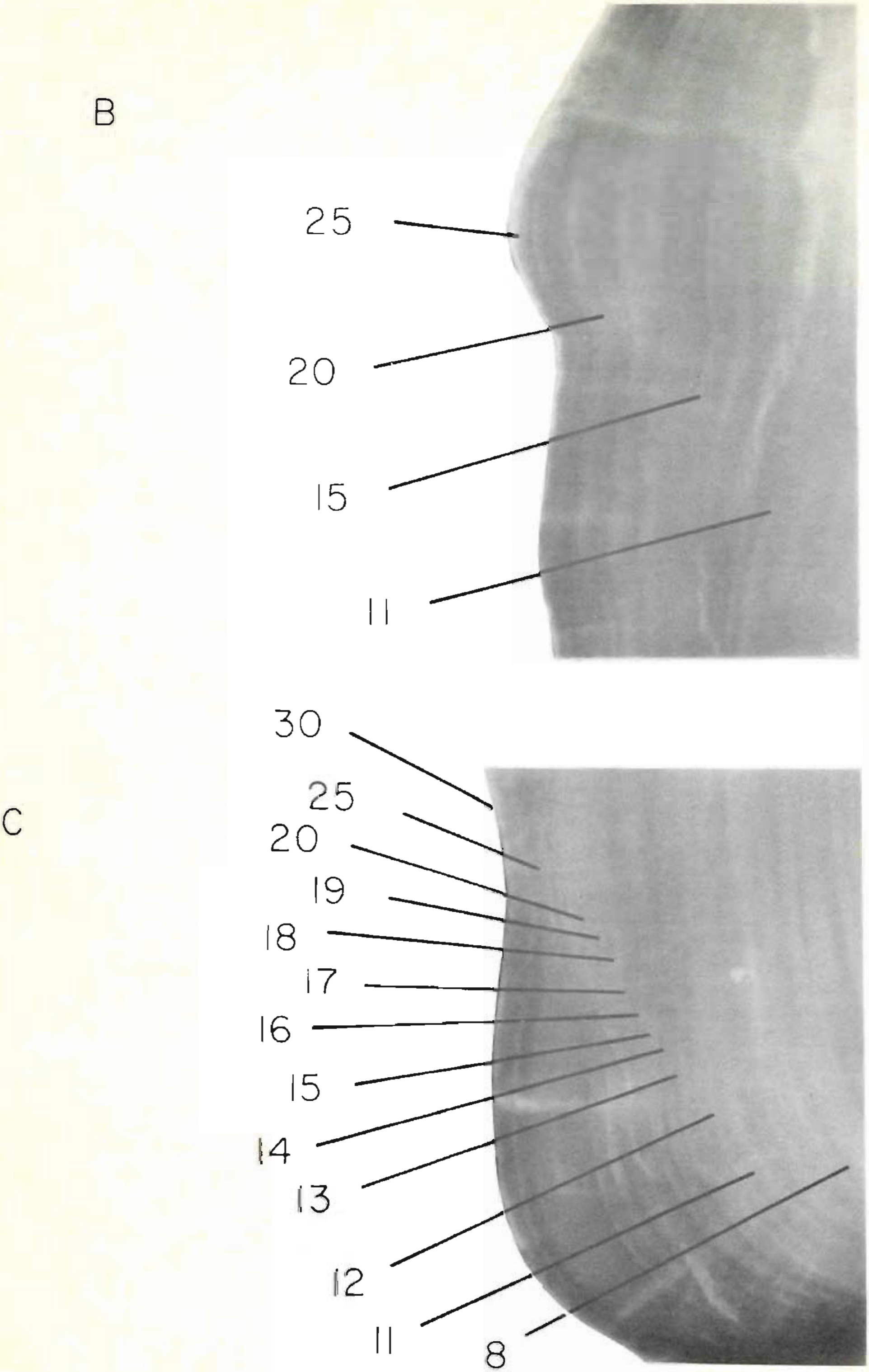


FIG. 65. Continued.

