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"Age Determination in Biology:
A Retrospective and Presentation of
Contemporary Methods"

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Age Readers Never die,
They only lose count.
—— Anonymous

Preface

Fisheries management is rich in examples of its interdisciplinary nature. Progress in the field can almost always be attributed to some technique or methodology adapted from fields ranging from the physical sciences and mathematics to biochemistry. Age determination, whether as an integral part of the management process or as an endeavor independent of management, is a representative example. The interdisciplinary substance of age determination is clearly demonstrated in the short table of contents for this volume where it is seen that the literature is drawn from a board range of biological areas and from the physical sciences and mathematics. An inherent consistency across the biological areas is forced by the nature of the data we are dealt and the commonality of the physiological processes involved in ageing. The physical sciences' role is more technological in the sense that, e.g., the use of the scanning electron microscope represents a step toward enhanced definition of the data, daily microgrowth lines. Mathematical and statistical methods usually enter in the interpretative or analysis stage where the data have been defined and conclusions must be drawn. It is in this stage that we confront the diversity or variability usually found in physiological and ecological data. The use of quantitative methods that are inherently probabilistic and methods based on widely accepted principles of growth have been combined in sophisticated algorithms to resolve patterns and thus allow the specification of age information in statistical terms.

An exception to the use of probabilistic methods at the interpretative or data analysis stage is when a sampling design for data collection is needed. This is often a serious consideration because age determination is one of the most expensive processes carried out in ecological and/or management studies. The not uncommon desire to make statistically significant statements in the face of both high biological variability and high cost makes a compelling case for statistically valid data collection.

Obviously, each of the multiple fields that contribute to age determination has its own specialized literature, together representing scores of journals. It is for this reason that we felt that a synthesis of methods and results across species and disciplines would have value under one cover. Some of this literature was examined, in fact, as the preliminary stage in a project frequently thought impractical by usual methods, age determination of tropical marine fish. The literature survey was useful in the identification of alternative body parts that might be used for ageing, alternative methods for defining and analyzing data and finally, for the validation of the data. Generalizing from this, we expect that this synthesis will be of special value to readers who wish to ask questions which are new for "their" species, but have been considered by others working with a different group of animals or plants. We are aware that we may have missed potentially valuable methodologies or applications. We would welcome recommendations, critiques, information, reprints, etc., to make revisions or updates as representative and inclusive as possible. Contributions would be greatly appreciated and acknowledged.

Vincent Gallucci and Donald Gunderson note that this work is in large part the effort of Dr. Lai, first as a Ph.D. student and then as a postdoctoral student.

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Introduction

Age determination is fundamental to the study of population dynamics (Odum 1959, Roughton 1962, Bagenal 1974). Its importance can be illustrated by the well-known population dynamics model in fisheries proposed by Russell (1931). The schematic diagram of Russell's model can be presented as:

Recruitment (R) ⟶ Resource ⟶ Natural Death (D)

Growth (G) ⟶ Stock ⟶ Harvesting (H)

Let P_1 be the stock at the beginning of a particular year and P_2 be the stock at the end of that year, the relationship of the stock with the four input/output paramenters is

$$P_2 - P_1 = R + G - D - H.$$

The study of population dynamics and stock assessment has focused on the estimation of the rate parameters for these processes via experiments and population modeling. Although many length-based population models have been proposed in the recent studies, the necessity of growth parameters in these models is still required for accurate estimation (Jones 1984; Lai and Gallucci 1987, 1988). Traditionally, these population parameters are estimated by age-structured models which rely on the age data to project the age composition of the population along time, e.g., VPA (Gulland 1965), cohort analysis (Pope 1972), Catch curve analysis (Chapman and Robson 1960), Leslie matrix model (Leslie 1945, Keyfitz 1977), etc. Although other approaches such as tagging experiments and survey samplings can also estimate the values of parameters, age determination is still the most commonly used, and perhaps most accurate, of the applicable methods. Therefore, the comment of Bagenal (1974): " Age determination is the central part of fish population dynamics".

When age determination is carried out by counting annual, seasonal, or daily growth marks (rings) on hard (bony) tissues, the method is called the "direct ageing method" and the age obtained from this method is called "chronological age". In contrast, morphological and/or physiological characteristics, e.g., larva, fry, juvenile, adult for salmon, and instar for crustaceans, are used to separate the life history of animals into a series of distinct stages. Others have used statistical techniques to separate mixtures of length-frequency distributions or to estimate age based on the growth model of a population and the length of an individual. Two of the best known statistical techniques based on length-frequency analysis (LFA) are MIX (Macdonald and Pitcher 1979) and NORMSEP (Hasselblad 1966). Methods such as raising the fish, tagging experiment, etc., are sometimes used to build a growth model in which the size of the fish is dependent on age. With the use of statistical calibration techniques, age can be predicted once the size of an individual is known. Since these methods are not counting growth marks from the hard structures, they are called "indirect ageing methods". Other indirect ageing methods include the use of heavy metal content, ageing pigment, isotope content, multivariate analysis, etc., which are all based on established statistical models.

The organization of this report mainly emphasizes the methods and applications of age determination for fishes. However, the development of ageing methods for fish has not reached its full potential and may not have as long a history as that of the other organisms. One of the examples that can be used to illustrate this argument is that paleoecological applications using the growth records stored in trees, bivalves, corals, and barnacles were developed and evaluated long ago. However, fish otolith microstructural analysis is just beginning. In fact, many biochemical or genetical ageing methods used in fishes were originally developed for the other organisms. Although this paper does not provide a detailed description of all the techniques and

practices for all ageing methods, it is my intention to provide useful information from other areas that can be adapted to fishes.

Age determination is a science and an art. Problems with age determination are frequently the source of blame, rather than the sampling or modeling techniques when stock assessment and fishery management fail. Indeed, age determination is a subjective area dependent upon the individual age reader. Inconsistency and non-repeatabilty between readings, even for a validated ageing method, is a central obstacle to making age determination a fully scientific area. Therefore, one part of this paper is devoted to the inconsistency problem.

Age determination is labor-intensive, costly, and routine work. This paper also provides a review of sampling designs for age-length keys and the techniques to estimate mean length-at-age and age composition from the age-length key. Alternatively, these parameters can be estimateded from the statistical analysis of length-frequency distributions. These methods have become a hot topic in current reseach, thus, a review of these methods is organized into a separate chapter.

It is also important to point out that age determination in fishery research aims directly at stock assessment and fishery management. A long-term age determination data base is required for any fishery management agency. Therefore, we summarized our personal opinions in the discussion to explore the question: how to organize an ageing laboratory to carry out the responsibilities of fishery management.

Age Determination for Plants

The age of trees has probably been of interest since before recorded history. It is believed that the first person to try to determine tree age is Theophrastus who described that the diameter of trees increased with time but apparently did not look at ring patterns (Studhelter 1955, 1956) . Keen (1937) reported that Leonardo da Vinci was the first one to recognize that trees grow periodically and annually. He also suspected the relationship between the width of rings and the moisture of corresponding years. Age determination of trees provides information about certain past events, e.g., timing, evaluating the influence of environmental conditions, and appraising successional patterns, range condition, and productivity (Roughton, 1962). Recent applications of tree ring analysis have played a significant role in forest management. The field of tree ring ageing, interpretation and application has become a scientific school called "dendrochronology" or "dendroclimatics".

Counting Tree Rings on Cross Section

Ring counting is the oldest and most commonly used method for age determination. Conifers and other trees growing in most temperate zones form rings corresponding to well-marked warm and cold periods or seasons. Even in semitropical climates trees will show distinct and easily counted rings if there are conspicuous wet and dry seasons (Chamberlain 1932, Schulman 1956).

The use of the terms "annual ring" or "annulus" may be a misconception. Ferguson (1962) stated that evidence of annual nature is seldom present in ring counting studies. This question has existed since Duhamel (1758) observed that trees that were known to be 20 years old did not necessarily have 20 rings, and trees of 10 years of age often had more than 10 rings (Studhelter 1955). The issue has legal as

well as scientific ramifications as can be seen from the court battle bewteen Smith (1882), a lawyer, and Child (1882), a botanist. Smith said:

> "It will be very difficult to convince an old surveyor, or an old lawyer who has tried many of these land cases, that each concentric ring, on an oak-tree at least, does not indicate a year's growth only of such trees. " (cited for Roughton 1962)

Recent evidence supports the position that growth rings may not be formed annually and periodically. (U. S. Forest Service, Forest Products Lab. 1955, Fritts 1976, Vasiljevic 1961). Despite these arguments over growth rings, the most moderate point of view is that: the activity of the cambium is commonly periodic and the xylem produced during one growth period constitutes a growth layer. In transverse sections of stems and roots, such layers are referred to as growth rings. If the growth is definitely seasonal and occurs once during a season, the growth layer and the growth ring may be called the annual layer and the annual ring respectively (Esau 1953).

Summarizing the work of many authors, possible explanations for abnormalities that would make it difficult to determine the yearly identity of growth rings are: missing, "omitted," or "absent" rings (Schulman 1956), or partial rings (Fritts 1976), or locally absent rings (Schulman 1956, Galdwin 1941, Douglass 1922, 1946). Some reasons why missing rings occur are: (i) lack of food manufactured in the crown under suppressed conditions (Turberville and Hough 1939), (ii) drought (Schulman 1941, Hawley 1941), (iii) injury by fire (Fritts 1976), (iv) extreme cold (Keithley 1931, Andrews and Gill 1939), (v) insect blight (Bailey 1925, Hurch 1949), and (vi) smoke or frost injury (Fritts 1976). Another possibility is that acid rain will prohibit tree-ring formation (Jonsson and Sunberg 1972, refer to Fritts 1976).

Double rings (Stokes and Smiley 1968), multiple rings (Glock 1951), or false rings (Vasiljevic 1961) are caused by multiple waves of combined activity within a growth season. The causes of false ring formation are still not well understood. Some

species form false rings due to droughts during the middle of the growing season or bad climate. However, some species have genetic problems (Stokes and Smiley 1968). Stokes and Smiley (1968) described methods for detecting such abnormal rings.

Samples from the base stem are usually taken by a "T"-shaped drill and do not sacrifice the tree. The preparation of samples is important to ring counting. Stokes and Smiley (1968), Galdwin (1942) and many other authors have described the preparation methods for particular purposes.

Estimating Physiological or Morphological Age Class

This method applies "size-age" relationships to aged trees. Shrubs are often categorized into seedlings, immature or young, mature, and over-mature or decadent on the basis of appearance (Interstate Deer Herd Committee 1951, 1954, Dasmann and Blaisdell 1954, Dasmann and Hjersman 1958, Mustard 1959). Similarly, seedlings, saplings, poles, mature, and over-mature have been used to describe trees based on diameters at breast or stump height which may be correlated to age (Gates and Nichols 1930). However, this method has been criticized as inadequate by many authors (Roughton 1962). Site factors and competition confound this method since young plants may grow larger in favored situations than mature plants in less favorable sites. Roughton cited two examples: (i) Baker (1923) found that the growth rate of aspen and conifer varied according to the law of chance regardless of the age and average size of trees present; and (ii) Brady (1939) reported an extreme case of dwarfing in ponderosa pine growing under unfavorable conditions.

Bud Scar and Branching Node Counting

Bud petals fall away as buds open and leave scars on the stems. Most of these scars are left by terminal buds and each of them marks the place at which a terminal bud began its development in a spring season (Fuller and Tipps 1950). Stallard (1929) used these scars to age pine trees up to 19 years old. However, as stumps grow, bark cracking and weathering prevents the aging of large stems. Whittaker (1961) reported that this method is reliable but subject to error including the occasional failure of older branches to produce shoots and multiple shoots produced by twigs.

Tree Ring Analysis

Tree ring specimens are usually obtained from the base at 1.4m above the ground. In the past, the tree-ring series was used to build a climatic response model which correlates growth-ring width variation to historical climate variation, so that the study has been called dendrochronology or dendroclimatics. The remarkable success of dendroclimatic reconstructions has been seen throughout the world (e.g., most recently, Blasing and Duvick 1984; Cook and Jacoby 1977 and 1983; Fritts et al. 1979) and thus the age determination of trees becomes a by-product of tree ring analysis. The latter study by Fritts et al. also provides critical calibration of C^{14} dating which was developed by Willard F. Libby at the University of Chicago (Hitch 1982).

Dendrochronology is concerned with the study of the chronological development of tree rings and the reconstruction of local climates over many thousands of years. Simply, it is based on four assumptions (Stoker and Smiley 1968):

1) Trees add one ring for each growing season; hence, it is called "annulus".
2) Although the total seasonal growth is the result of many interacting factors, such as genetic and environmental, only one environmental factor must

dominate in limiting growth.

3) This growth limiting climatic factor must vary in intensity from year to year and the rings faithfully reflect such variation in the width. Although the ring width is not necessarily directly proportional to environmental factors, the ring should narrow down in drought years and appear noticeably wider in rainy years.

4) The variable environmental growth-limiting factor must be uniformly effective over a large geographical area.

The method of matching growth rings from recently growing trees and collected ancient logs is called "cross-dating" (Fig. 1). After cross-dating, a pattern plot of relative variation of tree ring width can be built, which is then related to a master chronological plot. Back-dating of past climates is done by a regression model (Fritts 1976). For details of this back-dating method, see Roughton (1962), Fritts (1976), Stoker and Smiley (1968), and Hughes et al. (1982).

The most recent tree ring analysis has tried to use the historical record of forest growth that reflects changes with time in site factors, such as competition, tree and stand age, fire and other disturbances, and climate, to develop a climatic response model that indicated whether forest productivity declines are related to climate or other influences (e.g., Hepting 1963; Cook et al. 1987; Johnson and McLaughlin 1986; Eckstein et al 1983; Eckstein et al 1984; Hornbeck and Smith 1985; Cook 1985; Johnson et al 1987; McLaughlin et al 1987; Federer et al 1987; Hornbeck and Smith 1985; Hornbeck et al 1986; Johnson and Siccama 1983 and 1984; McLaughlin 1985; Scott et al 1984; Siccama et al 1982; Weiss et al 1985).

Climatic response models are typically based on ordinary least squares (Cook et al 1987) for a multiple regression equation or a principle component regression (see Fritts 1976 for detailed description). Other methods such as generalized least squares (Koutsoyiannis 1977) or ridge regression (Draper and Smith 1981) can also be used.

More recently, a time series model has been applied in the building of a climatic response model (e.g., Cook 1985, Clendenen et al. 1978). A Kalman filter method has also been applied to tree ring analysis (van Deusen 1987; Visser and Molenaar 1987). A general summary for time series analysis application is given in the age determination of bivalves section.

Radioactive Isotope Dating

C^{14} dating developed by Willard F. Libby in the late 1940's represents a high technological and scientific approach to historic dating. The principle is that C^{14} is created by cosmic rays bombarding nitrogen or C^{13} in the atmosphere and is absorbed by living things. It is stored in the body as organic material until the organism dies. C^{14} decays with a constant half-life time which is about 5730 years (Libby 1983). The proportion of C^{14} remaining in the organic material provides a measurement of the age of that material. The assumptions are that the material has not been contaminated with C^{14} and the proportion of C^{14} in the atmosphere is constant over time.

Using radioactive elements in trees for the analysis of variation in past climates depends on the existence of isotopes of stable elements. According to the IAEA monthly measurements of O^{18} and duterium ratios in the rain water, seasonal variation occurs in such a way that the amount of isotopes decreases in winter precipitation when water vapor distills from cold oceans, and increases in summer precipitation when vapor distills from warm oceans. The same effect was found in the successive seasonal layers of ice in the Greenland and Antarctic ice caps. When surface water and air temperatures are low, the amount of heavier isotopes found in ice layers is depleted. Therefore it is assumed that long-term changes in precipitation caused by changes in climatic temperature are well-documented in polar ice caps.

Libby (1983) applied these concepts to tree thermometry: "For temperate regions, the history of the surface temperatures of the seasons is stored in the glaciers of those regions, but glaciers have random advances and retreats which spoil the orderly sequence of the historic yearly ice layers. However, the history of the surface temperatures of temperate oceans should be stored in the rings of trees which grew in the temperate regions of the world and which subsisted on precipitation distilled from those oceans. Each tree ring should contain some kind of annual value of the isotope ratio in the precipitation of the year corresponding to the ring."

Tree growth is an anabolic process that produces new wood and can be generalized by the following chemical reaction:

$$CO_2 + H_20 \rightarrow \text{wood} + O_2$$

In trees which grow on rain water, isotope variation in rings should be a climate indicator because the isotope composition in rain and CO_2 varies with temperature.

Using C^{13}/C^{12}, O^{18}/O^{16} and D/H ratios, Libby successfully related the variations of isotope ratios to the variation of temperature of recent years and then used the relationship to predict past temperature variation and climate periodicity.

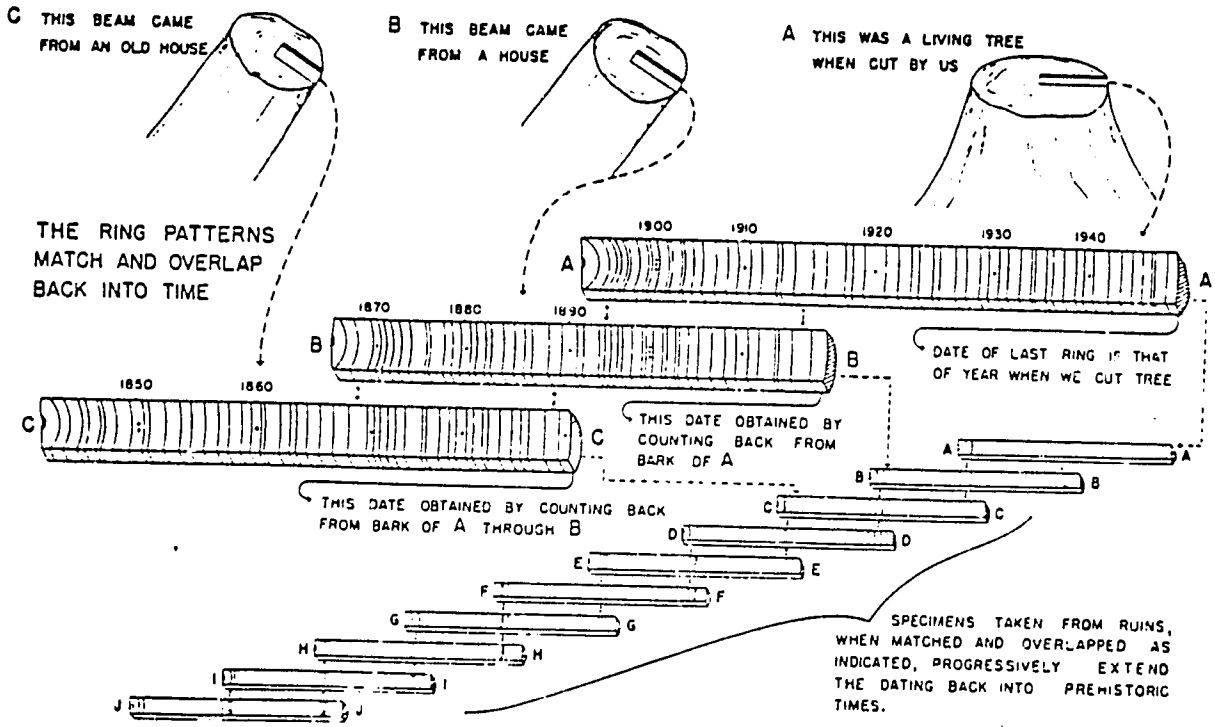


Fig. 1. (From Stock and Smily 1968)
The conceptual diagram of cross-dating technique.

Age Determination for Barnacles

Darwin (1854) first proposed that the ridges on the outer surface of the barnacle shell reflect a growth pattern which was related to the molting cycle. Subsequent studies have uncovered that the internal growth patterns in the shell can be identified as associated with the influences of molting, feeding, tidal immersion, tidal level and temperature. Also, many factors such as age, season of death, and growth rate, may be determined from growth patterns found within shells. Therefore, the studies of shell structures may provide important information for both ecological and paleoecological events (Bourget 1980).

The macrostructure of a barnacle shell is illustrated in Fig. 2. Typically, there are up to eight overlapping calcareous wall plates (called parietes for plural, paries for singular) and two bipartitie opercular valves that are used to close the aperture formed by the ring of parietes. The cone-shaped shell sits on a membranous basal disk that may or may not be calcified. Wing-like secondary structures, alae and radii, at the margins of prietes are used to increase the strength at junction of the plates.

The barnacle shell grows in height by adding material at the basal edge of the paretes and in girth by adding material at the margins of radii and alae (Fig. 2). However, some species such as *Tretclita* and *Purgoma* have a complete concrescence of the parietes. The growth proceeds entirely through addition of increments at the basal margin. For a detailed growth pattern for concrescent and nonconcrescent shells, see Crisp and Patel (1967) and Bourget and Crisp (1975b).

There are two processes involved in the deposition of a new material to the plates (Bourget 1980): the formation of cuticular tissue by the epithelium at the basal margin of the shell and the secretion of calcium onto this newly formed cuticle. Fig. 3 shows that a cuticular slip is produced by specialized cells underlying the base near the edge of the shell at each molting cycle. The secretory activity of that part of the

epithelium is synchronous with the premolting period. During the molting cycle, this newly formed slip of cuticle is stretched out at the margin and later becomes progressively filled with calcite secreted by the epithelium underlying the inner region of the parietal plates. The secretory activity of the epithelium of the mantle lining the shell is slightly influenced by the molting cycle. The pressures developed in the body fluids would push part of the new cuticle out of the calcified edge of the shell (Fig. 3 region A). Sequentially, the old cuticle, produced during the preceding intermolt period, becomes stretched and eventually breaks. The stretched part of the new cuticle, which is presumably sufficiently elastic to hold until the next intermolt period, then constitutes the template on which $CaCO_3$ is subsequently deposited.

To examine the internal growth bands (in terminology of Bourget 1980) a thin section is made from the shell. Bourget (1977) showed two different arrangements of growth bands (Fig. 4). In some species of Balanidae, where a shell sheath is present, the secretory activity of the epithelium underlying the shell is not continuous along the entire inner surface of the shell (Fig. 5) Then there are two series of growth bands, those forming the lower part of the paries and those forming the sheath. Those shells growing uniformly along the inner surface have much thinner bands than shells growing in two separate regions. In practice, uniformly deposited bands of chthamalids are too thin (< 1μm) to be precisely measured. In addition, the formation of intra- and inter-laminate figures and canals within the parietes complicates the interpretation of growth bands.

Bourget and Crisp (1975a) reported that barnacles in the field or photoperiod-controlled laboratory animals produce about two bands per day regardless of light regime. In the same paper, they also reported that the definition of growth bands is very poor in immersed animals compared to those under immersion-emersion cycle. However, such a difference in band contrast would not occur in the continuously immersed animals. Therefore, one must be careful in analyzing shell sections and not

be biased by the contrasting successive growth bands. Bourget and Crisp (1975c) also showed that shell growth continues during nonfeeding periods but at a diminished rate, and growth stops immediately following emersion and is resumed on immersion. Other factors such as food, wave action, barnacle orientation, water flow, competition, season, breeding, age, molting, tide, and temperature are known to affect growth rate and thus the band width.

The analysis of growth band measurement has been carried out with time series analysis (Trump and Bourget 1980). This modeling technique has been used to describe the relationship between band width and molt, tidal, weather, solar, and other environmental cycles complicated by noise from aperiodic occurances in the environment.

Another growth feature of the barnacle shell is that external growth ridges appear on the outer surface of different shell plates (Fig. 6). A summary of these growth ridges are listed in Table 1 of Bourget (1980). The most recent results indicate that the fine surface ridges cannot be related to any environmental factor, nor can they be considered as reliable age indicators. Bourget and Crisp (1975 a,c) showed that hirsute ridges are formed 6-12 hrs before ecdysis. But they are not reliable since they do not necessarily form at each molt. Winter rings may be used for age determination (e.g., Kuznetsov and Mateeva 1949; Petersen 1966; Crisp 1954; Klepal and Barnes 1975b). However, Petersen points out some drawbacks: older animals produce smaller winter rings and the rings on the older portion of the shell tend to wear away. It is necessary to establish the first winter ring on the shell prior to ageing. This is not easy in arctic and subarctic regions because of heavy abrasion of the shell by ice.

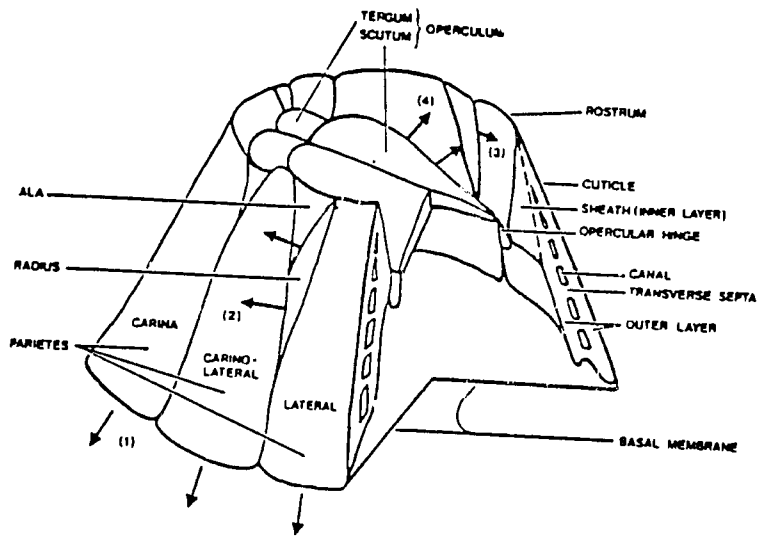
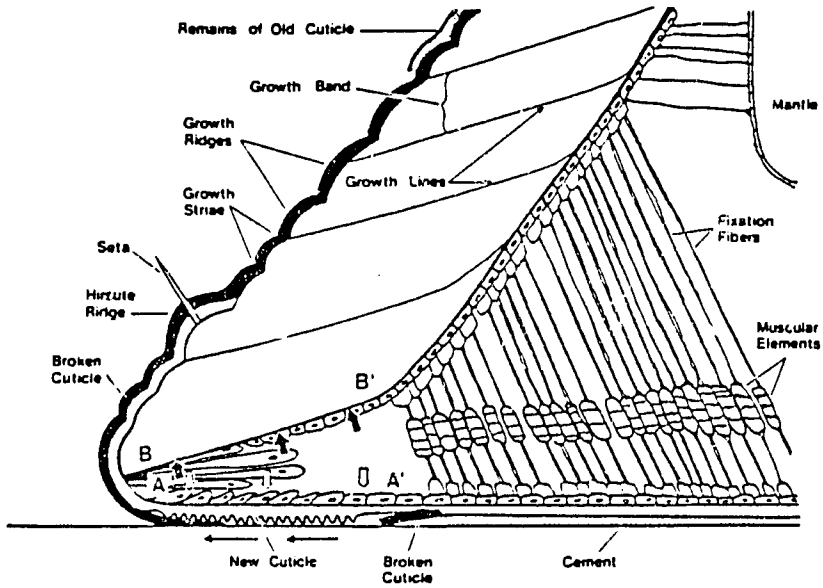


Fig. 2. (From Bourget 1980)

Surface and cutaway view of a typical barnacle shell. Arrows indicate directions of growth: (1) downward growth at the basal margin; (2) growth at the margin of the radii; (3) growth at the margin of the alae; (4) growth at the margin of the operculum.



Growing edge at the basal margin of the barnacle shell plate. The cuticular covering does not show the different constituent layers. Each cuticular scale is produced during one molting cycle. Scales overlap one another on the outer surface of the shell plate. (⟶) Direction of movement of the newly formed cuticular slip; (⇨) region of formation of the cuticle (A–A'); (➡) region of $CaCO_3$ deposition (B–B'). After Bourget and Crisp (1975a).

Fig. 3. (From Bourget 1980)

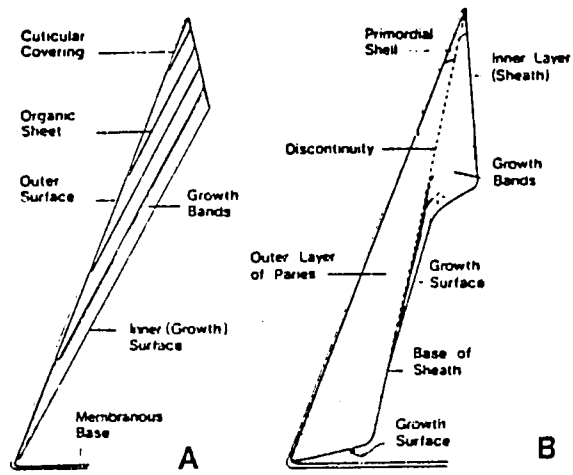


Fig. 4. (From Bourget 1980)

Distribution of the growth bands as seen in radial sections of a barnacle shell. (A) Bands continuous along the inner surface of the shell in *Chthamalus*; (B) bands discontinuous near the basal margin of the shell plate in *Balanus*. Note the absence of sheath in *Chthamalus*. After Bourget (1977).

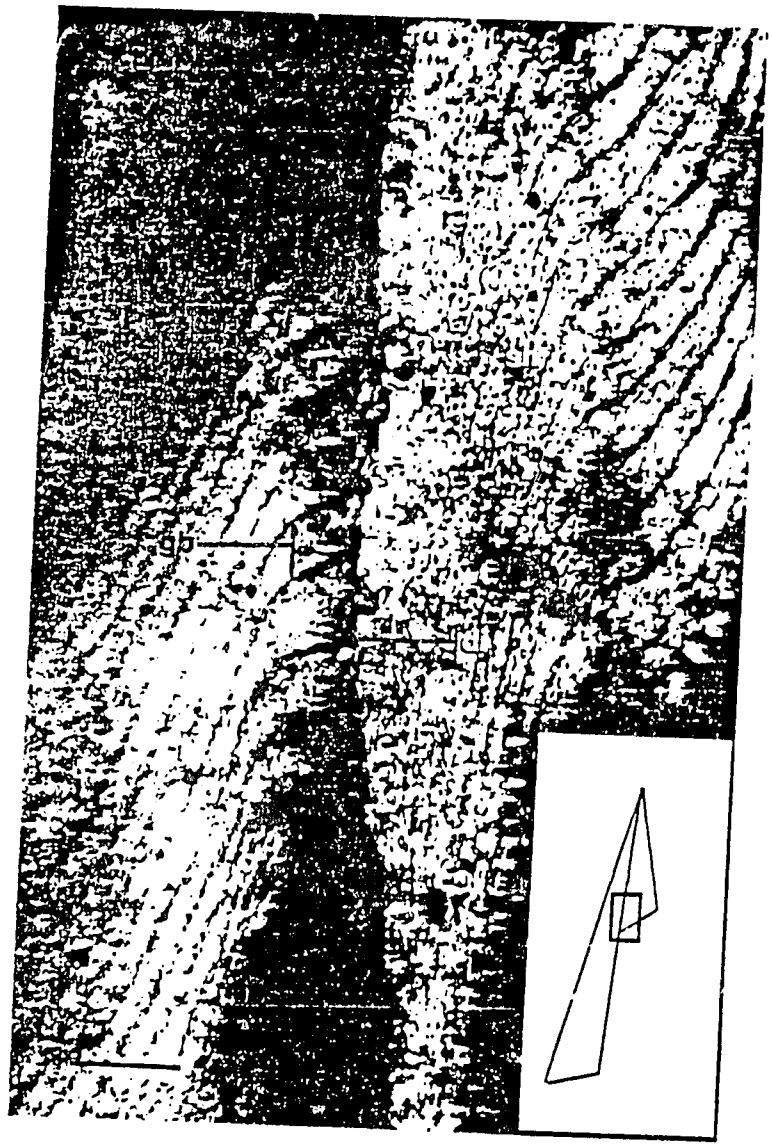


Fig. 5. (From Bourget 1980)

Growth bands (gb) as seen in thin section of *Balanus balanoides*. The figure shows the discontinuity (di) at the junction of the outer layer (ol) and sheath (sh). Scale bar: 50 µm The inset shows the location of the photograph on the shell section.

