# Age and Growth Assessment of Fish from Their Calcified Structures—Techniques and Tools[1]

JOHN M. CASSELMAN[2]

## ABSTRACT

**Age and growth assessment of fishes from their calcified structures has been used widely for many years, and forms the basis of most of our present-day fisheries management decisions. However, the results of these assessments have not been validated adequately, even in the confines of freshwater, let alone in the oceanic pelagic environment. Five major categories of endeavor should be pursued to improve and refine this science and its practical application: *Interpretation, validation, collaboration, automation,* and *innovation.* Examples of the techniques and tools associated with these approaches are presented in this overview, usually for freshwater species; however, they can be, or have been, applied equally well to oceanic pelagic fishes.**

**Interpretation of age and growth assessment can be refined and improved by using fluorochrome labels, which provide marks in the calcified structure that permit temporal and spatial orientation. A universally acceptable terminology is needed. Validation of age and growth studies should become routine. Fluorescent markers and tag-recapture are the most useful; however, comparisons of different calcified structures, and other, more indirect tests such as fitting growth models (e.g., von Bertalanffy) can be helpful. Collaboration through exchange programs can produce "reliably" aged reference material now that technology exists to facilitate the transfer of this science. Automation and mechanization of routine age and growth assessment are required. The physical and chemical properties of fish calcified tissue, as revealed by electron microprobe X-ray analysis, substantiate that, as in forestry X-ray densitometry, this approach can be used to mechanize and computerize age and growth assessment of fish. Innovation is necessary to develop new and more powerful techniques that can be used to determine age accurately and precisely. Otolith microstructure has greatly increased precision, and new biochemical (e.g., aspartic acid racemization analysis) and radiometric (e.g., analysis of uranium decay series nuclides $^{226}$Ra and $^{210}$Pb) techniques have the potential to make age determination truly objective.**

## INTRODUCTION

Age assessment of fish from their calcified structures is a vital component of most of our present-day fisheries management decisions. Even though this knowledge is used widely, validation of the accuracy of the estimates frequently has been relegated to low priority and often has not even been attempted. Validation should be an essential and routinely performed part of every study that involves the extraction of data from the calcified structures of fish. Although this critical problem is universal (Carlander 1982; Beamish and McFarlane 1983), it has not been adequately addressed even in the confines of freshwater, let alone in the oceanic environment. Probably one of the greatest challenges in validation of age and growth assessment is presented by the large oceanic pelagics such as tunas, billfishes, and sharks. These species are difficult to sample, highly mobile, and have extensive geographic ranges, often encompassing tropical as well as temperate oceans. Nevertheless, the basic principles of age and growth assessment of fish are similar regardless of species and environment, and the practical problems of assessing age and growth of large oceanic pelagic fishes are generally similar to those of other species.

Although comprehensive tests of the reliability of interpretations are few, they indicate that the complexity of the problem has been oversimplified. Some procedures previously considered to be reliable, especially those involving the scale method, are now suspect and under certain conditions have led us to erroneous assumptions. These inconsistencies have affected the confidence that can be placed on this important component of fisheries science. Increased effort is needed to refine, improve, and validate all aspects of the science and technology of age and growth assessment of fish.

If we are to address this problem thoroughly, we must start by considering some very basic problems. For example, inconsistent and ambiguous terminology persists, making communication and comparison of results difficult. This has hindered our ability to transfer science, to better understand the problems, and to develop universal theories explaining the factors causing check and zone formation.

The forum provided by international workshops and symposia, such as the one reported in these proceedings, helps to focus attention and coordinate efforts to resolve such universal problems as those associated with terminology and validation (Brothers 1983). Other international workshops and symposia held in recent years to examine the problem of age determination of fishes (Zoological Society of Slovakia 1968; Bagenal 1974; Everson 1980) not only contributed to better communications and understanding of the problem, but also stimulated additional research. For example, since the Reading symposium in England (Bagenal 1974), studies of otolith microstructure have contributed greatly to the precision with which age can be assessed.

The techniques and tools available to tackle this very fundamental fisheries problem are becoming more numerous, powerful, and sophisticated. Age and growth assessment is undergoing a technological revolution, as are many other fields

[1]Contribution No. 82-04 of the Ontario Ministry of Natural Resources.

[2]Ontario Ministry of Natural Resources, Research Station, Fisheries Branch, Box 50, Maple, Ontario, Canada L0J 1E0.

of scientific endeavor. Fisheries workers must be innovative and apply these modern techniques and tools more widely.

To improve and refine this science and its practical application, five major categories of endeavor should be considered: Interpretation, validation, collaboration, automation, and innovation. Each of these categories will be reviewed with examples of the available techniques and tools. Although many of the examples provided are for freshwater species, they apply equally well to oceanic species, especially the large pelagics, as indicated by examples from this workshop.

## INTERPRETATION

Ambiguous terminology has created confusion for those interpreting age and growth of fish. Sometimes results have appeared to be paradoxical when compared with those from other studies and structures. Inadequately defined, ambiguous terminology hinders our ability to transfer information and to develop universally applicable hypotheses. Where possible, we should communicate through a standard terminology (some standardization has been achieved in these proceedings—see Glossary), and if this is not available, then each term should be thoroughly defined.

Terms should describe conditions directly, not circumstantially. For example, optically different zones in fish calcified tissue other than scales should be described according to their structural appearance or light properties, e.g., translucent or opaque, not as "slow-growth-zones" and "fast-growth-zones" or "winter zones" and "summer zones," terms that assume that tissue with a certain optical appearance is deposited in association with slow or rapid growth or particular seasons. The interpretation of results has also been complicated by such ambiguous terms as "light and dark" or "black and white" when the method of illumination is not specified. The terms translucent and opaque should be used, because in the definitions of these terms the type of illumination is implicit—it is transmitted. Translucent means the tissue allows the transmission or passage of light, whereas opaque means that it does not allow the transmission of light, or is impervious to light rays. These terms are preferred and can be used regardless of the method of illumination, because even in reflected light the transmission and absorption of light energy are important.

If it is necessary to describe zonation in reflected light, then the opaque zone does not transmit light energy but reflects it, so the zone appears white or the color of the illuminating light. In reflected light, this zone would be referred to as a reflective zone. In reflected light, the translucent zone allows light to penetrate and be absorbed by the tissue or to pass through the tissue and be absorbed into the background, hence this zone appears darker. This zone would be referred to as an absorptive zone (Casselman 1974).

The term hyaline is acceptable, but has several disadvantages. Although it indicates that the type of tissue is glasslike, vitreous, or free of inclusions, it also means clear or transparent. Calcified tissue is not transparent, but is translucent. Also, the term hyaline has no direct opposite that can be used to describe the "opaque" condition, a term frequently used in juxtaposition. Hyaline explains the nature of the material, whereas opaque explains its light properties.

Terms such as ring, band, mark, and circuli (when not referring to scales) should not be used unless they are adequately described. For example, when talking about ring formation, it is impossible to know the properties (light, structural, or otherwise) of the zone being described. When referring to fish scales, I prefer the term check, which means a break or change in the uniform configurations of the circuli.

Not only is some of the terminology in use ambiguous, but in some cases it is also, by definition, incorrect. For example, the term annulus simply means concentric ring. There is no connotation of yearly in the Latin definition of annulus and it should therefore not be confused with the term "annular." However, the term annulus has become a common and accepted term in age assessment. For purposes of age assessment, the annulus (annual mark, year mark) can be defined as a mark that is subjectively located, sometimes very precisely for "back calculation," on or in a calcified structure; is associated with the distal edge of a concentric ring in the form of a check on the scale or a translucent zone in other calcified structures; is found along the entire structure; and is considered to separate the check or zone associated with the principal annual cessation or reduction in growth from the tissue deposited when growth resumes or increases. Two successive annuli are usually considered to demarcate one calendar year of calcified tissue growth.

Age assessment of fish from their calcified tissue is conducted by systematically interpreting (usually the optical appearance) either a whole or sectioned structure, starting at the focus or origin and examining all regions outwards to the edge. The structure may be treated and examined by different techniques. Nevertheless, the interpretation involves an examination of various checks and translucent zones in terms of their continuity or extent, location, and the quality of the tissue in and about them. The significance of these checks and zones is then judged according to criteria that are based on the definition of the annulus. The checks and zones associated with annuli are differentiated from those considered to be formed at other times and influenced by other factors. Generally, the checks and zones associated with annuli are those that are found throughout the structure and are separated by zones (usually more opaque) associated with growth. The growth zones between annuli usually have characteristics that indicate rapid growth followed by decreasing growth. When specific types of checks and zones are known to be associated with annuli, then the assessment is more objective.

Pseudoannuli, or false annuli, are similar to annuli, but are associated with checks and zones that are somewhat incomplete and irregular, are found in only one part of the structure, and often not in all structures. Although they are sometimes prominent, they are not associated with the check or zone that forms during the "principal annual cessation or reduction" in growth that produces the annulus.

Applying these interpretations results in an age that should be considered to be estimated, assumed, assigned, or assessed. Rarely are the criteria for distinguishing the various types of checks and zones sufficiently precise, or are the techniques adequately validated or even verified so that it can be said beyond reasonable doubt that we have "determined" age. Determination of "true" (correct) age without errors by these techniques from the calcified structures of all fish will probably always elude us. We must recognize and accept the limitations of the method. Age assessment as currently practiced is strongly subjective. Interpretation can, however, be greatly improved and refined.

When interpreting calcified tissue, it is essential that the examination and description of checks and zones be thorough, and that this information be recorded so it can be evaluated according to objective criteria to obtain age. All too often, age assessment is just a simple enumeration. Regardless of how regular and distinct the checks and zones appear, they should be interpreted in terms of well-defined criteria. Unfortunately, these criteria have not been adequately developed for most structures and species.

If the checks and zones associated with annuli are indistinct, variable in appearance, or coalesce (most frequently at the edge) as a result of decreased growth rate with increased age, then the assessment will be difficult, repeatability will be poor, and results will be inconsistent. Under these conditions the problem should be acknowledged, and the interpretation should be qualified by ranking the degree of confidence. For example, one system provides the number of annuli, a coded description of the edge of the structure (from Casselman 1978, Appendix J), and a numerical ranking (from 1 to 10) of the degree of confidence that can be placed in the assessment. All too often in the past, interpreters accepted the responsibility of providing an age estimate regardless of the difficulty and without indicating any measure of the degree of confidence they placed in the assessment. Unfortunately, even though interpreters examined structures and their images in considerable detail, often nothing more than an estimate of age was provided for further analysis.

An interpretation of a structure provides an estimated or assessed osseological age; however, this may not be the chronological or calendar age of the fish. Since calendar age is required for most fisheries work, it is essential that the relationship between the osseological age and the calendar age be known. If they are the same, the interpretation is valid; if not, the osseological age must be qualified, corrected, or rejected.

In order for an interpretation to be objective and unbiased, no information should be used when the initial interpretation is conducted. It should be made independent of time of capture, length of the fish, and even size of the structure, if possible. The first annulus should not be located by size or, for scales, by number of circuli. This procedure forces all the results to conform to some preconceived interpretation that may not apply to the sample being examined.

Errors in age interpretation undoubtedly occur and, within limits, are acceptable; however, random error is not as important as systematic error (Powers 1983). Serious systematic errors can develop when scales or other structures of fish that are very slow growing, or do not grow, no longer continue to grow and to record age according to normally recognized criteria. Under these conditions, the method results in fish being underaged. This problem is more common than has generally been thought. Indeed, some species may be much older than has heretofore been considered (Beamish 1979). Similarly, certain parts of a structure may continue to grow and indicate age, whereas in other parts, growth may be reduced to a level at which checks and zones are not delineated annually, hence do not represent the actual calendar age.

Specific methods of interpreting age from the various calcified structures have been reported in detail in the literature. However, this overview will refer to only those works selected to describe the various methods or considered important to age determination of oceanic pelagics.