SQUALIDAE

SPINY DOGFISH *(Squalus acanthias)* (Ketchen 1975)

There has been little information published regarding the age and growth of dogfish because of the difficulty in aging this species. Kaganovskaia (1933) and Holden and Meadows (1962) were among the first to attempt to age dogfish and Ketchen (1975) expanded on their methods by using the second dorsal spine for aging. Despite the development of an age determination technique there is little evidence of laboratories applying it for routine age determinations. There also is no evidence that the method has been validated for all age-classes in the population.

Dogfish possess two spines, one anterior to each dorsal fin. Each spine runs from the vertebral column, passes through the dorsal muscles and the skin, and runs obliquely upwards and backwards to end in a point. The posterior or second dorsal spine is used for aging the fish as it is larger and less subject to wearing (Fig. 66). The spine is triangular in cross section. The exposed portion is covered by an enamel layer on the two convex faces. The enamel-covered anterior faces contain a series of dark bands and ridges which extend from the base of the exposed spine towards the tip. Each of these dark bands that also form ridges is considered an annulus. Unpublished information collected by this laboratory suggests that the dark bands are formed by a concentrating of the enamel layer as a result of a slowing down of spine growth.

In this laboratory we routinely age about 2000 spines a year. The following description is the method currently used for aging this species.

Make three cuts to remove the spine, one on each side of the anterior edge and the third down the back side of the spine between the fin and the spine. All cuts should extend to the base of the spine where it attaches to the vertebrae. Care must be taken that the enamel is not damaged when attempting to remove flesh from the spine during this extraction operation. Place spines in heavy kraft coin envelopes and freeze until they can be dried 3–5 d at room temperature. Remove spines from the envelopes, and scrape and cut off the dried flesh and skin (once again care should be taken that the enamel is not knicked or removed). Rub briskly with a commercial wiping cloth to clean and polish the spines to "hi-lite" the annuli.

The spine base, that part embedded in the dorsal musculature, contains no annuli and is used to hold the spine while cleaning and reading. Spines are examined under low power (10X, 12X) with direct lighting from above. Each spine is hand held under the microscope. All reasonable distinct raised bands and broad faint bands are counted. Spacing between the bands and shadows is usually irregular. The least distinct bands are usually laid down during the first 5–7 yr, whereas annuli that form in latter years are more raised and compact. Annuli may form in a series of small tight bands and must be counted individually, not as groups or a series of groups.

Spine wear and calculation of annuli in the worn area is described in detail by Ketchen (1975). Spine wear occurs at the tip where either the enamel is worn completely off or only partial shadows of the ridges remain. The point at which the last complete annuli can be counted is referred to as the "no wear point." Here, the diameter of the spine is taken with a direct reading from a Vernier calliper (precision 0.05 mm). The diameter is applied to a curve generated by Ketchen (1975) and an estimate of the number of annuli that were lost by wear is made directly from the curve and added to the already counted number of annuli to produce a final age.

Although the method remains to be validated, a preliminary tagging study involving OTC-injected fish indicates that the amount of annual spine growth estimated by this method formed beyond the OTC mark in a fish that had been at liberty for 10 mo (Fig. 67).