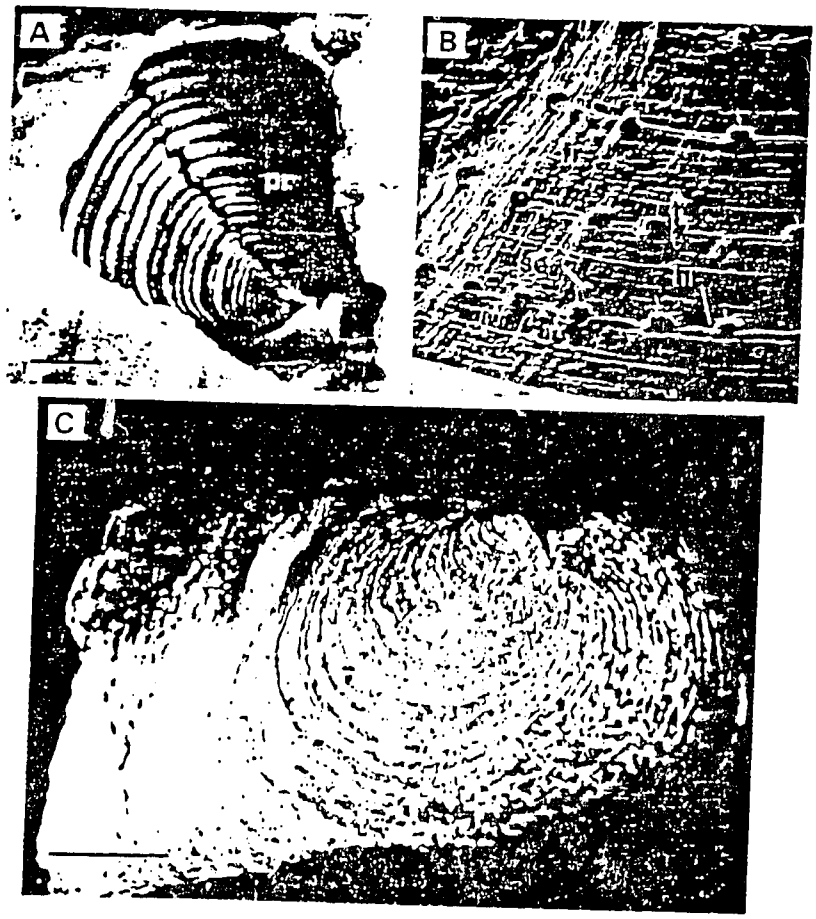


Fig. 6. (From Bourget 1980)

Growth ridges on the outer surface of the shell plates. (A) Scanning electron micrograph of prominent ridges (pr) on the opercula of *Balanus balanus*. Scale bar: 2 mm. (B) Scanning electron micrograph of fine surface ridges (fr) interspaced with more prominent hirsute ridges (hr), bearing setae (se), on the lateral paries of *B. balanoides*. From Bourget (1977). (C) Photomicrograph of the undersurface of the calcareous base of *Balanus tintinnobulum*. Scale bar: 5 mm. Supplied by C. Muir, Department of Zoology, The University, St. Andrews, Scotland.

Age Determination for Corals

The Phylum Coelenterata (Cnidaria) contains many colonial taxa with calcareous skeletons which are secreted from the organisms. It is well-known that the physical and chemical factors are capable of affecting the physiology of corals, and thus, the secreted mineralized skeletons are capable of preserving long-term (annual) and short-term (solar day) growth records of the colony. The Scleractinia, and the extinct Rugosa and Tabulata, are of special interest because the former is the dominant metazoan in the modern reefs and the latter two were widespread and common in the Paleozoic. The Scleractinia contain well-preserved internal growth banding, while many paleozoic corals have external epithecal ridges that reflect growth periodicity (Dodge and Vaisnys 1980).

The growth characteristics of corals has been reviewed by Wells (1957), Yonge (1963), Stoddart (1969), Buddemeier and Kinzie (1976), and Dodge and Vaisnys (1980). An annual coral growth feature was established by Knutson et al. (1972). They compared nuclear bomb test induced autoradiographic bands in coral skeletons from Eniwetok with density bands shown in X-radiography. The results confirmed that the density banding, high and low density bands, are formed annually. Dodge (1980) has reported that X-radiography is the most useful method for the growth study of coral skeletons. First, the coral is sliced into a rough slab using a common masonry saw and then thin slices of about 0.5cm are cut with a geological rock saw. The coral slice is exposed to X-rays with 100Kv. The radiograph is examined for counting growth rings of the coral. A X-radiograph is shown in Fig. 7. For more detailed preparation processes, see Dodge (1980).

The $CaCO_3$ staining method has been used to study the time of growth-band formation. Barnes (1972) used Alizarin red to stain living corals in the field and

laboratory. After a period of time for growth, the X-radiographed density banding was related to the stained reference datum.

The applications of a coral growth study is to reconstruct the past environmental events and to understand the relationship between the growth of extant species and environmental perturbation. From extinct and extant corals, the history of the earth rotation (Rosenberg and Runcorn 1975; Scrutton and Hipkin 1973) and the relationship of coral's physiology and present and past environmental changes (Buddemeier and Kinzie 1976; Vaisnys and Dodge 1978) have been constructed.

A general application of coral growth features in ecology and paleoecology have been described in Dodge and Vaisnys (1980). Vaisnys and Dodge (1980) also used the results of age determination to construct an age-frequency distribution for the coral reef population in Bermuda.

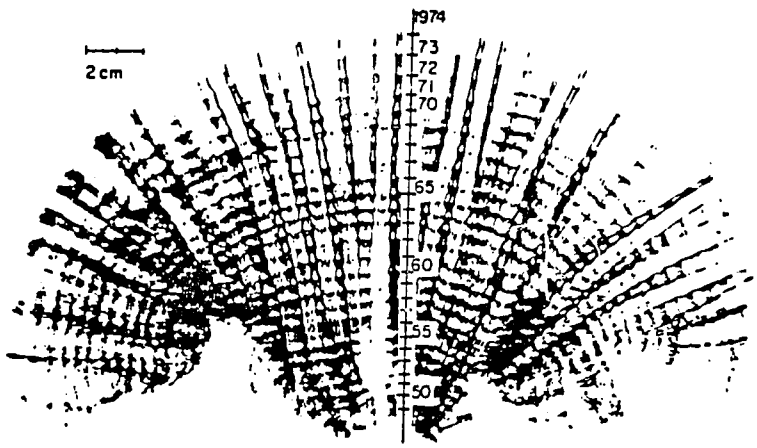


Fig. 7. (From Dodge 1980)

X-radiograph positive of medial section of *Diploria labyrinthiformis* from Castle Harbor, Bermuda, collected live on May 17, 1974. The annual density-band couplets (dark areas are of greater density on the X-ray positive) are circumferential, while the axial structures radiating from the coral base are the traces of corallite (polyp) walls in the skeleton. A typical band-width transect is shown where annual growth is measured from the top edge of a high-density band portion to the top edge of the next older, lower, high-density band portion. Bands have been assigned to their respective years of formation.

Age Determination for Cephalopods

Squids have been aged using mantle length frequency analysis (Squires 1967, Summers 1971, Mesnil 1977, Lange 1980, Ishii 1977). Summers (1971) used the probability paper method developed by Buchanon-Wallaston and Hodgson (1929), Harding (1949) and Cassie (1954). Ishii (1977) used the mantle length frequency distribution associated with the study of allometric growth and Lange (1980) employed the statistical method developed by Hasselblad (1966) to analyze the length frequency distribution.

More recently, age determination of squid has been done from growth rings in the statoliths (Hurley and Beck 1979, Hurley, Drew and Radtke 1979, Lipinski 1978, 1980). Clarke (1965) found "growth rings" in the beaks of *Moratenthis iggens* and Yashuda (1951), Yagi (1960) and Choe (1963) found daily age marks on the shells of cuttlefish.

Beak

Age determinations of *Lologo vulgaris* (Tinbergen and Verwey 1945) and *Todarodes sagitatus* (Fredriksson 1943), and several other species (Wirz 1963) have been studied with beaks but no success has been reported.

Clarke (1965) described the method of counting "microring," or "growth lines," on the lower beak of *Moroteuthis ingens* (Fig. 8A). The lower beaks found in the stomachs of whales were collected and cut on the left side of the rostral tip and the medial surface of the lateral wall and the exposed edge viewed by oblique reflected light. Ridges radiate from the rostral tip to the inner and posterior along the free edges of the wall. Undulations and microrings run roughly parallel to these free edges (Fig. 8B). The beak composition is secreted by an epithelium which adds chitinous material

on the posterior side of the wings and on the lateral wall. As the growth proceeds, the secreting area expands so that the chitinous material overlaps the edges of the growing surface. This overlapping gives rise to the microrings on the medial surface of the lateral wall. The width of the microrings occurs in a series of "cycles" that run from the rostral tip to the free edges of the lateral wall. Often the two earliest cycles do not have step-like microrings but only undulations, but as the growth proceeds, undulations occur with microrings. Utilization of microring depends on the ability to relate their formation to time; Clarke, however, was not able to establish the time required for a cycle, only that the time of the cycle formation is 6 or 12 months. Thus, if the lateral wall is worn away at the rostral tip to any appreciable extent, age determination based on the count of microrings and cycles will not be reliable.

Shell

The formation of the stripe pattern on the shell of cuttlefish has been interpreted to be periodic with age information (Appellof 1893, Naef 1928, and Yasuda 1951). Yagi (1960) showed that the shell stripe pattern of *Sepia esculeuta* (Fig. 9) is periodic and increases with age. Yagi found that the relationship of age and number of stripes on the ventrum of the shell can be described by

$$t = 18.4 \times 1.03^{y} \qquad \text{for } 100 < t < 300 \text{ days}$$

where t is age and y is the number of stripes. For the whole life history, a sigmoid curve can be used to depict the relationship

$$\log_{10} \frac{y}{a-y} = b(t - c)$$

where, a, b, and c are parameters. Using this relationship, Yagi estimated that the formation of the first stripe is about 1.5-2 days after hatching. The periodicity is about 9 days after 300 days. The average periodicity is about 3.5 days over the whole life. Choe (1963) observed distinct Wurstlamellen on the dorsum and in the striated area of the ventrum of the shell. He also showed that periodicity of stripe formation of *Sepia esculeuta* is affected by nutrition, salinity, oxygen content, and temperature.

Statoliths

Statoliths are paired calcerous structures located within a statocyst in the ventro-posterior region of the skull. They have similar structure and function with teleost otoliths and are composed of aragonite (Dilly 1976, Clarke 1978, Hurley and Beck 1979).

Methods used to extract statoliths are dissection (Dawe et al 1985) or dissolving the skull in bleach (Clarke 1978; Spratt 1978; Hurley and Beck 1979; and Lipinski 1980). The dissection method involves cutting the statocyst to expose the paired statoliths in their maculae. Hurley and Beck (1979) use sodium hypochlorite as the bleach solution, but the bleaching effect makes the statoliths of short-finned squid (*Illex illecebrousus*) unreadable (Dawe et al. 1985). Once the statoliths are extracted they can be preserved in gelatin for a long time (Spratt 1978). Squids caught for this purpose have to be preserved in ethanol or by freezing (Dawe 1981). Formalin preservation should not be used because statoliths dissolve in acid, even if it is very weak (Hurley and Beck 1979, Kristensen 1980).

Statoliths can be viewed under the microspcope. The premounting treatments are immersion in distilled water for approximately 24 hrs, trypsin-papain for 0.25-24 hr at 35^{o}C, absolute alcohol, or glycerin. The purpose of the premounting treatment is to

prevent the thin outer membrane from becoming dry, which renders it opaque to transmitted light. Mounting media can be Permount, Protexx, Cover Bond, EPON, Euparal, Eukitt, and Canada Balsam. Rings are viewed using a compound microscope as a series of dark and light bands (See Fig. 10 for example). Kristensen (1980) showed that organic material was important to form dark bands.

Lipinskii (1978) suggests that daily increment rings are formed in the nuclear region and monthly basis rings are formed in the outside region of the "juvenile statolith". He found that the frequency of ring formation closely approximated a diurnal periodicity. Hurley et al. (1979), ICNAF (1978) and Spratt (1978) found that back calculation consistently underestimated mantle length. This indicated that the number of rings underestimated the elapsed days.

Hurley and Beck (1979) compared back-calculated mantle lengths to actual lengths from length frequency distribution and think there may be confusion with the presence of mixed age groups within a single year class. Kristensen (1980) suggested that rings formed in the nuclear region may have a different interpretation than those formed in the outer region. Failure to detect rings may be due to the preparation technique (Hurley and Beck 1979, Hurley et al. 1979), or because ring formation is affected by physiological, nutritive, and hydrographical factors (Clarke 1965, Choe 1963, Hurley and Beck 1979). The validity of age determination of squids from statoliths has been carried out on known-age squids and with vital marking by oxytetracycline, strontium, or other antibiotics (Dawe 1981). Nevertheless, a recent study by Dawe and Beck (1985) concluded that further age validation is needed.

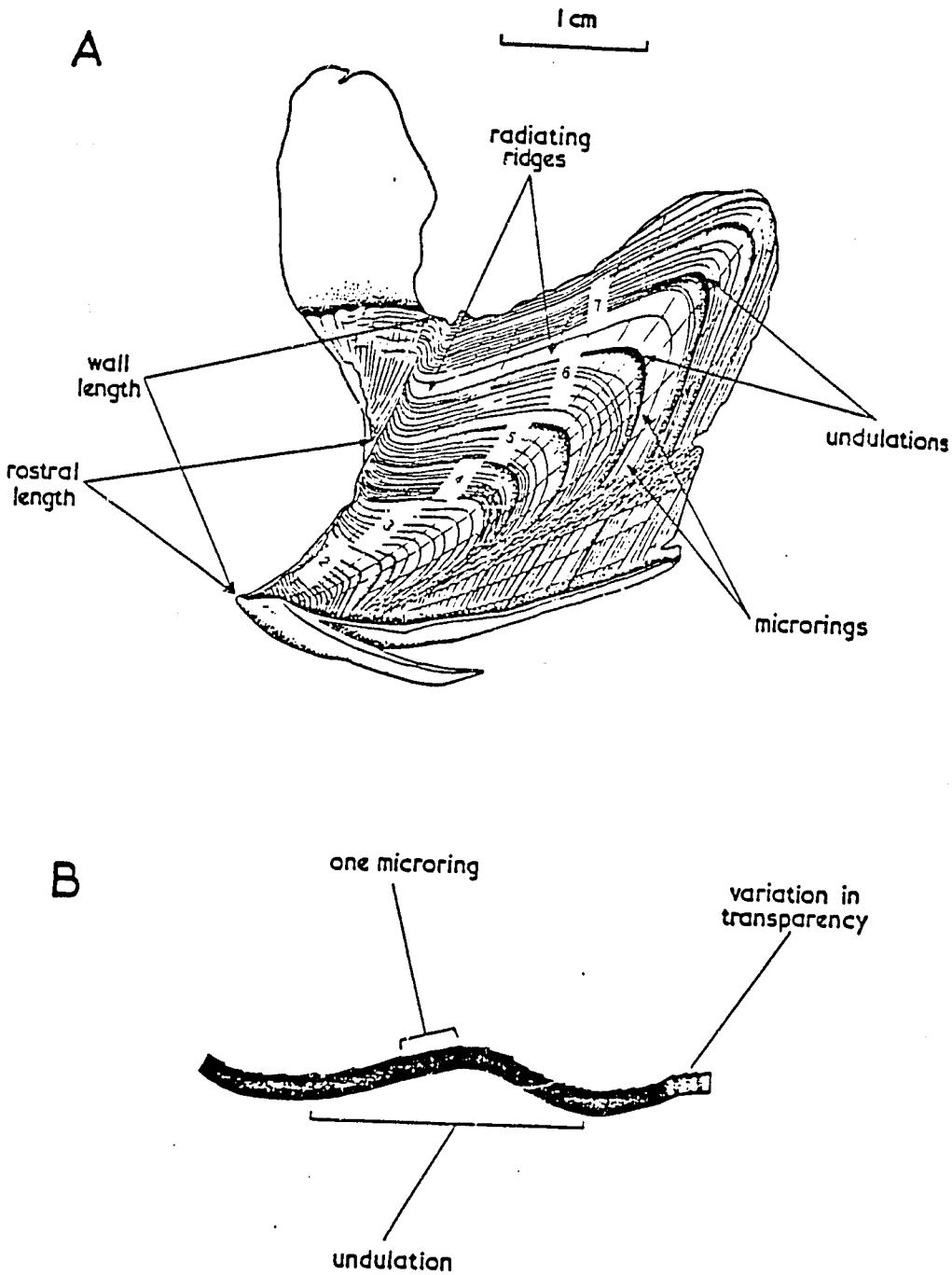


Fig. 8. (From Clarke 1965)

The diagrams show the microrings in the lower beaks of Monateuthis ingens.

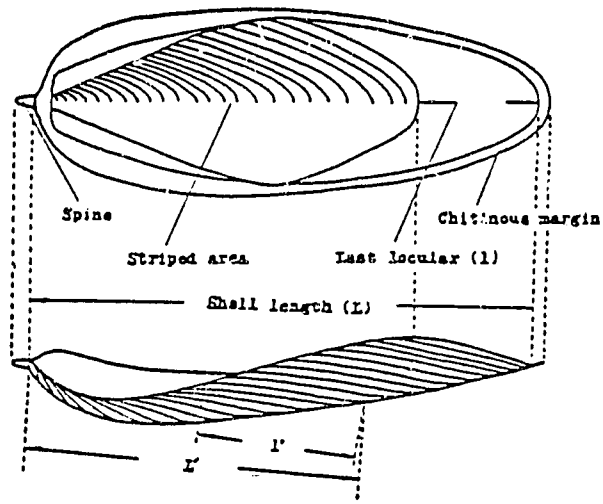


Fig. 9. From Yagi 1960)

Shell of *Sepia esculenta*. Upper: ventral view, Lower: lateral view of median longitudinal section. L': shell length at some stage of growth, l': last locular length at the same stage as "L'".

Locular index is calculated by $\frac{l}{L} \times 100$ or $\frac{l'}{L'} \times 100$.

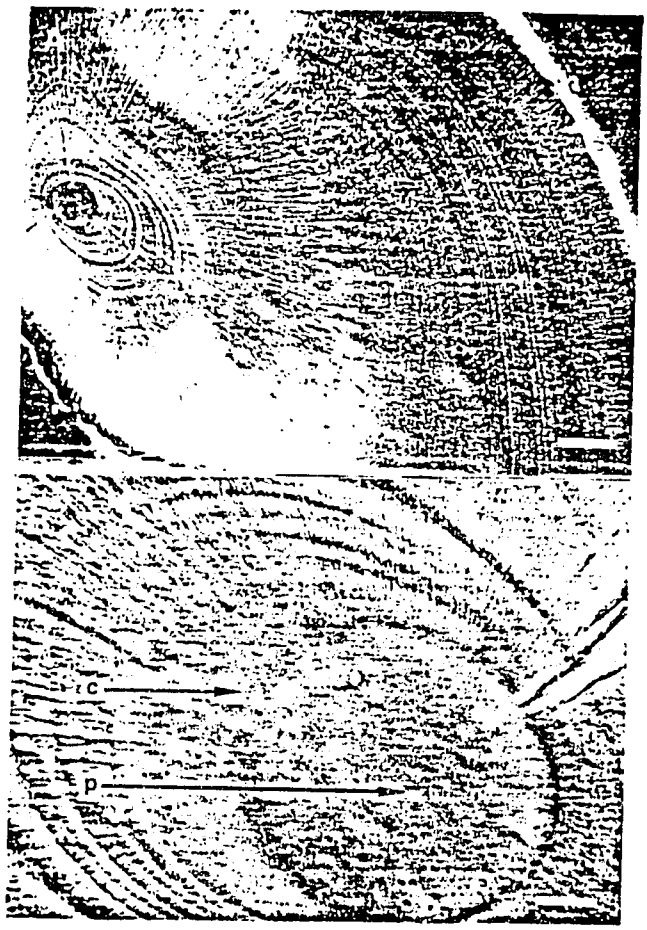


Fig. 10. (From Dawe et al. 1985)

Scanning electron microscope micrograph of acid-etched increments on the ground surface of a statolith from a 19-cm *I. illecebrosus*: (A) view of the plane through the core region (bar = 34.4 μm), and (B) increments in the immediate vicinity of the core (c) and primordium (p) (bar = 4.7 μm).

Age Determination for Bivalves and Gastropods

The growth pattern of the external surface of molluscan shells has long been a research subject in biology and paleontology. Lutz and Rhoads (1980) listed the early citations in this field. Hedgepeth (1957), Moore (1958), Hallam (1965) and Clark (1970) provide excellent discussions on the interpretation of growth patterns on the external surface of molluscan shells, as well as the effects of various environmental variables on shell growth and surface morphology. Recently, Taylor et al. (1969, 1973) and Rhoads and Lutz (1980) have made contributions to the understanding of shell structures, mineralogy, and the growth of bivalves.

Weymouth (1923), Weymouth and Thompson (1931), and Shuster (1957) described the internal shell structure which has been the basic reference for many age and growth studies of bivalves. There are four general layers within the bivalve: periosteracum; outer prismatic layer, inner mother-of-pearl layer; and nacrious layer. The outer prismatic layer is interrupted by alternative opaque and translucent lines of growth. These lines have continuity from a hinge tooth into the umbo and terminate at the external valve surface rings. The annual rings can be identified from these surface ring patterns.

Annuli have been identified on the external surface with and without polish. Rope and Jearld (1986) reported the validity of ageing the Atlantic deep-sea scallop (*Placopecten magellanicus*). Typically, dark coloration with interrupted shades indicate the annuli on the valves. Under a microscope the valve surface reveals fi·· · concentric circuli which are more dense near the annulus.

The difficulty encountered in interpretation of shell surface growth features arises from an inability to distinguish spawning and disturbance lines from an annual pattern (Lutz 1976). Saloman and Taylor (1969) reported that the age of *M. Mercenaria* determined from sectioned shells is older than when aged from the external surface by

Belding (1912). Ropes (1984) used acetate peels (for a detailed description of methods see Ropes 1987) to age the ocean quahog, which was formerly considered impossible to age (Murawski et al. 1982), by the rings on the external surface. Ropes (1984) validated the age of ocean quahog by marking clams and concluded that this species can be as old as 100 years. Ropes and Jearld (1986) also showed that the resilium in the hinge of the Atlantic deep-sea scallop reveals annual growth marks which are equal to the ring count on the external surface of values.

One theory of the microgrowth increment of the molluscan shell is based on the Lutz-Rhoads hypothesis. During aerobic metabolism, molluscs deposit $CaCO_3$ in the form of aragonite or calcite, together with organic material. Aerobic metabolism is usually associated with periods of active pumping during high tide in well-oxygenated waters. As the concentration of dissolved oxygen falls, such as in the internal microenvironment created by shell closure, anaerobic respiratory pathways are employed and the level of acidic end products within the extrapallial fluid rises. The acid is gradually neutralized by the dissolution of shell $CaCO_3$, leading to increases in concentration of Ca^{+2} and other end products within the extrapallial and mantle fluid. As a result of decalcification, the ratio of acid-insoluble organic material to $CaCO_3$ increases at the interface between the mantle and shell. The end product of this cyclic process is one growth increment (Lutz and Rhoads 1980).

Microgrowth increments have been used in (1) geophysical studies for defining Phanerozoic changes in the rate of earth rotation (Berry and Barker 1968, 1975; Pannella and MacClintock 1968; Pannella 1972, 1975; Dolman 1975; Rosenberg and Runcorn 1975); (2) ecological and paleoecological studies for assessing the effects of various biological and environmental stress (Pannella and MacClintock 1968; Rhoads and Pannella 1970; Kennish 1975 a, b, c, 1978, 1980; Berg and Hilbish 1985); (3) archecological studies for reconstructing settlement patterns of prehistoric harvesters (Coutts 1970, 1975; Ham and Irvine 1975; Clark 1977, 1979; Koike 1973, 1975).

In contrast to the microgrowth increment analysis that has become a standard for the study of bivalve shells (Hurley et al. 1987, Clark 1968, 1969, 1974), neither internal nor external rings on gastropod shells draw attention because of difficulties associated with a coiled shape. The commonly used cross-section method for bivalves cannot show all microincrements and the relative spaces between lines may not be consistent over the whole cross-section because the cut surface may not intersect the microincrements at right angles. For the slightly coiled or limpet-like shells, such as the South American*Concholepas concholepas*, cross-section of the shells do show promise (Adlerstein 1987).

Although the purposes of age determination for management purpose are different from that of paleoecological studies that use microincrements in shells, they share similar hypotheses. First, both types of studies must validate that the microincrements are deposited periodically, and second, that deposition of microincrements is controlled by certain environmental factors that are the determinant factors of shell growth. Time series analysis is one reasonable approach to these studies since the data collections can be regarded as equally spaced time series. The method is summarized in the following section and illustrated by examples from Adlerstein (1987) and Lønne and Gray (1988).

Time Series Analysis for Microincrement

1. Snapshot, smoothing and detrending

Suppose that a series of N microincrements in a shell are defined and the widths are measured as $\{X_t \mid t=1, 2, ..., N\}$. A snapshot of the time series, X_t vs t, gives a view of the relationship of microincrement width and time step. Most commonly, this snapshot of microincrement widths will show a seasonal fluctuation and a growth trend. However, neither the snapshot nor the original data provides good view of the

relationship. Lønne and Gray (1988) demonstrated the application of a smoothing procedure to their microincrement widths of *Cerastoderma edule* using a composite running median (Velleman and Hoaglin 1981). Growth trend can be removed by growth curve fitting, and the seasonal effect by differencing (Chatfield 1975; Priestley 1981). A Kendall's rank order correlation can be used to test if the data contains a trend (Legendre and Legendre 1983).

Figure 11 is the data of *Cerastoderma edule* presented in Lønne and Gray (1988). One can see the periodic fluctuation of microincrement widths along time in the smoothed and detrended data plot. In contrast, Figure 12 is a snapshot of the original microincrement widths for *Concholepas concholepas* (Adlerstein 1987, Fig. 13A). No smoothing and detrending procedures are required in this data set. There are 11 major peaks and several minor ones and the number of increments between the adjacent peaks is approximately 15-18.

2. Autocorrelation function

The procedures just described are used to remove trend and seasonal variation from a set of data. It remains to model the variation in the residuals of the modified time series data by means of a "stationary" stochastic process (refer to Chatfield 1975; Priestley 1981 for the terminology of stationary and stochastic processes). Simply speaking, a time series is said to be stationary if there is no systematic change in mean and variance.

The properties of a time series are found by the estimation of the autocovariance, R(k), and the autocorrelation coefficient, ρ(k), at lag k, for $0 \leq k \leq N$:

$$R(k) = \frac{1}{N-k} \sum_{t=1}^{N-k} (X_t - \bar{X})\,(X_{t+k} - \bar{X})$$

$$\rho(k) = R(k)/R(0)$$

The autocorrelation coefficient $\rho(k)$ measures the correlation between observations of the series at different distances. If the time series $\{X_t\}$ is purely random (white-noised), $\rho(k)$ will be approximately a normal distribution, $\mathcal{N}(0, 1/N)$, and 95% of its confidence interval is approximately $(\frac{-2}{\sqrt{N}}, \frac{2}{\sqrt{N}})$. The plot of $\rho(k)$ vs k is called a correlogram. One should refer to a textbook such as Chatfield (1975, Ch. 2) for the interpretation of a correlogram since this is the basis of the methodology used to investigate the following properties of time series: (1) randomness; (2) short-term correlations; (3) alternating data; (4) stationarity; (5) seasonality; and (6) outliers.

Autocorrelation investigations of *C. edule* and *C. concholepas* are shown in Figures 13 and 14, respectively. The autocorrelations of *C. edule* has the maximum at lags 15 and 28 and thus the microincrement widths show a distinguishable fluctuation of period 14, i.e., cycles of multiples of 14 are distinguishable. The maximum positive autocorrelations of *C. concholepas* occur at every 14 or 16 lags, which is very close to the observation in the snapshot. Alternatively, since we need to quantify whether the periodicity is statistically significant, we may perform analyses discussed below.

3. Spectral analysis

The first question is whether a harmonic relationship exists. To address this, one describes how variation in a time series may be accounted for by cyclic components at different frequencies. This procedure is called spectral analysis and is based on inference from a spectral density function.

A discrete Fourier transformation on data $\{X_t\}$ at a given frequency ω_k is required. Let $Z(\omega_k)$ be defined as the Fournier transform of $\{X_t\}$ at a given frequency ω_k.

$$Z(\omega_k) = \sum_t X_t e^{-i\omega_k t}$$

where $k=1,2,...[\frac{N}{2}]$; $\omega_k=2\pi f_k$ for $f_k=\frac{k-1}{N}$; and $[\frac{N}{2}] = \frac{N}{2}$ if N is even and $\frac{N-1}{2}$ if N is odd; and $i=\sqrt{-1}$. The real and imaginary parts of $Z(\omega_k)$ respectively are

$$A(\omega_k) = \sqrt{\frac{2}{N}} \sum_t X_t \cos(\omega_k t)$$

$$B(\omega_k) = \sqrt{\frac{2}{N}} \sum_t X_t \sin(\omega_k t).$$

The periodogram ordinate is defined for all ω_k in the interval $[-\pi, \pi]$ as

$$I(\omega_k) = \frac{2}{N} \left| \sum_t X_t e^{-i\omega_k t} \right|^2 = A^2(\omega_k) + B^2(\omega_k)$$

where $A^2 + B^2$ is called the amplitude at lag k. Priestley (1981, Lemma 6.1.1) shows that

$$I(\omega_k) = 2 \sum_{s=-(N-1)}^{N-1} R(s)\cos(\omega_k s) = 2\left\{ R(0) + 2 \sum_{s=1}^{N-1} R(s)\cos(\omega_k s) \right\} = 2\pi f(\omega_k)$$

where, $R(s) = \frac{1}{N} \sum_{t=1}^{N-|s|} X_t X_{t+|s|}$, is the (biased) sample autocovariance of X_t; and $f(\omega_k)$ is called (power) spectral density function.

Then plot the periodogram ordinates at the "standard frequencies" ω_k and test the value of the largest observed peak, $I_{max}(\omega_k)$. Fisher's g statistic can be used to test if the peak I_{max} is statistically significant,

$$g = I_{max}(\omega_k) \Big/ \sum_{k=1}^{[\frac{N}{2}]} I(\omega_k)$$

Although the periodogram is used in the equation, the periodogram $I(\omega_k)$ can be substituted by spectrum $f(\omega_k)$. The distribution of Fisher's g statistic (Priestley 1981, p. 408 ff) is

$$p(g > z) \sim \frac{1}{2} \exp\left(\frac{-Z}{2}\right).$$

This test can be extended to test for the second largest periodogram ordinate by

$$g' = \frac{I_{second}(\omega_k)}{\left(\sum_{k=1}^{[N/2]} I(\omega_k)\right) - I_{max}(\omega_k)}$$

and the next largest ones as $g^{(r)}$. The distribution for $g^{(r)}$ is

$$P[g^{(r)} > z] = \frac{N!}{(r-1)!} \sum_{j=1}^{a} \frac{(-1)^{j-r}(1-jz)^{N-1}}{j(N-j)!\,(j-r)!}$$

where a is the largest integer less than $1/z$, r is the r^{th} largest $I(\omega_k)$.

Figure 14 also shows the plot of spectral density against frequency for *C. concholepas*. There is a statistically significant peak at frequency of 0.0682 that corresponds to the periodicity that is located between 15 to 16 microincrements.

4. Bivariate processes

To investigate the influence of tides on the microincrement pattern, Lønne and Gray (1988) collected time a series of data on the immersion time of animals in sea water and on tidal height. From the results of the univariate analysis presented previously the maximum positive autocorrelation gives a cycle of 28-29 tidal periods (Fig. 15). Later, they calculated a cross-correlation function to examine the relationship between these two time series. In contrast to Lønne and Gray's (1988) approach, Adlerstein (1987) constructed a time series of hypothetical tidal height data and used this tidal data to do a cross-spectral analysis with the microincrement width data. The

cross-spectral analyses in both studies are to find the correlation between the two time series. This type of analysis is called "on an equal footing" by Jenkins and Watts (1968, p322). This section describes the method for this type of analysis.

Suppose that two time series, $\{X_t \mid t=1,2,...,N\}$ and $\{Y_t \mid t=1,2,...,N\}$, are observed simultaneously over the same time period, then the sample cross-covariance $R_{xy}(k)$ and cross-correlation functions $\rho_{xy}(k)$ at lag k are

$$R_{xy}(k) = \sum_{t=1}^{N-k} (X_t - \bar{X})(Y_{t+k} - \bar{Y}) / N \quad \text{for } 0 \le k \le N$$

and

$$= \sum_{t=1-k}^{N} (x_t - \bar{x})(y_{t+k} - \bar{y}) / N \quad \text{for } -N \le k \le 0,$$

and

$$\rho_{xy}(k) = \frac{R_{xy}(k)}{\sqrt{R_{xx}(o)\, R_{yy}(o)}}$$

Two properties of $\rho_{xy}(k)$ are of especial interest: (i) $|\rho_{xy}(k)| \le 1$ and (ii) $\rho_{xy}(k) = \rho_{xy}(-k)$. For the two uncorrelated series of white noise it can be shown that (Jenkins and Watts 1968)

$$E(\rho_{xy}(k)) \cong 0$$

$$Var(\rho_{xy}(k)) \cong 1/N$$

so that the values outside the interval $\pm 2/\sqrt{N}$ are different from zero at the 95% significant level.

The other useful statistic in cross-spectral analysis is the (squared) coherency which is given by

$$\gamma_{xy}(\omega) = [C^2_{xy}(\omega) + q^2_{xy}(\omega)] / f_x(\omega) f_y(\omega)$$

and

$$0 < \gamma_{xy}(\omega) < 1.$$

The functions $f_x(\omega)$ and $f_y(\omega)$ are called the power spectrum of the X and Y time series defined previously as the Fourier transform of the autocovariance function. The functions $C_{xy}(\omega)$ and $q_{xy}(\omega)$ are called co-spectrum and quadrature spectrum, which are the real and imaginary parts of the cross-spectrum $f_{xy}(\omega) = C_{xy}(\omega) - i\, q_{xy}(\omega)$ where $i = \sqrt{-1}$. The estimation procedures for $C_{xy}(\omega)$ and $q_{xy}(\omega)$ are given in Chatfield (1975). The coherency measures the linear correlation between the two time series at frequency ω and is analogous to the square of the usual correlation coefficient in linear regression analysis. The closer $\gamma_{xy}(\omega)$ is to one, the more closely related are the two time series at frequency ω.