The scale method has been used widely in fisheries, and involves a systematic interpretation of the checks (breaks or changes) in the configurations of the circuli located on the outer surface of the scale (Regier 1962; Carlander 1974; Casselman 1978). The scales are easily removed and magnified either as whole scales or, if thick, as their cellulose acetate impressions. Interpretations have appeared to be straightforward and in some cases even simple; however, when replication is attempted, results are often inconsistent, and verification studies indicate that in older and slow-growing fish, scales underestimate the true age and may be unreliable (Beamish and Harvey 1969; Erickson 1979; Mills and Beamish 1980). A thorough test of the validity of the scale method will demonstrate that in some cases the bias can be great and the method misleading. The scale method has been used frequently for the tunas (Yabuta et al. 1960; Bell 1962a, b; Yukinawa and Yabuta 1963, 1967; Yang et al. 1969; Yukinawa 1970) and Fourier series analysis has been used to determine the time of annulus formation (Nose et al. 1955). The scales of tuna appear to present many of the same problems in age assessment as do those of other fishes (Yabuta and Yukinawa 1963) and are not suitable for ageing billfishes or sharks. Regardless of species, the scale method should be treated with caution and should be avoided if the fish are suspected to be very slow growing or old, because the method will not provide comparable age assessments across a broad range of ages.

The otolith (sagittal) method, which can involve interpretation of either macro- or microzonation, has been used extensively in marine fisheries because workers have recognized that the fish were old and age could be assessed more easily by this method. It is now being applied more widely in freshwater fisheries. The otolith method (macrozonation) involves the recognition and interpretation of translucent zones, which are associated with annuli (although some prefer to enumerate opaque zones). Sagittae can be examined whole or can be fractured, ground and polished, sectioned, stained, charred, acid etched, or otherwise prepared for examination (Blacker 1974). Their removal, preparation, and examination are more complicated and sometimes more difficult than for other methods and also necessitate killing the fish. Nevertheless, the results for many species are more reliable because the interpretations more closely approximate "true" age than those obtained by other methods, especially for old fish (Beamish 1979; Erickson[3]). Results from the otolith method are generally more consistent because zonation is usually more distinct and more easily recognized, even in older fish, than with some other methods. However, this may not be the case for the giant (old) Atlantic bluefin tuna, *Thunnus thynnus*, because estimated vertebral age appears more accurate than does estimated otolith age (Lee et al. 1983).

Detailed microstructure (microzonation) exists in the otoliths of many species, making them especially powerful tools that can reveal even the daily age of the fish (Pannella 1974, 1980; Brothers et al. 1976). The otolith method has been applied to age assessment of tunas (Uchiyama and Struhsaker

[3]Erickson, C. M. 1982. Age determination of Manitoba walleyes using otoliths, dorsal spines, and scales. Manuscr. submitted to North Am. J. Fish. Manage. Department of Natural Resources, Fisheries Branch, Biological Services Section, Winnipeg, Manitoba, Canada R3H 0W9.

1981) and has been examined by using mark-recapture techniques and tetracycline labels to place a temporal and spatial orientation mark on the otolith (Wild and Foreman 1980). The otoliths of the sailfish, *Istiophorus platypterus*, as well as of other istiophorids, contain not only internal zonation, but also external ridges that appear to correspond to age, at least in young fish (Radtke and Dean 1981; Radtke 1983). The otolith method has been applied extensively to the large oceanic pelagic fishes in the present proceedings (Brothers et al. 1983; Hurley and Iles 1983; Lee et al. 1983; Radtke 1983; Wilson and Dean 1983). If fish are suspected to be old, this method appears more useful (see exception, Lee et al. 1983) and zonation should be interpreted along the region of maximum growth, or longest radius.

The fin ray (soft ray) or spine (spiny ray) methods are similar, and offer several advantages over otoliths and other bony structures. These structures can be removed easily, and it is not always necessary to kill the fish or significantly mutilate the carcass (Beamish 1981). The method is especially useful because, like scales, fins can be removed from the fish at time of tagging and compared with the corresponding structure removed at time of recapture. The rays are usually thin-sectioned near the base (Batts 1972; Jolley 1974; Beamish 1981) or the cut surface can be smoothed and illuminated indirectly to expose internal zonation (Deelder and Willemse 1973). Surface examination of whole spines has been used successfully to assess age of spiny dogfish, *Squalus acanthias* (Ketchen 1975). Although rays are useful, there are disadvantages. In older fish the core can undergo resorption and become vascularized, obscuring and even eliminating the first few zones. This would result in an underestimation of age. In old fish, fin rays in some ways are similar to scales because, like checks on the edge of scales, the distal translucent zones may be so close together that they appear to coalesce, making optical resolution and correct age assessment difficult or even impossible. This method is now being used more widely on many species, including oceanic pelagics, as illustrated by its wide application in the present workshop (Antoine et al. 1983; Berkeley and Houde 1983; Cayré and Diouf 1983; Compeán-Jimenez and Bard 1983; Gonzáles-Garcés and Fariña-Perez 1983; Johnson 1983).

The centrum (vertebral) method has not been used widely, although it is an important technique for age assessment of cartilaginous fishes such as rays and sharks (Stevens 1975; Thorson and Lacy 1982) and has been used for several species of tunas. The removal and preparation of vertebrae are more difficult and time-consuming than some of the other methods reported here, and necessitate killing and mutilating the fish. The method involves the surface examination of whole or sectioned centra. The centrum may be viewed in white light, cleared (e.g., cedarwood oil), stained (e.g., alizarine, silver nitrate, or treated in numerous other ways to enhance zonation and facilitate its interpretation (Galtsoff 1952; Cailliet, Martin, Kusher, Wolf, and Welden 1983). This method is examined in detail in this workshop (Cailliet, Martin, Kusher, Wolf, and Welden 1983; Cailliet, Martin, Harvey, Kusher, and Welden 1983; Johnson 1983; Lee et al. 1983; Schwartz 1983) and has been validated with known age (Lee et al. 1983) and partly known age material by using the location of "tagging marks" (Casey et al. 1983) and tetracycline labels (Holden and Vince 1973; Gruber and Stout 1983).

The flat bone method involves either a microscopic, or most frequently a macroscopic, examination of the optical zonation in large, relatively flat bones. This method does not usually involve sectioning or grinding, although the latter may be used to increase light transmission. Fluorescent light enhances optical zonation better than does incandescent light. Incident light with the bone viewed against a dark background appears to be better than transmitted light. The method has been relatively widely used in freshwater fisheries, and has many of the advantages of the other methods (Casselman 1979), although it necessitates killing the fish. The method is especially useful for growth estimation (Casselman 1978). Many types of bones have been used in this method, although opercula (Le Cren 1947; Frost and Kipling 1959), cleithra (Casselman 1974, 1978), and branchiostegals (Bulkley 1960) are probably the most useful. The use of these structures in age assessment of oceanic pelagics has not been adequately documented, although Prince[4] reported that the opercula of the marlins show no conspicuous optical zonation.

Regardless of the method used, age and growth assessment of fish from calcified structures involves an interpretation of growth recorded in the tissue. It is necessary to recognize growth reductions and cessations associated with annual major stoppages that occur at the same time each year, and to distinguish them from those that occur at other times and for other reasons.

Detailed studies that attempt to decode the complete chemical, physical, and physiological record of growth and environmental change in skeletal material of aquatic organisms, as described in Rhoads and Lutz (1980), are rare but have been attempted on some fish, e.g., northern pike, *Esox lucius* (Casselman 1978), and are proposed for others, e.g., lemon shark, *Negaprion brevirostris* (Gruber and Stout 1983). Correct interpretation of this osseological record depends upon a thorough understanding of the factors and physiological processes that influence its growth and check and zone formation. When initially building expertise and acquiring reference information for the accurate interpretation of calcified tissue, it is necessary to understand the environmental requirements of the species.

Temperature is one of the most important factors influencing growth, and the optimum temperature for maximum somatic growth is probably the most useful single value. Although this value varies with species, it can be estimated easily if the final preferendum is determined (McCauley and Casselman 1981). Other major factors affecting growth, such as feeding rate and reproductive cycle, are more difficult to measure, and can be elucidated only by detailed studies in the laboratory and natural environment.

When studying growth in relation to check and zone formation, it is necessary to study the seasonal growth cycle. This is best done in the natural environment and on indigenous fish for which growth history is known. Hence, it is necessary to use mark-recapture techniques and to place temporal and spatial orientation marks (labels) in the calcified structures. Such studies not only provide the scientific basis for age and growth assessment, but also provide reference material that can be used to improve subsequent interpretations.

[4]Prince, E. D., Fishery Research Biologist, Southeast Fisheries Center Miami Laboratory, National Marine Fisheries Service, NOAA, 75 Virginia Beach Drive, Miami, FL 33149, pers. commun. 1982.

Although many types of chemicals have been used to label calcified tissue, fluorochrome labels using an antibiotic such as tetracycline appear to be the best, and have the added advantage of being therapeutic and prophylactic. These fluorochromes are deposited at all sites of calcification, and are visible as a fluorescent band when exposed to ultraviolet light. In studies on northern pike in the natural environment, Casselman (1978) tested intraperitoneal, subcutaneous, and intermuscular injections. An injectable solution of oxytetracycline hydrochloride, which contained 100 mg/ml Liquamycin,[5] marked scales and bones best when the fish were injected intraperitoneally with dosage rates of 25 to 50 mg/kg body weight. However, the type of injection and dosage rate depend upon many factors, including growth rate, type of tissue being marked, type of mark desired, and required longevity.

Tetracycline has been used to elucidate the seasonal growth cycle of the calcified tissue and body of northern pike (Casselman 1978). Shown in Figure 1 are the seasonal dynamics of the qualitative growth of cleithra and scales, and the quantitative linear growth of the body, cleithra, and scales of northern pike tagged and recaptured throughout the year in a small, shallow lake. Checks and translucent zones associated with annuli (tissue type 1) were deposited during late winter and early spring (Fig. 1B). Widely spaced circuli and opaque cleithral tissue (tissue type 4) were deposited during early and midsummer. Most rapid cleithral and scale growth occurred during early summer (Fig. 1D), coinciding with the optimum temperature for growth (Casselman 1978). These data substantiate that the annulus formed on the scales and in the cleithra at approximately the same time, when growth was slowest, and only once each year. Maximum and minimum growth rates of both structures and the body coincided seasonally. During rapid growth, the scales grew linearly at a faster rate than did the bones, and both grew at a faster linear rate than did the body. The opposite appeared to be true during slow growth.

For purposes of estimating body growth from calcified structures ("back calculation"—see Smith 1983), it has frequently been assumed that growth of the structure is isometric or can be mathematically transformed so that it appears to be. However, considering the seasonal cycle of northern pike (Fig. 1C), isometric growth was only a transitional stage that rarely, and possibly never, occurs. During rapid growth, growth of both structures was positively allometric; during slow growth it was negatively allometric. This relationship was always more extreme in scales than in cleithra. These allometric growth differences substantiate that when interpreting growth from a structure, we are describing only the growth of that body part and not necessarily the growth of any other part or the fish as a whole. Growth should be compared on a relative, not an absolute, basis. Back calculation of body size at age, which has been conducted widely and not tested adequately, should be carefully reexamined and attempted only with valid ages.

