

Evaluating Fish Growth by Means of Otolith Increment Analysis: Special Properties of Individual-level Longitudinal Data

R. Christopher Chambers and Thomas J. Miller

ABSTRACT: Otoliths have unique utility in augmenting information about fish life histories. Estimates of an individual's size at prior ages and at life-cycle events, and of environmental effects on growth can potentially be obtained from measurements of sequential increments on otoliths. These multiple measures constitute a longitudinal record of growth for a fish. Such data are structurally distinct from size-at-collection and size-at-age data because of interdependencies of within-individual observations. Univariate statistical models, which assume independence of observations, are generally inappropriate; multivariate methods are preferred for hypothesis testing when data are drawn from within and among individuals. A set of otolith increment measurements from larval Atlantic menhaden, *Brevoortia tyrannus*, is used to demonstrate these principles. Within an individual menhaden, observations on increment radii are shown to be interdependent, as are those on distances between successive increments. The rankings of larvae by the sizes of their otolith radii and their short-term growth rates were found to generally persist through the 15-d period analyzed. Two cohorts of menhaden were tested for differences in their individual-level pattern of otolith increments using a modified multivariate analysis of variance. A significant, though temporary, reduction in growth in one of the two cohorts was revealed. Traditional univariate analyses were less sensitive to details of growth patterns within and among cohorts.

Introduction

Knowledge of the age structure, growth, and developmental rates in fish populations is fundamental to estimating demographic parameters and evaluating ecological processes. Fortunately, fish retain a record of growth in several anatomical structures: scales, otoliths, and bone. These structures have identifiable banding patterns or rings of several possible periodicities that reflect the punctuated nature of growth. Among these structures, otoliths provide a record that is often the clearest and of the highest potential temporal resolution. The regularity of deposition of otolith rings (hereafter called increments) provides a means of aging fish. Moreover, a repeatable relationship generally exists between otolith size and fish size, which allows a fish's size at a previous age to be estimated

by use of this relationship and the distance from the otolith center to the increment corresponding to that age.

The range of inferences based on otolith structure was significantly enhanced with the determination by Pannella (1971) that otolith increments are deposited daily. Hypotheses could be evaluated concerning the timing and effect of events operating early in a fish's life, a period when mortality is greatest (McGurk 1986) and variations in rates of growth and development could be particularly significant (Chambers and Leggett 1987; Houde 1987). We argue, however, that due to the application of inappropriate statistical methods, prior studies based on measurements of multiple increments per otolith have likely overlooked important details of within-cohort and among-cohort differences in patterns of fish growth.

In this paper we consider the analysis of size-at-age data derived from otoliths. First, we identify the structure typical of otolith-based data. Next, we emphasize the special properties of measurements taken on multiple features of individual otoliths. We make an important yet largely neglected distinction between data that represent growth records of individual fish and general size-at-collection and size-at-age data. We then consider options for comparing individual growth in two or more cohorts of fish. And, lastly, we use a sample dataset of otolith increments to analyze the patterns of individual growth, and compare growth of fishes from two cohorts. All issues and methods discussed apply generically to growth data, be it growth of otoliths, scales, or fish measured at daily or annual frequencies. We assume in subsequent discussions that the preparation and reading of the otoliths are free of error or are of known error.

Properties of Otolith Data

The variety and number of applications that use otolith observations have proliferated over the last decade as evidenced by papers in this and other volumes (Summerfelt and Hall 1987; Stevenson and Campana 1992). The otolith features that are quantified depend on the specific application. Each set of features has a characteristic data structure (Table 1). Otolith applications can be separated into a general dichotomy based on whether single or multiple features are measured per otolith. This distinction, in turn, determines whether univariate or multivariate statistical methods are appropriate for hypothesis testing. While this statement seems self-evident, we will argue that a common problem in otolith-based growth studies is a failure to match statistical models to the data structure and desired inferences, a practice that has compromised the evaluation and interpretation of results in these studies.

Measurements of multiple otolith increments in growth studies (Table 1, Usage 6), result in a data structure similar to that used in morphometric analyses for discriminating fish stocks (Table 1, Usage 5). Multiple features are measured per otolith in both applications: distances from otolith focus to increments (increment radii) or between adjacent increments (increment widths) in growth studies, and perimeters, areas, and various points of homology in morphometric analyses. Multivariate statistical methods are appropriate in both situations and, indeed, are commonly applied to morphometric data. These methods are decidedly absent, however, from analyses of increment-based growth data. We suggest that this is a fundamental shortcoming of fish growth studies in general and otolith-based studies in particular. As elaborated below, this shortcoming could be due to either a failure to retain the data at their original individual level of resolution or an application of more traditional, yet inappropriate, univariate statistical methods to data that, technically, contain repeated

Table 1. Otolith applications, measurements, and data structure. Each usage has a specific set of measurements with characteristic data structure. A fundamental dichotomy in data structure exists between applications that are based on single and multiple observations per otolith. The appropriate statistical model is one that matches the data structure and the research question.

Usage	Otolith Measurement	Data Structure
1. age estimation and validation, age at collection or event, hatch-date frequency distribution	counts of increments	univariate (ordinal)
2. otolith size-fish size relationship, back-calculation function, size at life-cycle event	size of otolith (diameter, radius, area, mass), size at check	univariate (continuous)
3. age validation, mark-recapture studies	presence or absence of marks (chemical, thermal marks)	univariate (categorical)
4. environments experienced	elemental constituents	univariate and multivariate (continuous)
5. stock identification and discrimination	morphometrics (perimeter, areas, points of homology)	multivariate (continuous)
6. growth reconstruction, sizes at prior ages and at life-cycle events (e.g., hatching, settlement)	radii or widths of multiple increments, radii at checks	multivariate (continuous, sequential)

measures on individuals. Potential consequences of these practices include a loss of information on the pattern of individual growth and a distortion of hypothesis testing due to assignment of inflated degrees of freedom in statistical tests.