Figure 16 shows the cross-correlation between immersion time and tidal height in Lønne and Gray (1988). There is a common fluctuation with a period of 28 days as shown by the distance between the two maximum positive cross-correlation coefficients. The displacement of the positive maximum from zero indicates a phase shift between the 2 oscillations of 14 periods. This is the same periodicity as the microincrement widths shown in Fig. 13. Therefore, Lønne and Gray (1988) concluded that the tidal patterns are the dominating influence in the formation of microincrements in *C. edule* shells.

The cross-spectral analysis on microincrement width and daily tidal height was also carried out for *C. Concholepas*. The cross-correlation function is sinusoidal with the maximum positive values at lags 3 and 18. The fact that the first maximum cross-correlation coeffient is not at lag 0 indicates a phase shift of 3 time steps between the two time series. The second maximum value at lag 18 indicates that both time series have periods about 15 which coincide with the previous analysis on microincrement widths. Also, the maximum coherency is 0.89 at lag 15. This confirms that the daily tidal pattern influences the microincrement deposition and that each microincrement represents daily growth.

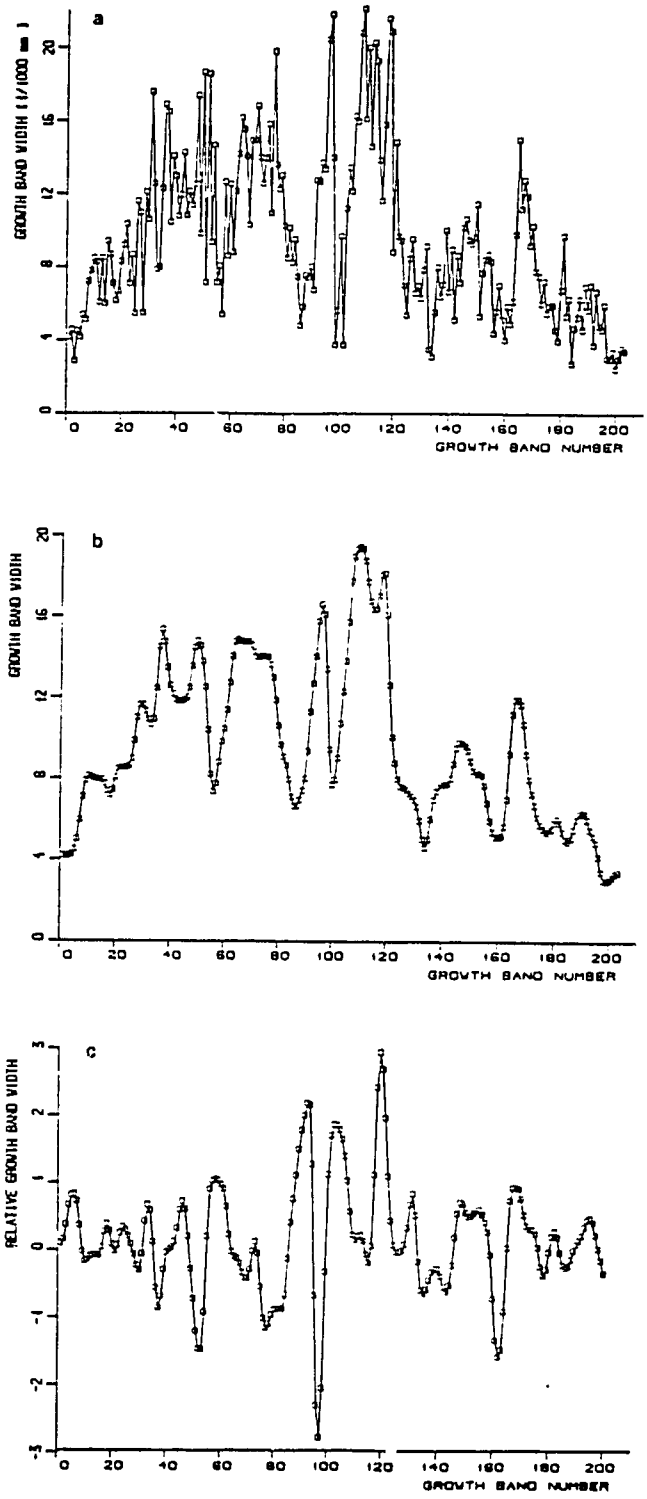


Fig. 11. (from Lonne and Gray 1988)

Cerastoderma edule. Microgrowth band widths of shells from level A. (a) Original data. (b) Smoothed data. (c) Smoothed and detrended data

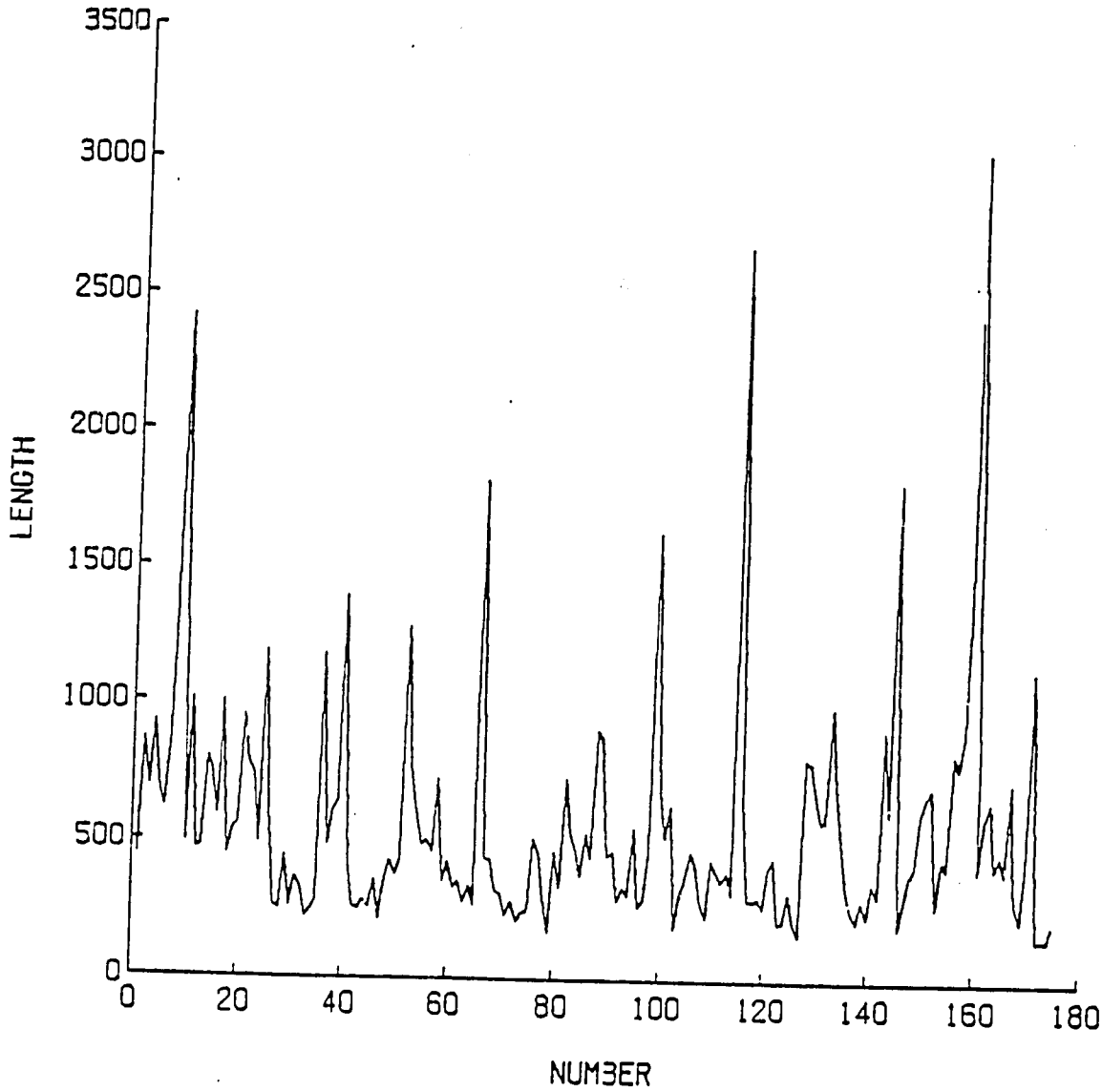


Fig. 12. (From Adlerstein 1987)
Microgrowth band widths of C. concholepas shell.

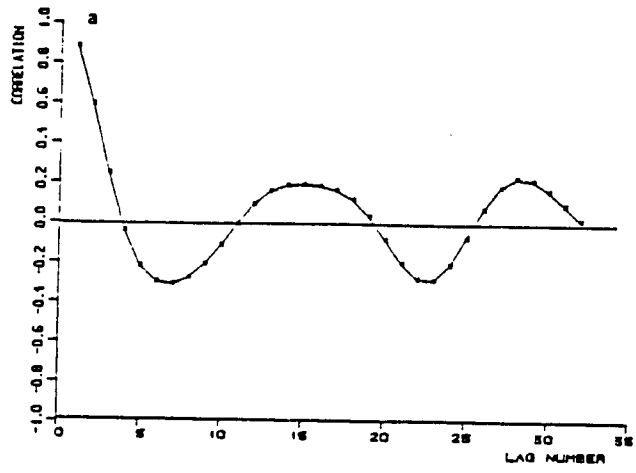


Fig. 13. (From Lonne and Gray 1988)

Cerastoderma edule. Autocorrelation of growth band width data. (a) From level A. Maximum positive correlation is at periods 15 and 28, giving a fluctuation of period 14.

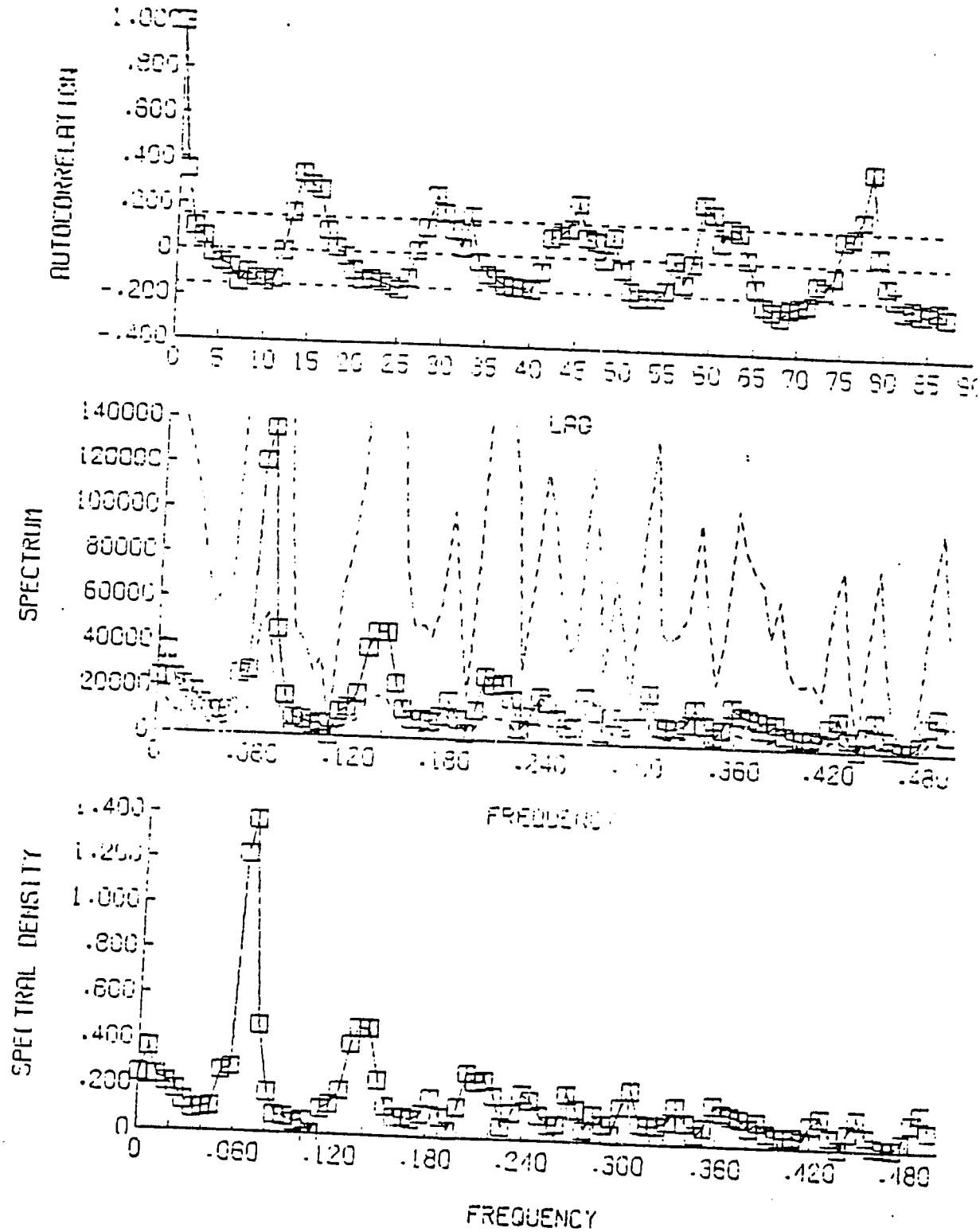


Fig. 14. (From Andlerstein 1987)

Autocorrelation, spectrum and spectral density of growth band width data of C. concolepas shell.

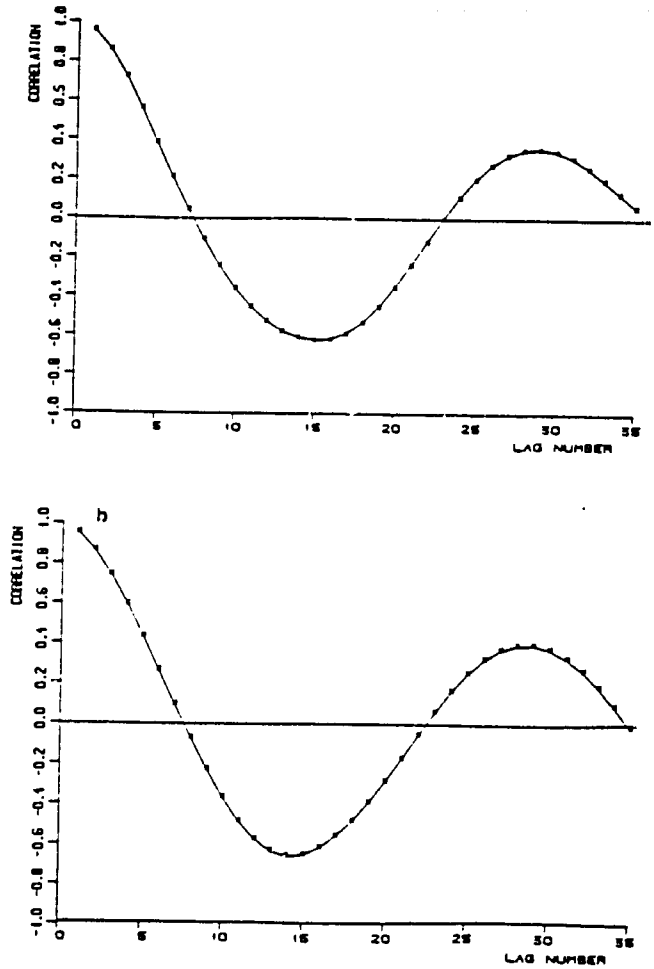


Fig. 15. (From Lonne and Gray 1988)

Autocorrelation of tidal data from level A. (a) Immersion time. (b) Tidal height. Maximum positive correlation occurs in both cases at period 28 to 29

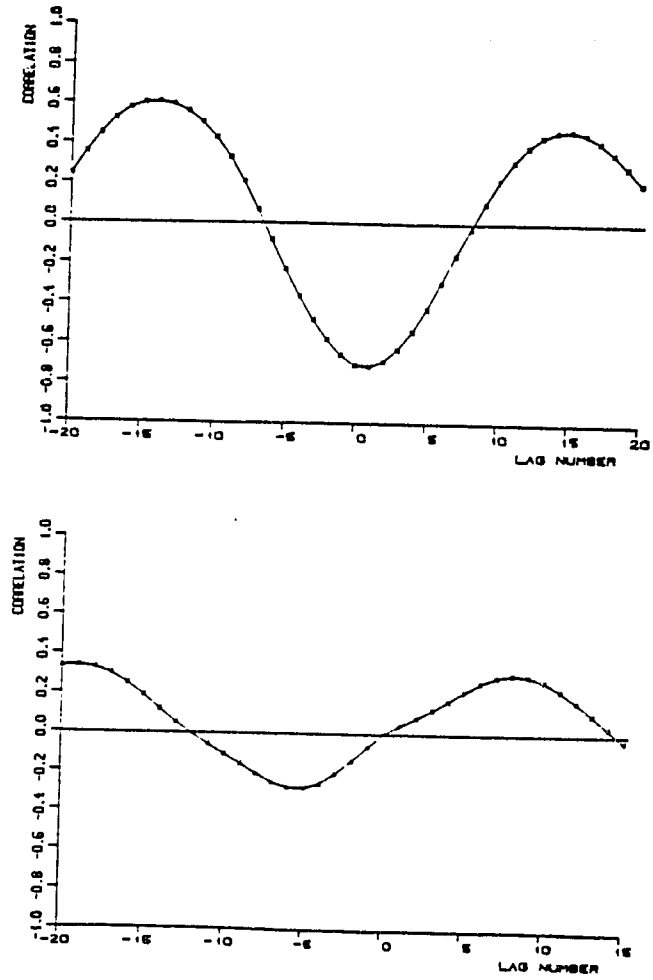


Fig. 16 (From Lonne and Gray 1988)

Cross-correlation between time immersed and maximum tidal height. (a) Tidal level A. There is a common fluctuation with period 28 as shown by the distance between the 2 positive maxima. The displacement of the positive maximum from zero indicates a phase shift between the 2 oscillations of 14 periods. (b) Tidal level B. There is a maximum positive correlation with period 23, and a displacement between the 2 oscillations of 4 to 5 periods

Age Determination for Crustaceans

Age determination of crustaceans is difficult due to their discrete growth pattern (Mackay and Weymouth 1934) and lack of permanent hard parts in the body as age indicators. Most crustaceans (especially decapods) spend their life in a molt cycle which means that they are of constant size for a period of time and then abruptly increase their size during another period.

Drach (1939, 1944) and Drach and Tchernizrotzeff (1967) examined the morphological and cuticular change associated with molting. They divided the molt cycle into four basic periods, five major stages and several substages. Characteristics of a standard molt stage for crabs and lobsters are given by Warner (1977), Aiken (1980) and Aiken and Waddy (1987). The number of molts does not directly correspond to age since there can be many molts per year, or less than one per year depending on species, age, sex and habitat (e.g., see Orensanz and Gallucci 1988).

Many attempts have been made to determine physiological and chronological age, but none of these provide sufficient resolution of age for crustaceans. In general, length frequency analysis, counting the number of segments of aesthetases on antennae and antennules, and counting the number of lamellae in the endocuticle are the commonly used methods.

General Approach

A general approach of the crustacean age determination is based on the relationship between molt and growth. Although Kurata (1961, 1962) was not the first to use this method, his contribution is that he reviewed various aspects of the relationship between molt and growth to age and size and developed a general rule to describe molt and growth sequences throughout the life span.

Methodology of age determination of crustaceans includes two variables: increment per molt (growth rate per molt) and duration of intermolt period (i.e., molt frequency in a given period, commonly a year). Figure 17 which is adapted from Orensanz and Gallucci (1988) shows the growth of *Cancer magistar* in three nursery areas. Body size increases after each molt and shows an instar growth pattern throughout the life history in the nursery. The duration between molts is shorter in the juvenile stage than in the adult stage. Figure 17 is an example of how environmental effects influence the age of sexual maturity, growth as measured by the duration of each molt and the number of molts.

1. Growth per molt

Dyar's rule (1890) that an animal grows by a fixed ratio at each molt (from Gaines and Campbell 1935) has been re-examined and is now known as Brook's law and says that each stage increases in size at each molt by a fixed percentage of its length during the early growth, which is approximately constant for the species and sex (Hanaoka 1944, 1952). Counter examples to both of these rules have been found in many growth studies of crabs and others (Kurata 1962).

There are many other models describing growth per molt, such as Hanaoka (1944, 1952), Ito (1953), Kurata (1962), Somerton (1980), Mauchline (1976), and Easton and Misra (1988). The most widely used model is developed by Hiatt (1948), and hence is called Hiatt's diagram. The diagram plots growth increment data in form of pre-molt size vs. post molt size. Although Hiatt did not provide a mathematical expression, Kurata (1960, 1962) and Butler (1961) expressed the Hiatt diagram as two intersecting straight lines as

$$Y = a + b\,X \quad \text{for } X \leq X^*$$

$$Y = c + d\,X \quad \text{for } X > X^*$$

where X and Y are pre-molt and post-molt sizes, X* is the specified value of pre-molt size chosen near the size where there is a decrease in slope of the first line in the Hiatt diagram. This is usually associated with the molt where sexual maturity occurred.

Kurata (1961) examined many crustaceans and found that the Hiatt plot is linear except for the breakpoint. The animals which are smaller than X* are thus called juveniles, and otherwise, adults. Kurata (1962) used a Hiatt diagram to investigate the factors that determine the length of the intermolt period and showed that temperature and size were most important. He found several relationships. For example, the length of the intermolt period increased directly as the cube of the body length, or logarithmically as the body length, or in relation to successive molt period. The logarithm of the intermolt period also decreased with increasing log of temperature. Kurata used the Hiatt diagram to derive growth constants through linear regression analysis. From the regression line, $Y = c + dX$ where X and Y are pre- and post-molt sizes respectively, the maximum size (L_∞) is calculated from the equation: $L_\infty = c / (1-d)$. This computation is similar to the Walford plot frequently used in the estimation of von Bertalanffy growth curve parameters, except that the time step is not 1 year. In fact, $K = -\ln d$, is similar to a Brody coefficient in the von Bertalanffy growth model, with the dimension of "per instar". These growth constants, in conjunction with factors describing the intermolt period at successive molts, are used to describe the sequence of growth and molting throughout the life history of a crustacean. Butler (1961) and Wilder (1963) have applied similar approaches for *Cancer magister* and American lobster *Homarus americanus*, respectively.

Mauchline (1976, 1977) criticized the fit of a straight line on a Hiatt diagram. In fact, fitting a linear regression equation to data in a Hiatt growth diagram assumes that the growth factors (i.e., the percentage increase in body length) at successive molts described by the line are constant. This assumption had been accepted by Kurata (1962) and many others for decapod and other species, but Mauchline (1976)

demonstrated exceptions in calanoid copepods and ostracods. Mauchline argued that the growth factor decreases logarithmically against body size or molt number. This log decrease in growth factors at successive molts would produce points that conform to a hyperbola instead of a straight line on a Hiatt diagram.

The general equation of this hypobola is:

$$(X - X_O)(Y - Y_O) = k \text{ or}$$

$$Y = \frac{k}{X - X_O} + Y_O$$

where X and Y are the pre- and post-molt sizes respectively and X_O, Y_O, and k are parameters to be estimated. Using non-linear least squares to estimate the parameters, the maximum length is calculated as:

$$L_\infty = \frac{X_O + Y_O}{2} \pm \sqrt{\frac{X_O + Y_O}{2} - X_O Y_O + k}$$

In additional to Mauchline's nonlinear model, Wilder (1953, 1963) used the allometric growth curve, $Y = aX^b$, to described the growth of American lobster (*Homarus americanus*). This equation can be linearized by a log-transformation making linear regression possible.

Somerton (1980) re-evaluated the linear and non-linear models on a Hiatt diagram and proposed an effective method to test the position of the breakpoint (X*) and thus the point of presumed sexual maturity. The linear model may be improved by two modifications: (i) Constraining one of the lines to intersect the other at X* eliminates the difficulty in using the equations to predict post-molt size. (ii) Allowing X* to be a free parameter, which is estimated along with the other parameters of the model, eliminates

the subjectivity involved with choosing a value of X*. With these modifications the model is expressed as

$$Y = a + bX \qquad \text{for } X \le X^*$$

$$Y = Y^* + D(X - X^*) \qquad \text{for } X > X^*$$

where $Y^* = a + bX^*$. To estimate the parameters, initial values of X* are chosen and estimates of slopes and intercepts of the two lines are obtained using linear regression. Then X* is revised a small amount and the parameters are re-estimated until a value is found which minimizes the residual sum of squares pooled over both lines.

The measure of goodness of fit, chosen to compare the hyperbolic and linear models, is the coefficient of variation of the residual:

$$CV = \frac{1}{\bar{Y}} \sqrt{RSS/N - K}$$

where RSS is the residual sum of squares, N is the number of data points, K is the number of parameters in the model, and Y is the mean of post-molt size. A smaller CV indicates a better fit. The choice of whether a linear model or a hyperbolic model is a better fit can be tested by Wilcoxon signed rank statistic

$$H_o : E(CV_{\text{straight line}}) \ge E(CV_{\text{hyperbola}})$$

vs. $$H_a : E(CV_{\text{straight line}}) < E(CV_{\text{hyperbola}})$$

where E indicates expected value. Of course, Somerton's modified model can also compared to Kurata's two straight-line models and the one straight-line model. The inference statistic is a generalized F-statistic (Weisberg 1983, Drapper and Smith 1981, Somerton 1978).

Easton and Misra (1988) criticized the use of linear growth model for crustacea because of a lack of a biological basis since the models (Hiatt 1948 and Somerton 1980) are (i) highly empirical, (ii) the b-value represents the average rate of change of the growth increment over the range of sizes of the sampled animals, (iii) the a-value shares the same characteristic of the b-value, and (iv) if a- and b-values are estimated by each instar, they are highly correlated (Hersh 1931) and also dependent on the unit of measure. Hersh (1931) contendes that the b-value and X (premolt length) are correlated (in fact, Hersh argues that X is a periodic function of b with decreasing amplitude instead of the linear form in Misra). Misra (1957) described the relationship between b and X as

$$b = a_0 + a_1 X$$

and then derived a new growth equation

$$Y = b_0 X^{a_0 + a_1 X} e^{b_1 X}$$

where Y is the post-molt length, X is the pre-molt length, a_0 is a constant equivalent to the b in allometric growth model, and a_0 and b_1 are constants. Allen's PRESS statistic for testing both goodness-of-fit and predictive ability within the range of the data (in Appendix 1 of Easton and Misra 1988) was used to evaluate the fit of the Misra model.

Instead of relating post-molt size to pre-molt size, other useful linear growth equations (Mauchlin 1978, Aiken 1980) are:

(1) log intermolt period on a measurement of body length or on successive molt number

(2) log growth factor (%) on a measurement of body length or on successive molt number; log of the cubic function of measurement of body length (log L^3) on log age (days).

(3) log intermolt period (days) on the cube root of Body weight (g).

(4) log increment in weight (g) on log body weight (g).

(5) log body weight (g) on log age (days).

Another approach for determination of growth per molt is mode tracing in the length frequency distributions (Poole 1958, Farmer 1973, Ito 1970). Tagging experiments also produce information on growth per molt (Squire 1970, Cooper and Uzman 1971), but Kurata (1962) criticized tagging:

> "the evidence of the intervals between and within molts procured by this method is negative in nature,.... tags are lost with ecdysis, the growth cannot be directly determined, ...such a kind of tagging method inevitably cause some damage in the integument of the animals."

Nevertheless, Ennis (1971), Wilder (1963), Cooper (1970) and Scarratt (1970) demonstrated that the natural growth of lobsters was not affected by a sphyrion tag and that tag retention was high.

2. Molt frequency

A study of molt frequency is done in terms of the determination of the length of the intermolt period. Mauchline (1977) summarized the methods for this study and assessed them to be of variable quality. Some of this study consists of observations on a few molting individuals (Poole 1958) in a restricted size range. Kurata (1962) reviewed molt frequency for various crustaceans and found that temperature is the main influential factor. He found that the linear relationship between the logarithm of length of the intermolt period against the logarithm of temperature and the number of days between molt is a cubic function of the carapace width of crab. Applying these two relationships, he successfully developed a model to estimate the age of crabs. Orensanz and Gallucci (1988) developed this approach in further detail.

3. Age Determination

Kurata estimated theoretical values for the number of days between successive molts and transformed this to cumulative values at each instar. Then, based on the average monthly temperature in a year, he calculated a so-called "effective cumulative temperature" for each month and year after hatching. Applying the effective cumulative temperature and cumulative days between molts, the age of the crab could be determined.

Donaldson, Hilsinger and Cooney (1980) used the data on growth per molt and fit a Hiatt diagram to tanner crab. They calculated hypothetical size after molt from the data and matched them with size frequency data to show the frequency of molt and growth per molt, from which the age of the tanner crab could be estimated. Farmer (1973) applied this approach by examining the size frequency distribution and percentage of molts in Norway lobster from monthly collections. He plotted mean carapace size against age to estimate a growth equation of the lobster. Butler (1961) used tagged Pacific crab to estimate growth per molt and applied a Hiatt diagram to determine instar size. Matching this with a size frequency distribution, he estimated the age of Pacific *Cancer* crabs.

In summary, the difficulty in ageing a wildstock of crustacea lies in estimating a size-at-instar schedule. This is done either by rearing the animal in a laboratory to simulate the natural environment or by the size-frequency distribution (SFD) analysis proposed by Orensanz and Gallucci (1988). The method of SFD analysis is described in more detail in the section of Length Frequency Analysis. Orensanz and Gallucci (1988) also describe the difficulties encountered in back-calculation to determine the size-at-earliest-instar schedule from the Hiatt diagram for the field samples and the difficulties encountered when more than one brood occurs in a single-year class (bimodal recruitment). Orensanz and Gallucci reported that the second brood of *Cancer magister* settled in Garrison Bay in late summer (August) while the first brood settled

in May. However, the second brood was smaller in size and reached Juvenile instar J7 or J8 by the spring of year 2. In contrast the first brood had reached Juvenile instar J9 to Adult instar A1 by the same period and were generally sexually mature. If the magnitude of the second brood is large enough, the estimation procedure would lead to a serious bias.

Number of Lamellae in Cuticles

Yano and Kobayashi (1969) aged *Gaetice depressus* with this method. They collected the crabs at an intermolt stage C4 (defined as Drach 1939) and fixed the specimens in 10% neutral formalin. A shell piece of about 1.3 x 2.3 mm was removed from heart zone of the carapace. The piece was dehydrated through a series of 70-90% ethanol, embedded in methacrylate and ground to about 50 μ in thickness and viewed under compound microscope. Yano and Kobayashi (1969) described their results as:

(1) The number of lamellae in the newly formed and completed endocuticle was found to increase with the increment of the carapace length, irrespective of sex and number of molts.

(2) A stepwise increase of 7-8 lamellae corresponding to the growth of the carapace could be found.

(3) Total thickness of endocuticle increases with the growth of the carapace regardless of sex difference.

Farmer (1973) followed this method to age Norway lobster with a small modification in the preparation of specimens. The tissue was embedded in ester wax and sectioned to 6 mm and stained with Mallory's triple stain to differentiate between the endocuticle and the underlying tissues. After plotting the number of lamellae against carapace length and the thickness of endocuticle against carapace length, Farmer

found no useful relationship and, as a result, concluded that this method can not be used in instar determination.

Number of Aesthetase

Howes (1939) tried to use the relationship of aesthetase number against body size of *Idetea viridis* for instar determination. Naylor (1955) examined total number, mean length and size range of *Idotea emarginata.* He found nothing that could be used for instar determination.

Farmer(1973) also tried this method again in Norway lobster. He examined the relationship between the number of aesthetase bearing segments of the expodite of antennule against carapace length. He concluded:

> "The number of instars through which a specimen has passed cannot be determined from the number of aesthetase bearing segments: the range of variation is too wide. It was not possible to follow the change in the number of segments at ecdysis. The work may be possible for small stages but insufficient for older animals."