## VALIDATION

Numerous methods have been used in an attempt to validate age assessment of fish (Brothers 1979, 1983). However, most of these methods are indirect. The most powerful direct evidence for testing validity is obtained by examining structures from known age fish, e.g., stocked fish (Cable 1956), or partly known age fish that have lived in the natural environment or have been reared in captivity under natural or seminatural conditions. Partly known age fish are those that have been captured, marked (e.g., tag and fluorochrome label), and released, then subsequently recaptured so that the duration of the mark-recapture period is known. Although fluorochrome labeling is one of the most precise ways to perform this test (Casselman 1978), it is possible to remove some structures, e.g., scales and fins, at time of tagging and compare them with those obtained at time of recapture. It is also sometimes possible to see "handling marks" on structures when fish are recaptured. Although these artificial labeling techniques have been known for many

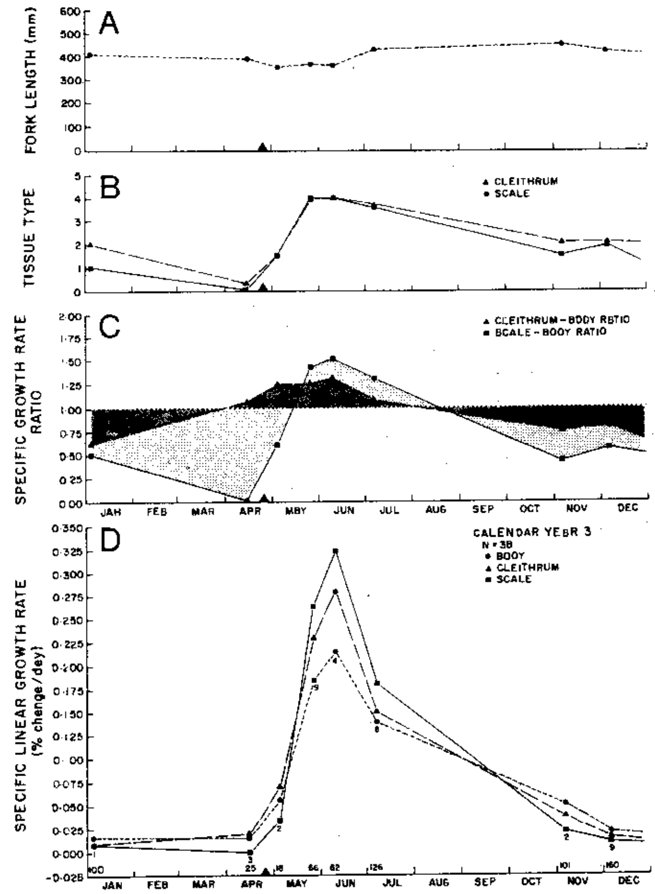



**Figure 1.—Seasonal dynamics of qualitative growth of cleithral bones and scales, and quantitative linear growth of body, cleithra, and scales of 38 northern pike, *Esox lucius*, in calendar year 3 from Smoky Hollow Lake, Ontario. Sexes are combined: 26 males and 12 females. Results are averaged by month of midpoint of the mark-recapture period and plotted on the mean day, except the samples for May which are separated by growth rate (fast or slow). Dark triangles on the X axes indicate the spawning period. A) Mean fork length of pike for the mark-recapture period. B) General classification of the type of calcified tissue deposited during the mark-recapture period. As the ranking of the tissue type increases, the associated circuli on the scales appear more uniform and widely spaced, and bony tissue in the cleithra appears more opaque (Casselman 1978). A check or translucent zone of type 1 is usually associated with annuli, and type 2 with pseudoannuli. C) Relative growth of calcified tissue and body. A ratio of 1.00 indicates isometric growth (dotted line). Shading indicates the deviation from isometric growth (dark—cleithrum; light—scale). D) Specific or instantaneous linear growth rates of body, cleithra, and scales during the mark-recapture period. Number of individuals is indicated below the data set. The number of days in the mark-recapture period is indicated on the X axis.**

[5]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

years, they are only now becoming more widely used in fisheries. For fish from the oceanic environment, the tetracycline method of validation has been used for centra (Holden and Vince 1973; Gruber and Stout 1983) and otoliths (Beamish and Chilton 1982). Wild and Foreman (1980) also used tetracycline to validate the occurrence of daily microstructure in otoliths of tunas.

Validation of age assessment should be a routine part of every study. Because a method has been shown to be valid under certain circumstances and for certain species, it does not necessarily mean that it can be assumed to be valid under all conditions. A different set of circumstances, such as change in growth rate, would necessitate a reevaluation of the method.

Many of the methods used in validation are often used independently to assess age. Some are strongly circumstantial but still provide good corroboratory evidence. Some of these, such as length-frequency and modal progression analysis, have been used to examine the validity of assessments made on oceanic pelagics (Yabuta and Yukinawa 1957; Le Guen and Sakawaga 1973).

One procedure frequently used to check assessed age is to compare ages assessed independently from different calcified structures from the same fish. The structures most often used in age and growth studies of the large oceanic pelagics are illustrated in Figure 2, and a thorough description of these, along with collection procedures, is provided by Prince and Lee (1980).

Comparisons of these types do not validate age assessment; they simply provide a measure of agreement and give some indication of the degree of confidence that can be placed in the interpretations. This comparative procedure would be better termed verification.

Numerous studies have shown that when ages of different structures are compared, perfect agreement over a broad range of ages is unlikely. This is especially true for muskellunge, *Esox masquinongy*, when scales are involved (Fig. 3). Cleithral age assessment of muskellunge from the St. Lawrence River has been validated over the entire age range of the species (Casselman[6]). Ages attained from scale interpretations agree well with cleithral interpretations up to approximately age 10. However, in older fish, scales contain fewer recognizable annuli than do cleithra. The edges of these scales appear eroded and have characteristics indicating resorption (Casselman 1979).

Much of the evidence used to evaluate the reliability of various methods and structures has come from verification studies rather than from direct validation (Beamish and McFarlane 1983). From these verification studies, it appears that sections made from otoliths (along the line of maximum growth) are most reliable, whereas scales, especially those from older fish, are least reliable. Other structures and methods of interpretation appear to fall intermediate in these comparisons. Johnson (1983) found sections of centra and first dorsal spines of little tunny, *Euthynnus alletteratus*, to give good agreement (96%). Lee et al. (1983) indicated that vertebrae tend to underestimate age, and enumeration of all zones in otolith sections seemed to overestimate the age of Atlantic bluefin tuna. However, the interpretations of vertebrae more closely approximated the partly known age of one very old giant bluefin tuna.

Although the tendency in verification studies has been to assume that the structure that provided the oldest assessment was the most reliable, this may not always be the case. Lee et al. (1983) were unable to reject the hypothesis that two translucent zones were deposited in the otolith of Atlantic bluefin tuna each year after first maturity. Otoliths of other species have also been shown to contain multiple zonation, e.g., lake

[6]Casselman, J. M., Research Scientist, Ontario Ministry of Natural Resources, Fisheries Branch, Research Section, Box 50, Maple, Ontario, Canada L0J 1E0. Unpubl. data, 1976.

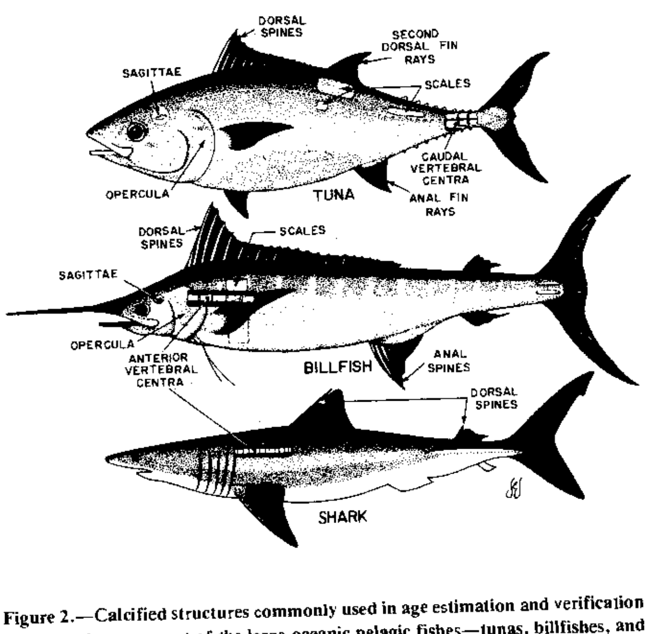



Figure 2.—Calcified structures commonly used in age estimation and verification and growth assessment of the large oceanic pelagic fishes—tunas, billfishes, and sharks. The approximate location of the sagitta in the cranium is illustrated. In addition to these hardparts, the crystalline lens of the eye, teeth, scutes, maxillaries, pterygiophores, cleithra, and various other flat bones, especially those of the opercular series, are used in some freshwater species.

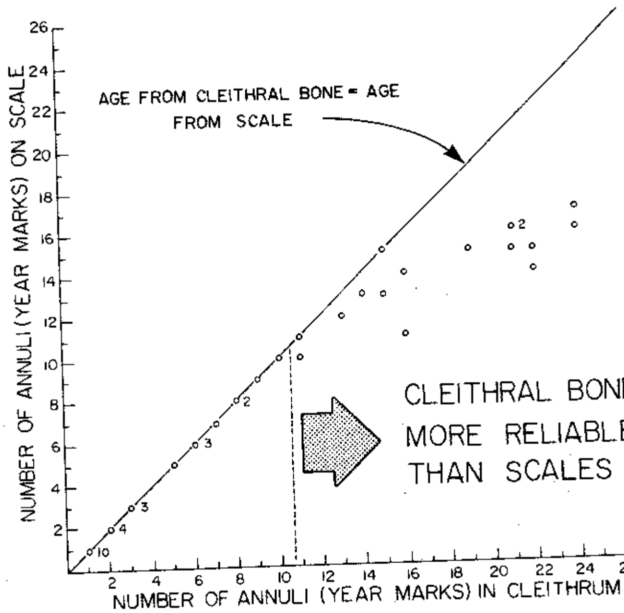



Figure 3.—Relation between number of annuli on the scales and in the cleithra of 42 muskellunge, *Esox masquinongy*, collected during 1965-75 from the St. Lawrence River, Ontario. When the data set represents more than one individual, the number is indicated. For fish older than age 10, the cleithral bones are more reliable for age assessment than are the scales.

herring, *Coregonus artedii* (MacCallum[7]), and European eels, *Anguilla anguilla* (Deelder 1981), which if interpreted literally, overestimated the "true" age of the fish. In verification studies, the simple recognition of more checks or zones associated with "annuli," hence older age, may not necessarily make the method or structure better.

One of the major problems with verification studies, as currently practiced, is that the results of comparison depend entirely upon the methods used for interpreting age. Specific criteria for recognizing annuli should be provided so that bias associated with interpretation can be evaluated.

Growth data obtained from age assessments should be credible. Compilation of data sets of size at scale age published for four species of fish from the province of Ontario provided evidence suggesting that older lake trout, *Salvelinus namaycush*, had been underaged by the scale method (Casselman[8]). When these growth data were applied to the von Bertalanffy growth model, the resulting parameters for mean asymptotic fork length for northern pike (98.3 cm); walleye, *Stizostedion vitreum*, (62.2 cm); and lake whitefish, *Coregonus clupeaformis*, (56.8 cm) were realistic (Fig 4). However, the mean asymptotic fork length of 145.3 cm for lake trout was unrealistic and overestimated the maximum observed length (Martin and Olver 1980) by approximately 26%. Considering the age range of these lake trout data and the ecology of the species, the von Bertalanffy growth model should apply. This model will not

[7]MacCallum, W., Assessment Unit Leader, Lake Superior Fisheries Assessment Unit, Ontario Ministry of Natural Resources, 435 James Street, Box 5000, Thunder Bay, Ontario, Canada P7C 5G8, pers. commun. 1982.

[8]Casselman, J. M. 1982. Growth response to over-exploitation. Unpubl. manuscr., prepared for Report of Strategic Plan for Ontario Fisheries, Working Group No. 15. Ontario Ministry of Natural Resources, Fisheries Branch, Research Section, Box 50, Maple, Ontario, Canada L0J 1E0.

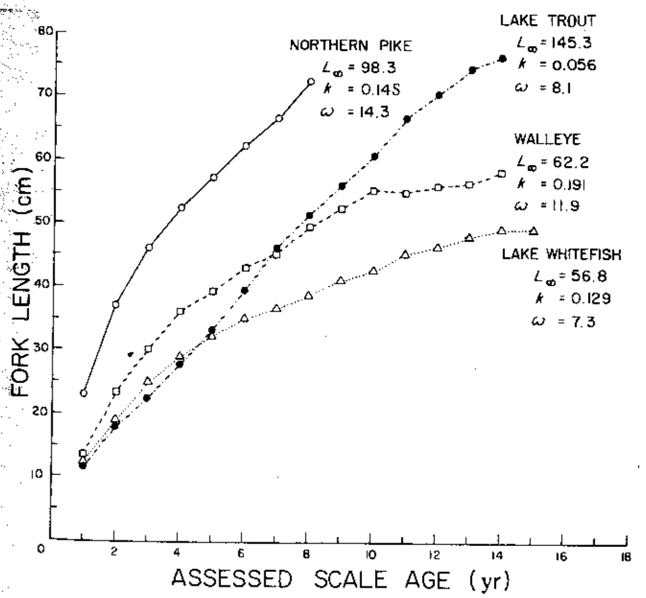



**Figure 4.—Mean fork length at assessed age estimated by the scale method for four species of freshwater fish. The von Bertalanffy growth parameters are provided ($k$ = growth coefficient, $L_\infty$ = asymptotic length). $\omega = k \cdot L_\infty$ (Gallucci and Quinn 1979). Size-at-age data are the means of published data from numerous Ontario populations: Northern pike, *Esox lucius*, $N$ = 18; lake trout, *Salvelinus namaycush*, $N$ = 42; walleye, *Stizostedion vitreum*, $N$ = 38; and lake whitefish, *Coregonus clupeaformis*, $N$ = 15.**

apply if fish grow through several growth stanzas later in life because of a change in diet or environment, e.g., eels, *Anguilla* sp. (Sparre 1979), or if the older individuals undergo a major increase in growth rate because of increased exploitation of the population. It is possible that some of these conditions might have affected these fallacious results for lake trout. However, the principal reason is that the scale method underestimates the age of old lake trout (Casselman unpubl. data 1982). After approximately age 6, the scale method applied to lake trout fails and with increasing age, this species is increasingly underaged by this method. This alone could explain the undiminished growth of older lake trout and the resulting unrealistically high asymptotic length.

