CHAPTER 1
Development and Application of the Otolith Increment Technique

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History of the Otolith Increment Technique

Growth rings have been used to age fish for a long time. Annual rings in vertebrae were used to age eels as long ago as 1759 (Hederstrom 1959). Scales were first used to age fish in 1888 (Carlander 1987). Otoliths have been used to age fish since Reibisch first observed annular ring formation in Pleuronectes platessa in 1899 (as reported in Ricker 1975). However, counting annuli was not useful in estimating the age of young fish which haven’t yet formed their first annulus or for tropical or deep-sea adult fish for whom growth is more constant and annulus formation less certain. The daily increment technique solved these problems by permitting the estimation of daily age.

The daily increment technique was developed in the early 1970’s and has gained wide acceptance during the last twenty years. Pannella (1971) observed approximately 360 fine increments between the otolith annuli of temperate water fish. These increments were postulated to be daily changes in the microstructure of the otolith. Pannella (1974) observed what seemed to be daily increments in adult tropical fish and also observed that these patterns followed a larger, 14-day cycle that coincided with lunar behavior patterns. It did not take scientists long to see the value of this new technique. Within five years, Struhsaker and Uchihaya (1976) validated daily increment formation in young Hawaiian naupia by holding field-captured larvae and juveniles in the laboratory, developing growth curves and using this information to show growth rate differences between reef areas. During the same time, Brothers et al. (1976) realized the usefulness of this technique to determine age and timing of life history events in larval and juvenile fish. Their enthusiasm for the technique brought it to the attention of other investigators. Application of the otolith increment technique increased rapidly in the 1980’s when published studies using the daily aging method increased almost exponentially (Fig. 1).

Many of the currently employed applications of the technique were discovered in these early years, as were some of the difficulties. Taubert and Coble (1977) applied the technique to fresh water fish and found that shortened day length and low temperatures resulted in the apparent cessation of daily increment formation in sunfishes. In 1978, investigators reported that two important estuarine fish, Atlantic silversides and mummichogs, also formed daily increments (Barkman 1978; Radke 1978). Barkman, comparing different otolith types, found that both the sagitta and lappillus could be used in ageing, but that the asteriscus was unreliable. This is not surprising, for in many species it is formed later in development. By 1979, Methot and Kramer had fit Gompertz growth curves to field-caught anchovy to obtain in-situ growth rates. Many of the issues that are covered in this monograph (reliability, choice of otolith, application in the field, formation of subdaily increments, lack of resolution) were encountered in these early studies. Although Pannella began using the technique to define annuli in adults, most investigators have since used the technique for the early life stages which previously could not be reliably aged.

Overview of Age and Growth Techniques

The knowledge of age and growth is fundamental to fishery science. In the early life stages, information on age structure can be used to clarify the effects of changes in the environment on growth and survival, and can result in an improved understanding of factors affecting recruitment success. In adults, knowledge of age and growth is used to determine the effect of fishing on the stocks, the efficacy of management policies, to understand life history events, and to maximize yield while still ensuring the future of the resource.

Age and growth can be determined by various methods: growing fish in confinement, raising fish from birth, examining hard parts which encode age information, and through biochemical tests. The usefulness of these methods depends on the habitat and the life history stage of the fish. In most instances, we need to determine the age of wild fish and to do so we
must examine calcified structures which contain age information. The structures which encode age information are bones (fin rays, vertebrae, cleithra, opercular bones), scales and otoliths. For most fish, otoliths have been the most reliable indicators of age. Otoliths show annual and, for younger fish, daily patterns and therefore form a permanent record of life history events. Otoliths also have an added advantage: experimental evidence shows no resorption of otoliths under stress conditions. This is not true of other hard parts. Scales have not proven to be as reliable because they can be lost and regenerated and because deposition ceases at older ages, thereby giving false age readings. In most instances, scales don’t record daily growth patterns and can’t be used to age fish under one year of age (for an exception see Szedlmayer et al. 1991). The prime advantage of using scales for ageing is that their removal doesn’t cause death of the fish and extraction is quick. Other bones can sometimes be as accurate as otoliths, but their reliability is species specific and their use for daily age estimation has not been documented. It is apparent from the literature that ageing based on otoliths is often more reliable than other techniques. The difficulty in ageing young fish by any means other than daily otolith increments demonstrates why this technique is so widely used.