Otolith increment data have a special covariance structure due to the fact that increments are deposited sequentially and successive radii increase in magnitude. These are characteristics of growth data in general. An extensive literature exists on statistical methods tailored to handle such data (Rao 1965; Timm 1975; Seber 1984). Appropriate methods include trend (growth), profile, and multivariate repeated measures analyses all of which are derivatives of multivariate analysis of variance.

Fish Growth, Otolith Increments, and Longitudinal Data

Fish growth plays a fundamental and often critical role in the analysis and management of fish populations. The importance of fish growth, combined with the frequent difficulty in obtaining reliable and representative observations of growth, emphasize the value of well-conceived sampling programs and extracting the most information from the data that are collected. A variety of models has been used to approximate fish growth (Ricker 1979) as has, we argue, a variety of data. The patterns and differences of growth of individual fish from one or several groups, sexes, sites, cohorts, or environments are often the focus of study (Fig. 1A). One valuable feature of otoliths, shared by scales and mark-recapture data in general, is that repeated measurements can be obtained that provide a record of growth for the individual. Repeated measurements are prerequisite to the analysis of individual growth. Such individual records have long been referred to as longitudinal data in the biometrical literature, dating back at least two centuries to when semi-annual measurements of the height of the son of Comte Philibert de Montbeillard were analyzed by Buffon in a 1777 supplement to his *Histoire Naturelle* (Scammon 1927).

The ability to estimate and analyze individual growth records is key to answering many questions of interest to fishery scientists. A short list of key questions includes: Do size advantages of fish persist, or is there evidence of compensation within a cohort? Do mortality rates vary with the patterns of individual growth? Does individual growth vary with time of hatching? Does growth differ among cohorts? and, Do environmental factors correlate with differences among cohorts in individual growth? Despite a widespread recognition of the value of otoliths for the purpose of estimating individual growth trajectories, prior studies have rarely retained the increment data or the back-calculated sizes-at-ages at their original individual level of resolution (for an exception, see Rosenberg and Haugen 1982). Indeed, common methods of handling individual increment data, such as averaging sizes across individuals within age-classes and modal progression, are those that have been applied to traditional size-at-age data collected at the annual level of resolution. These methods of aggregating information fail to capitalize on the unique properties of individual-level longitudinal records.

CROSS-SECTIONAL DATA, UNIVARIATE ANALYSES

Although individual growth is often the intended object of estimation in fish studies, the data used to derive such estimates are frequently of a qualitatively different level of resolution. Some

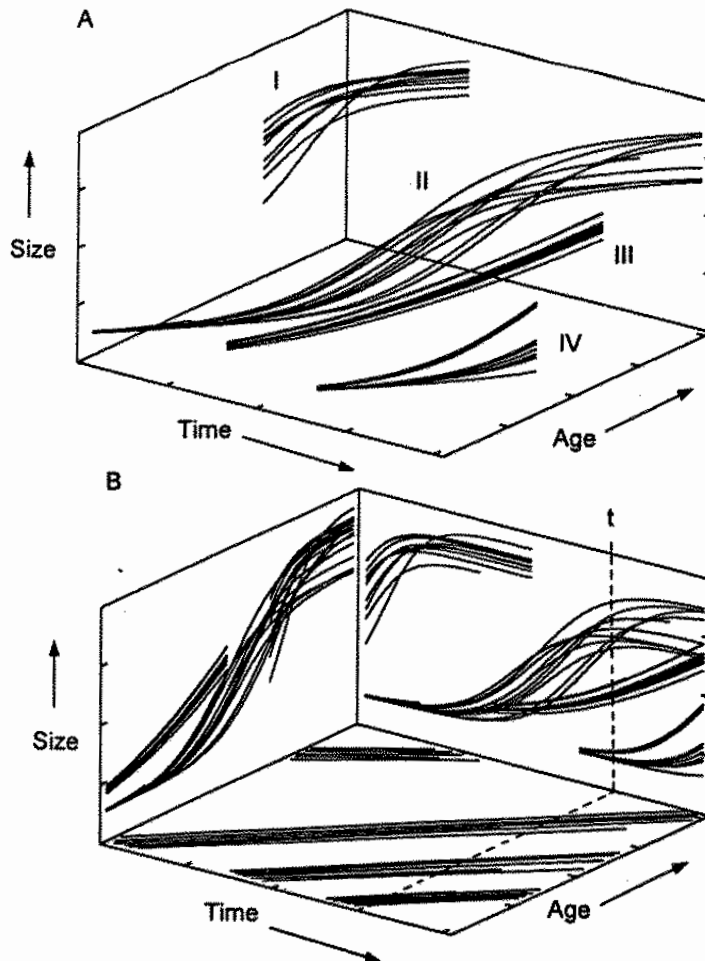


Fig. 1A) Growth trajectories in time-age-size space of four hypothetical cohorts of fish during a study program. The trajectories are generalized and may be viewed as either on a daily or yearly time scale. Initially, each cohort consists of 10 individuals, but several from each cohort die during the study. Cohort I is well established when the research program begins; cohorts II-IV begin during the research program. Individuals become invulnerable to sampling beyond a critical age, as shown by individuals in cohort I leaving the sampling space across the size-time plane. B) Projections of individual trajectories onto the component two-dimensional planes. Projections onto the size-time plane show size-at-time curves. It is clear that a sample at time t only includes individuals from cohorts II-IV. Projections onto the size-age plane show the growth of individual fish for the entire population regardless of cohort identity. It is important to note that the growth trajectories for any one cohort could differ from others. A sample at time t never cuts the size-age plane, therefore, a single sample taken at t cannot provide direct information about individual growth curves. These curves can be estimated using otolith increment information or other methods that provide repeated measures on individuals. Projections onto the time-age plane show age-at-time lines. By definition these projections are families of parallel lines at 45° angles from the time and age axes. The width of the family of lines for each cohort, and the length of individual trajectories on the time-age plane, provide information on hatch-date distributions and survivorship, respectively.

of the ways that this misidentification occurs are emphasized by projecting the growth trajectories from size-age-time space onto the component planes (Fig. 1B). Several approaches to data collection, reduction, and analysis have been used in otolith-based studies of fish growth, each varying in the degree to which individual growth is accurately represented. The most common approach uses one observation per fish—its size and age at the time of collection. Otoliths are used only to age the individual fish (Table 1, Usage 1). This data structure results whether the population is sampled once or repeatedly as long as the same individual fish are not measured twice. Using Fig. 1B, the sample is represented by the points of intersection of the growth trajectories of individual fish and the time of collection (time, t). Such data are referred to as cross sectional (Cock 1966; Sandland 1983). A function is then fit to describe the relationship between sizes (or mean sizes) and ages of fish at collection. If the cohorts in Fig. 1B represent year-classes, a function would be fit to size-at-age data for fish of ages 1 yr, 2 yr, and 3 yr (Fig. 2).