If the assessed scale ages of lake trout are corrected by verification using other calcified structures, then asymptotic length can be reduced by 25% (Casselman unpubl. data 1982). This is almost exactly the same amount by which the mean ultimate length of lake trout exceeded the observed values.

Unless it has been validated, the scale method should not be used for precise analyses of year-class strength and mortality rates of older individuals. In general, but depending upon growth rate, the scale method in freshwater fish is increasingly inconsistent from assessed ages 6 to 10. Beyond these ages, results are increasingly biased, tending towards an underestimation of "true" or calendar age. The scale method, however, is adequate for heavily exploited populations because these usually contain young, fast-growing individuals.

## COLLABORATION

Often, it is not possible to validate or even use different structures to verify age assessments. In such cases, several interpretations should be made to increase the precision of the estimate. Ideally, this replication should be done by several different interpreters. Repeatability provides a measure of the degree of confidence or reliability that can be placed in the assessments; this can be expressed as the "index of concurrence"—frequency of occurrence of the modal age (Casselman et al.[9]). If examinations are conducted by several interpreters who routinely assess age of the species by similar methods, then such collaboration can provide material that is "reliably" aged. Such exchange programs have been reported for otoliths (Blacker 1974) and sections of dorsal fin spines (Antoine et al. 1983).

In the past, it was difficult to transfer the information associated with each interpretation, hence the results were usually analyzed and summarized only in terms of age. If the exchanges depend upon an examination made directly from specimens, then the program is time-consuming, and if samples such as scales are supplied, different interpreters may use different specimens. Blacker (1974) and Antoine et al. (1983) eliminated these problems by circulating photographs that could be examined and annotated so that the interpretations could be related directly to the images used.

Microfiche reader-printers, which produce photographic prints directly from structures or their thin sections, have facili-

[9]Casselman, J. M. et al. (+ 13 participants). 1980. An exchange program used to examine the scale method of ageing yellow perch (*Perca flavescens*) from Lake Erie. Unpubl. manuscr. of an International Exchange Program, 136 p. Ontario Ministry of Natural Resources, Fisheries Branch, Research Section, Box 50, Maple, Ontario, Canada L0J 1E0.

tated such exchange programs. Prints from these machines can be made quickly and easily, and are relatively inexpensive. The Recordak Magnaprint Reader (Model PE-1A by Eastman Kodak, Rochester, N.Y.) uses the wet silver method to produce a negative image (Fig. 5) that is as good as those obtained by normal photographic procedures. These prints are of high resolution; even photocopies of the images are clear and have good contrast, and can also be interpreted easily. This hard copying technique can be used to obtain a permanent record of the interpretation to test consistency within and between interpreters and within and between samples and studies. Annotated hard copies, which document the interpretation and assessment, should be prepared routinely. These permanent records would make it possible to make corrections without reinterpreting the samples, if subsequent validation or verification proved the interpretations were incorrect or biased. These prints can also be used as training aids.

This hard copying procedure makes collaboration easier and more convenient. By circulating photocopies, collaborators can independently interpret, mark, and annotate the images. People seem more willing to participate in exchange programs and respond quickly when hard copies are used. Results can be more easily summarized and circulated, so that inconsistencies can be detected quickly.

Illustrated in Figure 6 are the summaries of the interpretations obtained in an international exchange program for a scale from a yellow perch, *Perca flavescens*, from Lake Erie (Casselman et al. footnote 9). In this particular exchange program, opercula and otoliths were used to verify the assessed scale age after the interpretations had been completed (Fig. 7). Although there was good agreement in the assessed age among the interpreters in this exchange program, there was some disagreement on the precise location of the annuli (e.g., Fig. 6. interpreter M, second annulus). Hence, different growth patterns were assigned to the same fish. Such exchanges demonstrate the types of problems that occur in routine age assessment. For example, in this study there was also considerable disagreement over the interpretation of the edge of the scale, a common problem when interpreting age from calcified structures.

Workers routinely conducting age assessment on the same species should participate in cooperative exchanges to standardize and test procedures. Now that quick and easy hard copying methods are available, the details of specific interpretations can be transferred easily and precisely.

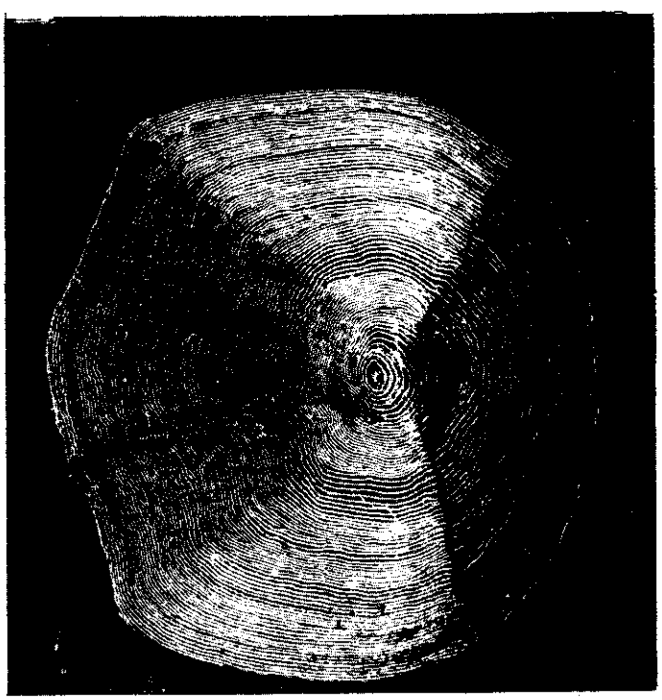


Figure 5.—Photographic print of the scale impression of a lake whitefish from Lake Mindemoya, Ontario. Fish was 361 mm total length, 320 mm fork length, and 460 g total weight. Estimated age 6+. Print is a negative image (10X) made directly from the scale used as a negative.

## AUTOMATION

Age and growth determination of fish will not become a truly objective science until the interpretation is quantified (see section on Interpretation) and the process can be mechanized and automated. Systems have already been developed to mechanize enumeration of circuli on scales (Mason 1974) and to automatically recognize checks and zones in scales and other structures by image analysis (Fawell 1974). Mechanization and automation of the scale method will not be accomplished easily because scales contain many types of checks that have been associated with annuli, and these vary in appearance throughout the different regions of the scale. However, other calcified structures, which contain fewer types of translucent zones associated with annuli, lend themselves more easily to automated procedures.

Although the most logical approach would be to use optical density in an automated system, a thorough understanding of the physical and chemical differences among checks on scales and among optically different zones in other structures could provide insight into differences that might be applied to detect seasonal growth patterns and perform automated analyses. The electron microprobe X-ray analyzer has substantiated that the translucent zone in fish calcified tissue is more heavily mineralized than adjacent opaque zones (Casselman 1974, 1978), and that calcium content is directly related to translucency (Fig. 8). Hence, in addition to optical zonation, fish calcified tissue contains corresponding chemical zonation. Even when calcified tissue appears to be optically uniform, elemental zonation corresponding to age can be shown to exist (Fig. 9). Microprobe analysis has also been used in analyzing centra of the spiny dogfish, revealing that calcium zonation occurs even in the relatively cartilaginous skeletons of sharks (Jones and Geen 1977). Although this method suggests possibilities for automation, line scan analysis with the electron microprobe is time-consuming and expensive.

Since fish calcified tissue shows mineral zonation and density that are directly related to translucency, X-radiography could be applied. Centra of elasmobranchs show differential zonation when X-radiographs are prepared by soft X-ray techniques (Cailliet, Martin, Kusher, Wolf, and Welden 1983).

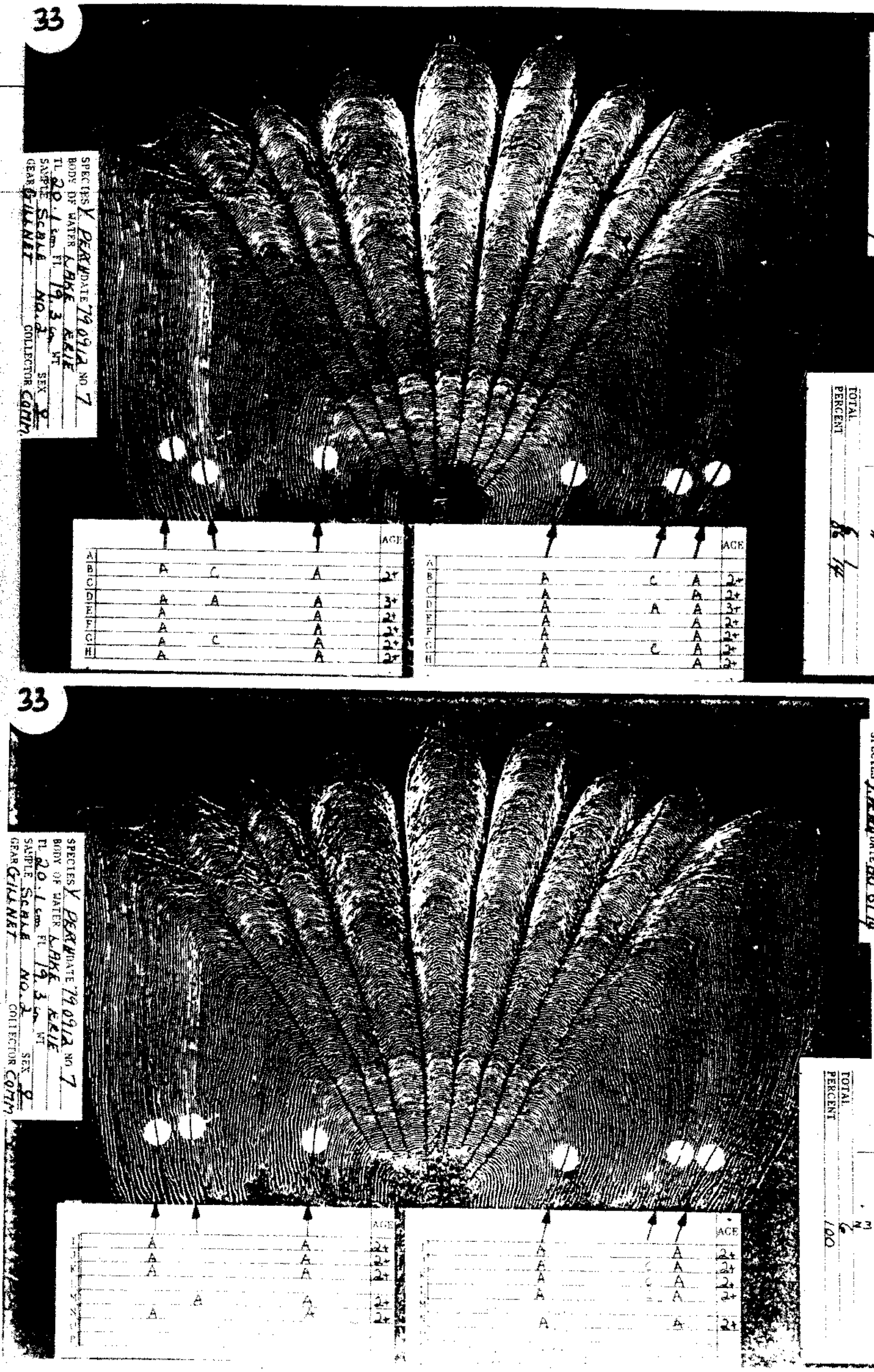
Figure 6.—A summary of the interpretations of the scale of a yellow perch, *Perca flavescens*, resulting from an international exchange program (Casselman et al. text footnote 9) used to evaluate scale age assessment of perch from Lake Erie. Assessments were originally made on negative images printed at 38X magnification. Results for interpreters B to H on top scale image and I to N on bottom scale image. The positions of all annuli (A) and checks (C) marked by each interpreter are indicated. Specific data on the fish and the program are superimposed on the prints (24X).

patterns
onstrate
ent. For
lisagree-
a com-
uctures.
he same
to stan-
sy hard
nterpre-

come a
ied (see
hanized
mech-
and to
d other
on and
ed eas-
ve been
rough-
alcified
zones
auto-

optical
ling of
scales
could
detect
alyses.
d that
heavily
1974,
anslu-
h cal-
Even
emen-
(Fig.
entra
occurs
Jones
ilities
nicro-

den-
raphy
ential
tech-
3).