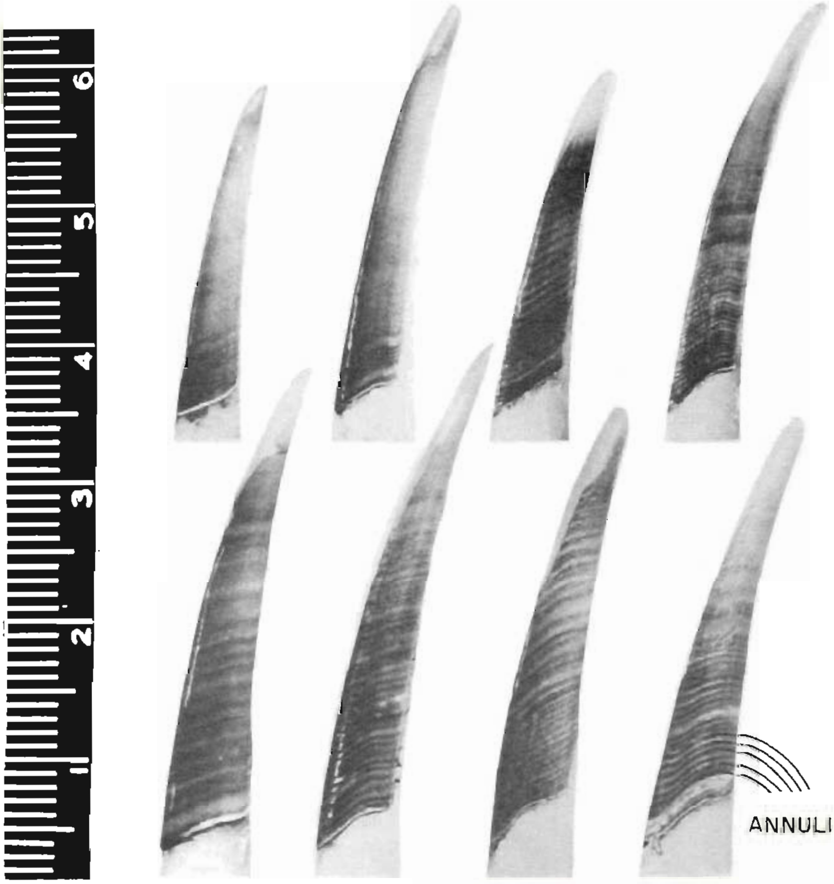


FIG 66. Lateral view of second dorsal spines from female dogfish (scale in centimeters). Fish length and estimated ages: (1) 39 cm, 2 yr; (2) 69 cm, 16 yr; (3) 82 cm, 24 yr; (4) 89 cm, 31 yr; (5) 93 cm, 22 yr; (6) 104 cm, 43 yr; (7) 109 cm, 42 yr; (8) 113 cm, 46 yr (all but No. 1 and 2 with ages calculated as per techniques described in text (photo courtesy Ketchen 1975)).