Some Concluding Remarks

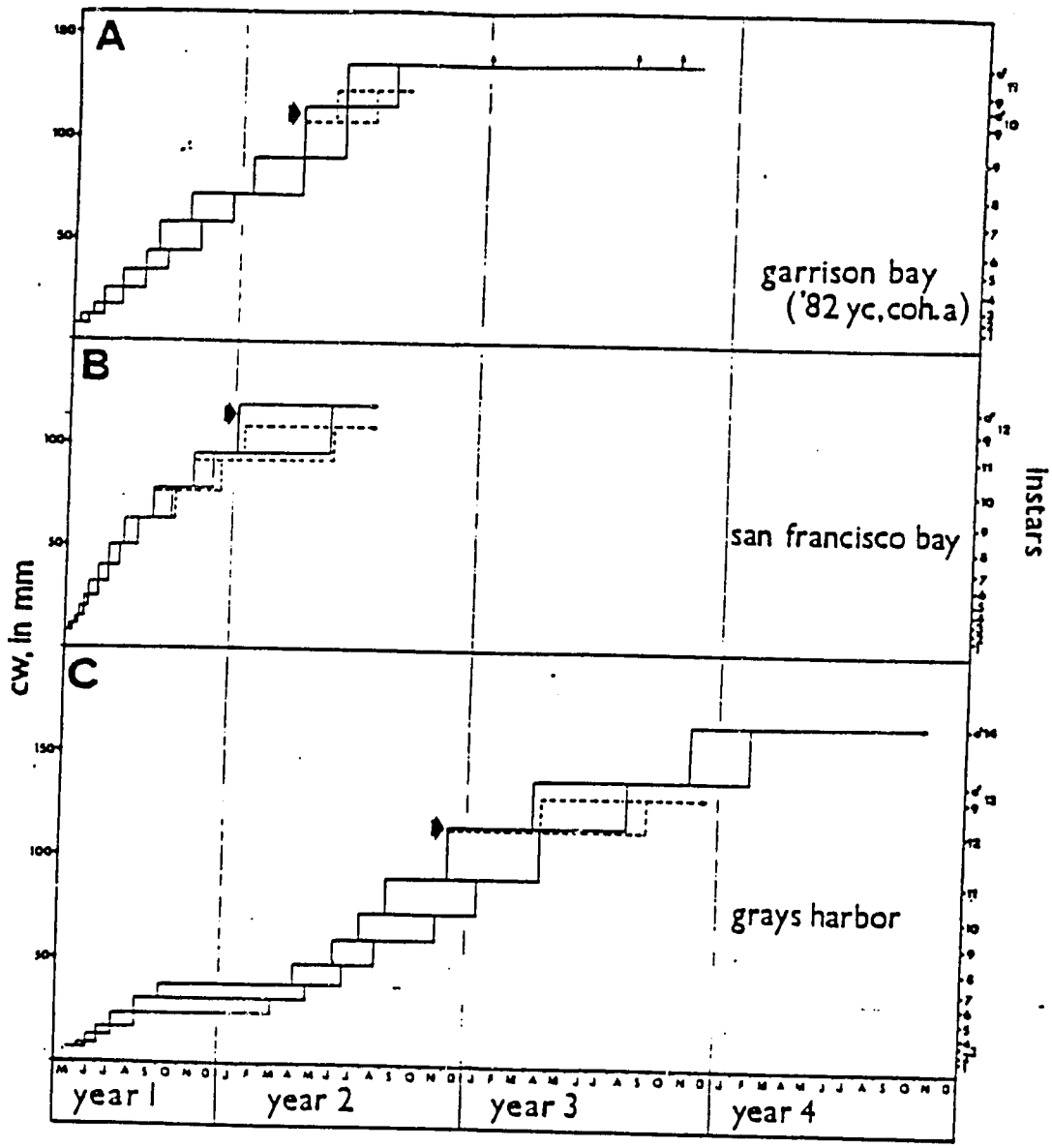
A great deal of research has gone into the development of ageing methodologies for crustaceans, but the results are not yet conclusive. One major obstacle has been the inability to define suitable structures that will retain age information. Although some direct ageing methods, i.e., the number of lamellae in the cuticle and the number of aesthatase, have been suggested, the results obtained from these methods are not entirely unsuccessful.

The Hiatt diagram is quite often used to study the growth of crustaceans. Kurata (1962) and others used this diagram with the estimated molt frequency to estimate

"age". However. there are pronounced variations in the growth rate of individuals within a species. The growth rate of an individual animal results from the interaction of molt frequency and the size increment at molt. Many studies (ref. Aiken and Waddy 1988) have reported that these two factors can be differentially affected by the physiological state of the animal and the environmental conditions that the animal encounters. Thus, it is possible for the individuals of any cohort to grow at widely differing rates. Therefore, superimposing a growth model and the molt frequency pattern may cause some serious problems.

Length-frequency distributions have frequently been used to determine the parameters of instar stages, but this method is mostly based for mode tracing without statistical or mathematical analysis. Orensanz and Gallucci (1988) applied the statistical technique of Macdonald and Pitcher (1979) and Schnute and Fournier (1980) constraining a mean-length at instar by the Hiatt diagram to estimate growth parameters and instar proportions. Although this method is a quantitative advance, the problem of estimating "age" remains.

Biochemical methods, e.g., lipid contents, RNA/DNA ratio, and lipofuscin and age pigment assay, have been applied to some species of Arthropoda (See Biochemical, Genetic and Radiographic Approaches). Although these methods probably provide the most direct estimate of "age", the problem resides in the calibration between wild stock and laboratory-raised, known-age animals.



Cancer magister, schematic representation of growth in three "nursery areas." A. Garrison Bay (this study); B. San Francisco Bay (from Collier, 1983, and Tasto, 1983); C. Grays Harbor (from Cleaver, 1949). Solid lines = juveniles and males; dashed lines = females; arrows = instar A1.

Fig. 17. (from (Orensanz and Gallucci 1988)

Age Determination for Terrestrial Mammals

Studies of age determination of terristrial mammals using teeth and bones have been well-documented in Klevezal' and Kleinenberg (1967). The following review is mainly based on their book.

Methods for physiological/morphological age

1. Degree of wear of teeth

This method is used for determining the age of carnivores and rodents in which the teeth are worn out as the animal ages. The age of Ungulata is determined by the time of replacement of milk teeth with permanent ones and by the degree of wear of permanent teeth. Fuller (1959) applied this technique on bison and was able to separate three age classes, "young adult", "adult" and "aged" bison. Flyger (1958) developed a technique of tooth impression as an aid in the examination of tooth wear in deer.

2. Rate of development of roots and the ratio of root to crown of tooth

Doutt (1942) used the relative breadth of the tooth canal to age Pinnipedia. Smirnov (1962) and Grakov (1962) used the relative breadth of fangs to determine the age of land carnivores (cited from Klevezal'). Montgomery (1964) applied the condition of tooth eruption to age raccoons within 110 days old.

3. Characteristics of skull and limb bones

Klevezal' and Kleinenberg (1967) stated that age be determined based on the external structure and dimensions of skull, weight of skull, rate of fusio of cranial sutures, character of the surface of limb bones and the rate of ossification of epiphyses. However, literatures citations are not found in their book.

4. Weight of lens of eyes

The eye lens grows continuously throughout the life. New lens fibers are proliferated by the growth and elongation of the epithelial cells. Because of the avascular nature of the lens and its density, those fibers at the center of the lens lose their cell membrane and become dense and rigid. Their cytoplasm loses water. It is apparent that, providing there is little individual variation in the growth rate of the lens within a species, the growth rate could provide a tool for age determination. Lard (1959, 1961) applied this method to cottontail rabbits and fox. Bayer et al. (1964) used this method on fur seal.

5. Ephyseal cartilage

Carson (1961) applied this technique in fox and gray squirrels by X-ray examination of the ephyses of the lower foreleg. The distal epiphyseal cartilage of the radius and ulna has three rather distinct thicknesses during the longitudinal growth of these bones. During the early stage of bone growth the cartilage is at its maximum thickness, 2/3 mm or more, and is represented by a light line of this width between the ephyses and diaphyses. During the intermediate stage of growth the cartilage is reduced to a thin disc having a width of about 1/4 mm. During the final stage of growth the ephyseal cartilage is gradually reduced from the intermediate level and is replaced by bone. Adults can be separated from young-of- the-year with this method.

6. Horn

In mature bison, Fuller (1959) described that the tips become blunted and shredded. This is caused by the new horns piercing through the old sheath which occurs in American bison at about 7 or 8 years old. The criteria is different in males and females.

7. Pelt size

Age of the harvest raccoon has been determined by the pelt surface area. Slate and Douglas (1981) use discriminant function analysis to classify raccoons as juvenile or adult from pelt size measurements. However, they cautiously recommend a refinement since they are inable to classify juveniles across the years. Moses and Boutin (1987) use logistic regression to classify raccoon (*Procyon lotor*) pelts to a juvenile or adult class.

Methodology for Chronological Ages

1. Method of using teeth

Although the shape of teeth is different for various mammals; in general, teeth are formed of a crown and a root. The crown sticks out of the jaw and is covered with enamel. The root is concealed in the tooth sack of the jaw and is coated with cement. In the ventral part of the tooth is a pulp cavity which consists of tooth pulp that contains odontoblasts, skeleton-formation cells, and gives rise to new dentine. It is believed in general that a new dentine layer is added onto teeth by a series of seasonal processes. This is the process by which indicators of growth history are formed.

Cement increases in thickness with time, and results from the formation of new layer by periodontium surrounding the root of tooth. Therefore, cement layer increases in a manner different from dentine. The earlier-formed layers are formed closer to the dentine and characterize the history of growth.

Mansfield (1958) and Ohsumi et al. (1963) indicated that the formation of a transparent band of the annual layer of dentine is different from the opaque band in the amount of mineral salts. The transparent band is always narrower that the opaque band and corresponds to decreasing growth rate of tooth and a rate of organic stroma

formation. The slow-growing zone is more calcified than the rapid-growing zone (Klevezal et al. 1967). However, there are different opinions about when the transparent band is formed in dentine and the cement of tooth. Most of the works indicate that transparent zone is winter or winter-spring growth. Christian (1956) assumed that the narrow band of E. fuscus is formed in the period of hibernation. Van Nostrand and Stephenson (1964) also support this assumption for Canadian beaver.

Mundy and Fuller (1964) are of a different opinion. They found that grizzly bears killed in spring show transparent bands and those killed in fall show opaque bands. Law and Cowan (1963) and McEwan (1963) related the seasonal changes in deposition of cement in teeth of Cervidae with the seasonal changes of feeding and isolation.

Preparation of teeth for age determination is dependent on species. Longitudinal half sections or thin sections are the most commonly used but they always give the least distinct figure. For those species which cease longitudinal growth earlier, cross sections through the middle of the tooth show all the growth layers (Klevezal' and Kleinenberg 1967).

A thin section can be cut and polished with fine grade paste or by simply grinding off superfluous parts until light can be transmitted. Acid etching to decalcify and produce a relief of ridges and grooves corresponding to layered structure has been suggested by many workers. The acids used include 10% formic acid, 5-7% nitric acid, 70% alcohol with 5% hydrochloric acid, etc. Hematoxylin or Delafild's hematoxylin can be used to stain the sections (Root and Payne 1984).

For viewing growth layers in cement, it is better to develop one lateral side of the root as compared with the opposite side because cement is not evenly deposited on the surface of the root (Mitchell 1963). Incisors of Asiatic wild ass and axis deers, and molars of Cervidae have been studied with this procedure. Acid-etching, decalcification and staining of cement parts are the same as dentine.

Sergeant and Dimlott (1959) used both thin and bi-sections of incisors to age moose. The polished surface of bi-sections were moistened with alcohol and examined with binocular microscope with reflected light.

2. Method of using bones

Bone is anatomically divided into compacta and spongiosa. The differentiated compacta consists messteal zone and inner and outer layers of generative plates. It is the outer generative plate which forms periosteal bone tissue (Maytyas 1955) in which the bone layer formed earlier is situated interiorly and those formed later are situated closer to the bone periphery.

Corresponding to seasonal changes of growth, the periosteal zone consists of layers of bony plates, separated by resting lines (Weinmann and Sicher 1947, Pritchard 1956), called adhesion lines (Klevezal' et al. 1967), which indicate the separation of annual layers of bone. The adhesion lines are formed simultaneously with the transparent band of dentine and cement (Klevezal' 1966).

Bony tissues can be freshly fixed in formaldehyde or alcohol, or dried without cleaning flesh, or boiled and cleaned of flesh and dried. However, bleaching with hydrogen peroxide is not suitable.

The adhesion lines can be seen in thin sections of the bone. Stained preparations of sections are found helpful. Thin sections can be made as follows: a plate of bone is cut and polished by being pressed with a cork to grindstones of increasing fineness of grain. Klevezal' et al. (1967) gives a detailed description.

Remarks

The preceding texts describe how annual growth layers are formed in dentine and cement of various teeth and in the periosteal zone of the bone. However, they are not all of equal value in age determination.

One of the greatest disadvantages for periosteal zones is that bone is subject to resorption and thus not suitable for age determination of older mammals. But in some species such as Rodentia, the resorption in the periosteal zone is so slow that there is no significant disadvantage.

Cement is sometimes better than dentine as the material for age determination because it is deposited outside the tooth and without growth limitation. But in most of the cases, the growth layers are narrower than that of the dentine and less well-pronounced. Some species form growth layers in one of their tissues, such as *Lagomorpha* and voles, and their ages can be determined only in the periosteal zone of the bone.

For those mammals in which growth layers are formed in all three tissues, such as Insectivora, Carnivora, and some Rodentia, simultaneous use of the teeth and the bone is recommended because the growth layers in the periosteal zones may not be evenly developed (Klevezal' and Kleinenberg 1967). These authors also used various sections of mandibles from different mammals to demonstrate the presence of different growth patterns and the relationship to how mechanical load is distributed.

Age Determination for Marine Mammals

Age determination for marine mammals, Catecea, Pinnipedia, and Sirenia, has been emphasized by zoologists of this century because of the heavy pressure that whaling industries have put on these species. In the past, physiological measures such as body length, translucency of the eye-lens and the number of corpra albicans, and chronological criteria such as teeth, baleen plate bones, and ear plugs have all been used in age determination.

Methods Related to Physiological Ages

1. Corpora lutea or corpora albicans

After ovulation, an ovulated follicle develops to form the corpus luteum, a hormone secreting gland. Instead of the disappearance of the corpus luteum in many mammals, corpus albicantia is formed and remains throughout the rest of the life span of whales. Because the ovulation rate and age of puberty are considered constant, the number of corpora albicans provides a possibility for age determination for whales.

This method is based on the assumptions that the ovulation rate and the age of puberty can be estimated. Ovulation rate is estimated from checking recently ruptured follicles and from developing corpora lutea. Rice and Wahlman (1971) reported that a female gray whale usually ovulates in late November and early December. Usually there is about one ovulation every two years during the biennial breeding season, and about 40 days intervene between two successive ovulations.

Age at puberty refers to the age at which gam. 's are first produced, and age at sexual maturity is the age at which the animal reaches ..s full reproductive ability. In general, the two ages are assumed to be the same. Ruud, Jonsgard and Ottestad (1950) measured the percentage of sexual maturity at length and correlated this information

with the age determined from baleen plates. Most of the female blue whales reach sexual maturity at 5 years, and males between 4 and 5 years. Rice and Wahlman (1971) estimated age at puberty of gray whales by plotting the percentage of animals that had reached puberty against the number of growth layers in the ear plug. They reported the age at puberty of gray whale ranges from 5 to at least 11 years with an average of 8 years.

2. Color changes in crystalline lens

Nishiwaki (1950) first inspected this method for the Antarctic blue whale and the fin whale. During the 1947-48 whaling seasons, Nishiwaki examined variations in the color of the lens and found that the color changes from colorless or clear to light yellow and deep yellow. The color becomes darker with increase of body length, the number of corpora lutea and weight of the testis.

Nishiwaki developed instrumentation based on the idea that the darker the lens color, the more the light being absorbed by the lens. Therefore, when a predeterminrd amount of light is transmitted through a crystalline lens, some is absorbed and the outcoming intensity is recorded by a photocell, thus providing a measure of absorption.

Nishiwaki calculated the percentage of the amount of light absorbed by a crystalline lens (Nishiwaki called this percentage a "lenticular coloration" or "degree of coloration") and plotted it against the number of corpora lutea, the weight of the testis and the body length. The evidence supports that the percentage of light absorbed is related to the growth of the whale.

Because corpora albicans had been widely used and is thought to be reliable, Nishiwaki concluded that the increase in the number of corpora albicans is supposed to change in accordance with the passage of time, so that the change in percentage of light absorption should also change regularly with the passage of time, i.e., with age.

This method is subject to two sources of error: The translucency of the lens decreases with the time after the death of the animal so correction is needed and the method is not applicable to immature whales because of the absence of corpora lutea. This method has the same disadvantages as the method of counting corpora albicans. Based on the relationship between the percentage of light absorption and the weight of testis, Nishiwaki found that the weight of testis increased up to a certain age, after which the weight of testis do not change - irrespective of age.

3. Morphology of cranial traits

The dimension and weight of the skull and the rate of fusion of the cranial suture have been used as age indicators in mammals (Klevezal and Kleinenberg 1967). Stuart and Morejohn (1980) related the growth layer group (GLG) counts to the measurement of external, cranial and postcranial traits. They found that most of the measurements show a good relationships to the number of GLG in dentine, however, they are sexually dimorphic.

Methods Related to Chronological Age

1. Ear plug

Lillie (1910) is the first zoologist who found the ear plug in a whale, but application of the ear plug to age determination remained for Purves (1955) to examine. The growth layer in the ear plug consists of one dark and one light lamina. There is a controversy over the relationship between the number of growth layers and absolute age. Ichihara (1966) provided evidence that in immature fin whales, the rate of accumulation of ear plug laminae is irregular with variation from one to two annually. Roe (1967a, b) based on histological studies of monthly collections of ear plugs in fin whales concluded that one growth layer is produced each year in spite of

sex and maturity. Roe also found that light laminae are formed in summer and dark laminae formed in winter.

The collection of ear plugs is frequently difficult. Rice and Wolman (1971) described the ear plug of the gray whale as being soft especially in small animals, and difficult to remove without distortion or breakage. Some of the plugs have a fibrous, columnar, or amorphous structure in which no laminae is found and the percentage of readable ear plugs is low. Gray whale males more frequently show regular laminae than females, in which growth is interrupted by the reproductive cycle (Rice and Wolman, 1971).

2. Baleen plate

William Scoresby, Jr., in 1820 found that the ring on the surface of the baleen plate may indicate the age of whales. However, the possibility of this as an age indicator was rejected by Eschricht and Reinhardt (1866) and Tullberg (1883). Further efforts by Machinstosh and Wheeler (1929) and Wheeler (1930) were unsuccessful.

Ruud (1940, 1945) re-examined the histological structure and ring pattern of baleen plates of blue, sei, and fin whales and concluded that the growth of baleen plates is characterized by seasonal changes in thickness. Ruud developed a culture record machine to record the messages of thicknesses. Based on a set of baleen plates from one whale, the records are of the same nature except at both ends of the baleen plate. Ruud concluded that a definite number of small sculptures were formed each called a "growth zone" and each step of growth represented a year of growth. Later Nishiwaki (1950) carried out the same study on the Antarctic blue whale and fin whale and was led to the same conclusion.

However, the controversy over baleen plates continues. The baleen of a whale has two contrary growth processes. Growth is from one end and wear and tear occur at the other end. Rice and Wolman (1971) reported that there is rarely more than 5 or 6

years of growth represented in a baleen plate of a gray whale and most baleen plates show a lesser number of growth zones than the number of growth layers in the ear plug. This method has also been applied to the Order Mysticeti.

3. Teeth

Although zoologists of the 19th century had noticed the dentinal layers in whales, the importance of counting layers was highlighted by Scheffer (1950) and Laws (1952). The use of this method of age determination has increased rapidly, and by the late 1960's had been applied to most species of toothed marine mammals. A systematic account of the use of dentinal layers up to 1970 has been reviewed by Scheffer and Myrick (1980).

Widely used techniques for the preparation of teeth include:

(1) Bisection of teeth. Related techniques are acid-etching and highlight. Pierce and Kajimura (1980) applied this method in Cetacean teeth. Bisection can be done either by grinder or diamond saw. After polishing the half-sectioned teeth are put into 5% formic acid (Bow and Purday 1966). However, the concentration of acid and soaking time depend upon the volume of tooth and species. The etched surface is highlighted with jewellers rough. Cupric oxide is also found to produce good highlighting properties.

(2) Longitudinal or thin cross sections are polished and acid-etched.

(3) Decalcified and stained thin sections.

(4) Polarized light microscope for examination of thin sections (Myrick 1980). Polarized light and phase contrast associated with a compound microscope have been used to detect differential optical and crystallograhic features of translucent objects. The technique was first developed by Moorehouse (1959) and widely used in dental histological studies (Peyer 1968). Myrick (1980) applied this technique to examine teeth and periosteal bones from the 'pan' region of the

mandible and rostrum of premaxillary of Odontocetes. An undecalcified thin sections (about 250 μm) mounted on glass slides are prepared and viewed under a photomicroscope equipped with a rotatable polarizer and analyzer, a rotory stage, petrographic objectives, and a 1/4A (first order red) quartz plate.

(5) Acetate peels to replicate the etched surface of a tooth.

(6) Microradiography to visually resolve growth layer structures in a thin section (Hohn 1980).

(7) Scanning electron microscope (Hohn 1980).

Although the method of counting growth layers is accepted widely by researchers, the interpretation of growth layers is the main problem in age determination. Pierce and Kajimura (1980) summarized the reasons as:

(1) lack of knowledge of the biological and environmental factors controlling deposition of dental tissue;

(2) inability to routinely distinguish between annual growth layer and accessary bands which represent different growth rates within a year;

(3) lack of known-age animals for comparative growth layer analysis;

(4) lack of consensus on tooth preparation methods and interpretation of observed results.

To summarize, there is no absolute validation for age determination but many attempts have been made a resolving this problem, often leading to further controversies. Interpretations of growth layers are subjective. Kimura (1980) tested six experienced tooth readers for their criteria of counting growth layers of a known-age striped dolphin. The result revealed that even if the readers count the same age, they have different interpretations on growth layers. Although intercalibration is helpful to reach a consistent interpretation between readers, the problem still remains unresolved: Are growth layers formed annually?

Scientists may employ time-marking of teeth in live animals with tetracycline, so that growth during a known elapsed time can be identified in teeth collected subsequently (Nielsen 1972), Milch et al. 1958, Harris et al. 1962, Mansson 1967). The advantages of using tetracycline are that toxicity is comparatively low; small doses are enough to mark the teeth and bone; and it can be given orally (Perrin and Myrick 1980). Tetracycline-marked teeth should be stored in the dark, dry, in ethenol or in cacodylate buffer. Formalin is avoided. Examination is under a microscope equipped with UV illuminator system.

Other chemicals such as Procion red-88N (Prescolt et al. 1968, Goland and Grand 1968), alizarin red S (Hoyte 1960, Lowe 1971, Klevezal and Kleinenberg 1967), haemotoxyline (Klevezal 1980), and lead acetate (Yagi et al. 1963) can be used as time-marking. Klevezal and Kleinenberg (1969) noticed that annual layers of dentine are deposited in odontocete teeth before the pulp cavity is filled. The time to cease deposition differs among species, between individuals and among the teeth of the same animals. Gurevich et al. (1980) applied tetracycline by injection to four common dolphins. One of these animals did not deposit the tetracycline mark at all. If this is the case, the age determination by dental growth layers is inaccurate. The other three animals deposit tetracycline marks in different teeth and did not accurately deposit growth layers as age increased. Gurevich et al. also proved that a yearly growth layer includes two opaque and two translucent zones; and one periosteal growth layer composed of one opaque and one translucent zone.

4. Bones

Nishiwaki, Ohsumi and Kasuya (1958) used the mandible of the sperm whale for age structure. Hay (1980) examined the periosteal bone of the anterior position of the mandible and found that the periosteal layers are deposited throughout most of the life of Norwhale. This technique is helpful to age the animals when embedded teeth are

occluded. However, internal resorption and the removal of early growth layers by osteoclast activity reduce the reliability of age determination, especially in old males.

General Discussion

As mentioned previously many ambiguous points remain unresolved about the use of teeth and bones in the age determination of terrestrial and marine mammals. Klevezal and Kleinenberg (1967) indicated that intensive mechanical load of dentine deposition on the surface of pulp cavity may cause inaccurate age determination from teeth. The deposition of growth layers does not occur when the pulp cavity fills with dentine before the last age. The case has been partially demonstrated by Gurevich et al. (1980) in the previous context. Intensive mechanical load can influence the deposition of dentine. Klevezal and Kleinenberg (1967) indicated that the upper parts of the pulp cavity of the molars of rodents (ie., striped field mice and Norway rat) have intensively deposited secondary dentine. Many accessary bands are found in these sections of the teeth and result in incorrect determination of the boundaries between the annual layers. Hay (1980) also found that dentinal deposition in norwhale embedded teeth ceased due to complete coverage of the root by cementum at the mean age of 15 to 16 layers in males and 13 to 14 layers in females. Incidence of dentinal occlusion increases after the sexual maturity of norwhale.

Using the growth layers in cementum is also subject to difficulties. Cementum is frequently narrow and without showing clear growth layers. For those teeth of thick cement, the accessary bands within the broad annual growth layers is the main source of error. Changes of permanent teeth are the other problem, especially in Ungulata. In this case, one should add a certain number of years elapsed before the formation of the first growth layer in permanent teeth.

Cross-sections of the mandible are the usually used ageing method. The sections are most frequently taken from the end of a tooth row. However, there is an important

drawback due to the unevenly developed periosteal zone. The periosteal zone reaches its maximum breadth on the buccal (external) side of the mandible in sable but on the lower margin in mink (Klevezal and Kleinenberg 1967) thus raising questions for the species as well. The processes of apposition and resorption under the influence of mechanical load can exert pressure on teeth and may lead to the appearance of numerous accessary adhesion lines, especially the inci and its adjacent mandible wall.

There is also difficulty in ageing tropical species. Kenyon and Fiscus (1963) found that there are distinct growth layers in the seal, *Monachus schauinslandii*. Peabody (1961) and Warren (1963) had examined the growth layers in bone of some tropical poikilothermic animals. Peabody linked the formation of growth layers to alternative dry and humid seasons in a year. However, in localities without contrasting seasons the layers may not form or form under the influence of the reproductive cycle. Despite many efforts, especially time-marking with tetracycline and other chemicals, scientists still do not have sufficient data to affirm the accurate age of some animals. The incorrect interpretation of growth layers and the composition of an annual growth layer group are the long-existing problems in age determination (Kimura 1980). Although intercalibration between readers is helpful to reach a consistent interpretation, accurate age reading still is not here.

Reading teeth with reference to biological information including relative age criteria can minimize factors (a) and (b) but it can also lead, through conscious or unconscious bias introduced by knowing how old the individual 'should be' based on length, sex, and other information, to a serious underestimation of biological variation and possibly even a completely invalid 'growth curve'. The following overall approach is recommended:

(1) Select the most appropriate tooth or bone, and develop a technique or techniques to demonstrate growth layer groups clearly.

(2) Describe the growth layer groups. If the sample permits, a subsample constituting an ontogenetic series consisting of large fetuses, neonates, and immature individuals, preferably of both sexes, based on body length should be assembled. This series can then be studied to determine the nature and thickness of, e.g., the prenatal dentine and neonatal line, and the growth layer groups in dentine and/or cementum can be described.

(3) The entire sample of tooth or bone preparations should then be read in random order. It may be a good practice to label preparations with random numbers (each corresponding to specimen number) while this is being done. Each preparation should be read independently several times by an observer, or several observers fully conversant with the results of (2), until adequate repeatability is achieved.

(4) If anomalies are found when the readings are checked against biological data, a careful check should be made for blunders, e.g., clerical errors or mislabelling. If these are not discovered, the preparation should be read again by an independent observer again without reference to the biological information, and if the anomaly persists, it should be accepted as probably reflecting biological variation.

Age Determination for Fish

Age determination of fish has been amply reported in this century. Although the methods and techniques are diverse, they can be categorized into several categories: indirect measurement includes raising and tagging methods; physiological aging methods such as morphological indicators, heavy metal contents in body tissue, radioactive element concentrations, and isotope dating; and chronological ageing methods which are based on counting annual growth rings on the bony tissues. Besides these, analysis of mixtures of statistical distributions in length-frequency distributions is frequently used as a substitution for age determination to estimate growth and mortality. This topic is reviewed in a separate section.

Age Determination by Raising Fish

Meyer (1878) used this method to estimate age and growth of herring. To apply this method, a growth model is first established from the cultivated animals. The model generally describes length as the function of age, i.e., age is the independent variable and length is the dependent variable. When the model is used to estimated age from the length of fish, the model must be rearranged such that length becomes the independent variable and age becomes the dependent variable. This statistical procedure is called calibration. Although there are many studies show that calibration based on linear regression is valid (Rosenbery and Pope 1987, Williams 1969, Scheffe 1973, Hunter and Lamboy 1981), none of them make a clear point about the validity of calibration based on non-linear regression model. The disadvantages of this method rise from the argument that the conditions of a culture farm are different from natural conditions. Also, techniques concerning the long-term cultivation of fish are frequently difficult, especially for off-shore species, and the method is time-consuming and costly.

Tagging Method

Age determination is one of the by-products of tagging experiments. Based on the growth rate between the times of release and recapture, the age of the tagged fish can be estimated. The assumptions of this method are that: tagging or marking does not interrupt the growth and normal activity of fish and the tagged recaptures cover enough of the life span of the species. The difficulties encountered in this method are mainly due to these assumptions: (1) Growth rate, mortality rate and activity of fish are influenced by tagging and marking, (2) Recaptures frequently take place over a short time after release, and the release period is not long enough to estimate the growth rate throughout life, (3) The number of recaptures does not cover the whole life history of the fish, and (4) It is frequently found that the length at recapture is smaller than that of release (Lai 1985).

Kirkwood (1983) proposed a maximum likelihood method to estimate the parameters for a von Bertalanffy growth curve using growth data from a tagging experiment associated with ageing data. He indicated that the use of age as a dependent variable in the non-linear regression to estimate the von Bertalannfy growth curve is not appropriate. As mentioned in the previous section, the calibration problem is a concern in using this method.

Morphometrics of Otoliths

Boehlert (1985) constructed a multivariate model by using age determined from the otolith break-and-burn technique as a dependent variable and otolith dried weight, otolith length, otolith width, the respective square and cubic terms of above measurements, and the interaction terms of otolith weight/length and otolith

length/width as independent variables. Age models of male and female *S. saebatae* were built by stepwise selection of regressors. When the ages obtained from these age models are used to compare with break-and-burn otolith ages, the difference is not significant. To build this kind of model, it is necessary to obtain ages of fish from any direct ageing methods and treat these age estimates as dependent variate. Therefore, if the ageing method is not validated, the result of this method is doubtful. Also, the differences of otolith morphometrics due to temperal and geological variations may not support an universal application for this method. Lai (1985) showed that discriminant analysis for sablefish otoliths can be used to distinguish the otoliths from different year classes in southeastern Alaska.

Ralston's Method

The daily increment was used only for juvenile fish. Ralston (1981) extended this method to adult fish in tropical areas. After preparation of an otolith section Ralston measured the distance (in microns) between the focus and antirostral margin with a calibrated microscope. Increments were read at selected points along the growth axis, wherever the increments could be inspected most clearly. At these selected points the density of increments was calculated from the number of increments divided by the segment length (L, the distance of counted increments at certain selected points). Assuming that each increment was formed on a daily basis which was small enough in comparison with the whole life span of the fish, the width of each increment can thus be represented by differential form df/dt. The inverse of df/dt (i.e., dt/df) is the density of increments measured by Ralston. Ralston investigated the curve of density against segment length and fit a linear regression model to it:

$$\ln(df/dt) = a - bL + e$$

where, e is random error term. After the parameters a and b are estimated, this differential equation can be solved as the following form:

$$t = \frac{\exp(-a)}{b} [\exp(bL) - 1].$$

Using this model, the age of fish (t) can then be predicted from the total length of an otolith (L).

Mercury content

The accumulation of mercury and other heavy metals is often noted. Forrester et al. (1972) reported that mercury content is as high as 1.96 ppm in the Strait of Georgia. Ketchen (1975) assumed that if the average annual net uptake per fish is constant, the mercury content existing in dogfish could work as an age indicator. According to the relationship of Hg content and body length calculated by Forrester et al. (1972), Ketchen back-calculated the age from length and projected the relationship of age against Hg contents. Unfortunately, the confidence limit of the fitted curve is too broad. Another drawback of this method is the Hg content in embryos. Actually, the embryos and pups of dogfish are not subject to the environmental seawater of high Hg concentration. Ketchen assumed that the mercury existing in embryos and pups was from the parents.

Scales

The ontogeny, growth and morphology of scales are described by Graham (1928), Chugunova (1959) and Kubo and Yoshihara (1968). The scales of bony fish

are characterized by bony ridges, and have been classified into ctenoid and cycloid. Teleost scales are composed of a central transparent basal plate (focus of scale) and mineralized outer layers. The outer surface of scale is deposited with bony-ridges (also called sclerites, ridges, or circuli) which form a series of concentric circles and alternate with valley-like depressions. The inner part of a scale is made up of layers of criss-crossing fibrous connective tissue (fibrous lamellae) which increase in number as the scale grows. The scale grows by sequential addition of platelets. Under the small first basal plate, another wider plate is laid down. On the margins emerging from under the upper basal plate, hyalodentine formations (circuli or sclerites) appear (Kubo and Yoshihara 1968). A fully developed set of wide and narrow sclerites constitute the annual growth zone.

Scales are generally collected from the area under the anterior part of the first dorsal fin and above the lateral line (Chugunova 1959), because these scales are larger than in the other areas. Scales of regular shape, growth rate, and size are important in some studies such as for the back-calculation of growth, for which scales from some locations are selected (Lander et al. 1964, Tesch 1968, and Yeh et al. 1977). Other factors such as better readability and the possibility of regenerated scales are also important to the selection of scales (Graham 1928).