**rch, *Perca*
man et al.
e Erie. As-
nification.
ale image.
ter are in-
the prints**

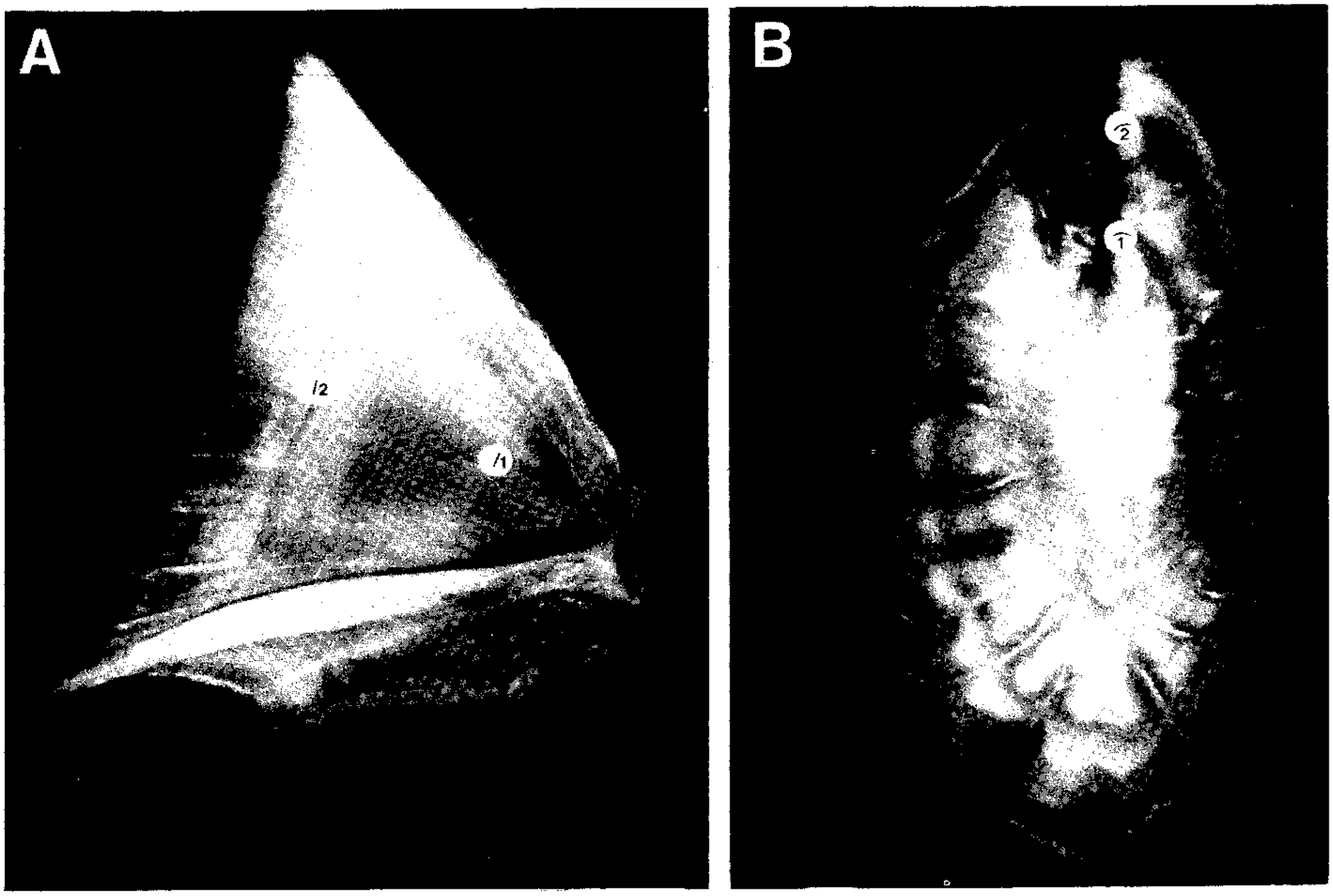



Figure 7.—Other calcified structures used ta corroborate age assessment af the scales used in the exchange program for Lake Erie yellaw perch. A) Opercular bone (3X); B) Whole otolith (11X). Both structures are fram the same fish fnr which scales are illustrated in Figure 6. Reflected light.

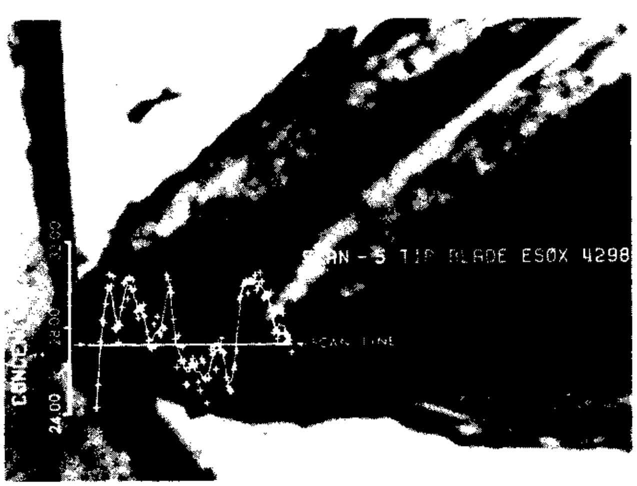



Figure 8.—Electron microprabe X-ray analysis for calcium across the optically different eones of a calcified structure. Calcium concentration (percent dry weight) determined by line scan analysis across the sixth translucent zone, seventh opaque zone, and seventh translucent zone (on the edge) of a thin transectional slice (thickness 158 μm) af the tip of a cleithrum of a northern pike. Actual distance of scan line is 900 μm. Transmitted light.

X-ray densimetric techniques have been used in dendrochronology, and systems have been developed that have totally automated and computerized tree-ring analysis and the age assessment of trees (Parker et al. 1973). Densimetric scanning techniques are directly applicable to age and growth assessment of fish because the optically different zones in fish otoliths and bony structures are also X-ray densitometrically different, and are remarkably similar to early and late wood in tree-ring formation (Fig. 10). This highly developed technology has been applied successfully to osseochronology of fish (Casselman et al.[10]). A typical example of an X-ray density scan of a radiograph made in a Hewlett Packard Faxitron Series X-ray System from a fin ray section of a lake sturgeon, *Acipenser fulvescens*, is shown in Figure 11.

[10]Casselman, J. M., M. L. Parker, and L. A. Jozsa. 1981. Osseochronology using X-ray densitometric techniques. Unpubl. manuscr, Ontario Ministry of Natural Resources, Fisheries Branch, Research Section, Box 50, Maple, Ontario, Canada L0J 1E0.

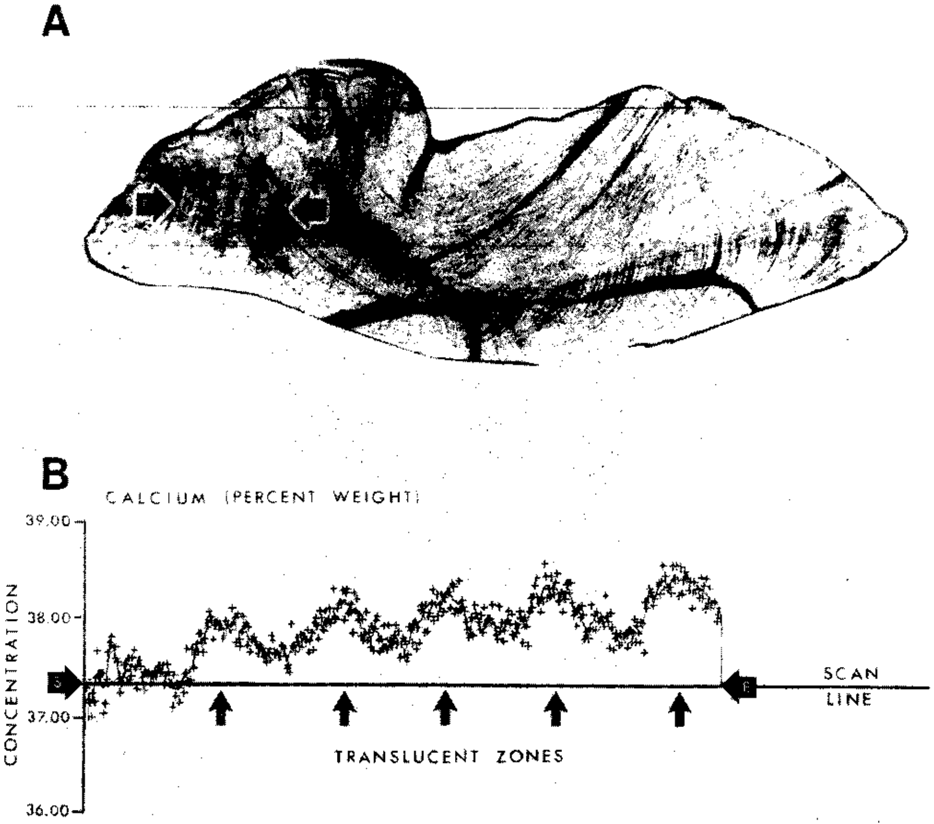



**Figure 9.—Electron microprobe X-ray analysis for calcium across a relatively translucent area of an otolith (sagitta) of a European eel, *Anguilla anguilla*. A) Cross section from the middle of the otolith of a fish from the Shannon River, County Tipperary, Ireland. Fish was 712 mm TL, 746 g TW. Arrows indicate the start and end of line scan analysis. Transmitted light; thickness 200 μm (59X). B) Calcium concentration as determined by line scan analysis. Arrows indicate the starting point (S) and end (E) of the scan, and correspond to those illustrated in A.**

## INNOVATION

In recent years, several innovative techniques have been applied to the problem of age determination. Otoliths of many species have been shown to have microstructure that suggests daily rhythmicity (Pannella 1974, 1980; Brothers 1979). Validation of the occurrence of these daily growth increments (Brothers et al. 1976; Taubert and Coble 1977; Wild and Foreman 1980; Radtke and Dean 1982) now makes it possible to examine and more precisely verify interpretations made by other, more subjective means.

Recently, daily increments in the otolith microstructure have been enumerated to verify the yearly periodicity of an "annulus" in the otoliths of largemouth bass, *Micropterus salmoides* (Taubert and Tranquilli 1982), and fallfish, *Semotilus corporalis* (Victor and Brothers 1982). Several techniques such as video enhanced light microscopy (Brothers et al. 1983) and scanning electron microscopy (Radtke 1983) have been used to increase the resolution of this microzonation. Acetate replication of the ground and hydrochloric acid etched surface of the otoliths provides useful imagery (Wild and Foreman 1980). Although the resolution is not as good as that obtained by electron microscopy, it is adequate and less costly. Detailed studies of otolith microstructure using electron microscopy and radioisotopes (e.g., $^{45}Ca$) will help elucidate the physical properties of microzonation (Watabe et al. 1982) and the physiological factors controlling incremental growth (Mugiya et al. 1981; Tanaka et al. 1981).