A function fit to cross-sectional data approximates individual growth only under restrictive assumptions, including the lack of size-biased sampling and mortality. This approach, therefore, is unacceptable when the detection of size-biased mortality within and among populations is a project goal. Biases in the derived growth estimates also result from the practice of aggregating all observations within a cohort into a single age group. Using the schematic data, the function derived from a fit to cross-sectional data does not equal the growth of individuals nor the average or variance in sizes-at-ages for any single cohort (Fig. 2B). Misrepresentation of individual growth is all the more likely when the fitted function is described as a "growth curve" without further elaboration as to whether it is intended to represent the average of individual growth curves, the growth curve of the average individual in the population, or some species-level growth curve. The tradition of referring to the functions used for fitting such data, such as the von Bertalanffy, as "growth models" or "growth curves" with "growth parameters" adds further confusion as to just what is being described and compared.

We conclude that the procedure of fitting a function to cross-sectional data, which seems to be the standard means of deriving growth curves in descriptive species accounts (e.g., Bagenal and Tesch 1978), is best considered as one that provides a quantitative relationship between fish size and age at collection. Importantly, this method precludes evaluation of the above questions or other hypotheses about individual growth trajectories. Because these data do not represent growth curves per se, we refrain from a discussion on methods of fitting and comparing functions for length-at-age data from two or more populations and only mention that a variety of linear and nonlinear models have been applied to such data (Ricker 1979; Ratkoswky 1990).

LONGITUDINAL DATA, UNIVARIATE ANALYSES

A second approach to otolith-based analysis of growth makes more extensive use of otolith information. Observations of fish size and age at the time of collection are enhanced by additional data on sizes at past ages, as inferred from the series of radii measured for increments of the otoliths (Table 1, Usages 1, 2, and 6). The data, therefore, consist of repeated measures on individuals for a sample from one or more cohorts—precisely the information required to evaluate hypotheses about individual growth. These data are then analyzed by means of univariate regression or analysis of variance (ANOVA) for purposes of evaluating environmental or other

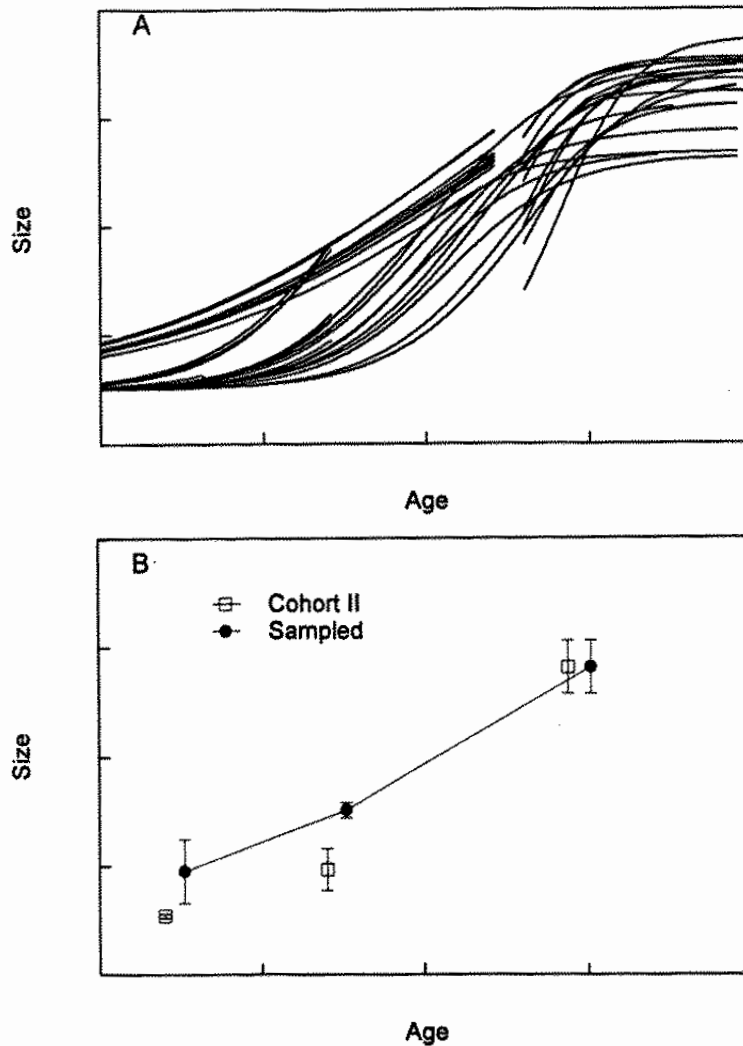


Fig. 2. A) Growth curves for the individual fish from Fig. 1. These are projections of trajectories onto the size-age plane. B) Size-at-age curves estimated from individuals sampled at time t (Fig. 1B). The age of individuals from the three cohorts was "estimated." Biases in the age estimation will also affect the growth rates estimated from these data. The age estimates, together with individual sizes at capture, were used to generate mean sizes-at-ages for the sampled population. Also shown are the actual sizes-at-ages for cohort II (Fig. 1). The points are displaced by 6 d to allow for easier visual comparisons. It is clear that a growth curve derived from the size-at-age estimates based on a single sample (i.e., cross-sectional data) is different from that based on a single cohort followed through time.