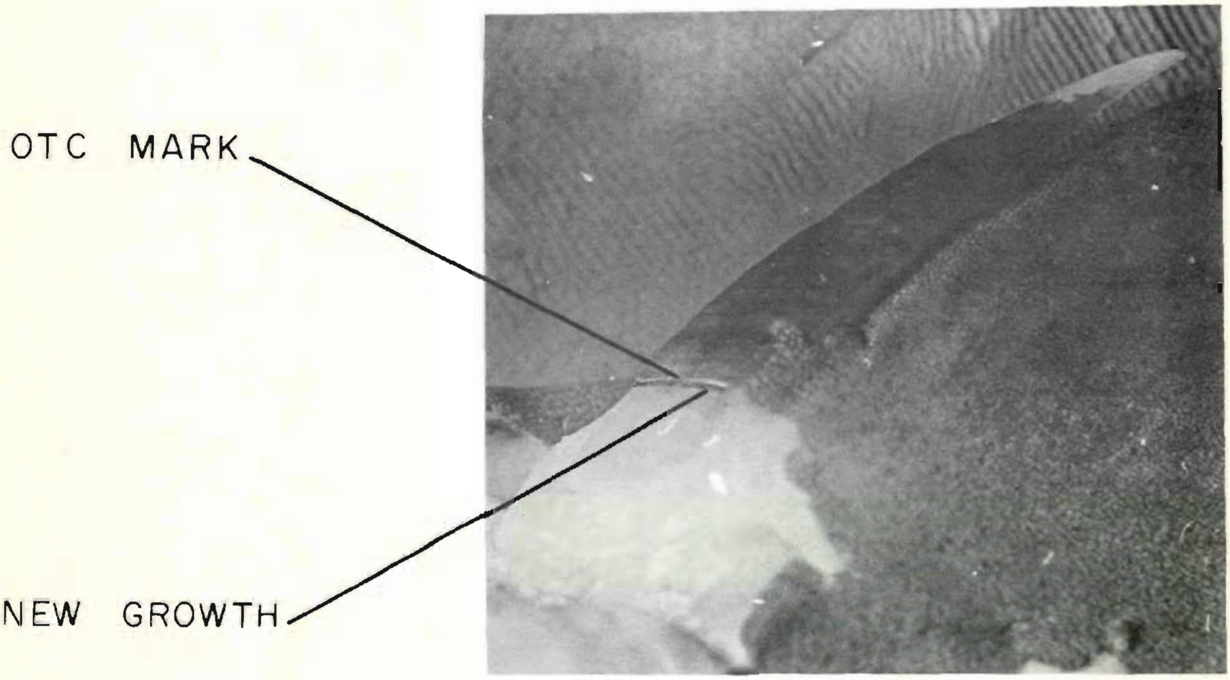


FIG. 67. A ♀ dogfish spine photographed with ultraviolet light, that was tagged and injected with OTC in April 1980, at a length of 101.1 cm and recovered February, 1981, at a length of 102.4 cm. The OTC mark fluoresces yellow under UV light. The new growth that formed beyond the OTC mark is similar to the amount of annual growth observed for most older dogfish (Fig. 66, No. 8).

Summary

This manual is a preliminary account of the procedures used in our laboratory to estimate the age of fishes. In an attempt to produce this first draft, some descriptions have been shortened and some material has been omitted. We expect to provide more detail about interpretations in subsequent drafts and add procedures that are still experimental or procedures that are applied to other species. We also intend to expand the description and discussion of analytical procedures.

We want to stress that many methods have not been validated for the particular species and most haven't been validated for individual stocks. In such cases, a number of structures should be examined to ensure that consistent readings are obtained. Even if consistency is obtained, accuracy can't be assumed until some form of validation is found for all age-classes in the population. In this laboratory we currently are attempting to validate a number of our procedures.

The range of ages produced by our methods is greater than previously determined for most of the species studied and the maximum ages obtained represent a significant change in our understanding of the possible life span of fishes (Table 1). For example, a rougheye rockfish (*Sebastes aleutianus*) was aged at 140 yr and this is one of the oldest recorded ages on record for any fish. Other species of fish were also found to be extremely old. In Table 1, 43% of the species range from 50 to greater than 100 yr and 48% are 29-49 yr old. Only 9% of the species have maximum ages of less than 20 yr.

Although it is possible to argue that our interpretations may not be correct for the species where the method hasn't been validated, it is equally possible to argue that previous interpretations had an even weaker basis for assuming accuracy. For some reason, possibly a consequence of the unquestioning use of scales, fisheries biologists seem to have had the impression that most fishes do not live very long and are usually in the age range of 5–15 yr. Often, when a structure was examined that had a growth

Table 1. Maximum ages obtained using the methods described in this report.

Family and common name	Scientific name	Maximum age (yr)
Anoplopomatidae		
Sablefish	*Anoplopoma fimbria*	53
Gadidae		
Pacific cod	*Gadus macrocephalus*	8
Pacific hake	*Merluccius productus*	23
Walleye pollock	*Theragra chalcogramma*	9
Hexagrammidae		
Lingcod	*Ophiodon elongatus*	21
Pleuronectidae		
Arrowtooth flounder	*Atheresthes stomias*	22
Rock sole	*Lepidopsetta bilineata*	25
Dover sole	*Microstomus pacificus*	45
English sole	*Parophrys vetulus*	22
Scorpaenidae		
Rougheye rockfish	*Sebastes aleutianus*	140
Pacific ocean perch	*Sebastes alutus*	90
Shortraker rockfish	*Sebastes borealis*	120
Silvergray rockfish	*Sebastes brevispinis*	80
Darkblotched rockfish	*Sebastes crameri*	47
Widow rockfish	*Sebastes entomelas*	58
Yellowtail rockfish	*Sebastes flavidus*	64
Bocaccio	*Sebastes paucispinis*	36
Canary rockfish	*Sebastes pinniger*	75
Redstripe rockfish	*Sebastes proriger*	41
Yellowmouth rockfish	*Sebastes reedi*	71
Harlequin rockfish	*Sebastes varigatus*	43
Sharpchin rockfish	*Sebastes zacentrus*	45
Squalidae		
Spiny dogfish	*Squalus acanthias*	66