Acetate replication has been used on otoliths of American eels, *Anguilla rostrata*, to provide new insights into the problem of age assessment of this species. It has been extremely difficult to interpret age of eels from the upper St. Lawrence River and Lake Ontario by using standard otolith procedures. By combining acetate replication and electron microprobe analysis of eel otoliths, it is now possible not only to assess the age more easily and consistently but also to describe the chronology of eel migration from the sea (Casselman 1982). Three types of optically different zonal patterns are observed in the acetate replicas (Fig. 12). One type is associated with the NUCLEUS and is comprised of broad, opaque zones separated by two to four translucent zones. Strontium-calcium ratios as determined by electron microprobe analysis substantiated that this tissue is deposited in the marine environment. Outside the NUCLEUS is a set of zones referred to as the zones of TRANSITION that are associated with migration up the St. Lawrence River. The opaque zones in this region are narrow and are separated by two or three broad translucent zones. Outside this region is the EDGE, which contains numerous broad opaque zones separated by very distinct, narrow translucent zones. The first opaque zone outside the zones of

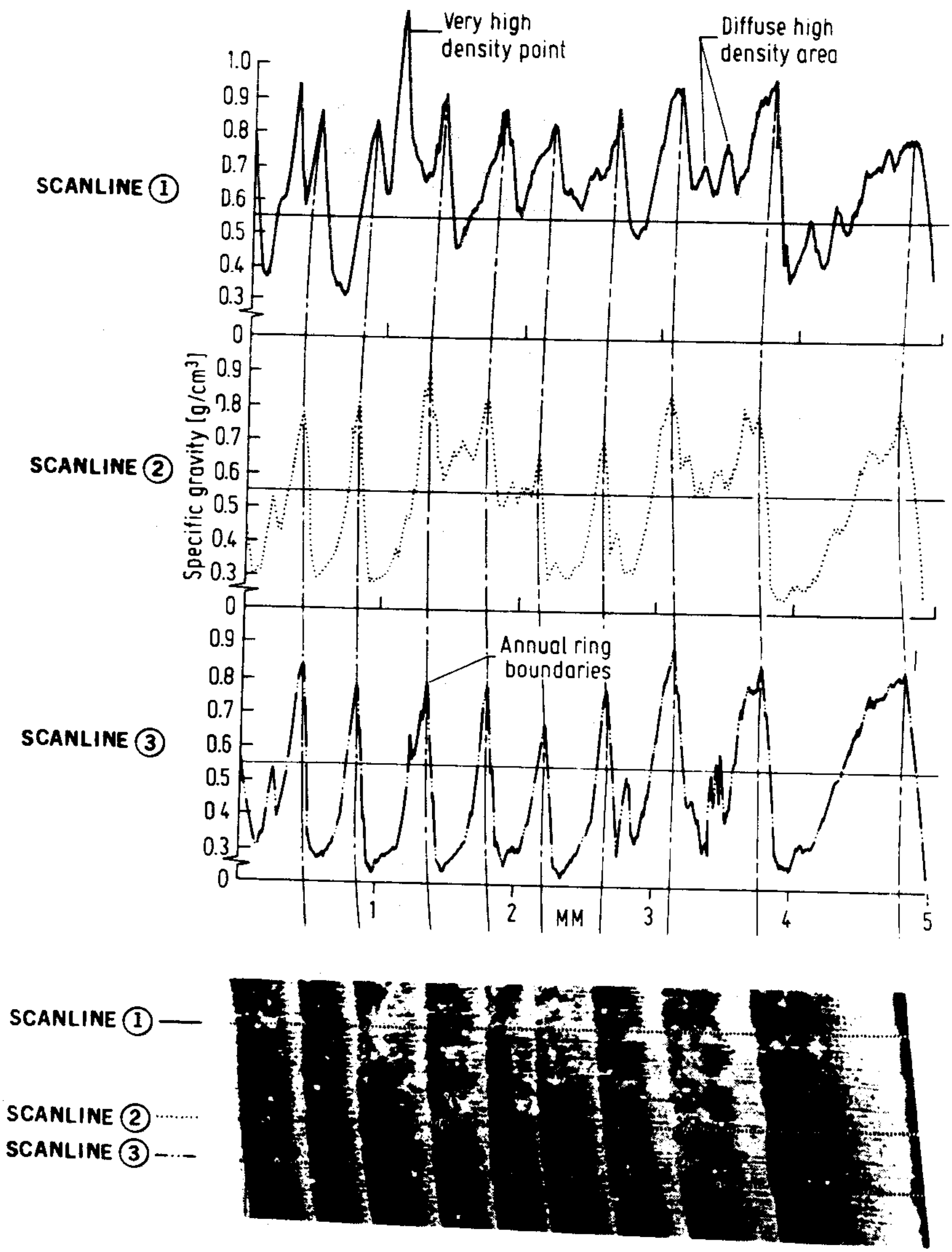



Figure 10.—Tree-ring density plots of three radial scans across a radiograph of a 2 mm thick transverse section of western hemlock, *Tsuga heterophylla*. The plots show the intra-ring density patterns of nine annual rings, as well as the relative density of two types of wood (earlywood—low density, and latewood—high density). Reproduced from Parker et al. (1974), figure 3, from Wood Science and Technology by permission of the authors and Springer-Verlag.

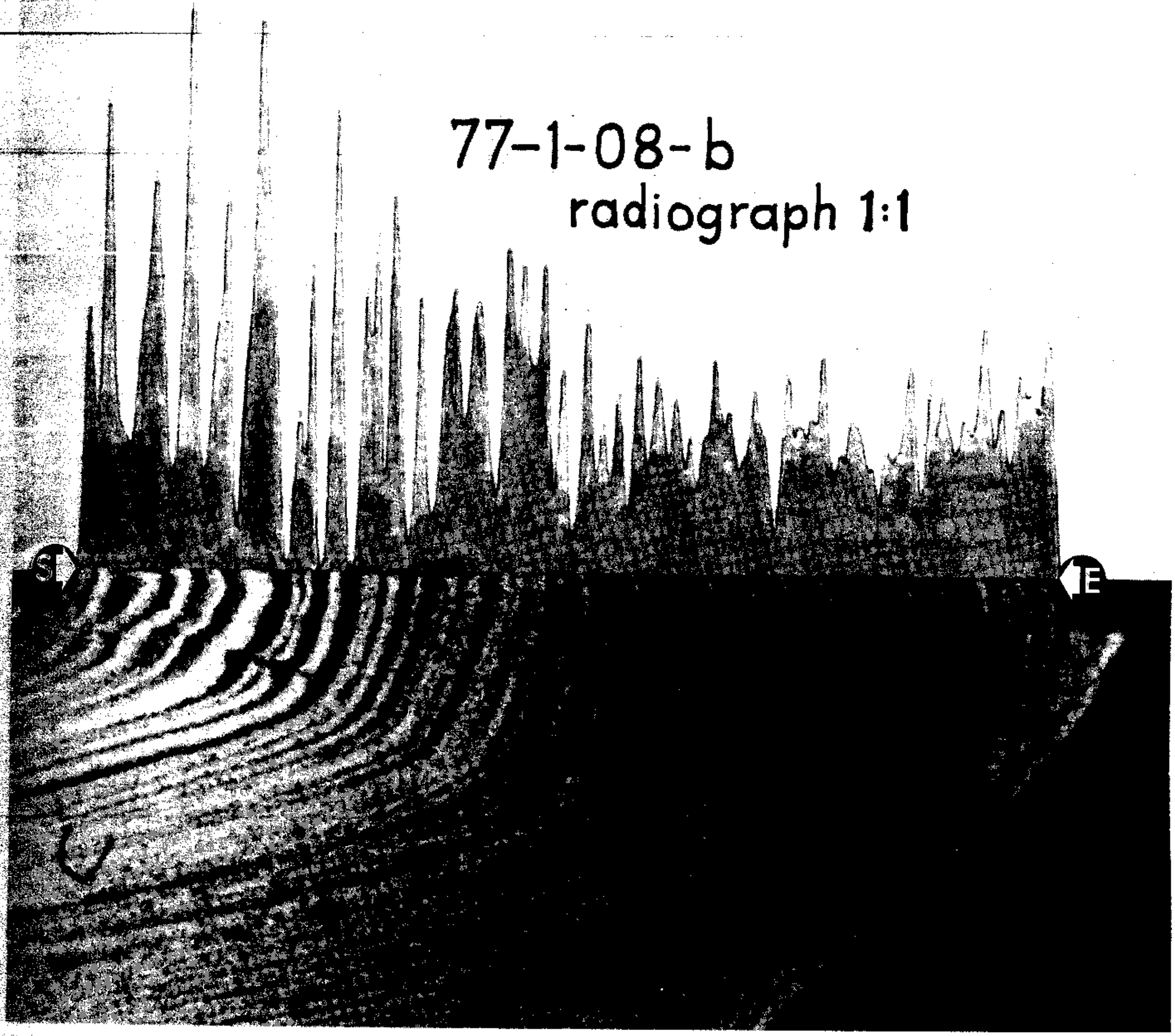



**Figure 11.—Calcified tissue density plot of a radial scan across a radiograph made from a portion of a thin cross section (250$\mu$m) of the first pectoral fin ray of a lake sturgeon, *Acipenser fulvescens*, from the St. Lawrence River. Fish was 160 cm TL, 44 kg TW, age estimated at approximately 60 yr. Zonation appears as it would in the fin section if it were viewed in transmitted light (25X). Light colored zones on the radiograph correspoad to the translucent znnes and have a high X-ray absorption and high density. The arrows mark the starting point (S) near the nucleus and the end (E) of X-ray density scan near the edge of the fin.**

TRANSITION is usually narrower than are subsequent opaque zones. This region is associated with life after migration either in the upper St. Lawrence River or Lake Ontario. Strontium-calcium ratios in this region of the otolith indicate life in the freshwater environment. The translucent zones associated with the EDGE are very distinct and easily recognized, so, by using acetate replication, it is now possible not only to assess the age of eels more reliably but also to determine precisely how long the fish has spent in each particular habitat. Strontium analyses helped Bagenal et al. (1973) discern similar migration information for brown trout, *Salmo trutta.* Calcified tissue contains valuable chemical information that can be used to improve and refine the interpretation of environmental growth history of fish.

Innovative biochemical methods have been developed to measure "instantaneous" growth rate of calcified tissue. This technique, which can be referred to as "scale growth index," measures the uptake of $^{14}$C-glycine incorporation by cells associated with isolated scales (Ottoway and Simkiss 1977, 1979; Adelman 1980). Although the technique and equipment are sophisticated, the method has potential for studying the factors causing check formation through a study of the growth rate of the scales.

Some biochemical techniques appear potentially useful for assessing age. One method, which is based on precise changes in the amount of insoluble protein in the crystalline lens of the eye, consists of two procedures: 1) Obtaining the appropriate lens fraction, and 2) quantitatively analyzing its protein com-

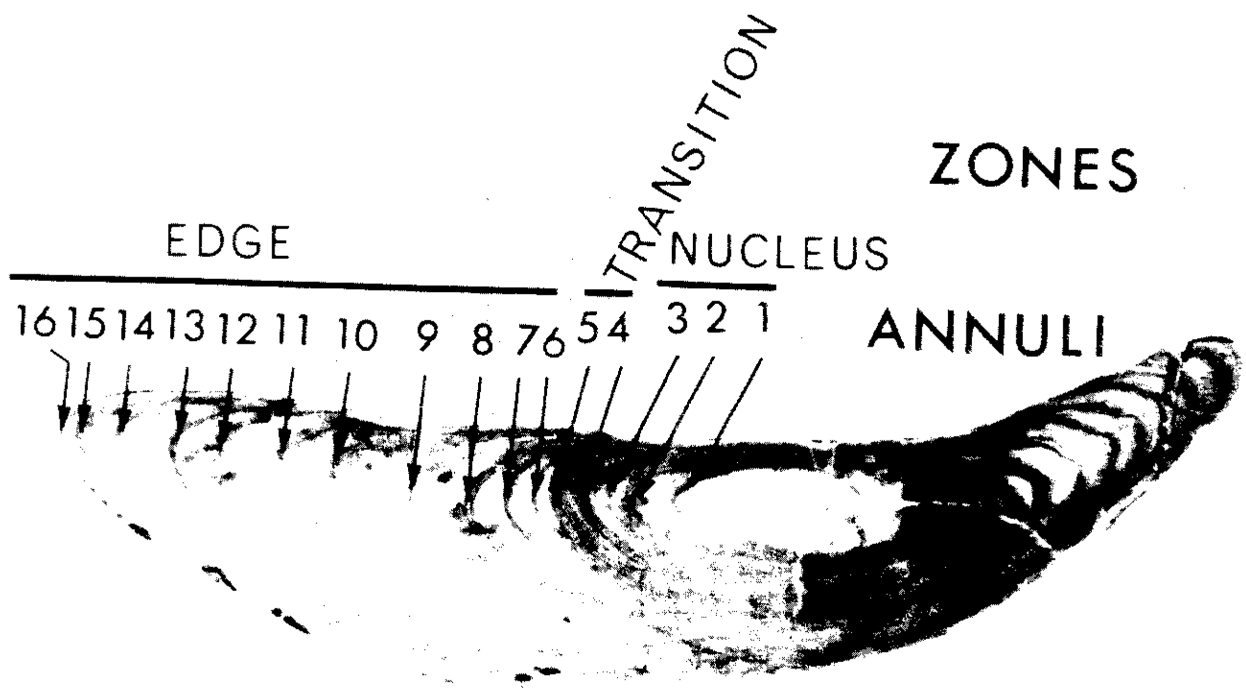



**Figure 12.—Photomicrograph of a cellulose acetate replica of a longitudinally ground and acid etched otolith (sagitta) of an American eel, *Anguilla rostrota*, from the upper St. Lawrence River. Fish was 800 mm TL, 1,210 g TW, assessed age 16 yr. Zonation appears as it would on the otolith surface if it were viewed in reflected light (50X). The zones (translucent) associated with annuli are indicated and are separated into the three distinct types of zonation. The first three translucent zones are of a similar type and are associated with the NUCLEUS. The fourth and fifth translucent zones are similar, broad, and different from preceding and succeeding zones, and are associated with TRANSITION, or migration up the St. Lawrence River (Casselman 1982). The remaining 10 translucent zones are similar, narrow, and are associated with the EDGE.**

position (Otero and Dapson 1972). Another involves amino acid racemization (Helfman and Bada 1976; Helfman et al. 1977; Masters et al. 1977), which consists of a comparison of the L and D isomers of aspartic acid. Proteins are initially comprised almost exclusively of L-amino acids. However, these change with time into their D-enantiomers at a rate that is proportional to temperature. With this technique, it would be necessary to know the thermal history of the fish. This may be possible in the future.