The most common method for scale preservation is to keep them in paper envelopes or booklets and to air dry them. Some workers keep scales in 50% alcohol or diluted formalin, or frozen in a refrigerator. It is important to use an appropriate preservation method. For example, scales of Pacific cod become soft and frigible when preserved in a low concentration alcohol (Lai 1985). Some staining techniques such as alizarin, ink, or DeFaures soulution (Kubo and Yoshihara 1968, Chilton 1970) have been proposed.

The methods of age determination using scales can be summarized as follows:

(1) Using scale impression or pressed scale. Either scale impression on acetate plate or scales pressed between two glass slides are viewed with microscope, projector, or microfische with transmitted light. Figure 18 shows the original design of the scale press used in Koo (1962).

(2) Using a polarized light microscope. Taylor (1916), Miller (1955) and Savage (1919) employed this technique. Takemura (1925, 1952) applied this technique on *Pleurogrammus azonus*.

(3) Using kimography or other sculpture instruments. The width of circuli are plotted against the number of circuli in order to identify the ring pattern. Koo (1955) applied this technique on red salmon with some modification. Let the length of Scale (S) be 100%. Koo divided the scale into a 5% area along the axis. Let D_i denote the distance of area by truncating the nearest circulus, the mean distance of each circuli is estimated as $d_i - D_i/(n-1)$ where d_i is the mean distance of circulus, n is number of circuli in the 5% area. If d_i is plotted against the area, the age of the fish is estimated by the peak of the curve.

(4) Hirschhorn's digitizing technique (1986, also Small and Hirschhorn 1986). Based on the facts that the scales are formed in the very early life of a fish and that scales should cover the entire body surface to protect fish from mechanical or biological injury, the area of the scale is the best way to project the growth history of fish. A photocopy of a scale is taken first, marking the position of identified annuli, the photocopy is traced on a digitizer to measure the areas of the whole scale and the successive annuli. Assume that the length of fish is proportional to the square root of scale area, the relationship between square root of the area of successive annuli vs. age can be represented by von Bertalanffy growth equation. Small and Hirschhorn (1986) demonstrated that this method can be used to detect false rings when the estimated L_∞

(in percentage related to the area of last annuli), e^{-k} and t_o are deviated substantially from normal range of the fish.

(5) Automatic scale reading machines. Mason (1973) tried to develop a semi-automatic machine for counting and measuring circuli on fish. He used a transmitted light source with a rheostat control, a microscope equipped with camera as an image dissector, and interfaced them with a digital computer to analyze the photo information which had been transformed to digital information. Van Utrencht et al. (1972) also used a photometric machine to analyze age structures including scales. The principle is to use a light source to scan the object, to transform the photon message into digital information, and to analyze the variation in the density of the scale with the interfaced computer. The National Marine Fisheries Service, Woods Hole, Massachusettes has a set of Guantimet image analyzers interfaced with a digital computer (Anon. 1981). The function of its software is to automatically examine fish scales by (i) identifying and determining circuli spacing. (ii) determining annulus and check locations. (iii) identifying edge type. The results of this instrument in reading haddock scales are: (i) Irregularities in scale growth pattern preclude the use of platelet shape. (ii) The detection of circuli yields acceptable results from annuli detection, however, the selected method utilizing the interspacing of circuli improved the accuracy of age determination. (iii) Irregularities in platelet shape introduced a significant number of false detections when this method is utilized.

The advantages of the scale method are: (i) The scale is easily prepared and preserved. (ii) Scales can be obtained without sacrificing the fish. (iii) Scales are easily read without very sophisticated techniques. (iv) Scales are almost two dimensional in growth, ie., they are nearly flat, and grow with fish size. Thus, they are the best way to project the growth history of fish.

The disadvantages of scales are due to their growth patterns. The major increases in scale size occur prior to maturity. After maturity the growth of most fish is

significantly reduced, especially in males which can affect the growth pattern of the scales (Lai 1985). For those fish which do not have obvious growth or cease to increase in size, the annuli on the edge of scales are indistinct or totally gone. For some species, especially tropical species, the annuli on scales are not visible. In recent years scientists have generally concluded that the scale method underestimates the age of fish. Lai (1985) has provided evidence that scale age is a consistent underestimator of fish age after maturity.

Fin rays and spines

Markert (1896) and Goodrich (1930) reported that dorsal spines of the spiny dogfish (*Squalus acanthias*) were composed of several different layers: beginning from the outermost, they are the enamel layer, pigment layer, outer dentine, middle dentine and inner dentine. The central cavity is also called the pulp cavity and a cartilaginous rod intrudes into it to form the base of the fin ray. Holden and Meadows (1962) suggested that the inner dentine layer is formed by a series of overlapping concentric cones laid down by the odontoblast, which forms a layer lining the pulp cavity (Fig. 19). The point of overlap is the enamel ridge that shows as the band of pigment on the external surface. Based on this theory, the most recently formed cone is represented in a cross section of the innermost ring or in longitudinal section of the ring at the base.

Ketchen (1975) extended this method to age spiny dogfish by using the second dorsal spine. He found that there are a series of dark bands and ridges in the lateral view of the second dorsal spine. Each of these dark bands is considered an annulus. Many others have since used this method to age the spiny dogfish (e.g., Kaganovskaia 1933, Soldat 1982, Chilton and Beamish 1982). However, the method has not been validated.

Beamish and McFarlane (1985) reported a different theory on the growth of dorsal spines of the spiny dogfish and validated their ageing method by OTC marking.

The longitudinal section shows that the dorsal spine consists of three major structural components: cartilage interior, stem, and mantle arranged from inside to outside. The stem consists of three (inner, middle, and outer) layers of dentine. When the mantle is scraped off with sandpaper, the appearance of growth pattern of the stem is the "cone bases" as described in Holden and Meadows (1962). Count of the alternating growth zones from the OTC injected specimens shows that the growth zones are formed annually. The cross sections of dorsal spines reveal growth checks shown only in the inner dentine, however, counting the rings is difficult.

Beamish and McFarlane (1985) reported that counting growth marks in the mantle is the most useful method. The mantle originates at the enamel gland and consists of an inner dentine layer, a midlayer of pigment, and an outer enamel layer. The mantle is laid down on the stem dentine. Pigment is produced by the melanophores at the inner surface of the enamel organ and is deposited between the enamel and the dentine. Dark pigmented ridges appear on the surface of the mantle. These ridges are used to estimate the age of dogfish. Holden and Meadows (1962) and Ketchen (1975) defined the annulus to be a darkened band. Beamish and McFarlane (1985), however, defined the annulus to be a darkened band or ridge or both because the ridges are darkened in most cases, however, "occasionally a ridge does form that is not darkened". The OTC marking methodology confirmed that this method is valid. Beamish and McFarlane (1985) recommanded that the mantle annuli be used for age determination. Since removing the mantle from dorsal spines is difficult and time consuming, counting annuli in the stem is not recommended.

In contrast to spines of dogfish, fin rays of bony fish are of two major kinds: spine (single rays) and soft-rays (segmented rays, often branched and always biserial). Most of the teleosts have two elements in each fin ray. The most recent growth layer is located at the outmost (Chilton and Beamish 1982). The growth pattern of this kind of

fin ray has not been studied in detail, however, a recent study using OTC marking method supports this theory (Beamish and McFarlane 1987).

The dorsal fin, pectoral fin, pelvic and anal fins are those which are commonly used (Kubo and Yoshihara 1968). The fins are cut through the articulation base and mucus removed, and the fins are kept in a paper envelope and air dried. Fin rays are mounted in resin and are cut using an electric saw. Sections of 0.4 to 0.8 mm are the most suitable thickness for age determination (Beamish and Chilton 1977). The thin sections are mounted onto a glass slide and viewed using a microscope with transmitted light. Lai (1985) showed that if the section of a fin ray is immersed in water within a clear petri dish and viewed with transmitted light under a microscope the result is a clearer, lower glare view of the section. Annual growth zones are identified by a translucent (light) band which is supposed to be a slow winter growth zone and a widely spaced dark zone corresponding to rapid growth. The center of the fin ray section is identified as a small translucent oblong or crescent-shaped area (Beamish 1981).

Deelder and Williemse (1966, 1973) developed another method using solid fin rays. After polishing the cut surface and drying it in the air for a few days, the fin ray was attached upside down to the polished surface under the microscope (Fig. 20). A small dark screen was put between the fin ray and the sidelight to control the quantity of incident light. Alternating dark and light bands can be counted if the screen is properly adjusted. Ketchen (1975) also applied this method to age spiny dogfish.

The advantage of using the fin-ray for age determination is that removal of fin-rays does not require the fish to be sacrificed, which may be the best method for the validation of ageing. A fin-ray can be taken at the time the fish is tagged and released. An additional fin-ray can be taken from the tagged fish when they are recaptured. Age assigned to the fish can then be verified by comparing the ages between time of release and recapture. The disadvantages are: (1) Interpretation of first annulus is difficult.

(2) They are difficult to handle, prepare and preserve. (3) When the oblique cut is made the outside annuli are distorted (Chilton and Beamish 1982). (4) The most important disadvantage is that this method is very time-consuming preventing the application to many readings.

Operculum and Other head bones

Some head bones with flat structure, eg., operculum, (LeCren 1947, Bordach 1955, Mitani et al. 1959, Cooper 1967, Fagade 1974), interopercle (Palman 1956), supra-occipital crest (Menon 1950) and cleithrum (Menon 1950), are commonly used in age determination. The bones have broad layers that are deposited during rapid summer growth and narrow layers that correspond to winter growth.

The operculum may be the most commonly used head bone. The operculum is easily removed with a scalpel by cutting the membranes between the preoperculum and the operculum and between the operculum and the branciostegal rays. The adhering tissue can be cleaned by hand by putting the operculum into hot water for a few minutes. Boiled water is avoided. For preservation, the operculum is kept in kraft paper envelopes and air dried.

Annuli on the operculum may be viewed with the naked eye or with a low power magnification microscope or magnifier. With transmitted light the broad layer is dark and the narrow layer appears transparent. With reflected light and the dark background, the broad layer is opaque and white, the narrow layer appears dark.

The advantages of this method are: (1) Easy-to-handle preparation and preservation. (2) Size of the operculum is always large and shows most of the annuli. The disadvantages are: (1) The method will sacrify the fish. (2) The first annulus is difficult to identify. (3) Accessory bands are frequently confused and difficult to detect from the annuli.

Vertebrae and Urostyle

Collected vertebrae can be kept in plastic bags and frozen. The vertebrae are boiled in water and removed from the adhering muscle. The vertebrae are soaked in 3% KOH solution to remove oil and remaining tissues, washed in tap water and air dried (Lai and Liu 1974, 1977). Three methods are used for examining the annuli:

(1) Examining the articulative fossae of vertebrae through a magnifying glass (Chugunova 1959). For this purpose, the vertebrae are embedded in wax with articulative fossae upward.

(2) Vertebrae are sawed or grounded lengthwise in the dorso-ventral direction. Half of the vertebrae is fixed on modelling clay with concave centrum upward. Annuli appear as ridges comprised of many irregular circuli.

(3) Thin section technique (van Utrencht et al. 1972). Longitudinal thin sections of vertebrae are obtained by grinding and polishing until they become transparent. The section is mounted with CEDAX after dehydration in alcohol and xylol mixture. A series of light and dark zones are found with transmitted light under a microscope. The dark zones are not uniform in their density and length, and represent the summer growth. The light zones represent the winter growth. van Untrencht et al. (1982) also tried to use a automatic photometric instrument for ageing the fish based on vertebrae.

Recently, Cailliet and Radtke (1986) used an electron microprob analysis and Welden et al. (1986) used a radiometric method to age elasmobranchs.

The disadvantages are: (1) They are difficult to handle, prepare and preserve. (2) It sacrifices the fish. (3) Collections of vertebrae should dissect the fish and many situations do not allow this process, especially sampling in commercial landings. However, verterbrae are the only useful part for ageing elasmobranchs.

Otoliths

There are three pairs of otoliths present in three optic capsules of teleosts (Fig. 21); the pair in the utriculus is lapillus, that in the saculus is sagitta, and that in the lagena is asteriscus (Lagler, Bardach and Miller 1962). These structures are calcareous and show growth zones. The sagittae are the largest otoliths in the fish and have been widely used for age determination. Therefore, the terminology of otolith is commonly referred to as sagittae.

Removing the otolith from the skull of fish involves cutting the fish across the occipital portion at a right angle to the line associating the joint of the operculum and the lateral line. Storage mediums of otoliths are 50% alcohol, glycerine and water mixture (50/50) plus a little thymol, fresh water and thymol, seawater with thymol, glycerine, glycerol, and xylol. For long-term preservation, otoliths are put into the refrigerator. It is important to keep them from infection by bacteria and fungi. Some workers have kept air dried otoliths in envelopes (Mosher 1954, Kao and Liu 1972), but this method always results in opaque otoliths and obscure age marks (Lalanne 1975). Readers must be aware, however, that there is no standard storage method for all fishes.

The chemical composition of otoliths has been studied by Fitch (1951) and Dannevig (1933). Most of the inorganic material is argonite ($CaCO_3$). Collagen constitutes the major part of the organic materials and Fitch (1951) found that the winter zone (translucent) contains more $CaCO_3$ than that of the summer zone. Irie (1960) used the Ca^{45} isotope to trace the seasonal deposition rate of $CaCO_3$ and found that the deposition rate of $CaCO_3$ is faster from the early summer to autumn than in the period from late winter to spring. The trend in the deposition rate of protein is the reverse of that of $CaCO_3$. Unfortunately, calcification in otoliths can be understood only in the framework of calcium metabolism which is highly complex.

The terminology used to describe seasonal growth zones on the surface of an otolith has been controversial and needs to be clearly defined. The zone corresponding to slow growth is called the hyaline zone. It is always called the dark zone because the zone appears dark under reflected light with a dark background. However, this zone is also called the translucent zone under transmitted light. The zone corresponding to faster growth is called the opaque zone and contains more $CaCO_3$. This zone is thought to be formed in the summer (Williams and Bedford 1974), and appears white under reflected light but is opaque, clouded or dark, under transmitted light.

Prepararion and examination methods for otoliths are diverse in the literature. Readers must select their method according to a tried-and-error routine. Brother (1987) gave a good summary of methods that have been widely used in otolith reading. In general, these methods involve one or several of the following procedures: examined whole otolith, grounded, cut, polished, broken, burned, stained, acid etched (normally with 1% HCl), mounted, or immersed into clearing agents. The following is a summary of commonly used methods to examine annual growth marks, and modifications should depend on the otolith's characteristics.

Surface reading

The otolith is immersed in water, glycerine, and 50% alcohol in a dark-background petri dish. The annuli viewed under a dissecting microscope with reflected light corresponds to the dark zone (winter growth zone). The summer growth zone corresponds to the opaque (or white) zone. If transmitted light is used, annuli are transparent (or light, translucent) and summer growth is opaque (the dark zones). Some thick otoliths must be grounded to allow better light reflection or absorption (Kao and Liu 1972). However, this method has been criticized because of the growth features of the otoliths. Otoliths of many species do not grow evenly in all dimensions. Kimura et al. (1979) showed that otoliths of old yellowtail rockfish do not show

growth zones on the distal (external side related to the body of fish) surface of otoliths, instead, the growth zones are shown on the proximal (internal side) surface. Chilton and Beamish (1982) also reported this phenomenon in many North Pacific species. Therefore, the surface reading technique is not valid for older fish and the thin section, break-and-burn, or other cross-section technique must be applied.

Break-and-burn technique

This technique was first developed by Christensen (1964) by applying the discovery of Dannevig (1956). Recently, it has been considered to be more precise than surface reading (Beamish et al. 1982, Chilton and Beamish 1982, Bennett et al. 1982). The otolith is broken or cut transversely across the nucleus (focus) using fingers or a saw. The broken surface is burned with an alcohol lamp until the color becomes dark brown. The broken-and-burned otolith is then put in soft model clay. The burned surface is painted with cedar or vegetable oil and examined under a stereomicroscope with reflected light (Fig. 22D). Narrow dark zones are considered to be annuli (winter growth zones corresponding to "dark" zones on the otolith surface with reflected light). The broad, light colored zones represent summer growth.

There are other methods used to read unburnt cross-sectioned otoliths (Fig.22). Lai (1985) found that break-and-burned otoliths can also be viewed by the method shown in Fig. 22C. The advantage of using this method is that the water medium provides homogeneous light absorbency and reduces the glare of burned surface when it is painted with oil.

Thin section

A thin section mounted in epoxy, resin, or wax is cut through the center of the otolith with a low speed electric saw. The thickness of the section should range from 0.4 to 0.5 mm to give the best appearance. The thin sections are mounted on glass

slides and viewed under a compound microscope with transmitted light. The annuli are identified by using surface readings.

It is also important to consider the orientation of the cut surface. Because of the asymmetrical growth of an otolith, with later growth appearing only on the interior (sucus) side, the transverse or oblique section is the most appropriate orientation to section.

Validation of Age Determination

The most important part of age determination is to validate the age-structure, the technique and the criteria used for chronological counting. The ages should be validated for each species, each unit stock, and each age-structure throughout the whole year-classes existing in a population. This procedure is difficult to carry out and thus is a negligible part of many research projects. The methods for the validation of age determination are summarized as follows:

(1) Periodic examination of annulus formation. Time of annulus formation can be determined by the following methods: (a) Probability of the appearance of an edged annulus (Maeda 1982, Lalanne 1975, Ogata 1956). (b) Measuring the relative marginal increment (Kubo and Yoshihara 1968, Lai and Liu 1974, 1977, Yamaguchi et al. 1972).

(2) Length-frequency analysis. A length composition is constructed of many age groups. Variation in fish length in each age group depends upon presumed theoretical density distributions, which frequently are normal distributions. Cassie's (1954) probability paper method, Hasselblad's (1966) NORMSEP, and Macdonald and Pitcher's (1979) MIX are the commonly used methods for separating mixed distributions (refer to Length Frequency Analysis for detailed summary). The

proposition of age groups and the mean length-at-age obtained from these methods can be compared with that obtained from direct ageing methods.

(3) Tagging experiment. The growth curve and growth rate determined from the age-structure are compared to those derived from tagging fish. Beamish and Chilton (1982) have applied this method to validate the age determination of sablefish.

(4) Tracing the strong year-class. The age composition obtained from age-structure (either by an age-length key or by random sampling) is constructed from several consecutive year classes. The year-class strength is then related to a survey or other biological evidence believed to produce a strong year-class. Lai et al. (1987) applied this method associated with the Mix Program (Macdonald and Pitcher 1979) to dismiss the scale method as invalid for the age determination of Pacific Cod.

(5) Comparison of growth curves to those of fish of known-age. Known-age fish can be raised in aquaculture fields. However, this method is not valid as shown in Lai and Gunderson (1987) since even a moderate ageing error will produce a growth curve similar to that without ageing errors.

(6) Oxytetracycline (OTC) marking. This method seems widely used in various species of animals. Beamish et al. (1983) indicated that counted annuli after OTC marks in sablefish otolith show good consistency between the age readings of readers who were either told or not told the time of marking. Beamish and McFarlane (1982) showed that a dosage over 0.75 mg/kg body weight was fatal to the sablefish and 0.25 mg/kg was suitable to sablefish and dogfish. This method may be subject to the following criticisms: (a) The effect of OTC on growth rate of fish is significant. (b) The number of OTC marked fish is not enough to cover all age-classes in the population and to do so would be very difficult. (c) The time-lag and effective duration of OTC is not clear. (d) The tagged fish must cover the whole life span of fish and be recaptured over a sufficiently long time period. Particularly, the criteria (a)-(c)

required substantial efforts in laboratory experimentation. Other chemicals such as acetozolamide and strontium have also been used (Brother 1987).

(7) Radioactive isotope: Bennett, Boehlert, and Turekian (1982) used Pb-210/Ra-226 measurement in otoliths to confirm the longevity of splitenose rockfish.

(8) Mercury content. Ketchen (1975) applied this method to validate the age determination of spiny dogfish.

(9) Morphometry of otolith. As described in earlier section, Lai (1985) found that otoliths of sablefish belonging to the 1977-year-class have a special shape and which was then quantified by discriminant analysis. Boehlert (1985) also used the morphometrics of otolith to carry out the age determination of *S. saebatae*.

Otolith Microstructural (Daily increment) Analysis

For many years, periodic growth patterns in the fish otoliths were studied from a taxonomic point of view or for the purpose of age determination. Although finer structures such as lammelae or subseasonal rings (Pannella 1980) have been mentioned in the literature (e.g., Hickling 1931, Irie 1960), the method did not attract great attention until Pannella (1971, 1974) discovered that the otoliths of some tropical and temperate fishes contained primary growth increments which seemed to be formed daily. The method offers a method of age determination for species where such data was previously not available. This finding is especially important for the tropical fishes for which age determination has been the source of more complaint than effort. Brother (1979) indicated that ageing by microstructures in otoliths seemed to be a promising method. Many studies have used the width changes in the microstructural increments associated with feeding and movement behaviors as an indicator of the life history stage of fish (Neilson and Geen 1982, 1984, Campana 1984). Brother (1984) provided a review of the uses of otolith microstructural examination for systematic

icthyology and speculated on the systematic applications of primordia number and type, as well as the increment width and appearence in taxonomic studies or for stock identofication. Uchiyama and Struhsaker (1981) found differences in growth rate of skipjack (*Katsuwonus pelamis*) between eastern and central Pacific based on the otolith microstructure. Brother (1979), Pannella (1980), and Gjosaeter et al. (1984) provide lists of fishes for which studies of otolith microstructure and growth have been studied.

Fish samples for microstructural study must be frozen or preseved in 95% ethanol. However, juvenile and larvae samples must be preserved with extreme care to avoid otolith degradation and dissolution. Any kind of formaline solution must be avoided. Also the pH value of storage medium must be checked frequently since some species' otoliths dissolve in a very weak acid solution.

For larger fish, otoliths can be extracted by the method described in the previous section. However, embryonic and larval fishes may need to be dissected under a microscope. Alochol or oil storage is probably more suitable for juvenile and larval otoliths. However, one must be aware of the possibility of degradation and dissolution in the storage medium and infection by bacterias and fungi. Brother (1987) reported that some oils become slightly acidic over time so mineral oil (some marble or $CaCO_3$ chips are added) is probably best.

The whole oil-mounted otolith can be looked at under a microscope at 400X or greater. The light source is an important factor in viewing the microstructures. Therefore, experiments on brightness, filter, filed and diaphram adjustment are neccessary. Otoliths can be mounted on glass slides with epoxy, polyester resin, Euparal, and other instant glues to facilitate grinding. Brother (1987) described the grinding by finger but a sophisticate ginding machine (Volk, personal communication) may also be applied. The grinding media are carborundum grit (400-800), metallurgical lapping film, aluminum oxide, etc. Following grinding, polish may be necessary. Frequently check the otolith under microscope until the appropriate features

of the growth increment are exposed. After grinding and polishing, otoliths may be etched by dilute HCl or EDTA solution to improve the view of the increments. The etching procedure is neccesary in SEM examination. Uses of acetate replaca techniques are frequently cited in the literature, however, Brother (1987) criticized these techniques because replicas only produce structures on the surface, and unless the ground surfaces are at right angles with all the increments, some growth increments may be missing in the replicas, making direct examination preferable. Brother (1987) also compared the advantages of using the SEM and the light microscope. He pointed that the light microscope is generally simpler and easier to use with greater tolerence of inappropriate grinding procedure. But the resolution of the light microscope may not be sharp enough to distinguish microincrement widths less than 1μm. In such a case, SEM is required, however, the preparation of thin section is more complicated, which includes sectioning, grinding, polishing, and coating with a 100-200Å layer of gold/palladium alloy. Besides these factors, SEM results are affected by tilt, accelerating voltage, and spot size, etc.

Interpretation of micro-growth marks is the central part of a current study. Otoliths are believed to be formed initially by one or more partially calcified primordia exocysted by the cells in the fishs' inner ear. Geffen (1983) and Neilson et al. (1984) reported fusion of primordia may occur before hatching; Mugiya et al. (1981) and Mugiya (1984) found diel variation in calcium depositions; and Dengens et al. (1969), Lowenstan (1981) and Ross and Pote (1984) reported that the organic matrix is a template for crystalization of calcium carbonate once the increment growth starts. Dumkelberger et al. (1980) found the differences in organic materials between the crystalline and protenaceous zones, which suggests that the organic matrix is probably subject to diel variation.

In some literature, the periodic increments are referred to as daily increments (Mugiya et al. 1981, Mugiya 1984) but environmental and physiological variables such

as photoperiod, temperature, feeding, growth, and endogenous circadium rhythm may disturb the deposition of increments. Campana and Neilson (1985) gave a complete review of whether these factors affect the diel cycle of increment formation or not. They also noted three important factors that cause problems in interpretation: constant temperature in the laboratory, otolith preparation and resolving power of microscope. The reader must refer to their paper for a detailed discussion of the controversial findings in the literature. Gjosaeter et al. (1984) listed fish species in which periodicity of increment pattern has been reported.

There is also a concern about when the first daily increment is deposited. Some species such as sockeye salmon, *Onchorhynchus nerka* , (Marshell and Park 1982), and Mummichog, *Fundulus heteroclitus* , (Radtke and Dean 1982) deposit the first daily increment before hatching but English sole *Parophrys vetulus* , (Rosenberg 1982) deposit the first daily increment after hatching.

Commonly used validation methods have been summarized in Geffen (1987). However, these methods are usually carried out in the laboratory and they are subject to the question of reliability when the results are used to compare wild and laboratory stocks. As stated above, there are many factors that can reduce the reliability of readings, therefore, validation must be designed to test that increment deposition is constant and predictable over time, that increment number reflects the age of a specimen, that increment deposition is independent of larval growth rate and feeding, and that increment deposition is controlled by the same natural phenomenon throughout the population. Thus, direct validation on wild stock needs to be developed. Some statistical methods, such as time series analysis, might be useful to correlate the increment width and daily environmental effects. This method has been successfully used in validating the deposition of daily increment in gastropod or bivalve shell (e.g., Adlerstein 1987 and Lønne and Gray 1988). Adapting this method to otolith daily increment seems promising.

In contrast to daily increments, checks (or discontinuities) can be found in otolith growth sequences when pertubation or stress affects the fish. These checks may occur at random or delimit incremental patterns of weekly or fortnightly periodicity (Pannella 1971, 1980, Campana 1984). Pannella (1980) and Campana (1983, 1984) reported that these checks may be formed due to the causes of sex maturity and lunar cycle. Other causes that interrupt otolith growth are discussed in Campana and Neilson (1985).

The application of otolith microstructure analysis has not yet reached its full potential for many animals such as corals, barnacles, and especially bivalves. The most obvious application is age determination and growth rate estimation. Ralston's (1983) method has partially explored the issue; however, many quantitative methods need to be applied. Many studies have modeled the growth as a state space model such that the growth increments at adjacent time steps are autocorrelated. When the observations are obtained, the Kalman filter method can be applied to the estimation of growth rate and in the prediction of future growth. This study is important for fish that grow rapidly in the first year, since the results provide information on how fish contribute to biomass production and potential yield in the earlier part of their life history.

The other potential application is to use the model that described the effect of environmental factors on the year-class strength and potential yield in past years to predict the future recruitment. Since much evidence supports the argument that the success of pre-recruitments is the critical factor in year-class strength, the prospect that year-class strength can be estimated prior to recruitment is an important concept in fishery management.

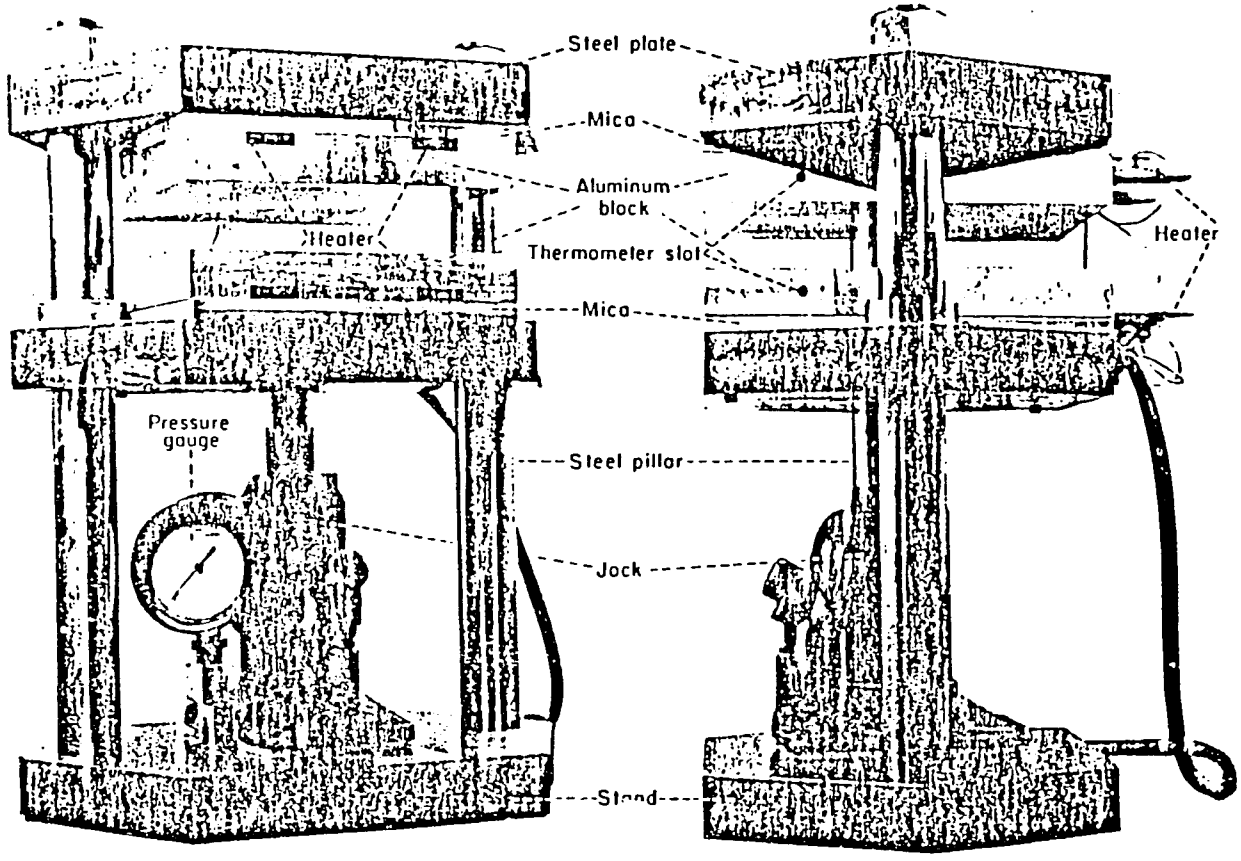
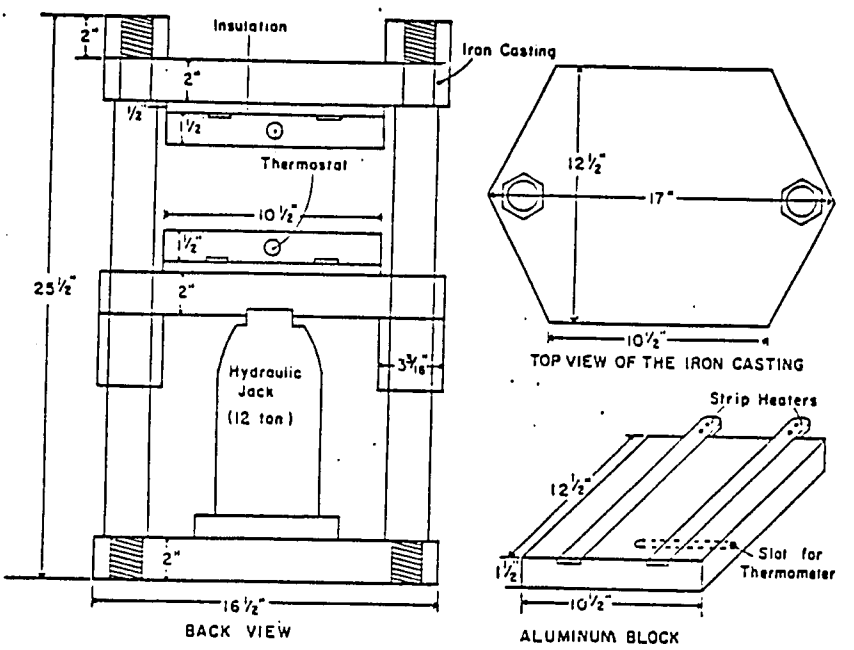


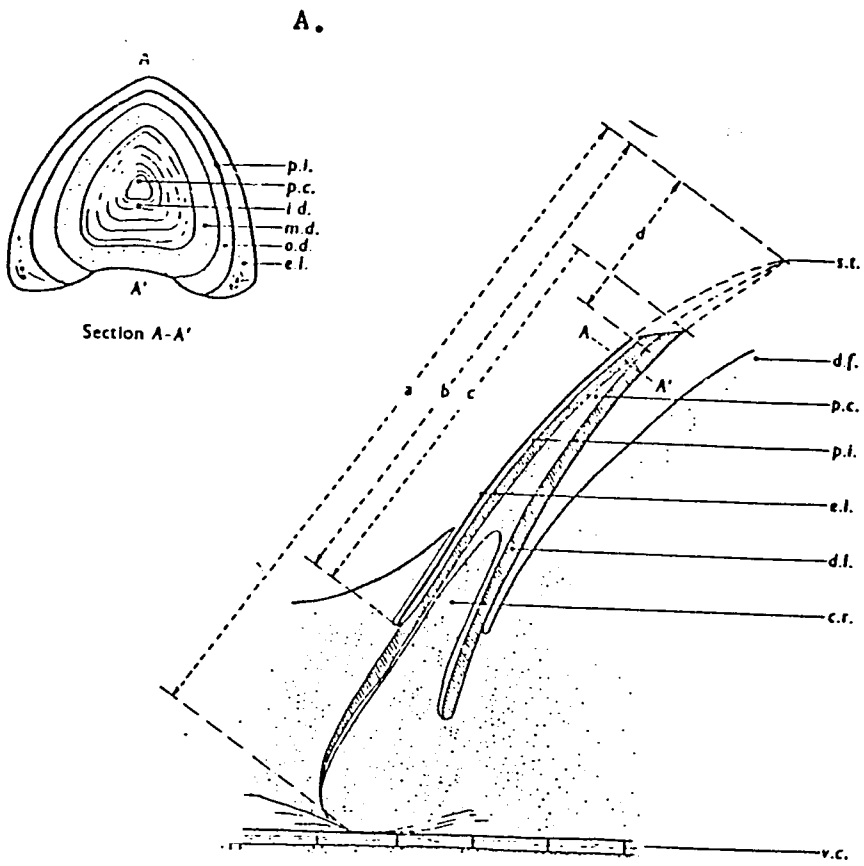
Fig. 18. Scale press and its structural components.