pattern that suggested the fish was older, the unexplained growth zones were called "checks" or it was assumed that more than one complete growth zone was deposited in a year. In our opinion, the prominent and regular growth patterns that we interpret as annuli cannot be identified as checks which are fragments of growth zones. Our interpretation has been validated directly for some age-groups of some species (Beamish and Chilton 1982; Cass and Beamish 1982; Archibald et al. 1982). Also, our conclusion that rockfish can be very old has been confirmed by analyzing the natural radionuclide concentrations in otoliths (Bennett et al. 1982). Therefore we suggest that until the procedures can be validated there is no reason to reject our interpretations and there is a better basis for assuming the fishes described in this report are older than previously thought.

The management implications of extreme longevity have been discussed by Leaman and Beamish (1982) and they conclude that failure to recognize that a species is long-lived will have important management consequences.

Finally, we want to stress that the process of estimating the age of fishes should never be taken for granted. All techniques are qualitative to some degree and continually require evaluation.

Acknowledgments

We wish to express our thanks and appreciation to Shayne MacLellan for her useful suggestions and able technical assistance with photography and drawings, to Cathy Manson for her daily growth ring study, and to Wendy Mitton, Dawn Barnes, Karen Charles, and all the staff of the Groundfish Program who contributed material for this manual. Dr K.S. Ketchen kindly reviewed the manual.

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Glossary

Accuracy — the degree of closeness to the true value

Age-group — the number of fish of similar age; the term age-group is not synonomous with year-class as aging errors may combine a number of year-classes in one age-group

Aging structures — any structure that can be used for age determination; the most common are scales, spines, fin rays, otoliths, vertebrae, operculum, and cleithrum

Annulus — any zone that forms once a year; frequently an annulus is identified as the zone that forms during or close to a period of no growth or slowing down of growth

Annual growth zone or pattern — all the growth on a structure which forms once a year

Checks — marks or growth zones or parts of growth zones that do not form annually but reflect various environmental or physiological changes

Edge of otolith near the sulcus — the edge of the otolith adjacent to the groove on the internal surface which contains the auditory nerve (Fig. 5)

External surface — the distal surface of the otolith (Fig. 5, 10)

Hyaline zone (translucent zone) — the zone that forms during or close to a period of no growth or slowing down of growth; this zone is often thought of as the winter zone and is frequently identified as an annulus; the zone appears clear under transmitted light and dark under reflected light; in younger fish it is narrower than the opaque zone but in very old fish it remains about the same width as the opaque zone

Internal surface — the proximal surface of the otolith (Fig. 5, 10)

Nucleus or center — origin of the aging structure, sometimes described as the larval or embryonic center; the term nucleus is used for otoliths and center is used for fin rays

Opaque zone — inhibits passage of light and forms part of the annual growth pattern and is usually the period of faster growth; this zone is often thought of as the summer growth; the zone appears dark under transmitted light and clear under reflected light; in younger fish it is wider than the translucent zone but in very old fish it remains about the same width as the translucent zone (Fig. 9)

Otolith lobes/fingers — as the otolith becomes older occasionally portions of it grow in the form of lobes or projections; this is common in rockfish otoliths (Fig. 54)

Plus growth — see opaque zone or summer zone

Population — a group of fish of one species that are not known to interbreed with another group of fish of the same species

Precision — the degree of reproducibility; it relates to the variability between readers or between readings

Reflected light — light that is shone onto the surface of an object from above or the side

Regenerated scale — a scale that forms when another scale has been lost (Fig. 1)

Stock — a group of fish of one species that may interbreed with another group of fish of the same species

Summer zone, summer growth — in the Canadian fishing zone it is a period of faster growth; it is sometimes referred to as plus growth (see opaque zone)

Transmitted light — light that is passed through the object from beneath

Validation of aging method — proving a given age is correct usually by means of a tagging or marking experiment

Winter zone, winter growth, winter rings — see annulus, translucent zone

Year-class — hatch or brood produced in a given year

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