influences on otolith and fish sizes. Either choice of statistical model treats otolith size at increment formation (e.g., increment radius) as the response variable. In a regression format,

$$s = \beta_0 + \beta_1 a + \sum_{i=1}^k \beta_i f_i + \epsilon \quad (1)$$

where s is otolith size, a is fish age at the time of increment formation, the f_i are optional embellishments to the model that would represent extraneous factors, such as temperature, year, density, location, fishing, and so on, evaluated for effects on otolith size, the β_i are parameters to be estimated, and ϵ represents unexplained variation in otolith size. In more compact matrix notation, which makes comparison with subsequent models more direct, Eq. (1) can be expressed as

$$s_{(n \times 1)} = X_{(n \times q)} \beta_{(q \times 1)} + \epsilon_{(n \times 1)} \quad (2)$$

where the notation parallels that in Eq. (1). The vector of otolith sizes, s , contains all n measurements taken on otolith size, X is a design matrix with a row for each of n measurements and $q = k + 2$ columns (the first column of X is a vector of 1s, the second contains the observed ages, and the remaining k columns are the measured extraneous factors), the parameter vector β has q elements, and ϵ is the unexplained deviations from the expectation $s = X\beta$. Interaction terms could be added to this model or the model could be parameterized for ANOVA. For both models the standard assumptions of common within-population variances and independent observations apply. Variants of this approach use change in size between successive measures, for example, increment widths (Δs), as the response variable (Weisberg and Frie 1987).

Employing a univariate model to analyze repeated measures data results in several undesirable consequences. First, information on the patterns within and differences among individual growth records is lost. Questions that require individual-level data, therefore, are no longer directly answerable. Second, data that originate from multiple observations per otolith cannot be assumed to be independent, hence the univariate regression and ANOVA assumption of independent errors is violated. Moreover, because statistical hypotheses are accepted or rejected based on the magnitude of the test statistic and the degrees of freedom (df), calculating the df from the total number of increments measured results in a significantly increased likelihood of committing a Type I error (falsely rejecting the null hypothesis of no among-groups difference). As a numerical example, say 20 fish are collected from each of two populations and 10 increment radii are measured on each fish. A univariate approach would treat these 200 measurements per population as 200 independent size-at-age observations (as if the trajectories for two cohorts in the size-age plane of Fig. 1B were replaced by 400 point observations). Further, say a three-parameter version of the univariate regression, Eq. (1), with parameters β_0 , β_1 , and β_2 is employed to test for population effects ($H_0: \beta_2 = 0$). In a univariate framework, the hypothesis of no population effect would be rejected at $F_{(0.05)} \geq 5.06$ based on 1, 397 df.

A further variant on the univariate approach explicitly treats age (Eq. 1) as a "repeated" factor in the statistical model and employs a repeated-measures ANOVA. Certain assumptions about the data structure apply to this procedure, such as compound symmetry of the variance-covariance matrix (Winer et al. 1991), that substantially restrict the frequency with which this procedure can be legitimately applied. When these assumptions are met, however, a univariate repeated measures

test does have greater power than a multivariate treatment of the data (McCall and Appelbaum 1973).

LONGITUDINAL DATA, MULTIVARIATE ANALYSES

A third approach also uses multiple increments per otolith (Table 1, Usages 1, 2, and 6). In contrast to the preceding method, the longitudinal record of sizes for each individual is retained throughout the analysis. Hence, the analysis is conducted at the same level of resolution as the biological hypotheses that address the growth of individual fish. Maintaining individual integrity and the concomitant covariance structure of longitudinal records could reveal, for example, evidence of growth compensation or depensation among other potential results.

Often the determination of whether groups of fish differ in the patterns of individual growth, or of environmental effects on growth, is the ultimate objective of growth studies. For linear size-at-age data, differences or effects can be evaluated by comparing slopes and elevations of the size-versus-age relationship for fish from different populations—the standard analysis of covariance (ANCOVA) with adjustments made to reduce the *df* to better represent the number of independent observations (e.g., Greenhouse and Geisser 1959; Huynh and Feldt 1970). Comparison of slopes and elevations alone, however, would be unlikely to detect short-term and nonlinear differences in growth. For example, a period of slow growth subsequently compensated for by faster growth could be significant biologically but may not change the coefficients of a linear growth model fitted to such data.

Several alternative methods exist for handling and comparing individual growth data. Polynomial and nonlinear models have been fitted to growth data and the estimated parameters compared singly or as a correlated set (Bernard 1981, 1982; Rodda 1981, Misra and Smith 1982). Alternatively, the size-at-age records for individuals could be retained as a response vector and compared directly. This method is particularly appropriate when the size-at-age observations per individual are few relative to the number of fish, as is the case in the example below in which sizes at six ages per individual are available for each of 42 fish.

Differences between groups in growth patterns, as represented by such records, can be evaluated by multivariate analysis of variance (MANOVA) and associated analyses of trends, growth, and profiles (Timm 1980). A general MANOVA model for handling longitudinal records, analogous to the regression model (Eq. 2), has the structure,

$$S_{(n \times p)} = X_{(n \times q)} B_{(q \times p)} + E_{(n \times p)} \quad (3)$$

where the rows of *S* are the size-at-age vectors $s = (s_1, s_2, s_3, \dots, s_p)$ for each *p* measurements on of *n* individual otoliths (or fish), the columns of *X*, as in Eq. (2), are the set of extraneous measurables (e.g., temperature, year, density, etc.) measured for each individual, *B* is a (*q* × *p*) matrix of parameters to be estimated, and *E* is a matrix of deviations from the expected value of $S = XB$. The sets of observations per individual, *s*, are assumed to be independent from one another, drawn from a multivariate normal distribution, and share a common variance-covariance matrix. Note that the sample size is now determined by the number of individual fish measured, not the number of measurements taken.