There are many important reasons to choose otolith microstructure to determine age and growth. The otolith is the only structure that consistently records daily events in the early life stages and annular events throughout life. With the advent of new computer image analysis systems, the task of increment identification, daily and annular counts and increment width calculations has been made quicker and more precise. With computer systems, data can be downloaded or plotted almost instantaneously. However, there are also drawbacks to otolith microstructure analysis. The fish must be killed to extract the otoliths. Also, otoliths can be difficult to read during certain life phases, such as at metamorphosis when accessory primordia may be laid down on the periphery of the otolith, and in older fish when increments are incompletely or compressed at the outer edges. Even with automation, the technique is time consuming, may call for specialized facilities (availability of computer-image systems and at times a scanning electron microscope) and does require training.

The daily increment technique is not limited to bony fishes. Volk (1986) has used the technique on statoliths of sea lampreys to determine annular age. Sea lampreys are agnathans and as such lack scales, bones and spines. Statoliths, which are analogous to otoliths, appear to be usable for age determination, and may prove useful for other invertebrates if daily ring deposition can be demonstrated. Hurley et al. (1985) have validated daily increment formation in the statoliths of short-finned squid and Jackson (1989) has used statolith microstructure to analyze the life-history events of a tropical cephalopod.

Information Obtained from the Otolith Increment Technique

The purpose of this section is to acquaint investigators using otolith microstructure with recent research results, beginning in 1985. For reviews of work accomplished prior to 1985, the reader is referred to Campana and Neilson (1985) and Jones (1986).

Age Determination

To estimate age from field-captured young fish, two pieces of information must be known: the age at first increment formation and the accuracy of increment counts. The age at first increment deposition is best determined through laboratory rearing. Alternatively, the age of increment initiation can be assumed to occur at hatch, at yolk-sac absorption, or at the stage at which a similar laboratory-reared species deposited its first increment. However, such an assumption may not be valid (Jones, 1986). If not, age estimates will be biased accordingly. The actual formation of daily increments has also been questioned. Several investigators have reported that increments were not formed daily or that deposition rates were related to growth rates (Geffen 1982; Lough et al. 1982; McGurk 1984). Geffen (1982) reported that increments were daily
only when the growth rate of larval Atlantic herring was ≥0.4 mm/day. McGurk (1987) also observed this problem when fitting growth curves to data derived from field-caught Pacific herring larvae. Nondaily (less than daily) ring deposition has been reported in several other species (Methot and Kramer 1979; LaRoche et al. 1982; Campana 1984). In these studies, use of scanning electron microscopy (SEM) provided no confirmation of nondaily deposition. However, Campana et al. (1987) reported that growth rates of 0.4 mm/day would still lead to the formation of increment widths that would be below the resolving power of the light microscope. Indeed, Jones and Brothers (1987) showed that narrow daily increments, deposited in the otoliths of starved striped bass could not be seen under the light microscope but were observed under SEM. Accordingly, deposition of daily increments appears to be a universal phenomenon under perhaps all but the most severe conditions. (For more complete discussions of sub-daily increments and the limits of light microscopy, see papers by Campana and Neilson, this volume).