Radioactive geochronology, which utilizes natural radionuclide ratios, appears to be one of the most potentially useful new techniques. This method has been used to examine growth rate of marine clams (Turekian et al. 1979; Turekian and Cochran 1981). Radiometric age determination has been used recently to confirm the longevity of splitnose rockfish, *Sebastes diploproa*, by measuring uranium decay series nuclides $^{226}Ra$ and $^{210}Pb$ in otoliths (Bennett et al. 1982). This is a truly objective procedure and signals that innovative techniques may revolutionize age and growth assessment of fish in the future.

The calcified structures of fish contain a great deal of additional information that should not be overlooked. They have been valuable tools in stock identification (Ihssen et al. 1981). Objective methods now exist for stock separation using Fourier series analysis to quantify the shape of calcified structures (Jarvis et al. 1978; Casselman et al. 1981). Characteristics in the calcified structures are strongly influenced by environmental conditions, but a genetic basis exists. Some calcified structures, such as scales, are probably more strongly influenced by environmental conditions than are others, such as otoliths (Casselman 1978). This may explain why otolith shape is a better discriminator than scale shape for lake whitefish stocks (Casselman et al. 1981).

## CONCLUSIONS

From this overview of the procedures, problems, and progress in assessing the age and growth of fish from their calcified structures, it is apparent that this science is now expanding rapidly and is going through a technological revolution. The techniques and tools, especially those pertaining to interpretation, validation, and automation, are becoming much more powerful and sophisticated. The problems have changed little, but the practical application is rapidly being improved, refined, and expanded. The wider use of fluorochrome markers, e.g., tetracycline, will greatly improve interpretation and will provide badly needed tests of validity for not only age assessment, but also growth evaluation. The major advances in microelectronics and computer technology in recent years signal that automated interpretation is feasible and inevitable. Although the procedures of age and growth assessment of fish from their calcified structures have remained virtually unchanged over the past 50 yr, there are now signs that the technology is starting to undergo major changes and is becoming increasingly specialized. There is evidence that the techniques and tools used in the future may be radically different from those used today. Innovations are being developed, such as biochemical methods of measuring instantaneous scale growth to provide a direct measure of growth rate, and radiometric age determination to provide a more objective age assessment. Such procedures could eventually eliminate subjectivity and make age and growth determination a truly objective science. Calcified structures also contain valuable information that can be applied to other fisheries problems. The quantification of the shape of calcified structures provides a powerful tool that permits stock identification from materials that are routinely collected for age and growth purposes.


## ACKNOWLEDGMENTS

E. D. Prince is to be congratulated for convening a very timely and stimulating workshop. I wish to thank E. D. Prince, E. B. Brothers, and A. Wild for their constructive suggestions and comments on this review. J. C. Javech drew the oceanic pelagics illustrated in Figure 2.


## LITERATURE CITED


ADELMAN, I. R.
1980. Uptake of [14]C-glycine by scales as an index of fish growth: Effect of fish acclimation temperature. Trans. Am. Fish. Soc. 109:187-194.

ANTOINE, M. L., J. MENDOZA, and P. M. CAYRÉ.
1983. Progress of age and growth assessment of Atlantic skipjack tuna, *Euthynnus pelamis*, from dorsal fin spines. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:91-97.

BAGENAL, T. B. (editor).
1974. The ageing of fish. Unwin Brothers Ltd., England, 234 p.

BAGENAL, T. B., F. J. H. MACKERETH, and J. HERON.
1973. The distinction between brown trout and sea trout by the strontium content of their scales. J. Fish Biol. 5:555-557.

BATTS, B. S.
1972. Age and growth of the skipjack tuna, *Katsuwonus pelamis* (Linnaeus), in North Carolina waters. Chesapeake Sci. 13:237-244.

BEAMISH, R. J.
1979. New information on the longevity of Pacific Ocean perch (*Sebastes alutus*). J. Fish. Res. Board Can. 36:1395-1400.
1981. Use of fin-ray sections to age walleye pollock, Pacific cod, and albacore, and the importance of this method. Trans. Am. Fish. Soc. 110: 287-299.

BEAMISH, R. J., and D. E. CHILTON.
1982. Preliminary evaluation of a method to determine the age of sablefish (*Anoplopoma fimbria*). Can. J. Fish. Aquat. Sci. 39:277-287.

BEAMISH, R. J., and H. H. HARVEY.
1969. Age determination in the white sucker. J. Fish. Res. Board Can. 26:633-638.

BEAMISH, R. J., and G. A. McFARLANE.
1983. Validation of age determination estimates: The forgotten requirement. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:29-33.

BELL, R. R.
1962a. A history of tuna age determinations. Proceedings of the symposium on scrombroid fishes, Part II, p. 693-706. Mar. Biol. Assoc. India.
1962b. Age determination of the Pacific albacore of the California coast. Calif. Fish Game 48:39-48.

BENNETT, J. T., G. W. BOEHLERT, and K. K. TUREKIAN.
1982. Confirmation of longevity in *Sebastes diploproa* (Pisces: Scorpaenidae) from $^{210}Pb/^{226}Ra$ measurements in otoliths. Mar. Biol. (Berl.) 71:209-215.

BERKELEY, S. A., and E. D. HOUDE.
1983. Age determination of broadbill swordfish, *Xiphias gladius*, from the Straits of Florida, using anal fin spine sections. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:137-143.

BLACKER, R. W.
1974. Recent advances in otolith studies. *In* F. R. Harden Jones (editor), Sea fisheries research, p. 67-90. Paul Elek (Sci. Books) Ltd., Lond.

BROTHERS, E. B.
1979. Age and growth studies on tropical fishes. *In* S. B. Saila and P. M. Roedel (editors), Stock assessment for tropical small-scale fisheries, p. 119-136. Proceedings of an international workshop held at the University of Rhode Island, Kingston, R.I., September 19-21, 1979. International Center for Marine Resources Development, U.R.I.
1983. Summary of round table discussions on age validation. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:35-44.

BROTHERS, E. B., C. P. MATHEWS, and R. LASKER.
1976. Daily growth increments in otoliths from larval and adult fishes. Fish. Bull., U.S. 74:1-8.

BROTHERS, E. B., E. D. PRINCE, and D. W. LEE.
1983. Age and growth of young-of-the-year bluefin tuna, *Thunnus thynnus*, from otolith microstructure. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:49-59.

BULKLEY, R. V.
1960. Use of branchiostegal rays to determine age of lake trout, *Salvelinus namaycush* (Walbaum). Trans. Am. Fish. Soc. 89:344-350.

CABLE, L. E.
1956. Validity of age determination from scales, and growth of marked Lake Michigan lake trout. U.S. Fish Wildl. Serv., Fish. Bull. 57:1-59.

CAILLIET, G. M., L. K. MARTIN, J. T. HARVEY, D. KUSHER, and B. A. WELDEN.
1983. Preliminary studies on the age and growth of the blue, *Prionace glauca*, common thresher, *Alopias vulpinus*, and shortfin mako, *Isurus oxyrinchus*, sharks from California waters. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:179-188.

CAILLIET, G. M., L. K. MARTIN, D. KUSHER, P. WOLF, and B. A. WELDEN.
1983. Techniques for enhancing vertebral bands in age estimation of California elasmobranchs. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:157-165.

CARLANDER, K. D.
1974. Difficulties in ageing fish in relation to inland fishery management. *In* T. B. Bagenal (editor), The ageing of fish, p. 200-206. Unwin Brothers Ltd., England.
1982. Age and growth of freshwater fishes. Briefs, Newsletter of Am. Inst. Fish. Res. Biol. 11(2):3.

CASEY, J. G., H. L. PRATT, Jr., and C. E. STILLWELL.
1983. Age and growth of the sandbar shark, *Carcharhinus plumbeus*, from the western North Atlantic. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:189-191.

CASSELMAN, J. M.
1974. Analysis of hard tissue of pike *Esox lucius* L. with special reference to age and growth. *In* T. B. Bagenal (editor), The ageing of fish, p. 13-27. Unwin Brothers, Ltd., England.
1978. Calcified tissue and body growth of northern pike, *Esox lucius* Linnaeus. Ph.D. Thesis, Univ. Toronto, Toronto, Ontario, 782 p.
1979. The esocid cleithrum as an indicator calcified structure. *In* J. Dube and Y. Gravel (editors), Proc. 10th Warm water Workshop, Spec. Publ. NE Div. Am. Fish. Soc., p. 249-272. Publ. by Minist. Loisir, de la Chasse et de la Pêche du Quebec, Dir. Recherche Faunique, Montréal, Qué.
1982. Chemical analyses of the optically different zones in eel otoliths. *In* K. H. Loftus (editor), Proc. 1980 North American Eel Conference, p. 74-82. Ont. Minist. Nat. Resour., Ont. Fish. Tech. Rep. Ser. No. 4.

CASSELMAN, J. M., J. J. COLLINS, E. J. CROSSMAN, P. E. IHSSEN, and G. R. SPANGLER.
1981. Lake whitefish (*Coregonus clupeaformis*) stocks of the Ontario waters of Lake Huron. Can. J. Fish. Aquat. Sci. 38:1772-1789.

CAYRÉ, P. M., and T. DIOUF.
1983. Estimating age and growth of little tunny, *Euthynnus alletteratus*, off the coast of Senegal, using dorsal fin spine sections. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:105-110.

COMPEÁN-JIMENEZ, G., and F. X. BARD.
1983. Growth increments on dorsal spines of eastern Atlantic bluefin tuna, *Thunnus thynnus*, and their possible relation to migration patterns. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:77-86.

DEELDER, C. L.
1981. On the age and growth of cultured eels, *Anguilla anguilla* (Linnaeus, 1758). Aquaculture 26:13-22.

DEELDER, C. L., and J. J. WILLEMSE.
1973. Age determination in fresh-water teleosts, based on annular structures in fin-rays. Aquaculture 1:365-371.

ERICKSON, C. M.
1979. Age differences among three hard tissue structures observed in fish populations experiencing various levels of exploitation. Man. Dep. Nat. Resour. MS Rep. 79-77, 31 p.

EVERSON, I. (editor).
1980. Age determination methods for Antarctic fish. Workshop on age determination of Antarctic fish. Cambridge, England. Cybium, 3rd ser., 1980(8):5-51.

FAWELL, J. K.
1974. The use of image analysis in the ageing of fish. *In* T. B. Bagenal (editor), The ageing of fish, p. 103-107. Unwin Brothers Ltd., England.

FROST, W. E., and C. KIPLING.
1959. The determination of the age and growth of pike (*Esox lucius* L.) from scales and opercular bones. J. Cons. Int. Explor. Mer 24:314-341.