Following a rejection of the hypothesis of no group differences in a general MANOVA, it may be of value to determine which groups differ from one another and which elements of the size-at-age vector are responsible for these differences. The identification of homogeneous subgroups is analogous to the familiar a posteriori analysis in univariate ANOVA. The second task, unique to multivariate hypotheses, is achieved by calculating the correlations between the original size-at-age value and the coefficients for the discriminant function that maximizes among-group differences (Timm 1975). These correlations, referred to as canonical loadings, increase in magnitude with the contribution of that element to the rejection of the null hypothesis of no group differences. Additionally, the univariate F-tests can be used to corroborate the determination based on the canonical loadings.

In repeated-measures MANOVA it is possible to evaluate differences in the measured variables within individuals and between groups, and in the interaction of individuals and group identity. For growth data it is generally expected, and of no special interest, that size increases with age within individuals (the age effect). The group effect (e.g., population differences) is likely to be of general interest. Moreover, the group \times within-individual interaction could reveal subtle differences in the pattern of growth during ontogeny for the groups being compared. Additionally, single-df orthogonal polynomial contrasts can be used to identify whether the groups' differences are of linear, quadratic, or higher order.

The MANOVA family of models explicitly accounts for dependencies among observations on the same individual. This means that the entire otolith (or fish), rather than each increment, is considered as the independent statistical unit. Using the previous numerical example, 10 otolith increments read from each of 20 fish drawn from two populations would yield a sample size of 40 size-at-age records. Evaluations, comparisons, and statistical tests are conducted on these 40 records as representative of the underlying growth of individuals from the two populations. The critical value of the multivariate test statistic, for example, Wilk's Λ , is based on this sample size ($n = 40$), the number of treatment levels ($k = 2$), and the number of parameters to be estimated ($p = 10$) as $\Lambda_{\text{crit}, 0.05 (p, k-1, n-k)}$. Approximate F-values for this test use df of $k-1, n-k$.

Several considerations and possible restrictions apply to the use of these multivariate models. For the most part, modifications to the model (Eq. 3) have been developed to accommodate these restrictions. First, the sample size, which in the current context is the number of otoliths or fish in the collection or experiment, needs to exceed the number of parameters to be estimated (Tabachnick and Fidell 1989). This is not likely to be a problem in most otolith studies. Note that these models, as in the example below, do allow for unequal sample sizes between groups. Nearly equal sample sizes do, however, ameliorate the effects of violations of the matrices' assumption of equal variance-covariance (Stevens 1986). Second, size-at-age vectors with missing values are generally dropped from the analysis as the default option in statistical packages. This practice results in a loss of information. Model modifications by Woolson et al. (1978) and multi-stage approaches (Laird and Ware 1982) offer a means of handling and including incomplete response vectors. Third, most methods require that the number of measurements per individual be the same and taken for the same set of ages. Obtaining sizes for the same ages for all subjects may not be a problem given the nature of otolith increment data. The equal-record-length restriction can be achieved by truncating the record lengths of individuals to that of the minimum response vector available for use, but this also results in a loss of information. The same-time restriction is relaxed in methods described in Hui (1984). The restrictions on both equal record length and intervals are obviated, however, in modifications to Eq. (3) that consider the repeated-measures data structure

in a two-stage random-effects model (Laird and Ware 1982). Lastly, multivariate procedures generally require access to computers and software packages. (They are not advised for calculators, which can serve for standard regressions and ANCOVA models.) MANOVA, profile, and repeated-measures analyses are available as procedures in SAS (SAS Institute 1987), BMDP (Dixon 1985), and SYSTAT (Wilkinson et al. 1992), among other statistical software.

Analysis of Otolith Increment Data - An Example

We sought an otolith-increment dataset to be used for two purposes: evaluation of the patterns of individual growth and comparison of two or more groups of fish that differed by some identifiable criterion. The following analysis of individual-level increment radii is performed on data from larval Atlantic menhaden, *Brevoortia tyrannus*.

Atlantic menhaden spawn during autumn and winter months in the shelf waters off the North Carolina coast. Discontinuous spawning can occur during the spawning season. Multiple spawning events can be inferred from collected larvae by back-calculating dates of hatching because increments are deposited daily following first feeding (\approx 3-4 d post-hatching, Maillet and Checkley 1989; Warlen 1992). Two menhaden spawning events, occurring early in 1986, were the source of data for the present analysis. The back-calculated dates place the modal days of spawning for these two events in late January and the middle of February for cohorts I and II, respectively.

A subsample of the original dataset of otolith-based sizes-at-ages was supplied to us by G. Maillet (Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada). The subsample represents a random draw of 30 menhaden larvae from each of two cohorts collected on February 14 and March 5-6, respectively. At the time of collection, larvae ranged in age from 17 d to 27 d and in size from 11.4 mm SL to 19.0 mm SL (Fig. 3). In order to increase the sample

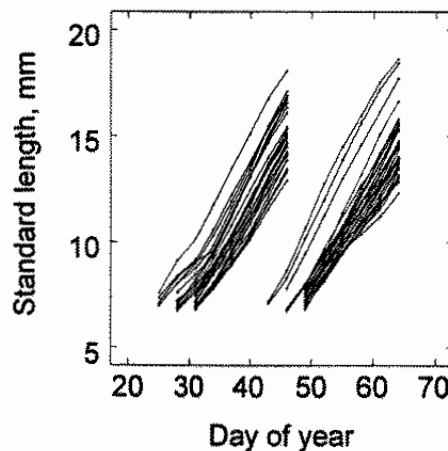


Fig. 3. Relationship between back-calculated length and day of year for a sample of 30 Atlantic menhaden larvae from each of two cohorts. Larvae were collected on February 14 and March 5-6 and ranged in age from 17 d to 27 d. Increment radii for every third day, starting at the otolith perimeter, were measured and used in the analyses.