**Growth**

Daily otolith growth, as measured with the width of each growth band, can be used to infer daily somatic growth. Two important relationships are used in fisheries: (1) the allometric relationship (an allometric relationship can be either isometric (proportional) or curvilinear) between the size of the otolith and the size of the fish, and (2) the growth rate of the individual fish or population expressed as length or weight increase per day. If the investigator intends to use retrospective growth analysis he must first establish the allometric relationship between otolith growth and fish growth. The allometric relationship can be a simple linear relationship (isometric) or a more complex curvilinear relationship depending on the species and the life history stage of the fish. In theory, once the allometric relationship between otolith size and fish size is known, then size at prior age can be back-calculated from the otolith alone (Fig. 2). In reality, such back-calculations are more complex to execute. First, the proper back-calculation algorithm must be used (see Campana and Jones, this volume, for further discussion) to insure reliable estimation of retrospective lengths. Second, when the relationship between otolith growth and fish growth is decoupled for individuals experiencing poor growth (Secor and Dean 1989), a single allometric relationship can no longer be used. Third, in asymmetric otoliths, the relationship between otolith size and fish size must be related to a standardized transect on the otolith along which increment widths can be measured. However, the usual case is that no single transect will be readable throughout the entire length of the otolith section (see Campana, this volume). By choosing a series of alternate paths, increment widths become a function both of the allometry, and more importantly, the changing angles at which the increment width is measured (Fig. 3). It would then be necessary to standardize these widths using their geometric properties. Such approaches have not yet been used, and until these problems are solved, use of increment widths are problematic in some situations.

Population (somatic) growth curves can be developed once individual ages and lengths are known. Growth data for the early life stages are commonly fit with a greater variety of curves than adult growth data (which are usually fit using the von Bertalanffy growth curve). During the larval and juvenile stages, population growth rate can be expressed using exponential (Struhsaker and Uchiyama 1976), logistic or Gompertz (Brothers et al. 1976; Lough et al. 1982; Laroche et al. 1982), or linear (Townsend and Graham 1981; Jones 1986) models. It is important to be wary of fitting curves that indicate an upper asymptote during the early life stages as it is obvious that growth does not
cease at this stage. Often, this leveling off of growth can be an artifact of sampling bias due to escapement from nets of fast growing larvae or juveniles (see Butler, this volume). Differences in growth rate over the entire life history of the fish can theoretically be correlated with environmental parameters. In practice, this can be difficult to do, as there is intrinsic heterogeneity within populations and subtle environmental changes can have cumulative effects on growth. At various life stages, young fish are differentially sensitive to changes in their environment. Environmental changes which may have profound effects on the larvae may be better tolerated by juveniles.

Traditionally, growth has been estimated by following the peaks in length-frequency data through time. This is difficult to do for individual cohorts because of the continuous production of eggs within the spawning season, confounded by heterogeneity of variance in growth. McGurk (1987) found that in fortnightly hatching of Pacific herring, length frequency modes can be followed when daily age information derived from otoliths is also available.

In recent years, more sophisticated techniques have been applied to growth estimation analysis. These techniques have included time series analysis, development of an age-temperature growth model (Campana and Hurley 1989), and cohort tracking techniques, among others. For example, Gutiérrez and Morales-Nin (1986) used time series analysis to estimate and forecast growth of sea bass in the laboratory. Their results showed that the growth response changed over different life stanzas. Juvenile sea bass were able to integrate their growth response to temperature events over previous days, whereas larvae did not, but rather experienced an imme-
mediate change in their growth rate. An application of hatch date groupings into within-season cohorts allowed Crecco and Savoy (1985, 1987) to show the significance of short-term hydrographic and meteorologic events on growth and survival of American shad. Savoy and Crecco (1988) were able to separate density-dependent and density-independent growth, and thus determine that year-class strength for these fish was established in the egg and larval stages. Their analysis demonstrates the power of the otolith increment technique for examining stock-recruit relationships.

Stock-specific differences in growth rates are important in the study of population dynamics. Watanabe et al. (1988), with the use of daily increment ageing, showed geographic differences in the growth of Pacific saury. Previous studies had estimated sauries could reach five years of age. Watanabe et al. presented the first clear evidence that this species is short-lived, a fact which was not clear from examination of length-frequency data. Additionally, western stocks were shown to grow significantly faster than eastern stocks. This paper is a clear example of the benefit of using daily ageing techniques to refine growth estimates in short-lived or tropical fish.

Knowledge of growth can also be used in aquaculture. Knowledge of growth rates of cultured versus wild fish are useful to the mariculturist who uses this information to determine the feasibility and, potentially, profitability of rearing fish in captivity. It is particularly important to determine whether growth can be accelerated within the first year of life and which conditions enhance growth. The application of the daily increment technique is not absolutely necessary for these estimates, but can be used to refine them.