Items needed for final assembly of the scale press:

Description	Quantity Needed
1. 12-ton hydraulic jack	1
2. Strip heaters: 115 volt, 500 watts	4
3. Hydraulic pressure gauge: 1-10,000 pounds, 3½ inches diameter.	1
4. Thermoswitches: range 100 F to 400 F, 115 volt, 10 amp.; 230 volt, 5 amp. Sensitivity ±0.1° F.	2
5. Ferrotype plates (photographic)	2

Fig. 18. Continued.



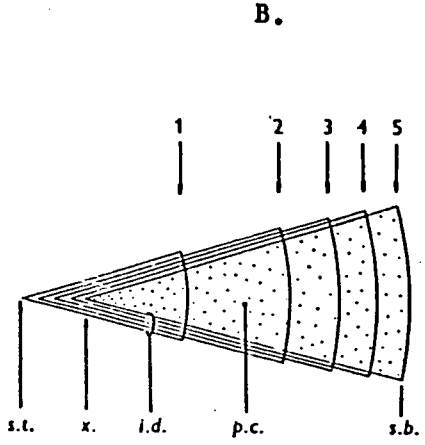


Fig. 19. (from Holden and Meadows 1962)

A. Diagram of a dorsal spine of *S. acanthias*, longitudinal and cross-section, modified from Markert, 1896, and Goodrich, 1930. *d.f.*, dorsal fin; *d.l.*, dentine layer; *e.l.*, enamel layer; *i.d.*, inner dentine; *m.d.*, middle dentine; *c.r.*, cartilage rod; *o.d.*, outer dentine; *p.c.*, pulp cavity; *p.i.*, pigment layer; *s.t.*, spine tip; *v.c.*, nerve cord in vertebral column.
Measurements: *a*, total spine length; *b*, corrected external spine length; *c*, uncorrected external spine length; *d*, distance from spine tip at which the pulp cavity becomes visible in longitudinal section.

B. Diagrammatic representation of inner dentine structure in the spine of a five year old fish. *i.d.*, inner dentine; *p.c.*, pulp cavity; *s.b.*, spine base; *s.t.*, spine tip; *x*, distance from spine tip at which ring number becomes maximally constant in serially cross-sectioned spines; 1, 2, 3, 4, 5, bases of successively formed cones, seen as rings in longitudinal section.

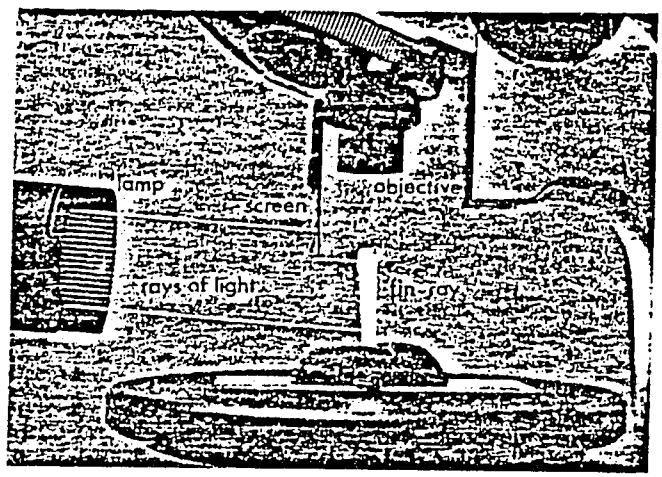


Fig. 20. Diagram shows the method of Deelder and Willemse (1973) for aging fin rays.

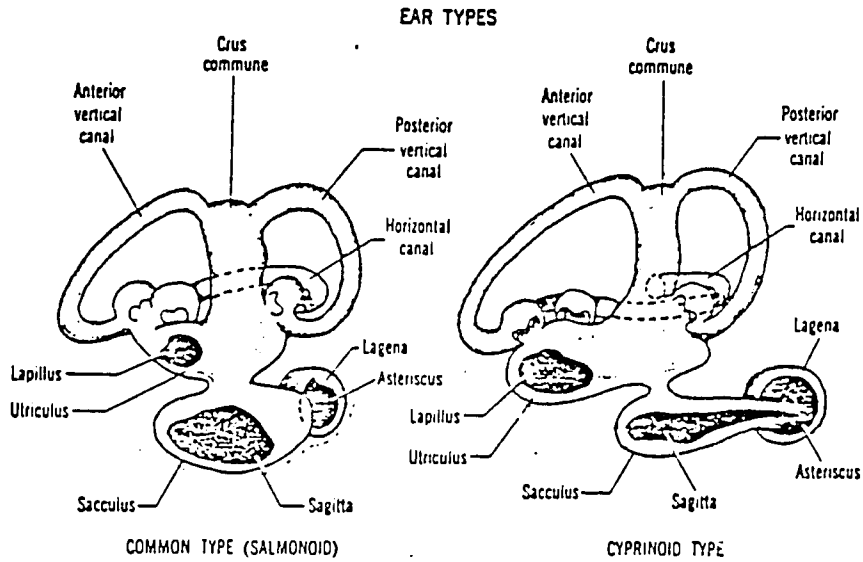


Fig. 21. Location of otoliths in the inner ear of teleosts. (From Lagler, Bardach and Miller 1962)

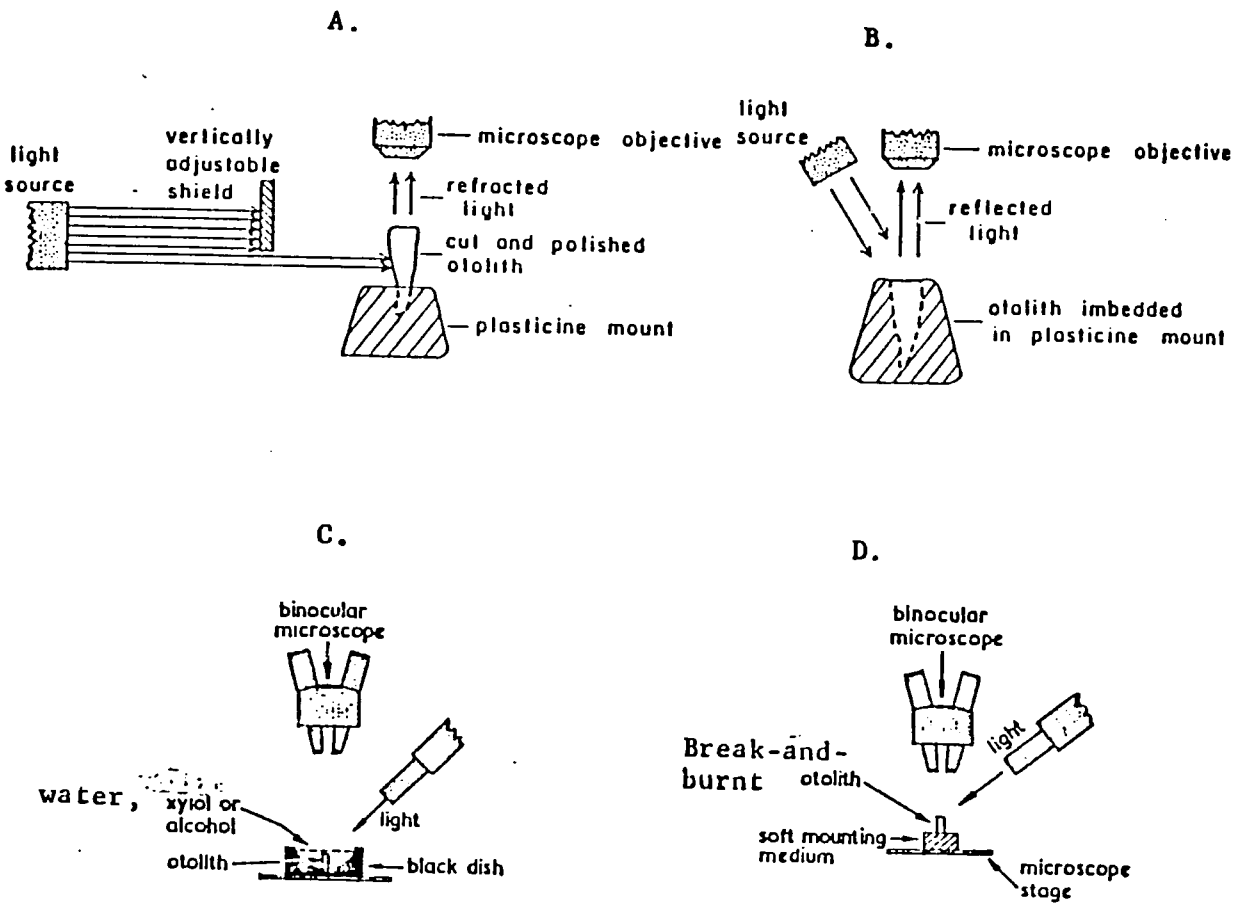


Fig. 22. Methods of aging cross sectioned otoliths. (A and B from Ketchen 1970; C and D from Gambell and Messtorff 1960)

Biochemical, Genetic, and Radiographic Approachs

Many new techniques related to age determination have been discovered during the past decade. Some of these methods are still developing, but they provide the possibility for age determination.

Amino Acid Racimization Dating

The amino acid racimization dating technique was originally developed for ageing fossils such as bones, shells and wood (Bada and Schroeder 1975, Master and Bada 1978, Schroeder and Bada 1976, Williams and Smith 1977). This method is based on the principle that: all of the amino acids (except glycine) exist in two different isomeric forms, D and L forms, whose rotational planes are seen to be of opposite directions under polarized light. Under equilibrium conditions, two enetiomers are in equal abundance but a living organism would maintain a disequilibrium condition because the organism utilizes the L-form only. After an organism dies or becomes metabolically stable, the biochemical reactions to maintain disequilibrium of two isomeric protein forms cease and the reaction called 'racimization' begins. That is, L-amino acids are converted to D-amino acids until an equilibrium state has been reached and the determination of the D/L ratio is a possible way to age the animal.

Bada, Brown and Masters (1980) extended the racimization of aspartic acid in teeth and the ocular lens nucleus of marine mammals. In this case, eye balls should be collected within 30 hrs of death and frozen. The dentine of teeth should be isolated and not boiled (Helfman and Bada 1976). Since the rate of amino acid racimization depends on temperature, the precise temperature of the environment where the teeth being collected must be known. To apply this method, the relationship of D/L ratio and age must be estimated from the animals with known age.

Microradiography

The principle of X-rays was given by Brain (1966): The absorption of X-rays by the atoms of a particular element is proportional to its atomic number. The rate of absorption depends on the types and relative proportions of the atoms present in its molecules. Therefore, different tissue components can be distinguished by differential absorption due to the composition and density of materials. Many applications of this method have been described in the previous sections.

The method is subject to error due to the correlation of changes in the degree of mineralization (radiopacity) with changes in structure (optical density) or in stainability (Perrin and Myrick 1980). Hohn (1980) used 150um thick sections of *Tursiops truncatus*, 600 um for Dugong and 50 um for *Phocoena phocoema.* The images of sections are examined with a compound microscope and transmitted light.

Casselman (1973) applied this method to examine lamental composition in relation to the zonation of cleithrum from pike. He related the results directly to differential calcification of the zones and confirmed that the translucent zone has a higher calcium content and is totally inorganic. Calcium and the total mineral content of both zones increases with age and a decreasing growth rate. Calcium content is relatively low in opaque zones and in the older and slower growing parts of the cleithra. Cailliet et al. (1983) applied this method to age sharks using vertebral centra.

Cellular Biology

Hayflick (1980) provided a detailed description of the physiological and biochemical reactions when cells age. Although the concept does not suggest an immediate and direct application of age determination, the results of cell culture provide

potential for age determination. Despite speed, the number of times that an embriotic fibroblast doubles its cell number in cell culture (doubling) is fixed at 50. The rate of doubling of human fibroblasts is inversely proportional to the age of donor. The latent period, the time that the fibroblast migrates out from the edge of cultivated tissue, increases with age and is found to be controlled by the nucleus.

Some evidence shows that the cultured cells have a finite capacity to divide. There is a kind of clock mechanism to control the cell function and capacity. There are three hypotheses to explain changes in cells under ageing:

(1) Over a period of time the information in the information-processing system represented by the transcription and translation of genetic messages in DNA into RNA and into enzymes and other protein mole ıles may be increasingly subject to error. Such error gives rise to faulty enzymes and leads to a decline in the cell's ability to function.

(2) It is known that only 4% of the information in the DNA of the cell nucleus is utilized by a given cell in its lifetime. The repeated sequences are normally repressed but if an active gene is extensively damaged, it is replaced by identical reserved genes. The redundancy of the DNA might provide insurance against the system's inherent vulnerability to random molecular accidents, thus lengthening the time before a sufficient number of errors can accumulate and confound the genetic message. Ultimately, all the repeated genes would be used up, errors would accumulate and physiological deficiencies leading to age changes would arise. This hypothesis implies that long-lived species should have more redundant DNA than short-lived species.

(3) There may be ageing genes that slow or shut down biochemical pathways in a sequential manner and lead to the predictable expressions of what is recognized as age change. The function of ageing genes might be analogous to the normal functional decline and death of cells that take place on a massive scale during the development of

an embryo. The fate of these cells is determined by "death clocks" that operate on a precise schedule. The same processes continue throughout life, operating at different rates in different tissues and leading to the normal age changes that increase susceptibility to disease.

Change of lipid contents

The lipid in many calanoid copepods have shown the importance of wax esters as reserve lipid. Lee (1974, 1975), Sargent et al. (1981), Kattner et al. (1988) reported that the lipid of copepods from the high latitudes may contain more than 90% of wax esters. Gatten et al. (1979, 1980) reported high proportions of wax esters in adults and copepodid V throughout the years. This ratio index becomes a good indicator for determination of copepodid stages.

Lee et al. (1972) investigated wax esters and triacylglycerols in different life stages of *Calanus helgolandicus*. Wax esters were not found in eggs, nauplii, and copepodid I, but increased from stage III to V. Lee et al. (1974) reported the lipid composition of *Euchaeta japonica* in different stages. Kattner and Krause (1987) reported the changes in lipid composition during the development of *Calanus finmarchicus s.l.* from copepodid I to adult. However, geographical, temporal and environmental factors will have a significant role in the results and must be taken into account when the method is applied to the determination of stage.

RNA:DNA ratio

A technique based on the ratio of RNA to DNA has been used to assess the relative growth rate of organisms without hard parts for age determination. The amount of DNA in any given species of organism is essentially constant and unaffected by environmental differences or nutritional conditions since DNA is the primary carrier of

genetic information (Lehninger 1975). The various forms of RNA changes as the rates of protein synthesis fluctuate during growth. Hence, the correlations between RNA/DNA ratio or RNA concentration and growth rate are found in a wide variety of organisms: e.g., hydrothermal-vent vestimentiferans (*Ridgeria piscesae* and *R. phaeophiale*) by Debevoise and Taghon (1988), fish by Buckley (1984), Bulow (1987), and some invertebrates by Sutcliffe (1970).

Lipofuscin and Age Pigment Assay

Lipofuscin or age pigment assay has been used as the age indicator (Ettershank 1983, 1984a, b, Ettershank et al. 1983, Lehane and Mail 1985, Mail et al. 1983, Sheldahl and Tappel 1974, Sohal 1981, Sohal and Donato 1978, 1979, Nicol 1987) for flies and euphasia. Ettershank (1983, 1984 a, b) used lipofuscin pigment fluorescence coupled with discriminant function analysis of 24 external morphological measurements of Antarctic krill (*Euphausia superba*) to separate a population of adult females into age classes. Nicol (1987) reported the limitations on use of the lipofuscin ageing techniques applied to copepod (*Calanus hyperboreus*), euphausiid (*Meganyctiphanes noevegica*), and squid (*Ilex illecebrosus*) due to the preservation medium. Formalin-preserved samples yield the highest fluorescence reading and increase with time spent in the preservative. Oguri (1986) applied the concentration of lipofuscin in interrenal cells to the age determiantion of anglerfish (*Lophius litulon*) and leopard shark (*Triakis scyllia*).

Electron Microprobe Analysis Technique

Cailliet and Radtke (1987) used this technique in sharks. A longitudinal thin section (about 1mm thick) was taken and scanned by electron microprobe to decide

calcium and phosphorus levels across the surface of the centrum. Cailliet and Radtke reported a distinct embryoic growth band followed by a peak in calcium and phosphorus associated with birth and first feeding. The elevated peaks are separated by reductive valleys representing the annual growth cycles.

Analysis of Precision of Age Determination

Estimates of the chronological age of fish depend upon the assumption that annual growth rings or annuli can be identified and counted in the age-structures. It is a general belief that fish that live in temperate or high latitudes are easier to age than tropical species. However, the current literature shows that errors in age determination prevail throughout the whole world. For young or juvenile fish of temperate species the appearance of an annulus may be very consistent and easier to interpret, but it becomes more complicated for mature fish. Thus, the problem in age determination is confounded by factors of human bias and fish physiology. The problems of validity and variation in age determination have been associated with age determination since the beginning of such studies. Fisheries scientists working on age determination have agreed to use precision to refer to the degree of repeatability (or reproducibility, agreement). Thus precision is related to the variability between readers, between readings, between age-structures, or between agencies, while accuracy relates to the degree of bias from the true age. This section will focus on the precison of age determination.

Many statistics have been used to compare the precision of age determination (e.g., Kimura et al. 1979, Campbell and Babaluk 1979). Since the difficulty of age determination increases with age, it becomes a general requirement that the statistic reflect this difficulty. The importance of this requirement has been illustrated by commonly used percent agreement statistics (Beamish and Fournier 1981). They point out that if 95% of age readings by two readers agree within ± 1yr for Pacific cod (*Gadus macrocephalus*), this is very poor precision since most commercial samples are comprised of only a few age classes (Note, Beamish and Fournier originally cited the scale age done by Kennedy (1970), but it is known that Pacific cod can be over 12-yrs old based on dorsal fin-ray age, see Lai (1985) and Lai, Gunderson, and Low (1987)).

However, if 95% of the age readings of spiny dogfish (*Squadus acanthias*) are within ± 5 yr, this can be an indication of good precision as dogfish may be as old as 60 yr with 30 age classes in the fishery. Thus, the use of a statistic is not independent of age would provide a better estimate of precison. In the following, we describe some statistics for measuring and testing the precision of age determination.

Chi-square Test

Suppose we have a sample of N fish aged by two readers (or two age-structures, two readings by the same reader, etc). The frequencies of these N fish are classified into k age classes as shown in the following table.

Reader	Age Class 1	2	...	k	Total
1	n_{11}	n_{12}	...	n_{1k}	$n_{1.}$
2	n_{21}	n_{22}	...	n_{2k}	$n_{2.}$
Total	$n_{.1}$	$n_{.2}$	...	$n_{.k}$	$n_{..}$

Then the chi-square test can be carried out as

$$\chi^2 = \sum_{i=1}^{2} \sum_{j=1}^{k} \frac{(n_{ij} - \frac{n_{i.}n_{.j}}{n_{..}})^2}{\frac{n_{i.}n_{.j}}{n_{..}}}$$

with (2-1)(k-1) degrees of freedom, $n_{..}=2N$, and $n_{1.}=n_{2.}=N$ for this example, but it can be expanded to m readers and $n_{1.} \neq n_{2.} \leq N$ when there are missing or unreadable otoliths. This method cannot reflect the difficulty of ageing as the number of age classes increase and is not valid when the number in any cell $n_{ij} < 5$.

Average Percent Error (APE)

The statistic used to measure the relative precision of age readings must be able to reflect the difficulty of ageing. Since the difficulty always increases with age, Beamish and Fournier (1981) proposed the average percent error (APE). Suppose that N fish are aged R times (i.e., R replicates). Let X_{ij} be the ith age reading for the jth fish, the average age based on R readings for the jth fish is

$$X_j = \frac{1}{R} \sum_{i=1}^{R} X_{ij}$$

Then, the average error (AE_j) in ageing the jth fish is a fraction of the average of the age estimates.

$$AE_j = \frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j}$$

The index of the average percent error is defined as

$$APE = \frac{1}{N} \sum_{j=1}^{N} \frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j} \times 100\%.$$

The problems of statistical inference about the APE , the probability distribution and test statistics, remain unaddressed. An example is shown in the next section.

Coefficient of Variation

Chang (1982) criticized the index of APE because the range of fish year-classes available to be aged increases in proportion to the average age of fish in the fishery, i.e., the standard deviation is proportional to the mean. With the same notation described in the previous section, the coefficient of variation is

$$V_j = \frac{1}{X_j}\sqrt{\frac{(\sum X_{ij} - X_j)^2}{R(R-1)}} \ .$$

The percent error contributed by each observation to the average age-class may be estimated by the index of precision (D_j) as

$$D_j = V_j/\sqrt{R}.$$

Then Chang proposed the average V and D for all fish aged as

$$V = \Sigma\, V_j / N \text{ and } D = \Sigma\, D_j / N.$$

Table 1 is the example used in Beamish and Fournier (1981) and Chang (1982). Reader 1 apparently has greater precision than Reader 2. However, there are several benefits of using V and D: (1) Variance is a better estimator than the absolute differences, as it is statistically unbiased and consistent. The estimated mean and variance converge to true estimators as sample size increases and the coefficient of variation shares these properties. (2) The index of precision can be used to show the percent error contributed by each observation to the average age determination for the jth fish. (3) The probability density function for V can be derived and tested as described in Zar (1974) and Sokal and Rohlf (1969), while the APE cannot. Therefore, for statistical purposes, one might choose V and D instead of APE.

Index of Variation and Regression Analysis

As mentioned in many studies, age determination is labor-intensive, time-consuming, and costly work. The problems associated with APE, V, and D are that these methods require at least three readings for each reader (or ageing method) and the represented sample size (which must be large) for the testing of fish if the population includes too many age classes. Also, these three statistics do not describe systematic

differences between two readings such as otolith vs. scale found in many species (Lai 1985). To overcome these problems, Lai (1985) developed the regression analysis and index of variation (IV).

The regression method used by Campbell and Babaluk (1979) compared the regression line of the two age readings against the 45° line through the origin. The fitted regression model is the full model, H_a: $Y = \beta_0 + \beta_1 X + e$, and the 45° diagonal line is the reduced model, H_0: $Y = X + e$, or $\beta_0 = 0$ and $\beta_1 = 1$. The generalized F-test (Weisberg 1980, Drapper and Smith 1980) was used to test systematic differences between the two age readings. If H_0 is rejected, there is a significant systematic error.

This test only shows the existence of a systematic difference between the two readings or, in other words, there are balances between positive and negative deviations of the two readings for all age classes. Nevertheless, this does not necessarily mean a high precision since the statistical test does not provide a description of the deviation of each of the two readings. High precision between two age readings must show a very "small" random error, i.e., the scattering plot of the two readings is not widely spreaded along the 45° diagonal line. In order to examine the degree of precision, an index of variation (IV) was calculated,

$$IV = 100\% \times \sqrt{\Sigma(Y - X)^2 \Big/ \frac{N-1}{2}(\bar{X}^2 + \bar{Y}^2)}$$

where, Y: tested age as dependent variable,

X: reference age as independent variable,

N: sample size, and

$\bar{X}$, $\bar{Y}$: mean ages of X and Y.

The statistic of IV is the residual mean square from fitting $Y = X$ to the data, and divided by the mean of the two estimated mean ages, $\bar{X}$ and $\bar{Y}$. Therefore, the value of IV is that it indicates the degree of variation of the two ages being compared and

measures the relative precision of the two age readings by taking account the distribution of ages in the sample.

If X represents the known (true) age of fish, the accuracy of reading can be measured by the coefficient of variation (c.v.) as

$$C.V. = \sqrt{\Sigma(Y - X)^2 / (N-1)\ \bar{X}^2}\ .$$

An example using Pacific cod in Lai (1985) illustrates this method. Table 2 shows that there are no systematic differences within the reader using coracoids, otoliths and dorsal-fin rays, but there is in the pectoral-fin rays and scales. The value of IV is 14%, 13%, 15%, respectively, for the coracoids, otoliths, and dorsal-fin rays. Therefore, with the validation and other precison tests, Lai et al. (1987) suggested that dorsal -fin ray method is the most suitable ageing method for Pacific cod.

Test of Percent Agreement by Log-linear Model

A statistical test for percent agreement by using a normal approximation (Snedecor and Cochran 1967, Fleiss 1977) can be employed but it is considered inappropriate because age classes may act as an important factor in the measurement of agreement. Therefore, Lai (1985) and Lai, Gunderson, and Low (1987) developed a log-linear model method to test the dependence of agreement (repeatability) upon the ageing methods and age class. For this statistical test, two readings on each fish by different ageing methods are required, but independent samples for each of the different ageing methods are OK.

The data from Lai et al. (1987) were used to illustrate the method. Table 3 lists the percent agreement by age class and by ageing method. In this study there are three factors, ageing method (M), age class (A), and repeatability (R). The sampling

model is product-multinomial, using the terminology of Fienberg (1981, Sec. 3-2), since the number of fish being aged is fixed for each ageing method after deleting unreadable or damaged age-structures. The aged fish were cross-classified into corresponding cells denoted by factors A and R.

Let y_{ijk} be the observed cell frequency for the ith ageing method, the jth row of age class. and the kth column of repeatability, and let m_{ijk} be the expected value of y_{ijk}. The general log-linear model (called the saturated model because it includes the highest three-factor interaction) for our three-way (5x8x2) contingency table is

$$\theta_{ijk} = \log(m_{ijk}) = \mu + \overset{M}{\lambda_i} + \overset{A}{\lambda_j} + \overset{R}{\lambda_k} + \overset{MR}{\lambda_{ik}} + \overset{AR}{\lambda_{jk}} + \overset{MA}{\lambda_{ij}} + \overset{MAR}{\lambda_{ijk}} \qquad (1)$$

where, as in the usual analysis of variance model, all effects sum to zero over any subscript.

Let θ be the marginal mean of θ_{ijk} over the subscript which is replaced by "+" to indicate averaging, then the parameters in (1) can be written as

$$\mu = \theta_{+++} \qquad \lambda_{ij} = \theta_{ij+} - \theta_{i++} - \theta_{+j+} + \theta_{+++}$$

$$\lambda_i = \theta_{i++} - \theta_{+++} \qquad \lambda_{ik} = \theta_{i+k} - \theta_{i++} - \theta_{++k} + \theta_{+++}$$

$$\lambda_j = \theta_{+j+} - \theta_{+++} \qquad \lambda_{jk} = \theta_{+jk} - \theta_{+j+} - \theta_{++k} + \theta_{+++}$$

$$\lambda_k = \theta_{++k} - \theta_{+++} \qquad \lambda_{ijk} = \theta_{ijk} - \theta_{ij+} - \theta_{i+k} + \theta_{i++}$$

$$- \theta_{+jk} + \theta_{+j+} + \theta_{++k} - \theta_{+++} .$$

Log-linear models are "hierachical", i.e., higher-order interaction terms can be included only if related lower-order terms are included. For example, λ^{MAR} is not included unless λ^{AR}, λ^{MR}, λ^{MA}, λ^{M}, λ^{A}, and λ^{R} are all included.

Once all expected cell frequencies (m'_{ijk}) are estimated, the goodness-of-fit for the selected model can be tested using the likelihood ratio test statistic

$$G^2 = 2 \sum_i \sum_j \sum_k y_{ijk} \log\left(\frac{y_{ijk}}{m_{ijk}}\right)$$

which has approximately a χ^2-distribution with degrees of freedom (df) = number of cells – number of parameters (Fienberg 1981, Sec. 3-3 and 3-4). The results from log-linear model for the data in Table 3 are listed in Table 4.

Using the partition property of G^2, we can decide whether an effect or an interaction should be included. In Table 3, for example, H_o: $\lambda^{MAR}=0$ can be tested by examining $G^2 = 28.08 - 0.00 = 28.08$. This is not significant at the 1% level (referred to a χ^2-distribution with df=28). Similarly, $G^2 = 20.79$ for H_o: $\lambda^{MA}=0$, which exceeds the upper 1% tail value of a χ^2-distribution with df=4, and is rejected. This means that λ^{MAR} will not be included in the model but λ^{MA} will. Hence, our best log-linear model is

$$\theta_{ijk} = \log(m_{ijk}) = \mu + \overset{M}{\lambda_i} + \overset{A}{\lambda_j} + \overset{R}{\lambda_k} + \overset{MR}{\lambda_{ik}} + \overset{AR}{\lambda_{jk}} + \overset{MA}{\lambda_{ij}} \qquad (2)$$

which implies that percent agreement was correlated with the age class and ageing method, and the estimated age frequencies differed because of the ageing method. However, these pairwise relationships between any two factors are unrelated to the third.

Because we are interested in the effects of the ageing method and age class on repeatability, it is reasonable to look at the ratio between agreement (k=1) and disagreement (k=2) for each combination of ageing method and age class, i.e., m_{ij1}/m_{ij2} for all i and j. The logarithm of this ratio is known as the logit model (Fienberg 1981, Chapter 6). The logit model for Equation (2) can be derived as

$$\text{logit}(i,j) = \log\left(m_{ij1}/m_{ij2}\right) = \omega + \omega_i^M + \omega_j^A \qquad (3)$$

where, the logit effects are:

$$\omega = (\lambda^{R}_{1} - \lambda^{R}_{2}), \quad \omega^{M}_{i} = (\lambda^{MR}_{i1} - \lambda^{MR}_{i2}), \text{ and } \omega^{A}_{j} = (\lambda^{AR}_{j1} - \lambda^{AR}_{j2}).$$

The effects (λ's) without factor R in Equation (2) are cancelled out by subtraction in Equation (3). The values of λ's can be obtained from the BMDP program and substituded into (3). The results show that there was a significant decreasing effect on agreement as age increased (Table 5). This indicated that percent agreement decreased with increasing age. Age determination using coracoids and dorsal-fin rays had a positive effect on agreement, which indicated that agreement of these methods was higher than the average of the five methods, but the other ageing methods had a negative effect, ie., agreement was lower than average.

ANOVA with Repeated Measurements

Suppose that at least two age readings are carried out by different ageing methods (or probably also by different readers) for total of N fish. This becomes an analysis of variance using a factorial experiment design with repeated measures on the subjects; that is, a single subject which is repeatedly measured by various methods. The ageing methods or readers are termed "within-subject factor" or "trial factor". The data from Lai et al. (1987) are used to illustrate this method.

A sample of 200 Pacific cod was aged twice by otolith, dorsal-fin ray, pectoral-fin ray, and scale. The ANOVA model was

$$X_{ijn} = \mu + \pi_n + M_i + (M\pi)_{in} + R_j + (R\pi)_{jn} + (MR\pi)_{ijn} + \varepsilon_{ijn}$$

where, X_{ijn} is the observed age of the nth fish by the ith ageing method and jth reading, and i=1,2,3,4 indicates ageing methods,

j=1,2 indicates the first and second age readings,

n=1,...,N indicates number of fish,

μ is the grand mean,

π is the effect between subjects (individual fish),

M is the effect of ageing method,

R is the effect of reading,

($M\pi$), ($R\pi$), and ($MR\pi$) are the two- and three-factor interactions of effects M, R, and π, and

ε is the random error.

The summary of this ANOVA model is shown in Table 6. The Q-statistic (Snedecor and Cochran 1967) was used to test the differences between the mean ages between readings and between ageing methods.

Table 6 shows that the effect of ageing method was significant for all fish older than age 3. There was no significant difference (5% level) between readings except for ages 5-6. This difference probably resulted from differences between readings for otoliths and pectoral-fin rays (Table 6). Mean square error (MSE) for the ageing method effect increased with age and was the predominant component in the within-subject variation for all age categories. Therefore, variability in age determination was mainly due to the ageing method rather than inconsistent annulus interpretation by the reader.