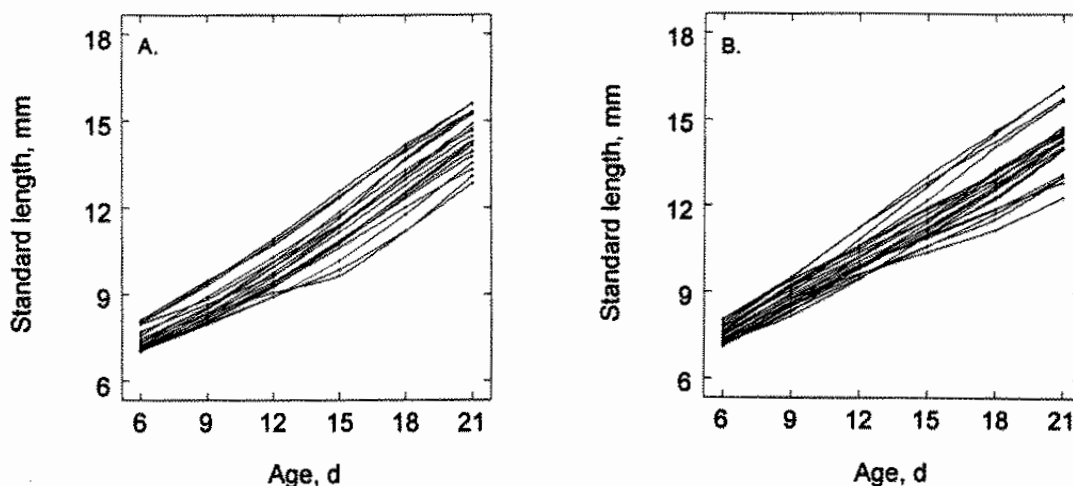


Fig. 4. Relationship between back-calculated length and age for cohorts I (A) and II (B) of Atlantic menhaden. The size-at-age trajectories are for individuals of ages 6-21 d post-hatching. The trajectories show a low level of crossing over during ontogeny (see also Table 2). The two cohorts were compared for differences in individual trajectories by a multivariate analysis of variance tailored to growth data (Table 3).

size of larvae for time series analyses in the original studies by Maillet and colleagues, every third increment radius was measured beginning from the otolith perimeter. The data provided to us for analysis, therefore, were otolith radii at collection and for every third increment (day) prior. Complete descriptions of the methods of fish collection and otolith preparation, and an analysis of the entire dataset from which the subset presently analyzed was drawn, appear in Checkley et al. (1988) and Maillet and Checkley (1989, 1991).

DATA PREPARATION

Increment records for each larva were not of the same length nor were they reported for the same 3-d intervals of age (Fig. 3). We, therefore, trimmed records to a standard period of 6-21 d post-hatching and used only those records for which observations were made on the same 3-d sequence (i.e., 6 d, 9 d, 12 d, 15 d, 18 d, and 21 d post-hatching). In addition, in order to increase our sample size, the series of otolith radii measured on larvae at ages 5 d, 8 d, 11 d, 14 d, 17 d, and 20 d were converted to ages 6-21 d by linear interpolation. This resulted in a final sample size for our analysis of 19 and 23 larvae for cohorts I and II, respectively (Fig. 4, Appendix I).

The statistical analyses were conducted on otolith sizes, s , as estimated by increment radii. Analysis of the changes in otolith sizes, s , was also performed for some tests as it can potentially present different insights into the growth process of larval fish. In particular, analysis of short-term growth (Δs) rather than size effectively removes the historical component embedded in size measurements because the previous observation of a fish's size has a large influence on its current size.

Preliminary inspection of the data revealed the variances increase with mean radii (Fig. 4). Transformation of the radii to their natural logarithms was conducted to equalize their variances (Table 2).

A good relationship exists between fish size and otolith size for menhaden (Maillet and Checkley 1991). Nevertheless, to avoid the hazards of back-calculation (Francis 1990) and environmentally dependent otolith size-fish size relationships (Mosegard et al. 1988; Reznick et al. 1989; Secor and Dean 1989), we analyze increment data directly. However, otolith sizes (radii), s , were converted to larval standard lengths, SL, for purposes of graphical exposition using the equation (Maillet and Checkley 1991):

$$SL = 4.829 (\exp (1.544 (1 - \exp (-0.024 s))))$$

which resulted in $r^2 = 0.95$ ($n = 1,085$).

COVARIANCE IN SIZE-AT-AGE

The pattern of otolith growth resulted in a positive covariance between radii across ages for both cohorts (Table 2). Size advantages established by the age of 6 d (the youngest ages analyzed) persisted for the 2-wk period considered (Table 2, Fig. 4). The lack of evidence of compensation within cohorts for ages 6 d to 21 d was consistent with the pattern observed for size-at-age data when all available ages were used (ages 3-27 d, Fig. 3). The mechanisms responsible for size differences expressed as early as 6 d post-hatching that persisted until at least 3 wk of age warrant further study. A rank correlation analysis of increment widths (Δs) rather than radii revealed that the pattern of individual trajectories was not simply a matter of propagation of early size advantages (Table 2). Rather, individuals of higher growth rate tended to maintain high growth rates, particularly so for ages ≥ 9 d. The demonstration that observations of increments from an individual otolith are interdependent underscores the need to consider such data in a multivariate framework for purposes of testing hypotheses about among-group differences.

COMPARING GROWTH TRAJECTORIES FROM TWO COHORTS

The hypothesis of no difference in size-at-age records of the two cohorts was evaluated with a repeated-measures MANOVA. This model has the same general form as Eq. (3) but with only one extraneous factor (cohort identity)

$$S_{(42 \times 6)} = X_{(42 \times 7)} B_{(7 \times 6)} + E_{(42 \times 6)} \quad (4)$$

where s_{ij} is the otolith size (radius) of fish j at age i , B contains parameters for the sizes-at-ages and the single categorical variable for cohort identity, and E is the matrix of deviations from the expectation of $S = XB$. By means of suitable contrast matrices, the repeated-measures format allows for testing of variations due to within-subject differences, between group (factor) differences, and the interaction of subject and group (Cole and Grizzle 1966). The model was evaluated using SYSTAT (Wilkinson et al. 1992).