Growth increment widths are used to determine somatic response to habitat change. Campana and Neilson (1985) point out the difficulties in estimating individual increment widths and daily instantaneous growth. It is quite difficult to accurately measure narrow increments. As stated previously, larvae and juveniles have different capacities to integrate environmental change and their physiological response, hence sudden changes in increment widths are less understandable in juveniles than in larvae. Before predictions of body growth can be drawn from otolith growth data, the nature of the relationship between the two must be known for the entire life time over which inferences are to be drawn. With metamorphosis, for example, the otolith may change its axis of growth. This will result in a change in the relationship between otolith size and fish size. See Campana and Neilson (1985) for further discussion.

Life History Events

Because the otolith records daily events, many life history events such as metamorphosis or a change in habitat will be reflected by changes in the width of increments and in the elemental composition of otoliths. Several investigators have realized that the timing of these events and associated growth changes can be estimated by examining otoliths for check marks and changes in composition. One early example is the study of recruitment from the pelagic larval to the demersal adult reef habitat of the bluehead wrasse (Victor 1982). Prior to this study, it had been hypothesized that recruitment was limited by the rate of adult mortality. Victor, however, demonstrated that recruitment was sporadic and related to environmental events. He was able to show this by back-calculating hatch dates for juveniles and for adults one year later. Similarly, the timing of outmigration of anadromous fish can be determined by observing the change in otolith increment width, leading to an evaluation of enhanced growth and survival resulting from this habitat change. Neilson et al. (1985a) were able to discern the timing of migration of chinook salmon from river to estuary using this technique and to determine the resultant growth enhancement. They were able to support the hypothesis that growth rates were reduced by density-dependent factors which occurred in the estuary, a hypothesis that was otherwise only testable with extensive mark-recapture efforts. Examination of daily increments permitted them to back-calculate the growth of individuals and to correlate this with population size and environmental factors. This study extended the use of otolith microstructure beyond simple assessment of age and growth to the testing of hypotheses of population structure, migration and behavior.

Recent studies have used the elemental composition of otoliths to infer the timing of day-to-day environmental changes or changes of physical habitat. For example, the change in the calcium/strontium ratio can be used in combination with increment number to estimate the dates of migration of anadromous and catadromous species. Radtke (1984) and Mulligan (1987) used stable-isotope and elemental composition techniques in an effort to enhance the understanding of stock identification. Mulligan (1987) and Mulligan et al. (1987) used the elemental composition of otoliths to discriminate river of origin in young-of-the-year striped bass and white perch stocks. Recently, Kalish (1989) has demonstrated that the wave-dispersive electron microprobe can provide accurate measures of five elements (Ca, Sr, Na, K and S) and is superior to the energy-dispersive microprobe.
for elemental analysis. Even though this procedure seems straightforward, it is as yet not fully tested for application to otoliths. For example, Radtke (1989) reported that a change in physiology could influence the chemical composition of otoliths. In cases where the elemental composition is both a function of the environment and the individual fish’s physiology, this technique will have limited utility. As yet not enough work has been done to evaluate this technique.

Recruitment

With the advent of the daily ageing technique investigators can now estimate individual birthdates (data necessary in recruitment calculations) and survival-at-age. The work of Savoy and Crecco (1988) and Crecco and Savoy (1987) is an example of the sophistication and relevance of such analyses. Natural mortality was calculated and partitioned into density-dependent and density-independent components. In these papers and in earlier ones, the ability to age fish with the daily increment technique, and the ability to then assign these fish to within year-class cohorts, led directly to the calculation of mortality (decrease in numbers-at-age) and to the definition of a quantitative relationship between mortality and environmental events with cohort abundance. These studies were done under especially amenable conditions; anadromous species are easier to assess than oceanic pelagics. Houde (1987), in his synthesis of recruitment variability, states that accurate calculations of mortality not only demand knowledge of age, but also demand unbiased and representative sampling of the life stages that are being studied. Savoy and Crecco (1988) worked in a river system which was amenable to such sampling. The marine and estuarine environments tend to be more heterogeneous and because fish change physical habitat as they mature, representative sampling can be difficult. Houde (1987) suggested that growth is intimately related to mortality and that growth estimates, which rely only on a knowledge of age and do not require estimates of abundance, can be used as surrogates for mortality estimates. However, this hypothesis has not yet been tested in a detailed manner.