Using the Q-statistic, the mean ages of the two readings were not significantly different except for age group 5-6 using otolith and pectoral-fin ray ageing methods (Table 7). Significant differences between ageing methods were found in all age categories except the youngest. Age readings from dorsal-fin rays and pectoral-fin rays were not significantly different for fish younger than age 6. Age readings from otoliths and pectoral-fin rays were not significantly different for fish older than age 7. Otolith readings were older than other methods for fish younger than age 6 but were younger

than dorsal-fin ray readings for fish older than age 7. Scale readings gave consistently younger ages than the other methods.

Table 1. (From Chang 1982)

Example of a set of walleye pollock (*Theragra chalcogramma*) ages using the fin-ray method (Beamish and Fournier 1981).[a]

Fish No.	Reader 1						Reader 2					
	1st	2nd	3rd	APE	*V*	*D*	1st	2nd	3rd	APE	*V*	*D*
1	7	6	7	0.0667	0.0866	0.0501	6	5	6	0.0784	0.1019	0.0589
2	7	6	6	0.0717	0.0912	0.0527	6	5	6	0.0784	0.1019	0.0589
3	6	6	5	0.0784	0.1019	0.0589	5	4	6	0.1333	0.2000	0.1156
4	4	4	4	0.0	0.0	0.0	4	4	3	0.1212	0.1575	0.0910
5	4	5	6	0.1333	0.20	0.1156	6	5	5	0.0833	0.1083	0.0626
6	3	3	3	0.0	0.0	0.0	3	3	2	0.1667	0.2165	0.1251
7	5	5	5	0.0	0.0	0.0	5	4	5	0.0952	0.1237	0.0715
8	4	4	4	0.0	0.0	0.0	5	4	4	0.1026	0.1332	0.0770
9	4	4	4	0.0	0.0	0.0	4	3	3	0.1332	0.1732	0.1001
10	7	8	7	0.0606	0.0787	0.0455	6	5	7	0.1111	0.1667	0.0963
11	7	7	7	0.0	0.0	0.0	7	5	6	0.1111	0.1667	0.0963
12	3	2	3	0.1667	0.2165	0.1251	3	2	3	0.1667	0.2165	0.1251
13	5	5	5	0.0	0.0	0.0	5	4	4	0.1026	0.1332	0.0770
14	4	4	4	0.0	0.0	0.0	5	2	4	0.3030	0.4166	0.2408
15	6	6	4	0.1667	0.2165	0.1251	6	4	5	0.1333	0.2000	0.1156
16	7	7	7	0.0	0.0	0.0	5	6	5	0.0833	0.1083	0.0626
17	6	6	5	0.0784	0.1019	0.0589	5	5	5	0.0	0.0	0.0
18	7	7	7	0.0	0.0	0.0	7	6	6	0.0702	0.0912	0.0527
19	8	7	7	0.0606	0.0787	0.0455	7	5	5	0.1569	0.2038	0.1178
20	5	5	5	0.0	0.0	0.0	5	4	5	0.0952	0.1237	0.0715
$\bar{Y}$				0.0441	0.0586	0.0339				0.1163	0.1571	0.0983

[a]Where APE is the average percent error using the index of Beamish and Fournier; *V* is the coefficient of variation; and *D* is an index of precision.

Table 2. Comparison of age readings made by Reader-1 using various aging methods.

	Coracoid			Otolith break-and-burn			Dorsal fin ray			Pectoral fin ray			Scale		
	M	F	C	M	F	C	M	F	C	M	F	C	M	F	C
Intercept	.3980	.4478	.4162	.7766	.4035	.5942	.3493	.3886	.3744	.9736	.7462	.8491	.8397	.7987	.8726
Slope	.9194	.9237	.9233	.8763	.9657	.9202	.9567	.9371	.9456	.8277	.8928	.8631	.8326	.7988	.8024
F (Y=X)	1.733	1.277	2.823	4.107	3.385	4.978	.911	1.630	2.147	6.276*	4.270	9.399*	6.555*	12.75**	16.24**
R^2	.91	.88	.89	.83	.82	.82	.84	.87	.85	.75	.79	.77	.70	.72	.70
IV	12.1	13.2	12.8	13.7	15.2	14.5	14.4	12.3	13.3	16.3	14.8	15.6	15.7	14.9	15.3
% agreement	63.04	62.26	62.63	55.96	57.14	56.58	65.21	70.37	67.96	54.00	56.2	55.19	60.19	64.10	62.22
% agree ≤5	81.82	63.64	72.73	68.29	71.15	69.89	83.66	76.92	80.00	72.34	68.00	70.10	64.52	73.91	69.19
% agree 6-10	47.83	60.00	54.72	46.97	45.31	46.15	58.62	69.23	64.23	38.46	48.33	43.75	33.33	28.00	30.00
% agree ≥11	0	100.00	50.00	100.00	66.67	80.00	0	25.00	12.50	0	0	0	-	-	-
% ± 1	81.52	77.36	78.79	73.85	74.37	74.12	76.53	82.41	79.37	71.50	74.11	72.64	80.56	81.20	80.00

*: significant at 5% level; **: significant at 1% level

M: male; F: female; C: combined

X: reference age; Y: test age

Table 3. (From Lai et al 1987)

Observed frequency of agreement between two age readings using different ageing methods. Percent agreement is shown in parentheses.

Method (*M*)	Age (*A*)	Repeatability (*R*)		
		Agree	Disagree	Total
Coracoid	under 2	9 (100)	0	9
	3	1 (50)	1	2
	4	10 (77)	3	13
	5	12 (60)	8	20
	6	18 (67)	9	27
	7	5 (45)	6	11
	8	3 (43)	4	7
	over 9	4 (40)	6	10
Total		62 (63)	37	99
Break-and-burn	under 2	9 (90)	1	10
	3	7 (64)	4	11
	4	8 (73)	3	11
	5	41 (67)	20	61
	6	35 (58)	25	60
	7	14 (39)	22	36
	8	6 (35)	11	17
	over 9	9 (41)	13	22
Total		129 (57)	99	228
Dorsal fin ray	under 2	9 (100)	0	9
	3	2 (67)	1	3
	4	13 (81)	3	16
	5	36 (77)	11	47
	6	40 (82)	9	49
	7	24 (69)	11	35
	8	9 (39)	14	23
	over 9	7 (29)	17	24
Total		140 (68)	66	206
Pectoral fin ray	under 2	8 (100)	0	8
	3	6 (75)	2	8
	4	18 (64)	10	28
	5	36 (68)	17	53
	6	24 (45)	29	53
	7	12 (43)	16	28
	8	10 (45)	12	22
	over 9	3 (25)	9	12
Total		117 (55)	95	212
Scale	under 2	9 (82)	2	11
	3	11 (61)	7	18
	4	60 (74)	21	81
	5	48 (64)	27	75
	6	11 (38)	18	29
	7	1 (11)	8	9
	8	0 (0)	1	1
	over 9	0 (0)	1	1
Total		140 (62)	85	225

Table 4. (From Lai et al, 1987)

Test for the independence of percent agreement (R) of age readings correlated to age-class (A) and ageing method (M).

Model	df	G^2	Probability
$\mu + \lambda_i^M + \lambda_j^A + \lambda_k^R + \lambda_{ik}^{MR} + \lambda_{jk}^{AR} + \lambda_{ij}^{MA} + \lambda_{ijk}^{MAR}$	0	0.00	1.0000
$\mu + \lambda_i^M + \lambda_j^A + \lambda_k^R + \lambda_{ik}^{MR} + \lambda_{jk}^{AR} + \lambda_{ij}^{MA}$	28	28.08	0.4603
$\mu + \lambda_i^M + \lambda_j^A + \lambda_k^R + \lambda_{ik}^{MR} + \lambda_{jk}^{AR}$	32	48.87	0.0285
$\mu + \lambda_i^M + \lambda_j^A + \lambda_k^R + \lambda_{jk}^{AR} + \lambda_{ij}^{MA}$	56	241.75	0.0000
$\mu + \lambda_i^M + \lambda_j^A + \lambda_k^R + \lambda_{ik}^{MR} + \lambda_{ij}^{MA}$	35	125.03	0.0000

Table 5. (From Lai et al 1987)

Estimated logit effects corresponding to the loglinear model in Equation (2).

Factor		Logit effect
constant (ω)		0.540
Age (ω_i^A)	≤2	2.204
	age 3	0.240
	age 4	0.676
	age 5	0.278
	age 6	−0.196
	age 7	−0.738
	age 8	−1.064
	over 9	−1.400
Age method (ω_j^M)	Dorsal fin ray	0.568
	Coracoid	0.096
	Otolith	−0.058
	Pectoral fin ray	−0.208
	Scale	−0.398

Table 6. (From Lai et al 1987)

Comparison of ageing variability of Pacific cod by ANOVA.

Dorsal fin-ray age	N		Between subject (π)	Within subject					
				Method (M)	$M\pi$	Reading (R)	$R\pi$	MR	$MR\pi$
1-2	8	SS	14.359	0.297	2.578	0.016	0.109	0.047	1.328
		df	7	3	21	1	7	3	21
		MS	2.051	0.099	0.123	0.016	0.016	0.016	0.063
		F		0.81		1.00		0.25	
3-4	19	SS	52.395	8.967	38.658	0.164	3.711	0.072	7.553
		df	18	3	54	1	18	3	54
		MS	2.911	2.989	0.716	0.164	0.206	0.024	0.140
		F		4.18**		0.80		0.17	
5-6	93	SS	270.495	166.154	273.721	1.840	26.285	1.122	74.253
		df	92	3	276	1	92	3	276
		MS	2.940	55.385	0.992	1.840	0.286	0.374	0.269
		F		55.85**		6.44**		1.39	
7-8	57	SS	217.244	344.018	267.232	0.219	26.031	0.921	56.829
		df	56	3	168	1	56	3	168
		MS	3.879	114.673	1.591	0.219	0.465	0.307	0.338
		F		72.09**		0.47		0.91	
9+	23	SS	339.457	498.283	198.717	0.035	21.652	1.869	53.130
		df	22	3	66	1	22	3	66
		MS	15.430	166.090	3.011	0.348	0.984	0.623	0.805
		F		55.16**		0.35		0.77	

** = significant at 1% level.

Table 7. (From Lai et al 1987)

...—Tests for differences between mean ages of Pacific cod using various ageing methods. Bracket and underline: not significantly different at 5% level.

Dorsal fin-ray age	N	Reading	Dorsal fin ray	Otolith	Pectoral fin ray	Scale	SD	df
1-2	8	1	1.375	1.375	1.375	1.250	0.063	7
		2	1.375	1.475	1.375	1.250		
	Method mean		1.375	1.425	1.375	1.250	0.124	21
3-4	19	1	3.842	4.211	3.842	3.579	0.147	18
		2	3.847	4.315	3.895	3.579		
	Method mean		3.895	4.263	3.869	3.579	0.194	54
5-6	93	1	5.505	5.774	5.430	4.581	0.078	92
		2	5.588	5.979	5.591	4.570		
	Method mean		5.547	5.877	5.511	4.576	0.103	276
7-8	57	1	7.386	6.561	6.614	5.000	0.128	56
		2	7.316	6.702	6.737	4.982		
	Method mean		7.351	6.632	6.676	4.991	0.167	168
9+	23	1	10.130	8.000	8.304	5.522	0.293	22
		2	9.913	8.261	8.130	5.304		
	Method mean		10.022	8.131	8.217	5.413	0.362	66

Growth Curves and Age-Length Key

Fitting and Testing the Growth Curves

One of the many uses of ageing data is the estimation of growth curves. Although there are many growth models in scientific research, the von Bertalanffy growth model is the most widely used one since the model fits many populations (Beverton amd Holt 1958, Allen 1971).

Prior to the availability of computers, the von Bertalanffy growth curve was traditionally fitted by the Walford plot (Gulland 1969, Ricker 1975). However, recent studies show that a non-linear regression produces better length-at-age data (Gallucci and Quinn 1979, Vaughan and Kanciruk 1987, Sandberg 1984). Also, statistical tests on growth curves are more theoretically sound than those based on the linear regression associated with a Walford plot (Allen 1962). Therefore, the discussion in this section is on the non-linear estimation of the von Bertalanffy growth curve.

The derivation of the von Bertalanffy growth model can be referred to in von Bertalanffy (1948), Beverton and Holt (1958), and Gallucci and Quinn (1979). More versatile models based upon the von Bertalanffy model can be found in Chapman (1968) and Schnute (1983). The von Bertalanffy growth model is written as

$$l_t = L_\infty \left(1 - e^{-K(t-t_0)}\right) \qquad (1)$$

where l_t is the length of a fish at age t (or mean-length for the individuals of age t), L_∞ is the asymptotic size, generally the maximum size an animal can reach, K is the Brody growth coefficient which describes the curvature of a growth curve, and t_0 is the age at zero length which in fact is an adjustment factor.

Assuming that N fish have been sampled for age determination and classified into A age classes of N_t fish, for t=1,..., A. The simplest analysis for this age data is to compute the mean length-at-age, $\bar{l}_t$, and variance s_t^2 for each age class. Then the Walford analysis (ricker 1975, Gulland 1969) is applied.

Fish, however, do not grow at constant rates either throughout their life history nor are rates between individuals the same. Therefore a stochastic von Bertalanffy growth model is written as

$$\bar{l}_t = L_\infty (1-e^{-K(t-t_o)}) + e_t$$

or

$$l_{ti} = L_\infty (1-e^{-K(t_i-t_o)}) + e_{ti}$$

where e is the random error and i = 1,2,...,N.

A non-linear least squares method (Gallucci and Quinn 1979) or maximum likelihood method (Kimura 1979) are the commonly used methods for parameter estimation. The difference between the two methods in the stochastic model is the assumption of random error. The maximum likelihood method requires an underlying assumption on the probability density distribution of e, frequently a normal distribution (denoted by N), but the least squares method does not. If $e \sim N(0,s^2)$, then the maximum likelihood method is reduced to a least square method (Kimura 1979). The least squares estimation is to find a set of parameters L_∞, K and to minimize the sum of squares corresponding to the previous stochastic models:

$$\sum_t (\bar{l}_t - E(\bar{l}_t))^2 \quad \text{or} \quad \sum_{ti} (l_{ti} - E(l_{ti}))^2$$

where $E(\bar{l}_t)$ and $E(l_{ti})$ are the expected prediction of length-at-age. However, these objective functions are based on the assumptions that

$$e_t \sim (0, s^2)$$

or

$$e_{ti} \sim (0, s^2)$$

i.e., constant variance over ages or individuals, and the sample size for all age groups N_t are equally represented in the population. If this is not the case, weighted least squares must be applied. For example, when $\bar{I}_t$ is used and its variance (s_t^2) varies with age t, and sample size N_t provides the information to compute $\bar{I}_t$, then the weighted least square function (Kimura 1979) is

$$\sum \left(\frac{N_t}{s_t^2}\right) \left(\bar{I}_t - E(I_t)\right)^2.$$

In fact, one can apply any value as a weighing factor w_t or w_{ti} to minimize the sum of squares

$$\sum_t W_t \left(\bar{I}_t - E(I_t)\right)^2 \quad \text{or} \quad \sum_{ti} W_{ti} \left(\bar{I}_t - E(I_t)\right)^2$$

where W_t or W_{ti} indicates the relative importance of $\bar{I}_t$ or I_{ti} in projecting a growth curve.

The commonly used non-linear least squares methods are numerical iteration based on Gauss-Newton algorithm (e.g., Marquardt 1963). These methods normally produce a by-product, an estimate of the large-sample covariance matrix which is the inverse of the "information matrix" and is evaluated at the convergence value of the LS estimate (Ratkowsky 1983, Bard 1974). The elements of the "information matrix" are simply

$$\frac{1}{s^2} \sum_{t_1 i} \frac{\partial f}{\partial Q_j} \frac{\partial f}{\partial Q_k}$$

where f is the von Bertalanffy growth model, Q represents the growth parameters, and s^2 is the estimated residual variance. Schnute (1983) proposed using Nelder and Mead's (1965) Simplex, a direct search method. However, the algorithm does not estimate the variance and covariance of the parameters.

Many statistical tests between growth curves have been proposed in the past, however, only two frequently used methods are summarized. The reader should see the original papers for further details.

1. W-method (Gallucci and Quinn 1979)

Gallucci and Quinn suggested the reparameterization of the von Bertalanffy growth model into

$$l_t = \frac{W}{K}\left(1-e^{-K(t-t_0)}\right)$$

where $W = KL_\infty$, which is the slope of the growth curve at t=0, i.e., the growth rate at the birth of an animal. The test of equality of W between two growth curves can be a c^2 -test of homogeneity for large samples or a t-test for a small samples. Note that W -test does not require an assumption about random errors.

2. Likelihood ratio test (Kimura 1980)

When random error $e_t \sim N(0,s^2)$, the maximum likelihood estimator can be obtained by maximizing the likelihood function

$$L = -\frac{N}{2}\ln(2ps^2) - \frac{1}{2s^2}S(L_\infty,K,t_0) \qquad (2)$$

where $S(L_\infty,K,t_0)$ is the sum of squares for the growth model. Taking $\frac{\partial L}{\partial s^2} = 0$ and $s^2 = \frac{S(L_\infty,K,t_0)}{N}$, the maximum likelihood method is reduced to a least squares

method. Suppose that we have two growth curves to be compared. The likelihood ratio test procedure is: (1) to fit the individual curve with separate parameter sets,

$$(L_{\infty 1}, K_1, t_{o_1}) \text{ and } (L_{\infty 2}, K_2, t_{o_2}).$$

and compute the likelihood values Φ_1 and Φ_2 using (2).

(2) If the hypothesis is $H_o: L_{\infty 1} = L_{\infty 2} = L_{\infty}$, then it fits the growth curve to the combination of two data sets with $(L_{\infty}, K_1, K_2, t_{o_1}, t_{o_2})$ and computes the combined likelihood value Φ.

(3) The likelihood ratio statistics are $T = \Phi - (\Phi_1 + \Phi_2)$. The statistic (-2T) has an asymptotic chi-square distribution (Dixon 1983) with degrees of freedom given by the difference in the number of estimated parameters between two hypothetical models (1) and (2). In this case df = 1.

Estimation of Age Composition and Associated Sampling Designs

Age-length key (ALK)

The most direct method to estimate age composition is probably a simple random sampling (SRS) technique. In other words, collect a large number of fish samples and determine their ages. The problem is that age determination is costly and labor-intensive. The accuracy of age composition from SRS is a great concern (Kimura 1977). Because of the difficulty and expense of age determination, a double sampling technique was developed: the first stage of sampling is to collect a large amount of fish for a length frequency distribution (called the length sample); the second stage is to subsample a small number of fish from each length stratum for age determination (called age subsample), which is very costly. After determining the

age of fish in age subsamples, the data are organized as a contingency table by age vs. length, i.e., an age-length key. Fredrikssen (1933) first developed this subsampling estimation. Tanaka (1953) applied the theory of a double sampling technique to derive the age-length key method that has been widely used in fisheries.

The proportion of fish at age i is estimated as $\hat{p}_i = \sum_{j=1}^{L} \hat{l}_j \hat{q}_{ij}$, where l_j is the proportion of fish in the jth length stratum, q_{ij} is the proportion of fish from the jth length stratum belonging to the ith age, and L is the number of length strata. The variance of estimated $\hat{p}_i$ is approximated by

$$Var(\hat{p}_i) = \sum_{j=1}^{L} \left[\frac{\hat{l}_j^2 \hat{q}_{ij}(1 - \hat{q}_{ij})}{n_j} + \frac{\hat{l}_j (\hat{q}_{ij} - p_i)^2}{N} \right]$$

Fisheries scientists frequently use two different subsampling schemes to obtain age-length data: 1) fixed age subsampling, in which the size of age subsamples in all samples and length strata is constant. That is, $n_j = n/L$ where $n = \sum_j n_j$ is the size of the total number of age subsamples, 2) random age subsampling, in which the size of the age subsample in each length stratum is proportional to the length sample size for all length strata. That is, $n_j = nl_j$.

To carry out optimum sampling, the precision of the estimated age composition over the entire age-length key is necessary. Kimura's (1977) Vartot is an appropriate error index defined as

$$Vartot = \sum_{i=1}^{A} Var(\hat{p}i) = E\left[\sum_{i=1}^{A} (\hat{p}_i - p_i)^2 \right]$$

Actually, $\sum_{i=1}^{A} (\hat{p}_i - p_i)^2 = (\hat{p} - p)'(\hat{p} - p)$ is the squared Euclidian distance between the points of true and estimated A-dimensional (A age classes) vectors, p and $\hat{p}$. This statistic is adequate for optimum sampling since the more accurate the estimated $\hat{p}$ the closer the two points, i.e., $(\hat{p} - p) \rightarrow 0$.

After algebraic manipulation (see Lai 1987), the Vartot for fixed and random age subsamplings can be expressed as

$$\text{Vartot} = \frac{a_1}{n} + \frac{a_2}{N}, \text{ for fixed age subsample}$$

and

$$\text{Vartot} = \frac{b_1}{n} + \frac{b_2}{N}, \text{ for random age subsample.} \qquad (3)$$

where $a_1 = \sum_{i=1}^{A} \sum_{j=1}^{L} [\hat{l}_j^2 \hat{q}_{ij}(1 - \hat{q}_{ij})]$

$$b_1 = \sum_i \sum_j [\hat{l}_j \hat{q}_{ij} (1-\hat{q}_{ij})]$$

$$a_2 = b_2 = \sum_i \sum_j [\hat{l}_j (\hat{q}_{ij} - \hat{p}_i)^2]$$

For an optimum allocation of n and N, we need a cost function which for simplicity we assume is a linear function of n and N. Let c_1 be the unit cost of observing the length of a fish, and c_2 be the unit cost of ageing a fish. The total cost for an age-length key, C, can be written as

$$C = c_1 N + c_2 n.$$

For an optimum sampling design in an ALK, we can either (i) find optimum values of n and N (denoted by n^* and N^*) to minimize the Vartot with a given amount of total cost C, or (ii) find n^* and N^* to minimize the total cost C with a specified

level of Vartot. These two choices can be carried out by the method of the Cauchy-Schawz inequality (Cochran1977, Kendal and Stuart 1977). To apply this method, we first write the product of (Vartot)(C) as:

$$(\text{Vartot})(C) = \left(\frac{a_1}{n} + \frac{a_2}{N}\right)(c_2 n + C_1 N), \text{ for a fixed age subsample.}$$

or

$$= \left(\frac{b_1}{n} + \frac{b_2}{N}\right)(c_2 n + C_1 N), \text{ for a random age subsample.} \qquad (4)$$

The optimum n^* and N^* can be obtained when they minimize the product value of Vartot and C. Following the algebra of the Cauchy-Schwarz inequality, the two equations in (4) can be represented as

$$\left(\frac{a_1}{n} + \frac{a_2}{N}\right)(c_2 n + C_1 N) \geq (\sqrt{a_1 c_1} + \sqrt{a_2 c_2})^2, \text{ for a fixed age subsample}$$

or

$$\left(\frac{b_1}{n} + \frac{b_2}{N}\right)(c_2 n + C_1 N) \geq (\sqrt{b_1 c_1} + \sqrt{b_2 c_2})^2, \text{ for a random age subsample.}$$

According to these equations, the lower bound of (Vartot)(C) is restricted by the RHS, i.e., the product (Vartot)(C) will be minimized when the equality of these equations holds. Expanding both sides and following some algebraic manipulations, Lai(1987) derived the optimum subsampling ratio:

$$r^* = \left(\frac{n^*}{N^*}\right) = \sqrt{\frac{a_1 c_1}{a_2 c_2}} \qquad \text{for fixed age subsampling.}$$

$$r^* = \left(\frac{n^*}{N^*}\right) = \sqrt{\frac{b_1 c_1}{b_2 c_2}} \qquad \text{for random age subsampling.}$$

The optimum subsampling ratio is the same for the two choices but the optimum set of n^* and N^* is dependent on the constraints specified in the two choices.

For choice (i) in which total cost is given, r^* is substituted into the cost function to obtain optimum size of the length sample:

$$N^* = \frac{C}{c_1 + c_2 r^*}$$

and subsequently, the optimum size of the age subsample is:

$$n^* = r^* N^*.$$

The minimum Vartot that can be reached based upon the given total cost is

$$\text{min. Vartot} = \frac{a_1}{n^*} + \frac{a_2}{N} \quad \text{for fixed age subsampling}$$

$$\text{min. Vartot} = \frac{b_1}{n^*} + \frac{b_2}{N} \quad \text{for random age subsampling}$$

For the choice (ii) in which a desired Vartot is preset, r^* is substituted into equation (3) of Vartot to obtain the optimum size of the length sample:

$$N^* = \frac{\left(\frac{a_1}{r^*} + a_2\right)}{\text{Vartot}} \quad \text{for fixed age subsampling}$$

and

$$N^* = \frac{\left(\frac{b_1}{r^*} + b_2\right)}{\text{Vartot}} \quad \text{for random age subsampling}$$

The optimum size of the age subsample is

$$n^* = r^* N^*.$$

The corresponding minimum total cost required to reach this preset Vartot is

$$\text{min } C = c_1 N^* + c_2 n^*$$

for either fixed or random age subsampling.

The necessary condition for optimum sampling is $0< r \leq 1$. Therefore,

$$\frac{c_1}{c_2} \leq \frac{a_2}{a_1} \qquad \text{for fixed age subsampling}$$

$$\frac{c_1}{c_2} \leq \frac{b_2}{b_1} \qquad \text{for random age subsampling}$$

must be held for the optimum sampling design. When equality holds, r=1 and the ALK degenerates to a simple random sampling for age samples.

In practice, we need a pilot sampling program or prior information for an age-length key to estimate the parameters a_1, a_2, b_1, and b_2. The results of Lai(1987) and others (e.g., Kimura 1977) are: (i) random age subsampling is superior to fixed age subsampling and also to simple random age sampling based on Vartot (Kimura 1977) and, (ii) keep the size of the length interval as small as possible (Tanaka 1953), but not so small that too many strata have small sample sizes that are too small.

Figure 23 shows the relationship of $D=\sqrt{Vartot}$ and total cost for three species, Pacific Cod, pollock and sablefish (Lai 1987). Whether a fixed or a random age subsample is used for the three species, it is obvious that D decreases rapidly until C=10,000 minutes, which is almost 70 working days. A point of diminishing returns is reached beyond this total cost and the curves become flatter for C greater than 10,000. These results indicate that setting precision at D=0.02, 0.025, and 0.03 respectively for Pacific cod, walleye pollock and sablefish, and using random age subsamples would represent a reasonable compromise between cost and precision. Increasing the total cost beyond this level will yield little or nothing from the ALK.

Alternative methods

The double sampling ALK decribed previously is based on: (i) two samples, length and age, come from the same population, and (ii) growth rates, gear selectivities, and age composition do not vary between the two samples. Westrheim and Ricker (1978) identified these serious limitations and illustrated that bias can arise if the conditions are not satisfied.

Clark (1981) and Bartoo and Parker (1983) made an important advance in technique by allowing age composition among samples to be non-constant. They imposed the "inverse age-length key" in which $\sum_{j=1}^{L} q_{ij}=1$ rather than $\sum_{i=1}^{A} q'_{ij}=1$ as in the ordinary age-length key. In this section, q_{ij} is the probability of an age i fish belongs to the jth length category. Reader must be aware that q_{ij} defined in this section is actually equivalent to that in the analysis of mixture distributions (e.g., Macdonald and Pitcher 1979). By such a formulation, the length frequency composition is the linear transform of age composition. In linear algebra terms

$$[l_j] = [q_{ij}]^T[p_i], \quad \text{or } \mathbf{l}=\mathbf{Q}^T\mathbf{p},$$

where l_j and p_i are specified as in the age-length key and T indicates the transform operation of a matrix. **Q** is called inverse age-length key. Bartoo and Parker (1983) solved this system of equations by ordinary least squares, while Clark (1981) used a restricted least squares technique in which each proportion is restricted to a non-negative value of p_i and $\Sigma p_i=1$. Both of these approaches assume that $[q_{ij}]$ is known although Clark later extended this to unknown $[q_{ij}]$ by using normally distributed length frequencies at age i, i.e., $\sum_{j=1}^{L} q_{ij}=1$.

The only uncertainty (random error) entering is through the observation of $[l_j]$. However, $[q_{ij}]$ is normally based on a relatively small sample size and is

subject to a higher uncertainty. The variance of the estimated $[p_i]$ based on regression analysis is not appropriate (note that neither Bartoo and Parker, nor Clark suggest a procedure to estimate of the variance).

A new development for estimating age distributions is the application of the EM (Estimation and Maximization) algorithm (Dempster et al. 1977). The work is mainly contributed by Kimura and Chikuni (1987) and Hoenig and Heisey (1987). The difference between the two is that Kimura and Chikuni (1987) treat the observed $[q_{ij}]$ as mixtures of emperical distributions of independent length samples, while Hoenig and Heisey (1987) apply a log-linear model. Both methods have the same assumptions: (i) age and length samples may be collected from the two populations, (ii) growth rate and gear selectivity may be different for the two populations, and (iii) two populations may have different age composition.

If the age and length of fish are estimated for the two populations (k=1,2), the two samples from each of these populations can be classified into an A x L x 2 contingency table shown as in Fig. 24 . A random sample collected from sample k=2 is aged and classified entirely by length and age categories and thus is called "complete data". In contrast, the other sample is collected with length measurement and is classified by length category with unknown age and thus called "incomplete data" (k=1). Then, the problem is to estimate the age composition **p** for the sample k=1.

To apply the EM algorithm, prior information about q_{ij} is needed. This can be obtained from the complete-data k=2 , i.e., from q_{ij2}, by assuming the probability that a fish of age i falls into length category j remains constant from sample to sample. Let $q_{ij2} = P\{j|i\}_{k=2}$ be the "probability of length j giving age i in the sample k=2 (Hoenig and Heisey 1987), viz, the inverse age-length key (Kimura et al called it an iterative age-length key). Also, let p_{12} be the probability that an animal from

sample k=2 has in age i, while P_{i1} be the unknown age distribution to be estimated for the sample k=1.

For the sample k=2, the probability that an animal lies in cell i, j is given as

$$P(i,j|\ k=2) = q_{ij2}\ p_{i2}$$

Thus, the distribution function of the "complete data" (k=2) is defined by

$$n_{++2}! \prod_{i=1}^{A} \prod_{j=1}^{L} \frac{(q_{ij2}p_{i2})^{n_{ij2}}}{n_{ij2}!}$$

based on a multinominal sampling with sample size fixed at n_{++2}. Similarly the distribution function for the "incomplete data" (k=1) can also be defined by substituting k=1. This is essentially the same as equations (3) and (4) in Kimura and Chikuni (1987).

Since the two samples are drawn independently, the joint distribution function is the product of the two respective distribution functions for k=1 and 2. That is,

$$\prod_{k=1}^{2} n_{++k}! \prod_{i=1}^{A} \prod_{j=i}^{L} \frac{(q_{ijk}\ p_{ik})^{n_{ijk}}}{n_{ijk}!}$$

The log-likelihood function is

$$L = \sum_{i=1}^{A} \sum_{j=1}^{L} (n_{ij1} + n_{ij2})\ \log\ q_{ij1} + \sum_{i=1}^{A} n_{i+1}\ \log\ p_{i1} + \sum_{i=1}^{A} n_{i+2}\ \log\ p_{i2}$$

where $q_{ij1} = q_{ij2}$, since q_{ij1} in "incomplete data" are first calculated based on known q_{ij2}. The maximum likelihood estimates from the likelihood function are

$$\hat{q}_{ij} = \frac{(n_{ij1} + n_{ij2})}{(n_{i+1} + n_{i+2})}$$

$$\hat{p}_{i2} = \frac{n_{i+2}}{n_{++2}}$$

$$\hat{p}_{i1} = \frac{n_{i+1}}{n_{++1}}$$

in which $\hat{p}_{i2}$ is fixed due to the random sample from "complete data" and n_{ij1} can be calculated by $n_{ij1} = \frac{n_{ij1}\, n_{ij2}}{n_{+j2}}$ as the initial value. The estimated cell counts in sample k=1 are

$$\hat{n}_{ij1} = \hat{q}_{ij}\, p_{i1}\, n_{++1} = \hat{q}_{ij}\, n_{i+1}.$$

This is the M-step of the EM algorithm.

The E-step updates the cell count in the sample k=1 by

$$n_{ij1} = \hat{n}_{ij1} \frac{n_{+j1}}{\hat{n}_{+j1}} = \frac{\hat{q}_{ij}\, n_{i+1}\, n_{+j2}}{\sum_{i=1}^{A} \hat{q}_{ij}\, n_{i+1}}$$

These procedures are carried out concurrently.

Instead of taking joint distributions of complete and incomplete data, Kimura and Chikuni (1987) started with the EM algorithm to find the ($\hat{p}_{i1}$) that maximized the likelihood function described in (1). Kimura et al.(1987) also showed that this procedure satisfies the previous unpublished work by Fukuda and Chikuni. The algorithm can be summarized as follows

(i) Initially set $\hat{p}_{i2} = 1/A$ where A is the number of age classes.

(ii) Use available age-length data to find an empirical length at age distribution $\hat{q}_{ij2}$, then, compute $\hat{q}_{ij1} = \hat{p}_{i2}\, \hat{q}_{ij2} / l_j$.

(iii) Calculate $\hat{p}_{i1} = \sum_j l_j \hat{q}_{ij1}$.