Table 2. Variances (diagonal, shaded), covariances (lower triangle), and rank correlations (upper triangle) of otolith increment data at different ages of larval Atlantic menhaden. Variances and covariances are for increment radii. Rank correlations are for increment radii (first entry) and the increase in radii over successive 3-d age intervals, referenced as $\Delta i-i+3$ (second entry). Rank correlations (Spearman's ρ) are significant at the $p < 0.05$ level (critical $\rho_{(42, 0.05)} = 0.31$) except where noted by *NS*. Significant rank correlations mean that the relative sizes or short-term growth rates of otoliths are maintained over the referenced age interval. Observations from the two cohorts were pooled for this analysis ($n = 42$); however, the pattern of rank correlations within each cohort was consistent with the overall pattern (see Fig. 4). Otolith increment radii were transformed to their natural logarithms prior to this analysis.

Otolith increment radii at: / growth over:	age 6	age 9/ $\Delta 9-12$	age 12/ $\Delta 12-15$	age 15/ $\Delta 15-18$	age 18/ $\Delta 18-21$	age 21
age 6/ $\Delta 6-9$	0.014	0.94/0.38	0.85/ NS	0.77/ NS	0.62/ NS	0.58
age 9/ $\Delta 9-12$	0.013	0.013	0.93/0.71	0.80/0.58	0.64/0.36	0.59
age 12/ $\Delta 12-15$	0.011	0.012	0.013	0.94/0.83	0.81/0.52	0.76
age 15/ $\Delta 15-18$	0.011	0.011	0.013	0.015	0.95/0.59	0.91
age 18	0.009	0.009	0.012	0.014	0.015	0.97
age 21	0.008	0.008	0.010	0.013	0.014	0.014

The individual size-at-age trajectories of the two cohorts differed significantly (Table 3). This was evaluated by the age \times cohort interaction in the repeated measures MANOVA, which was highly significant (Wilk's $\lambda = 0.49$, $p \leq 0.001$). The differences in the size records of the two cohorts occurred at ages 9 d and 12 d, which were identified by the canonical loadings, and corroborated by the univariate F-tests (Table 3). A visual comparison between the two cohorts of their mean sizes-at-age and mean increment widths emphasizes these differences (Fig. 5). Slower growth of larval menhaden in cohort I at ages 6-9 d, which is also revealed on the size-versus-date plot for Julian days 30-35 (Fig. 3), was associated with storm events in the original analyses by Maillet and colleagues (Checkley et al. 1988; Maillet and Checkley 1991). They hypothesized that these storms dispersed the zooplankton prey of menhaden larvae which in turn reduced larval consumption rates. There is evidence that cohort I regains parity with cohort II in their respective sizes-at-ages through faster growth between ages 12 d to 18 d (Fig. 5).

A comparison of MANOVA results with those from traditional univariate analyses is instructive. The highly significant differences in size-at-age records between the two cohorts revealed by the MANOVA (Table 3) were less apparent when the size-at-age records were treated

Table 3. Multivariate repeated-measures analysis of otolith increment radii. Upper panel: The hypothesis of no difference in size-at-age trajectories of the two cohorts is tested by the age \times cohort effect. The significant age \times cohort term reflects a lack of parallelism of the pattern of individual growth between the two cohorts. The age effect results from the increase in radii with age. Lower panel: The sizes-at-ages responsible for the rejection of the null hypothesis of no difference in size-at-age trajectories between the two cohorts in the MANOVA are identified by the magnitude of the canonical loadings. These are corroborated by the univariate F-tests for contrasts between the two cohorts for increment radii compared at common ages. Both criteria identify otolith radii at ages 9 d and 12 d as responsible for between-cohort differences (see Fig. 5). Abbreviations: df_h = hypothesis degrees of freedom; df_e = error degrees of freedom; Wilk's λ = multivariate test statistic; * = $p < 0.05$.

Source	df_h	df_e	Wilk's λ	F	p <
age	5	36	0.004	1676.4	0.001
age \times cohort	5	36	0.493	7.4	0.001

	Otolith radii at					
	age 6	age 9	age 12	age 15	age 18	age 21
canonical loadings	0.14	0.36	0.35	0.17	- 0.01	- 0.06
F-value from univariate tests for cohort I vs II ($df = 1, 40$)	0.88	5.75*	5.31*	1.30	0.01	0.162

as independent observations and analyzed via ANCOVA. The F-value resulting from the ANCOVA for the hypothesis of no cohort differences in their size-age relationships was 5.1, which would be claimed as significant ($p = 0.025$). Note that this p-value is more than 25 times larger than that from the MANOVA. Further, the univariate probability value is substantially influenced by the inflated df (1, 249), which follows from the assumption that all increment radii were independent.

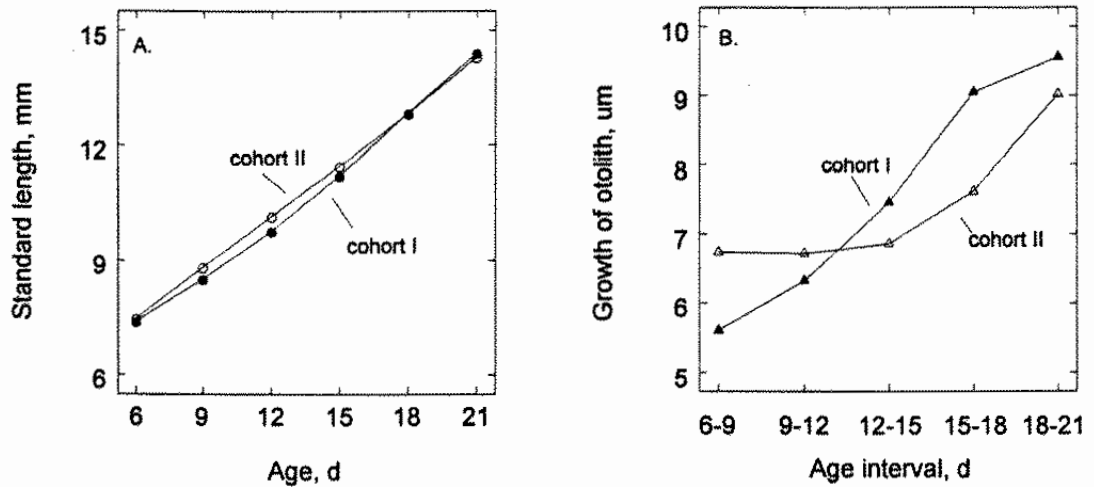


Fig. 5. Relationship between average back-calculated length and age (A) and between increase in otolith size and successive 3-d age intervals (B) for two cohorts of Atlantic menhaden ages 6-21 d post-hatching. Individuals of cohort I were significantly smaller than members of cohort II at 9 d and 12 d (Table 3). Initial growth rates were lower for larvae of cohort I, but there was evidence of compensation through accelerated growth between ages 12-18 d.