Stock Identification

It is possible that otolith microstructure may be a useful tool for separating sympatric populations of juvenile fish. Neilson et al. (1985b) used the number and position of primordia and the nuclear core size at first increment formation as criteria to separate stocks of juvenile steelhead and rainbow trout. Their results showed that temperature had a greater effect than stock origin. Although these results are discouraging with this species, the technique may be useful for stocks which develop under similar temperature regimes. For example, Campana et al. (1989 a,b) used otolith microstructure to monitor larval drift and thus infer stock structure.

Technical Considerations

This section will provide a brief overview of some important technical topics and issues which should be considered in preparing and executing studies using the otolith increment technique, many of which are covered in greater detail in the other papers in this monograph. Researchers who incorporate this information into their work will be rewarded by improved results.

Importance of Establishing Initial Objectives

A clear expression of objectives is fundamental to the design of any study involving otolith microstructure examination and analysis. The objectives should be specific and detailed. Once objectives are stated, the desired level of precision and the required sample size necessary to achieve it will be calculable. This prior planning is obviously valuable, but not always done. In age and growth studies, for example, the nature of the inquiry will affect the methods that are used and the number of samples that are needed. In regards to accuracy, mortality can be calculated with knowledge of relative age, without knowing absolute age (Essig and Cole 1986). In this case, if the estimate of age-at-first increment formation is wrong, the error will be constant for all calculations and will not alter the relative change in numbers-at-age. However, the timing of life history events relies on accurate estimation of hatch age (Brothers and McFarland 1981) and the age of first increment deposition must be known accurately. The need for a specified level of precision will dictate the number of samples that need to be taken, and the degree of replication of measurements that are necessary. The intrinsic variability in age and growth characteristics of the population to be studied, along with the magnitude of the differences that must be detected, will dictate the number of samples that are necessary. Careful specification of experimental design will allow calculation of the power of the test (Rice 1987; Peterman 1990). Developing hypotheses, setting objectives, and following through with careful experimental design are all quite time consuming. However, such an approach will maximize the chance of success in the research endeavor.

Importance of Setting Uniform Standards

There are advantages in standardizing research procedures. The importance of establishing defined stan-
and different gears will sample the populations differently. These topics are covered in the paper by Butler in this volume.

**Preparation of Otoliths**

Proper otolith preparation will enhance the investigators ability to see relevant microstructure. Selection of techniques used in preparing an otolith for microstructural examination is dependent, in part, on its size and on the type of analysis which is intended. It often comes as a surprise to the neophyte that otolith size can be species dependent as well as age dependent. Big fish do not necessarily have big otoliths. Once fish have been collected, the investigator needs to match the removal technique with otolith size. A beginning investigator, having read a paper which describes the apparent ease of handling otoliths 200 μm in diameter, can be unprepared to handle larvae such as a day old striped bass and remove otoliths which are only 10 μm in diameter. Additionally, samples destined for SEM examination will be handled differently than those to be prepared for light microscopy only. The investigator needs to be prepared for these contingencies. Methods of preparing otoliths are covered in detail in the paper by Secor et al. in this volume.

**Interpretation of Otolith Microstructure**

Estimations of increment count and increment width, measurements fundamental for modeling growth, rely on the investigator’s ability to interpret otolith microstructure. Important considerations include the choice of the “right” counting and measuring axis, selection of criteria for defining a daily increment, and image optimization through choice of a microscope and image analysis system. Difficulty can sometimes be encountered in trying to differentiate daily increments from other structures. There are specific criteria that the “increment” must meet. The literature presents the simple case of opaque/translucent zones. Increments, however, do not always conform to the simple light band, dark band model, but can have varying dark and gray areas in the middle. For a comprehensive summary of otolith microstructure, the reader is referred to Campana and Neilson (1985) and to the papers by Secor et al. and Campana in this volume.