(iv) If the maximum absolute deviation over ages between current and previous $\hat{p}_{i1}$ is less than a small constant, the optimum estimates are reached; otherwise repeat (ii)-(iv).

The asymptotic variance of $\{\hat{p}_{i1}\}$ can be calculated by the inverse of the information matrix obtained from the maximum likelihood methods. Kimura and Chikuni (1987) suggested using the coefficient of determination

$$R^2 = 1 - \frac{SS_{residual}}{SS_{total}} = 1 - \frac{\Sigma(\hat{l}_{j1} - \frac{n_{+j1}}{n_{++1}})^2}{\Sigma(\frac{n_{+j1}}{n_{++1}} - \frac{1}{L})^2}$$

as an index.

Again, we emphasize that the q_{ijk} represented the length distribution of a random sample of fish of age i. If q'_{ij2} is estimated in the standard age-length key manner ($\sum_{i=1}^{A} q_{ij2} = 1$), then q_{ij2} must transformed by

$$q_{ij2} = \frac{q'_{ij2}\, n_{+j2}/n_{++2}}{\sum_{j=1}^{L} q'_{ij2} n_{+j2}/n_{++2}}.$$

where, $\sum_{j=1}^{L} q_{ij2} = 1$.

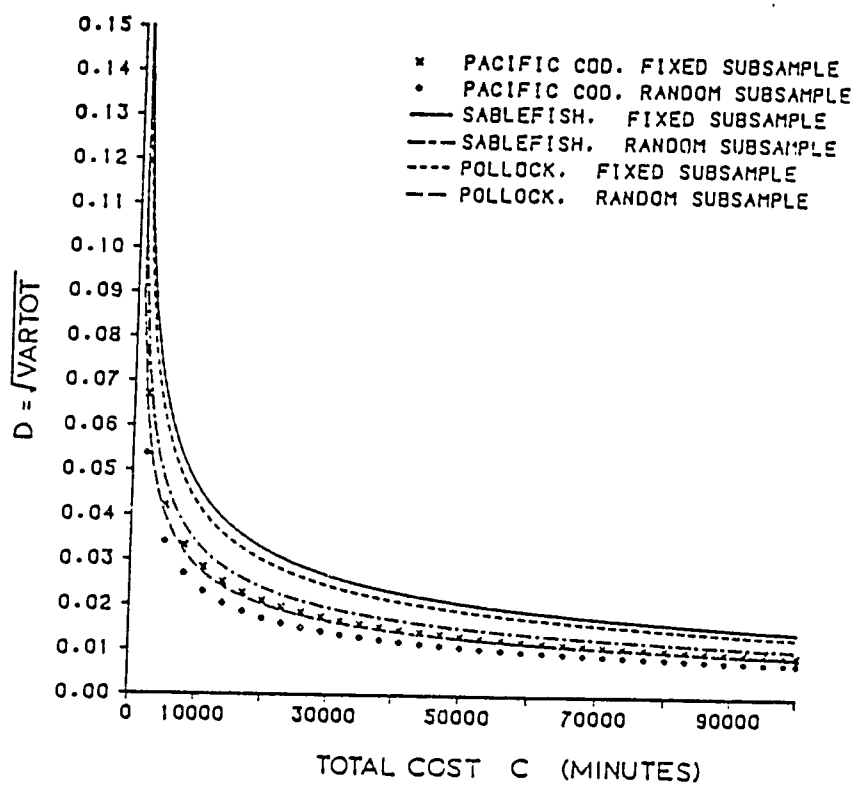


Fig. 23. (From Lai 1985)

The relationship of $D = \sqrt{Vartot}$ vs. total cost for Pacific cod, sablefish, and pollock with fixed or random age subsample. (from Lai 1987).

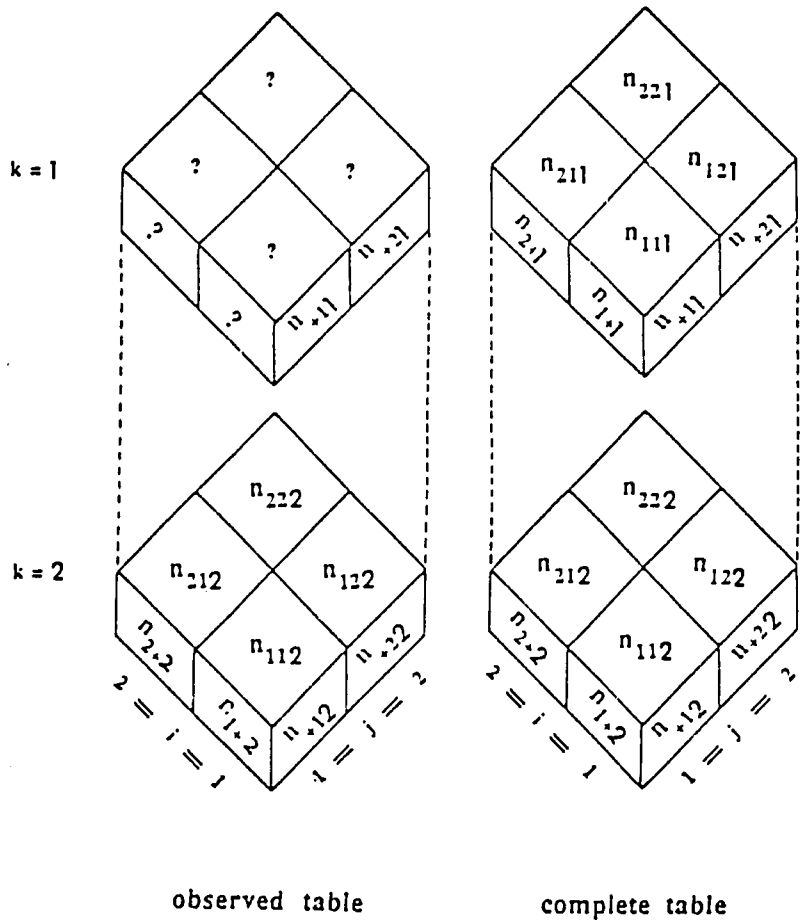


Fig. 24. Contingency table representation of age-length relationship. The observed length (k=1) and age (k=2) samples are represented in left where the age distribution is unknown (denoted by ?) and need to be estimated. If the data of age-length relationship is completely classified, the contingency table for length and age samples is represented in right. (After Hoenig and Heisey 1987).

Analysis of mixture distributions

Because of the difficulty and expense of age determination, estimating the age distribution and growth from length distributions has become very attractive. There are essentially four types of methods: (i) the age-length key technique; (ii) regression analysis using an empirical inverse age-length key as design matrix, length composition as a dependent variable and age composition as regression coefficients; (iii) EM (estimation and maximization) algorithm using an empirical inverse age-length key, and (iv) statistical analysis of finite mixture distributions on length-frequency data. The first three methods are reviewed in a later section. Here, we review the fourth method.

Conceptually, the method to analyze length frequency data goes back to Petersen (1891) who used a mode tracing method. However, he did not propose a statistical solution and did not apply it to depicting length-at-age of different cohorts passing through years. This method was revised by Pauly and David (1981) with the ELEFAN program, where the numerical evaluation of mode-lengths is constrained by growth structure. Pearson's (1894, 1915) mathematical solution for crab size frequency data, although thought unrealistic, is the first statistical approach to the problem. A probability paper technique based on the mixture of normal distributions has also been used (Harding 1949, Cassie, 1954). It is well-known that the logarithm transform of a normal probability density function becomes a hyperbolic curve. Buchanan-Wollaston and Hodgson (1929) and Tanaka (1962) have applied this method to separate the length-frequency distribution into an age composition. Solutions to the mixture problem that use likelihood methods are the focus of the recent studies (Hasselblad 1966, 1969; Macdonald 1969, 1975; Macdonald and Pitcher 1979; Kumar and Adams 1980). This method has been extended by Schnute and Fournier (1983) and Fournier and Breen (1985) by

constraints on growth structure and mortality of the cohort. In fact, a large number of articles with various methods and pdf decompositions have been published and readers can refer to textbooks by Titterington, Smith and Markov (1985) and Everitt and Hand (1981).

The general theory of the analysis for mixed length distributions can be stated as follows. If $f(x|\mu_i,\sigma_i^2)$ is the true length-frequency distribution function for the ith age component, the true length-frequency distribution for a mixture of A age components is

$$g(x) = \sum_{i=1}^{A} \pi_i f(x|\mu_i,\sigma_i^2)$$

where π_i is the proportion of age i fish in the population and μ_i, σ_i^2 are the mean length and variance of the age i component. The function is usually assumed to have a normal probability density, but other pdfs can be used.

Log-transform of a normal probability function

Although fitting a hyperbolic curve to a log-transform length frequency historgram was well-known, Tanaka (1962) derived the theory and applied the method to length frequency data for fish population dynamics. The probability density function of a normal distribution for a given age group is

$$f(x) = \frac{N}{\sqrt{2\pi}\sigma} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right)$$

where, N is the sample size, μ is mean length at the given age, and σ is the variance. The log-transformation is

$$\ln f(x) = \left(-\frac{1}{2\sigma^2}\right) x^2 + \left(\frac{\mu}{\sigma^2}\right)x + \left(\ln \frac{N}{\sqrt{2\pi}\sigma} - \frac{\mu^2}{2\sigma^2}\right)$$

$$= ax^2 + bx + c$$

Tanaka fitted the curve from the youngest age component and progressed to the oldest by giving values to μ's and σ's. He then evaluated the chi-square (χ^2) value of the previous trial. The iteration stops when the χ^2 value does not change substantially.

Hasselblad's Method or NORMSEP (1966, 1967)

Suppose that length distribution at any given age i is normally distributed and the observed fish length-frequency distribution is organized by a histogram. Then, the fish in $(x_j - 0.5, x_j + 0.5)$ is denoted to be in the x_j length category (x_j is mid-length with an interval of 1cm or 1inch). The probability of an age i fish being in the x_j category is q_{ij} and can be calculated as

$$q_{ij} = f[(x_j + 0.5-\mu_i)/\sigma_i] - f[(x_j-0.5-\mu_i)/\sigma_i]$$

where $f(x) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{X} \exp(-t^2/2)\, dt$. When q_{ij} is estimated, the probability of a fish being in length j, p_j, and with age i, π_i, can be expressed as

$$p_j = \sum_{i=1}^{A} \pi_i q_{ij}.$$

This is also the probability density function (pdf) of a length frequency distribution which is a mixture of normal pdf's. Then the likelihood function for the length-frequency distribution is

$$L = \sum_j n_j \ln(p_j)$$

Note that the equation is weighted by n_j, the observed frequency in the jth length category.

The method of Macdonald and Pitcher or MIX (1979, 1987)

Macdonald and Pitcher(1979) started with the overall pdf g(x) constrained by

$$\sum_{i=1}^{A} \pi_i = 1, \quad 0 \le \pi_i \le 1, \quad \mu_1 < \mu_2 < \ldots < \mu_A, \text{ and } \sigma_i^2 > 0. \qquad (1)$$

Then, the proportion of fish in the length interval (l_j, l_{j+1}) is

$$p_j = \int_{l_{j+1}}^{l_j} g(x)dx$$

In practice, the length frequency observations are grouped into a histogram. The observed p_j is

$$\hat{p}_j = n_j/n.$$

where n_j is the number of observations in the length interval j and $n = \Sigma n_j$. Macdonald and Pitcher suggest minimizing twice the Kulback statistic

$$-2n \sum \hat{p}_j \log(p_j/\hat{p}_j)$$

to estimate the parameters μ_i, σ_i^2, and π_i. They also discussed various other objective functions that might be used. These functions include minimum chi-square (MCS) and minimum modified chi-square (MMCS) which can be simply written in matrix form, $(\hat{p} - p)'C(\hat{p}_j - p)$, where C is a diagonal matrix with elements $\hat{p}_j/n$ for

MCS and $\hat{p}_j/n$ for MMCS. The variance of the estimates can be calculated from this quadratic form.

Constrained by growth and mortality

Hasselblad and Macdonald and Pitcher used only natural constraints listed in (1). These constraints have not been explicitly expressed in any tranform equations to be incorporated into the model. Kumar and Adams (1980) first formulated these constraints such that μ_i and σ_i^2 are estimated through transformed parameters. Kumar and Adams(1981) constrained the mean length at age i within $(x_{(1)}, x_{(2)})$. The transformed mean, variance and proportion are:

$$\alpha_i = \ln(\mu_i - x_{(1)}) - \ln(\mu_i - x_{(2)})$$

$$\lambda_i = \ln(\sigma_i^2/2)$$

$$\delta_i = \ln(p_i - b) - \ln(l_i - p_i) \quad \text{for } 1 \le i \le A-1,$$

where $b \le p_1 \le l_1$ and $b \le p_i \le (1 - b - \Sigma p_i) = l_i$ when $2 \le i \le A-1$. In this case, the parameters to be estimated are α_i, λ_i, and δ_i.

Schnute and Fournier (1980) constrained the mean length at age and standard deviation error according to a transformed von Bertalanffy growth curve

$$\mu_i = \mu_1 + (\mu_A - \mu_1)\frac{1 - k^{i-1}}{1 - k^{A-1}}$$

$$\sigma_i = \sigma_1 + (\sigma_A - \sigma_1)\frac{1 - k^{i-1}}{1 - k^{A-1}}.$$

When μ_1, μ_A, σ_1, and σ_A are knowm, only the parameter k needs to be estimated. The von Bertalanffy growth parameters are

$$L_\infty = \frac{\mu_A - \mu_1 k^{A-1}}{1 - k_{A-1}}$$

$$K = -\ln k$$

$$t_o = a_1 - \frac{1}{\ln k} \ln \frac{\mu_A - \mu_1 k}{\mu_A - \mu_1 k^{A-1}}$$

where a_1 is the age of the first components.

Fournier and Breen (1985) generalized the standard deviation of length at age i by

$$\sigma_i = a + b\sqrt{i},$$

where a and b are constants. They also assumed that length frequency samples come from a steady state population, such that the population is subject to exponential decline by age

$$n_i = R \exp(-Z(i-a_1))$$

where R is the observed number of fish in the first component of age a_1, n_i is the number of fish in age i, and Z is an instantaneous natural mortality rate.

Orensanz and Gallucci (1988) extend this method to analyze the size-frequency distribution (SFD) of crabs. In a previous sections on crustacea the size-at-instar of crabs has been described as part of discontinuous growth associated with molting. The growth increment is described by a Hiatt line. Therefore, the mean size at the ith instar is depicted by

$$\mu_i = a + b\mu_{i-1}$$

Starting from the first instar in the sample with mean size of μ_1, the mean size at the ith instar μ_i can be estimated. This step parallels the Schnute-Fournier approach. Then, assume that mean and standard deviation is linearly related,

$$\sigma_i = a + b\mu_i.$$

The program provided by Orensanz and Gallucci (1988) is similar to MIX (Macdonald and Pitcher 1979). The program estimates the proportions and mean size of all K instars included in the sample.

Discussion

The advantages of using constraints are that they reduce the number of estimated parameters and yield a well-defined response surface. Nevertheless, the difficulty of deciding on the number of age components A remains. Experience suggests that A is always smaller than the true number. Thus the user must make an intelligent choice among the methods, balancing the amount of biological information available against the disadvantages of a straight curve fitting approach. Introducing prior knowledge allows subjective biases to occur which are inevitable but must be guarded against. This problem is confounded by the degree of identifiability of the modes in the mixture of distributions. Behboodian (1970) showed that in the case of a mixture of two normal distributions, the condition $|\mu_1-\mu_2| \leq 2\min(\sigma_1,\sigma_2)$ is sufficient for the existence of a single mode. Bhattacharya (1967) showed that the plot of $\ln \{g(x_{j+1})/g(x_j)\}$ for the two adjacent length categories against x_j results in a series of straight lines with negative slopes, each corresponding to a component of the mixture. Fowlkes (1979) suggested the plot of $\{f(x_j-\bar{x})/\sigma - p_j\}$ vs. $(x_j-\bar{x})/\sigma$ where $\bar{x}$ and σ are sample mean and standard deviation and $p_j = (j-0.5)/N$. In the case of two components, the appearance is cyclical and nearly horizontal for only one component. Folwkes also suggested that a smoothing procedures will reduce local noise. However, a general procedure to determine the number of components has not been successfully developed yet.

Another problem arises from whether a mixture of normal or other distributions is appropriate for the length-frequency analysis. McNew and

Sumerfelt (1978) used the method of Macdonald (1976) to analyze 10 collections of large mouth bass. The results were compared to the age proportion derived from scale age. The age proportions derived by length frequency deviated significantly for an average error of 28%, but mean length and standard deviation of length at each group were not greatly influenced. Therefore, a more appropriate approach may be to use some age-length key and the EM algorithm of Kimura et al. (1987) or Hoenig et al. (1987). But the methods have not been evaluated.

Consequence of Ageing Errors on Population Dynamics

Concern over variability in age determination arose as early as in 1932 when Professor Johs Schmidt carried out studies to resolve the variability and to see how great or how small the differences would be between persons using the same materials. The results have been reported by Fredriksson et al. (1934). However, the effects of variability in age determination of fish population dynamics has not been assessed. Gulland (1955, 1958) reported that survival rate was significantly affected by over- and under-estimation of age, but was little affected by even 50% incorrect age determinations if the error was nearly random, i.e., random indicates a normally distributed error in age around the reference age.

Ricker (1969,1975) showed that (i) if the error in age reading is random and the magnitude remains the same at all ages, all mean lengths at age decrease; (ii) if the error in age reading is random and the magnitude increases with age, all mean lengths at age decrease but the magnitude may be reduced compared to (i); and (iii) when negative error exceeds positive error and this difference increases with age, the survival rate varies from a small over-estimate to a large under-estimate.

Brander (1974) examined the error in age determination of Atlantic cod by reassigning the age queried otoliths, and found that the reassigned age-length key gave very small differences in estimated abundance. The survival rate was increased between 0.1% and 0.2% if the proportion of misclassification was increased by 2.5% between successive ages.

Le Cren (1974) assessed the effect of errors in ageing on the production of fish populations. Unbiased erors in ageing, especially for older fish, would have little effect on production estimates. Estimates of juvenile production required data that would reveal errors in interpreting the first annulus. The unlikely systematic errors

arising from doubling or halving the true age would halve or double the production estimates.

Mortera and Levi (1983) examined the consequences of errors in age determination on cohort analysis using the data in Jones (1971). Their results are summarized as following.

	Fishing mortality	Abundance
ages 1+2 to age 1	over-estimated in early ages but under-estimated in olders	under-estimated
ages 1+2 to age 1 and ages 3+4 to age 2	same as above	same as above
ages 6 onward grouped	under-estimated in all ages	over-estimated
2, 5,10, 29% misclassi-ficationbetween a given age and the preceeding one	over-estimated at the given ages	under-estimated
2, 5,10, 20% misclassi-ficationbetween a given age and the successive one	under-estimated	over-estimated

Barlow (1984) simulated a misclassification matrix with the probability of random ageing error evenly distributed along the diagonal. Applying this matrix to a simulated age composition with known instantaneous total mortality rate (Z), Barlow showed that the estimated Z is subject to substantial bias even when the degree of ageing error is considered small.

In recent years, errors in age determination have been documented universally and fisheries scientists become more concerned about the problems that arise from these errors (e.g., Lai 1985, Lai and Yeh 1986, Lai et al 1987, Lai et al 1988, Boehlert and

Yoklavich 1984, Linfield 1974, Beamish and Chilton 1982, Beamish and McFarlane 1983, 1987, Clark et al 1986, Beamish 1979, Kimura and Targart 1979).

An example for the consequences of ageing errors

Age determination error can be classified into three types according to Lai (1985): (i) normally distributed error around their reference age, (ii) skewed error around reference age, but the mean difference is zero, and (iii) biases due to under-ageing or over-ageing. Lai (1985) and Lai and Gunderson (1987) carried out a simulation to investigate these errors in ageing using walleye pollock (*Theragra chalcogramma*) as an example.

In type (i) error the situation can be represented by the comparison of age readings between/within readers. The age of the ith fish tested (Y_i) is normally distributed with mean equals to its reference age (X_i) and standard deviation $S=CX_i$, where C is a coefficient of variation. Thus the error age can be modeled by $Y_i = R_iS + X_i$ where R_i is the random variation generated by a normal random number generator. Type (ii) error is generally found in break-and-burn vs. otolith surface ageing. It can be modeled by a (positively skewed) log-normal distribution with mean $(\ln Y_i) = \ln X_i$ and $S = C\ln X_i$. Type (iii) error is found in the cases for break-and-burn age readings vs. dorsal-fin ray, pectoral-fin ray, and scale age readings. The age readings by the latter methods are consistently younger than found from using the break-and-burn ages. The model that describes this type of error is $(Y_i-3) = g(X_i-3)$ for $X_i, Y_i > 3$. There is also a type (i) error associated with the model of type (iii) error such that the standard deviation is $S = CY_i$. When the gradient $g<1.0$ under-ageing occurs and $g>1.0$ over-ageing occurs.

A Monte Carlo simulation for the three types of ageing error was carried out by sampling from a collection of age-length keys. The percentage deviation from 200 simulations, of the estimates of growth parameters and survival rate for walleye pollock

are presented as $D_j = 100\% \times (K_j - K_0)/K_0$, where K_j is the value of estimated von Bertalanffy growth coefficient K from the jth simulation and K_0 is the reference value. The optimal instantaneous fishing mortality rate (F*) and optimal recruit age (t_c*) are also observed.

Table 8 shows that L_∞, K, and the survival rate does not change substantially (less than 10%) from type (i) error although K was substantially biased (>10%) in the case of type (ii) error. With the current t_c = 3yrs, F* was over-estimated for both type (i) and type (ii) errors. The bias in F* also increased with an increase in the C value (Table 9). Setting F=0.65, the estimated t_c* was smaller than that without ageing error and the bias increased with increasing C value. These results indicate that increasing C (i.e., increasing variability of ageing error) could substantially affect management decisions.

Figure 25 shows that the bias of L_∞ was <10% in under- and over-ageing errors, but bias in K could be substantial (>20%). Survival rate was substantially under-estimated with under-ageing errors and only slightly over-estimated with over-ageing errors. At t_c = 3yrs, F* generally increased with decreasing g, the gradient of bias between two age reading, especially when the value of C in the equation controls increases in standard deviation (Fig. 26). If F* was used to determine management policy, serious over-exploitation could result if the scales of walleye pollock (g=0.4) were used as the ageing method. Setting F=0.65, t_c* increased with increasing gradient (Fig. 27). This effect would contribute further to the over-fishing that would result from using scales.

Table 8. (From Lai and Gunderson 1987)

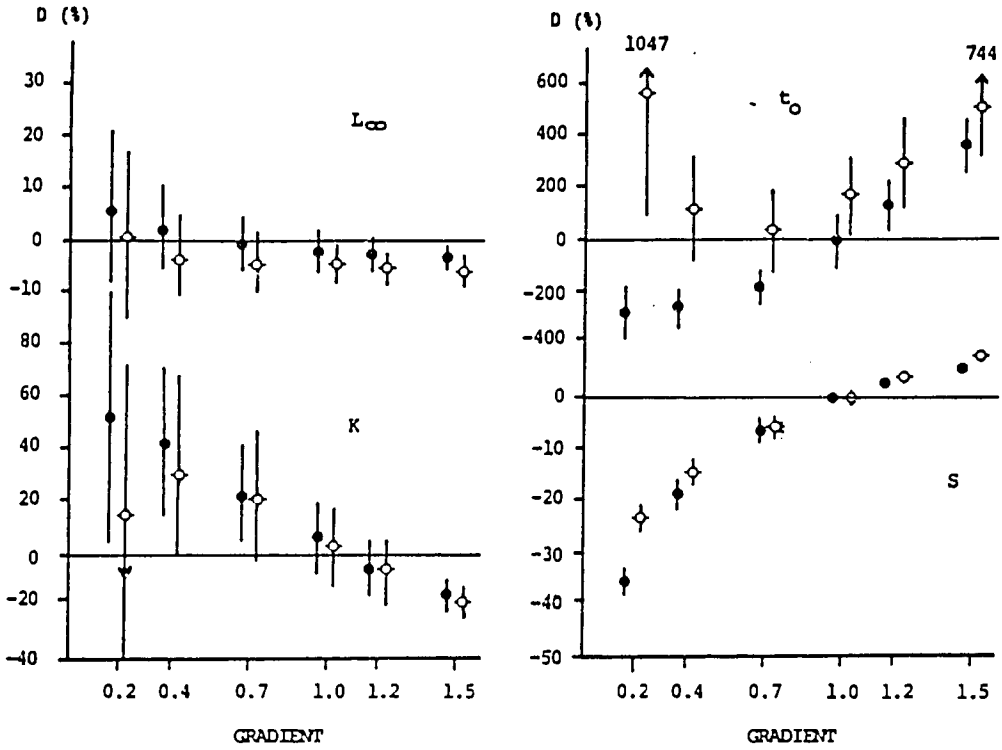
Percentage deviation (D) and 95% confidence interval (in parentheses) of population parameters with normally distributed and positively skewed ageing errors

Ageing error	C.V.	L_∞	K	t_0	S
Normally distributed	0.1	−2.4% (−6.4%,1.6)	5.6 (−8.2,19.9)	1.0 (−98.7,100.7)	−0.1 (−0.7,0.4)
	0.2	−4.4 (−8.7,−0.1)	5.2 (−12.1,22.5)	165.9 (4.4,327.4)	0.9 (−0.1,1.9)
Positively skewed	0.10	−8.4 (−12.0,−4.9)	17.5 (−2.3,37.2)	102.7 (−81.6,287.0)	0.7 (−0.5,2.0)
	0.15	−8.8 (−12.2,−5.4)	17.1 (−3.1,37.3)	182.8 (−19.7,385.2)	−0.4 (1.7,38.3)

Table 9. (From Lai and Gunderson 1987)

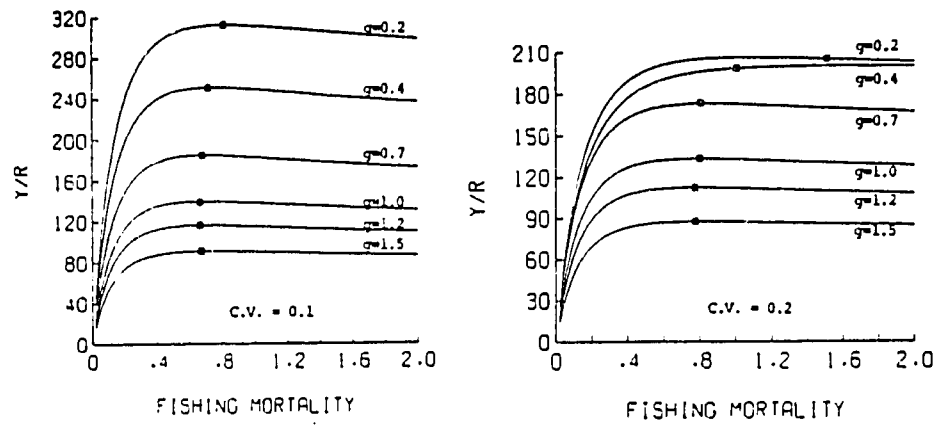
Estimated optimum fishing mortality rate (F^*, t_c=3 years) and optimum age at first capture (t_c^*, F=0.65) for walleye pollock, with normally distributed and positively skewed ageing errors

	C.V.	F^*	Y^*/R' (at F^*, t_c=3)	t_c^*	Y^*/R' (at F=0.65, t_c^*)
Reference	-	0.633	136.57	4.199	146.63
Ageing error					
Normally distributed	0.1	0.674	139.85	4.088	148.49
	0.2	0.748	133.11	3.905	138.55
Positively skewed	0.10	0.861	142.25	3.705	144.98
	0.15	0.928	141.05	3.601	142.23



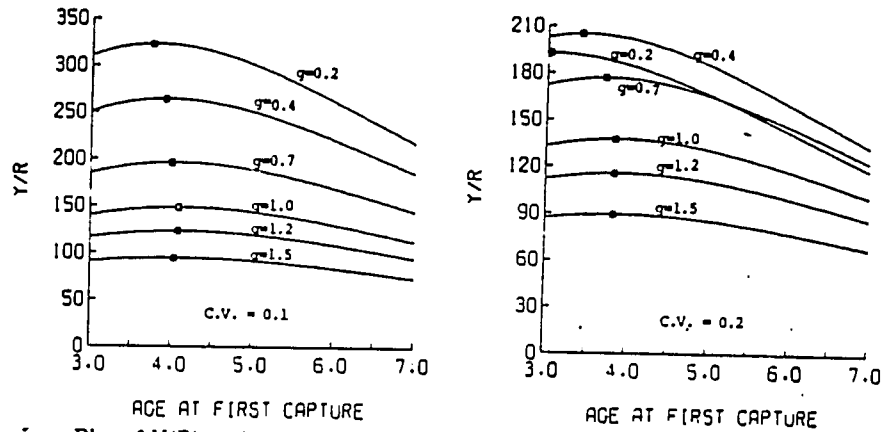
Variations of population parameters (D and its 95% confidence interval) subject to under- and over-ageing errors. (●, C.V. = 0.1; ⊕, C.V. = 0.2; | = 95% C.I.)

Fig. 25.. (From Lai and Gunderson 1987)



. Plot of Y/R' against fishing mortality for under- and over-ageing errors. ⊕ indicates the locus of maximum Y/R' on the yield curve. $t_c = 3$ years.

Fig. 26. (From Lai and Gunderson 1987)



. Plot of Y/R' against age at first capture for under- and over-ageing errors. ⊕ indicates the locus of maximum Y/R' on the yield curve. $\bar{F} = 0.65$.

Fig. 27. (From Lai and Gunderson 1987)

An Age Determination Laboratory

An age determination is known as a fundamental part of the study of fish population dynamics. Although many ecologists make age determination a first step in the estimation of growth and mortality rates, it is important to emphasize that age determination in fisheries research aims directly at the broader objectives of stock assessment and fishery management. Therefore, long-term and regular collection of age data from commercial fisheries is required for a detailed stock assessment using catch-at-age analysis. Age composition analysis, incorporating age data with catch and effort statistics, can be used to calculate biomass and to monitor the fluctuation of year-class strength under fishing pressure. This is a key point in the design of a fishery management plan and is important for evaluation of alternative management strategies.

Accordingly, an ageing laboratory has become an essential component of a fisheries management agency. In order to maximize the use of an ageing laboratory, it must be capable of developing methods for ageing various fish stocks, managing an age data base for supporting the requirements of research and management, and monitoring variability of year-class strength and growth.

The development of ageing methods generally starts with the search for useful ageing methods and follows with a series of validation studies. Since ageing methods are diverse and their utility varies between species, comparative studies and validation must be carried out to evaluate which method has the greatest accuracy and precision for each species. Although there are many biological and quantitative methods to validate ageing methods, several techniques have failed because they are not consistent over the entire life span of fish. Thus, direct validation using oxytetracycline injection is strongly recommended by most current studies. The tagged fish used in this study must cover all age classes in the population and must be recovered for a sufficiently long period in adequate abundances to be statistically significant.

Many studies in the recent literature have raised concerns about ageing errors due to the precision of age readings and the use of invalid methods. The latter problem can only be solved by the validation protocol described above. Precision, however, remains as a critical problem. To overcome this problem, a strict quality control over age readings is the only available solution. Therefore, age readers training workshops and cross-tests based on a double sampling method must be held on a regular basis to maintain and improve the quality of age determination whenever possible. When a new age reader is recruited, training and cross-testing between readers must be carefully designed. The principle advantage, therefore, of training workshops and frequent cross-tests is to make age readings consistent and ageing methods transferrable.

Since age determination is long-term, labor-intensive, and costly, statistical sampling designs that optimize have been developed. The methods are based on developing an appropriate age-length key from which a time series of length-frequency data can be collected and which can serve as an important supplement for stock assessment.

A computerized data base management system is needed to maintain the age data base. With computerization, new data entry, updating, estimation of growth and age composition, and analysis of the precision of age readings can be carried out easily and regularly. The data base needs to interface with other fishery statistics and be accessible to all researchers. In a long-term perspective, the ageing laboratory will play a significant role in the specification of protocols for data collection and sampling design.

When an ageing laboratory is fully operational, it can support preliminary monitoring of interannual variability of year-classes and growth. These results can be used to address the following issues:

1) Does the current fishing pressure depress some year-classes which have higher reproductive value?

2) Can the new year-classes support the existing exploitation rate after recruitment?

3) How does a year-class contribute to the fishery?

4) How do the environmental factors such as El Niño affect recruitment and potential catch?

With such information a management agency can predict future stock characteristics and adjust strategies to correspond to the predicted possible changes in abundance.

In addition to "traditional age determination", otolith microstructural (daily increment) analysis is often emphasized. This relates the growth in early life history to environmental factors. One of the possible applications in fishery management is to model the effect of environmental factors on year-class strengths and yield potentials in past years and then use the model to predict the future. The prospect of estimating year-class strength a half year to a year prior to recruitment is an attractive management concept that could guide and supplement survey estimates of abundance.

Finally, I emphasize again that age determination is an essential part of stock assessment and fishery management. The availability of a good long-term age data base, which is the input into catch-at-age analysis, will make stock assessment more accurate and a successful fishery management plan more likely.

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