General Conclusions

Otoliths in general, and their increment structure in particular, have unique utility in providing the researcher with insights into the history of individual fish. Information on past size and growth rates of larval fish are especially valuable because body size has often been assumed to play an important role in food consumption, predator avoidance, and the chances of survival in general (Miller et al. 1988; Bailey and Houde 1989). The individual records of sizes at past ages made available from otoliths are precisely the type of data that are critical for determining whether mortalities are at random or whether certain attributes are disproportionately represented in the population of survivors.

Along with the advent of techniques that supply information on the current and past status of the individual come associated challenges in how to best analyze such data. It is imperative that the statistical techniques employed match the level of resolution of the data and the inferences of interest (Chambers and Leggett 1992; Chambers 1993). From our assessment of otolith-based growth studies we conclude that a mismatch has developed due in large part to the failure to recognize that such data are derived from repeated measures on individuals. The mismatch between individual-level longitudinal data on one hand, and statistical techniques developed for single independent observations on the other, is not unique to fishery science. Similar accounts and suggestions for mitigating the problem, which may provide additional guidance for the analyst of longitudinal data in fisheries, have been documented in animal science (Gill and Hafs 1971), dendrochronology (van Deusan 1989), community ecology (Gurevitch and Chester 1986), physiological ecology (Potvin et al. 1990), and population biology (Bowen et al. 1992). Our

recommendations for analysis of otolith-based growth data are to first determine the data structure inherent to the application and then proceed with statistical methods that match that structure. Under restrictive assumptions these methods may be univariate, even for longitudinal growth data, but to fully exploit the information available from otoliths will likely require multivariate techniques.

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Appendix I. Otolith radii from two cohorts of larval Atlantic menhaden ages 6-21 d post-hatch used in the analyses presented in Tables 2 and 3. Radii were measured for every third increment.

Cohort Number	Fish Number	Day of Year @ Age 6 d	Otolith Radius (μm) at Age (d)					
			6	9	12	15	18	21
1	1	25	11.90	18.00	21.80	24.70	32.70	43.20
1	2*	26	13.13	18.23	23.73	31.00	39.87	49.67
1	3*	26	16.97	23.87	30.20	39.03	48.97	59.37
1	4*	26	14.93	19.73	24.80	32.53	41.40	50.40
1	5	28	11.50	17.60	24.60	33.50	43.60	54.40
1	6	28	11.60	16.40	21.10	25.80	32.80	41.80
1	7*	29	12.47	18.07	25.27	33.57	42.57	51.77
1	8*	29	11.67	16.63	22.77	30.70	40.10	50.30
1	9*	29	13.70	19.20	27.17	36.40	46.43	56.73
1	10*	29	11.97	16.83	22.97	30.43	39.33	48.43
1	11*	29	11.63	16.20	21.10	27.43	35.83	45.83
1	12	31	16.30	20.60	27.20	34.00	43.40	53.20
1	13	31	12.60	18.90	26.30	35.20	46.80	57.30
1	14*	32	14.30	21.07	28.10	35.63	44.40	53.10
1	15*	32	16.47	23.07	30.77	39.33	48.40	57.40
1	16*	32	16.57	23.53	31.33	40.30	49.70	59.30
1	17*	32	13.20	19.23	26.33	33.87	43.33	53.33
1	18*	32	12.10	17.33	23.07	29.70	37.17	44.57
1	19*	32	12.23	17.27	23.43	30.63	38.90	47.30
2	1*	44	13.93	21.17	30.67	40.67	51.80	63.53
2	2	46	15.50	23.80	32.90	42.70	52.40	63.30
2	3*	47	12.50	19.30	27.53	35.47	43.43	52.13
2	4*	47	12.07	18.87	25.93	30.97	36.37	43.40
2	5*	47	12.73	19.03	25.80	32.93	40.90	50.63
2	6	49	16.60	23.40	29.50	36.60	43.40	51.80
2	7	49	14.40	22.20	28.40	34.30	41.40	48.50
2	8	49	14.30	20.80	27.40	34.40	44.00	52.60
2	9	49	12.90	18.90	24.30	29.50	34.50	42.80
2	10	49	12.80	20.20	26.10	31.30	36.50	41.70
2	11	49	12.10	17.90	25.70	33.80	43.50	53.30
2	12	49	12.60	18.80	25.40	31.70	38.30	48.40
2	13	49	12.50	17.20	23.60	31.40	40.10	48.90
2	14	49	15.20	22.40	29.50	38.00	48.80	59.60
2	15	49	13.80	20.20	24.50	28.40	32.60	38.80
2	16	49	13.70	20.40	26.70	32.70	40.50	51.00
2	17	49	16.00	23.50	29.00	36.30	42.00	52.80
2	18	49	14.60	22.70	29.50	36.30	43.60	53.50
2	19	49	12.00	18.20	23.80	29.40	35.60	42.80
2	20*	50	16.67	23.97	32.83	41.37	50.10	60.30
2	21*	50	13.27	19.40	25.93	31.40	38.70	48.60
2	22*	50	13.27	19.63	25.73	32.27	40.43	50.13
2	23*	50	15.20	21.70	27.53	34.30	42.37	50.37

* estimated by linear interpolation from increment radii representing ages 5-20 d