Another difficulty in interpreting otolith microstructure is the presence of “subdaily” increments, which are formed at a greater than daily rate. Campana (1984) studied the effect of manipulated environments (constant light; fluctuating light; fluctuating temperature) on the formation of increments in
larval and juvenile plainfin midshipmen and found that subdailies occurred throughout both stages, but were more likely to occur in young larvae. This lability in entraining a diel rhythm was corroborated by studies of other vertebrates. Campana offers an excellent suggestion of using known-age/otolith size data to help forecast the likely width of increments, then using this information in determining the probable width of increments when it is hard to discern daily from subdaily increments. In addition to Campana’s studies (1983, 1984), several other authors have tried to deal with this problem (Pannella 1980; Neilson and Geen 1982). Uechiyama et al. (1986) were able to differentiate subdailies in dolphins by changing the optical focus. Eckmann and Rey (1987) have demonstrated that subdaily increments in coregonids can be produced by manipulating environmental conditions in the laboratory. Research to date has not shown whether this is a problem for fish of all ages or only during certain life history stages. It is usually easier to discern increments in field-captured larvae and juveniles. The constancy of the laboratory environment tends to produce fainter increments. However, even the clarity of field-captured young fish otoliths depends on how well they are prepared (see Haake et al. 1982 and Secor et al., this volume). Staining techniques have been developed to enhance annuli (Bouain and Siau 1988). Such techniques have not yet proven useful in enhancing microstructural increments. There are also limits to the resolving power of the light microscope, even with the best preparations (clean otoliths, ground to the core, well etched, blue light source), because the light microscope is limited by the size of the smallest wavelength of the visible spectrum (see Campana, this volume). Very fine increments, smaller than 1µm, are usually only a problem in slow growing fish. However, if fine increments are not recognized, this will lead to the incorrect conclusion that increments are non-daily, underestimate age, and overestimate growth rate. These fine increments are visible with SEM, but very few studies (Jones and Brothers 1987; Castonguay 1987) have used SEM when increments appear to be non-daily or growth too fast.

Validation of Daily Increment Formation

Beamish and McFarlane (1983) emphasized the need to validate age in studies of adult fish which require age-based growth and mortality estimation. This requirement also applies to the use of daily increments, but with the added difficulty of validation in the first months of life. Some of the standard techniques used for adults, such as marginal increment analysis, are rarely used with daily increments because of the difficulty of resolving marginal increments, while other techniques such as mark and recapture are made far more difficult by the high natural mortality which occurs during the larval stage in many species. Chemical marking techniques can be used effectively in both lab and field situations. The paper by Geffen (this volume) discusses the techniques which are used to validate daily increment formation in the early life history stages.

Sources of Error

An important consideration in any carefully designed otolith study is the evaluation of potential sources of error that have been introduced during collection, preparation, or interpretation of material. Under certain circumstances bias can be corrected (see Campana et al. 1987 for a discussion of how to correct for unresolvable increments), but in other circumstances the magnitude and direction of the bias may not be estimable and may cause fallacious conclusions to be reached. It is a good practice to first be aware of potential errors which can occur and guard against their introduction. The paper by Neilson (this volume) discusses these sources of error and recommends procedures for avoiding them.

Statistics and Data Analysis

Statistical analysis is often conducted after completion of the research study, with no prior planning. Yet, to insure success, experimental design and analysis should be considered carefully as a first step, as the foundation of the research. Proper preparation entails appropriate random or systematic sampling, estimation of sample size, and selection of the appropriate analytical tests prior to implementation of research. Selection of the appropriate statistical test is important. For example, precision and accuracy of counts and increment widths need to be tested. When comparing the precision among counters and accuracy of increment counts between counters, it is not appropriate to use simple t-tests. A multiple series of t-tests will yield significant results in 5% of the studies (at an α level of 0.05) by random chance alone, when no real biological differences exist. Additionally, since variances are usually related to the magnitude of the mean age, real differences can be obscured when sample sizes are small. Analysis of variance (ANOVA) or analysis of covariance (ANCOVA) are far more powerful and appropriate tests. One approach to testing for differences in precision has been presented by Chang (1982).