GALLUCCI, V. F., and T. J. QUINN II.
1979. Reparameterizing, fitting, and testing a simple growth model. Trans. Am. Fish. Soc. 108:14-25.
GALTSOFF, P. S.
1952. Staining of growth rings in the vertebrae of tuna (*Thunnus thynnus*). Copeia 1952:103-105.
GONZÁLES-GARCÉS, A., and A. C. FARIÑA-PEREZ.
1983. Determining age of young albacore, *Thunnus alalunga*, using dorsal spines. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:117-122.
GRUBER, S. H., and R. G. STOUT.
1983. Biological materials for the study of age and growth in a tropical marine elasmobranch, the lemon shark, *Negaprion brevirostris* (Poey). U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:193-205.
HELFMAN, P. M., and J. L. BADA.
1976. Aspartic acid racemisation in dentine as a measure of ageing. Nature (Lond.) 262:279-281.
HELFMAN, P. M., J. L. BADA, and M. -Y. SHOU.
1977. Considerations on the role of aspartic acid racemization in the ageing process. Gerontology 23:419-425.
HOLDEN, M. J., and M. R. VINCE.
1973. Age validation studies on the centra of *Raja clavata* using tetracycline. J. Cons. Int. Explor. Mer 35:13-17.
HURLEY, P. C. F., and T. D. ILES.
1983. Age and growth estimation of Atlantic bluefin tuna, *Thunnus thynnus*, using otoliths. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:71-75.

IHSSEN, P. E., H. E. BOOKE, J. M. CASSELMAN, J. M. McGLADE, N. R. PAYNE, and F. M. UTTER.
1981. Stock identification: Materials and methods. Can. J. Fish. Aquat. Sci. 38:1838-1855.

JARVIS, R. S., H. F. KLODOWSKI, and S. P. SHELDON.
1978. New method of quantifying scale shape and an application to stock identification in walleye (*Stizostedion vitreum vitreum*). Trans. Am. Fish. Soc. 107:528-534.
JOHNSON, A. G.
1983. Comparison of dorsal spines and vertebrae as ageing structures for little tunny, *Euthynnus alletteratus*, from the northeast Gulf of Mexico. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:111-115.
JOLLEY, J. W., Jr.
1974. On the biology of Florida east coast Atlantic sailfish, (*Istiophorus platypterus*). *In* R. S. Shomura and F. Williams (editors), Proceedings of the International Billfish Symposium, Kailua-Kona, Hawaii, 9-12 August 1972. Part 2. Review and contributed papers, p. 81-88. U. S. Dep. Commer., NOAA Tech. Rep. NMFS SSRF-675.
JONES, B. C., and G. H. GEEN.
1977. Age determination of an elasmobranch (*Squalus acanthias*) by X-ray spectrometry. J. Fish. Res. Board Can. 34:44-48.

KETCHEN, K. S.
1975. Age and growth of dogfish *Squalus acanthias* in British Columbia waters. J. Fish. Res. Board Can. 32:43-59.

Le CREN, E. D.
1947. The determination of the age and growth of the perch (*Perca fluviatilis*) from the opercular bone. J. Anim. Ecol. 16:188-204.
LEE, D. W., E. D. PRINCE, and M. E. CROW.
1983. Interpretation of growth bands on vertebrae and otoliths of Atlantic bluefin tuna, *Thunnus thynnus*. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:61-69.
Le GUEN, J. C., and G. T. SAKAGAWA.
1973. Apparent growth of yellowfin tuna from the eastern Atlantic Ocean. Fish. Bull., U.S. 71:175-187.

MARTIN, N. V., and C. H. OLVER.
1980. The lake charr, *Salvelinus namaycush*. *In* E. K. Balon (editor), Charrs: Salmonid fishes of the genus *Salvelinus*, p. 205-277. Junk Publ., The Hague, The Netherlands.
MASON, J. E.
1974. A semi-automatic machine for counting and measuring circuli on fish scales. *In* T. B. Bagenal (editor), The ageing of fish, p. 87-102. Unwin Brothers Ltd., England.
MASTERS, P. M., J. L. BADA, and J. S. ZIGLER, Jr.
1977. Aspartic acid racemisation in the human lens during ageing and in cataract formation. Nature (Lond.) 268:71-73.
McCAULEY, R. W., and J. M. CASSELMAN.
1981. The final preferendum as an index of the temperature for optimum growth in fish. *In* K. Tiews (editor), Proceedings of a world symposium on aquaculture in heated effluents and recirculation systems, Vol. II, p. 81-93. Heenemann, Berlin.
MILLS, K. H., and R. J. BEAMISH.
1980. Comparison of fin-ray and scale age determinations for lake whitefish (*Coregonus clupeaformis*) and their implications for estimates of growth and annual survival. Can. J. Fish. Aquat. Sci. 37:534-544.
MUGIYA, Y., N. WATABE, J. YAMADA, J. M. DEAN, D. G. DUNKELBERGER, and M. SHIMIZU.
1981. Diurnal rhythm in otolith formation in the goldfish, *Carassius auratus*. Comp. Biochem. Physiol. 68A:659-662.

NOSE, Y., S. TOMOMATSU, K. MIMURA, and Y. HIYAMA.
1955. A method to determine the time of ring-formation in hard tissues of fishes, especially for the age determination of Pacific tunas. Rec. Oceanogr. Works Jpn. 2(3):9-18.

OTERO, J. G., and R. W. DAPSON.
1972. Procedures in the biochemical estimation of age of vertebrates. Res. Popul. Ecol. 13:152-160.
OTTAWAY, E. M., and K. SIMKISS.
1977. "Instantaneous" growth rates of fish scales and their use in studies of fish populations. J. Zool. (Lond.) 181:407-419.
1979. A comparison of traditional and novel ways of estimating growth rates from scales of natural populations of young bass (*Dicentrarchus labrax*). J. Mar. Biol. Assoc. U.K. 59:49-59.

PANNELLA, G.
1974. Otolith growth patterns: an aid in age determination in temperate and tropical fishes. *In* T. B. Bagenal (editor), The ageing of fish, p. 28-40. Unwin Brothers Ltd., England.
1980. Growth patterns in fish sagittae. *In* D. C. Rhoads and R. A. Lutz (editors), Skeletal growth of aquatic organisms: Biological records of environmental change, p. 519-560. Plenum Press, N.Y.
PARKER, M. L., G. M. BARTON, and L. A. JOZSA.
1974. Detection of lignans in western hemlock by radiography. Wood Sci. Technol. 8:229-232.
PARKER, M. L., J. SCHOORLEMMER, and L. J. CARVER.
1973. Computerized scanning densitometer for automatic recording of tree-ring width and density data from X-ray negatives. Wood Fiber 5:237-248.
POWERS, J. E.
1983. Some statistical characteristics of ageing data and their ramifications in population analysis of oceanic pelagic fishes. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:19-24.
PRINCE, E. D., and D. W. LEE.
1980. Bioprofiles sampling manual for oceanic pelagic fishes. U.S. Dep. Commer., NOAA Tech. Memo. NMFS SEFC-55, 20 p.

RADTKE, R. L.
1983. Istiophorid otoliths: Extraction, morphology, and possible use as ageing structures. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:123-129.
RADTKE, R. L., and J. M. DEAN.
1981. Morphological features of the otoliths of the sailfish, *Istiophorus platypterus*, useful in age determination. Fish. Bull., U. S. 79:360-367.
1982. Increment formation in the otoliths of embryos, larvae, and juveniles of the mummichog, *Fundulus heteroclitus*. Fish. Bull., U.S. 80:201-215.
REGIER, H. A.
1962. Validation of the scale method for estimating age and growth of bluegills. Trans. Am. Fish. Soc. 91:362-374.
RHOADS, D. C., and R. A. LUTZ (editors).
1980. Skeletal growth of aquatic organisms: Biological records of environmental change. Plenum Press, N.Y., 750 p.

SCHWARTZ, F. J.
1983. Shark ageing methods and age estimation of scalloped hammerhead, *Sphyrna lewini*, and dusky, *Carcharhinus obscurus*, sharks based on vertebral ring counts. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:164-174.
SMITH, C.L.
1983. Summary of round table discussions on back calculation. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:45-47.
SPARRE, P.
1979. Some necessary adjustments for using the common methods in eel assessment. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 174:41-44.
STEVENS, J. D.
1975. Vertebral rings as a means of age determination in the blue shark (*Prionace glauca* L.). J. Mar. Biol. Assoc. U.K. 55:657-665.

TANAKA, K., Y. MUGIYA, and J. YAMADA.
1981. Effects of photoperiod and feeding on daily growth patterns in otoliths of juvenile *Tilapia nilotica*. Fish. Bull., U.S. 79:459-466.

TAUBERT, B. D., and D. W. COBLE.
1977. Daily rings in otoliths of three species of *Lepomis* and *Tilapia mossambica*. J. Fish. Res. Board Can. 34:332-340.

TAUBERT, B. D., and J. A. TRANQUILLI.
1982. Verification of the formation of annuli in otoliths of largemouth bass. Trans. Am. Fish. Soc. 111:531-534.

THORSON, T. B., and E. J. LACY, Jr.
1982. Age, growth rate and longevity of *Carcharhinus leucas* estimated from tagging and vertebral rings. Copeia 1982:110-116.

TUREKIAN, K. K., and J. K. COCHRAN.
1981. Growth rate of a vesicomyid clam from the Galápagos spreading center. Science (Wash., D.C.) 214:909-911.

TUREKIAN, K. K., J. K. COCHRAN, and Y. NOZAKI.
1979. Growth rate of a clam from the Galápagos Rise hot spring field using natural radionuclide ratios. Nature (Lond.) 280:385-387.

UCHIYAMA, J. H., and P. STRUHSAKER.
1981. Age and growth of skipjack tuna, *Katsuwonus pelamis*, and yellowfin tuna, *Thunnus albacares*, as indicated by daily growth increments of sagittae. Fish. Bull., U.S. 79:151-162.

VICTOR, B. C., and E. B. BROTHERS.
1982. Age and growth of the fallfish *Semotilus corporalis* with daily otolith increments as a method of annulus verification. Can. J. Zool. 60:2543-2550.

WATABE, N., K. TANAKA, J. YAMADA, and J. M. DEAN.
1982. Scanning electron microscope observations of the organic matrix in the otolith of the teleost fish *Fundulus heteroclitus* (Linnaeus) and *Tilapia nilotica* (Linnaeus). J. Exp. Mar. Biol. Ecol. 58:127-134.

WILD, A., and T. J. FOREMAN.
1980. The relationship between otolith increments and time for yellowfin and skipjack tuna marked with tetracycline. [In Engl. and Span.] Inter-Am. Trop. Tuna Comm. Bull. 17:509-560.

WILSON, C. A., and J. M. DEAN.
1983. The potential use of sagittae for estimating age of Atlantic swordfish, *Xiphias gladius*. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 8:151-156.

YABUTA, Y., and M. YUKINAWA.
1957. Age and growth of yellowfin tuna (*Neothunnus macropterus*) in Japanese waters by size frequencies. [In Jpn., Engl. abstr.] Rep. Nankai Reg. Fish. Res. Lab. 5:127-133.
1963. Growth and age of albacore. [In Jpn., Engl. abstr.] Rep. Nankai Reg. Fish. Res. Lab. 17:111-120.

YABUTA, Y., M. YUKINAWA, and Y. WARASHINA.
1960. Growth and age of yellowfin tuna. II. Age determination (scale method). [In Jpn., Engl. abstr.] Rep. Nankai Reg. Fish. Res. Lab. 12:63-74.

YANG, R. -T., Y. NOSE, and Y. HIYAMA.
1969. A comparative study on the age and growth of yellowfin tunas from the Pacific and Atlantic oceans. [In Engl.] Bull. Far Seas Fish. Res. Lab. (Shimizu) 2:1-21.

YUKINAWA, M.
1970. Age and growth of southern bluefin tuna *Thunnus maccoyii* (Castelnau) by use of scale. [In Jpn., Engl. synop.] Bull. Far Seas Fish. Res. Lab. (Shimizu) 3:229-257.

YUKINAWA, M., and Y. YABUTA.
1963. Age and growth of bigeye tuna, *Parathunnus mebachi* (Kishinouye). [In Jpn., Engl. abstr.] Rep. Nankai Reg. Fish. Res. Lab. 19:103-118.
1967. Age and growth of bluefin tuna *Thunnus thynnus* (Linnaeus), in the North Pacific Ocean. [In Jpn., Engl. abstr.] Rep. Nankai Reg. Fish. Res. Lab. 25:1-18.

ZOOLOGICAL SOCIETY OF SLOVAKIA.
1968. The international conference on ageing and growth of fishes. Mimeographed invited lectures, Smolenice, Czechoslovakia, 148 p.