Proper definition of the sampling frame and the sampling unit for otolith studies will help avoid incorrect
inference and pseudoreplication (*sensu* Hurlbert 1984). The sampling frame can be defined as the entire population of interest. Say we are interested in understanding the growth of bay anchovy in Chesapeake Bay. The spatial limits of the sampling frame would be the entire bay and the temporal aspect would be the time period over which the age group occurred in the bay. If we are interested in the earliest life period occurred in the sampling frame would consist of all bay anchovy of, say, up to one month of age. Theoretically, the entire area of the bay where these fish occur must be sampled, unless a smaller region can be demonstrated to represent the entire bay. The elucidation of the sampling unit is more complex. If one could choose any individual fish with equal probability to any other fish, then the sampling unit is the individual fish. If not, then the sampling station or perhaps the nearest tow might be the sampling unit, depending on the sampling design. Determination of the sampling unit is not a trivial exercise because the sampling unit determines the degrees of freedom used in statistical tests. For example, all otoliths from one fish are related to each other. When two otoliths are examined from the same fish, in statistical terms, only one observation has been made. The average of the two otolith counts would be the observation. By understanding the sampling unit we also avoid the problem of pseudoreplication (Hurlbert 1984). Pseudoreplication occurs when the observations or measurements are not independent from each other and are not true replicates.

Several categories of analysis are common to otolith daily increment analysis: modeling of growth, back-calculation of hatch dates, and estimation of mortality. These analytical methods are covered in detail by Campana and Jones, in this volume.

**Looking Ahead**

Over the past 10 years, the otolith increment technique has been used with increasing sophistication not only to determine age and growth, but to unravel conditions affecting recruitment, the timing of life history events and even stock differentiation. Recruitment has historically been examined from the standpoint of mortality rate in relationship to the ambient environment and, recently, the relationship between growth, mortality and year class strength (Houde 1987). While the second approach has been widely discussed, it has not been well tested. Otolith analysis provides the means by which this approach can be tested. The otolith increment technique has already been applied to the issue of larval drift and stock identification (Campana et al. 1989 a,b; Thresher et al. 1989), but these are only the first of many studies to be expected. The limitations on such studies are defined not so much by the difficulty in obtaining age-structured data, but by its incorporation into an appropriate spatial analysis. An increasingly popular use of the otolith increment technique is the analysis of hatch date distributions in relation to dates of egg production; results of this type of study provide a means for evaluating which factors influence the survival of young fish. With the recent discovery of growth rate effects on the fish:otolith relationship (Secor and Dean 1989), quantification of increment width autocorrelation (Gutiérrez and Morales Nin 1986), and a new approach to growth back-calculations (Campana 1990), there is a reasonable potential for vastly improved back-calculations at the daily level.

Recent technological developments promise major advances in the interpretation of otolith microstructure. One such recent development is the use of the electron microprobe to quantify the elemental composition of otoliths. Elemental analysis may be used in the future to link the larval fish to its natal water mass, and to define environmental conditions during previous growth phases (Radke 1984; Kalish 1990). Elemental analysis can be used for stock identification, while isotope analysis can be used in temperature and food web studies. These techniques rely on the assumption that the otolith records conditions under which the fish grew, perhaps even on a day-to-day basis. However, this technique has yet to be refined sufficiently for routine reliable use (Kalish 1989). New advances in underwater acoustic methods may one day permit the fishery scientist to accurately estimate the size and extent of larval fish aggregations, which, when combined with estimates of age, could improve our knowledge of mortality during the early life history stages. In the interim, chemical mass-marking of the otoliths of hatchery-reared fish is providing new opportunities for determining short-term in-situ mortality rates. On a more speculative front, recent advances in instruments such as scanning tunneling microscopes and NMR scanners could conceivably be used to map (in 3-D) the microstructure of an otolith without any prior sample preparation.

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