Larval Fish Age, Growth, and Body Shrinkage: Information Available from Otoliths

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External and internal examination of Atlantic cod (*Gadus morhua*) otoliths for macrostructure and microstructure, by light and scanning electron microscopy, indicated daily rhythmic patterns. The first daily increment developed the day after hatching. Sagittae changed shape from spherical to oblong at 20d and to crenulated at 50–60d old. Cod were reared at three temperatures (6,8 and 10°C), to provide a range of growth and developmental rates. Distinctive marks formed at yolk-sac absorption, initiation of feeding and settlement.It was possible to determine age and growth rate from otolith analyses. The relationship between otolith length and fish size was independent of growth rate; it followed a quadratic function for the smaller individuals (<6.5 mm), and it was linear in individuals greater than 25 mm. Larval fish shrank considerably at death. The magnitude of shrinkage was dependent on larval length, and the elapsed time between death and fixation. Immediate fixation in ethanol resulted in minimal shrinkage. The relationship between fish length and otolith diameter may be used to correct for shrinkage associated with collection and death.

L'examen interne et externe de la macro et de la microstructure d'otolithes de la morue franche (*Gadus morhua*), par microscopies optique et électronique à balayage, a révélé la présence de rythmes quotidiens. La première augmentation quotidienne est apparue le jour après l'éclosion. Les sagittas, sphériques, sont devenues oblongues à 20 jours et crénelées à 50–60 d. Trois groupes de morues ont été élevés à des températures différentes (6, 8 et 10°C), ce qui donne diverses vitesses de croissance et de développement. Des marques caractéristiques sont apparues lors de la résorption du sac vitellin, du début de l'alimentation et de la descente vers le fond. L'analyse des otolithes a permis de déterminer l'âge et le taux de croissance des larves. Le rapport existant entre la longueur des otolithes et la taille des poissons était indépendant du taux de croissance; ce rapport avait la forme d'une fonction quadratique pour les poissons plus petits (<6,5 mm) et était linéaire pour les poissons de taille supérieure à 25 mm. Les larves de poissons ont rétréci de manière considérable à leur mort. L'importance du rétrécissement dépendait de la longueur de la larve, ainsi que du temps écoulé entre la mort et la fixation. La fixation immédiate à l'éthanol a permis d'obtenir un rétrécissement minimum. Le rapport existant entre la longueur des poissons et le diamètre des otolithes peut servir à corriger le rétrécissement lié à la récolte et à la mort.

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The discovery of microincrements in otoliths — analogous to annual increments, but formed on a daily basis — has permitted the ageing of larval fish. Daily growth increments are displayed in the structures of many animals (Neville 1967), and their formation in fish otoliths was first postulated by Pannella (1971, 1974). Subsequent work by Brothers et al. (1976) verified daily increment deposition for several fish species reared from eggs in the laboratory. Since then numerous studies have dealt with daily increments in reared and wild fish (e.g. Campana and Neilson 1985; Jones 1986a).

Although daily increments occur in most species studied, validation of the frequency of increment deposition is a necessary prerequisite for age and growth studies that use otolith microstructure. Three methods of validation are: (1) the use of known-age fish; (2) sequential sampling of a population in which neither age-selective migration nor age-selective mortality occurs; and (3) the introduction of dated marks onto the otoliths. The first method is the preferred one, especially in the study of larval fishes, since increment formation is initiated at different ages depending on the fish species (Neilson and Geen 1982, 1985; Brothers et al. 1976; Radtke and Dean 1982). In the present study, daily increment deposition was validated in Atlantic cod (*Gadus morhua*) using laboratory-raised larvae and juveniles. Precise ageing provides accurate estimates of growth rates, making other larval fish population parameters more reliable.

Besides permitting age determination, otoliths can provide other information about larval fish biology and ecology. Otoliths have been used to determine temperature histories (Radtke 1989; Radtke et al. 1989) and migration patterns (Radtke et al. 1988; Radtke and Morales-Nin 1989). In this study otolith microincrements are analyzed for life-history changes in larval and juvenile cod, and increment width is related to growth rates.

Numerous authors (e.g. Blaxter 1971; Theilacker 1980; Hay 1981; Leak 1986) have demonstrated a difference in measurements between laboratory and field-collected specimens due to shrinkage. Larvae, in particular, are susceptible to shrinkage. Although most studies have focused on the effect of preservatives on fish size (e.g. Blaxter 1971; Schnack and Rosenthal 1978; Tucker and Chester 1984), death itself may be the major cause of shrinkage (Theilacker 1980; 1986). In the present study postmortem shrinkage in cod is analyzed in individuals ranging

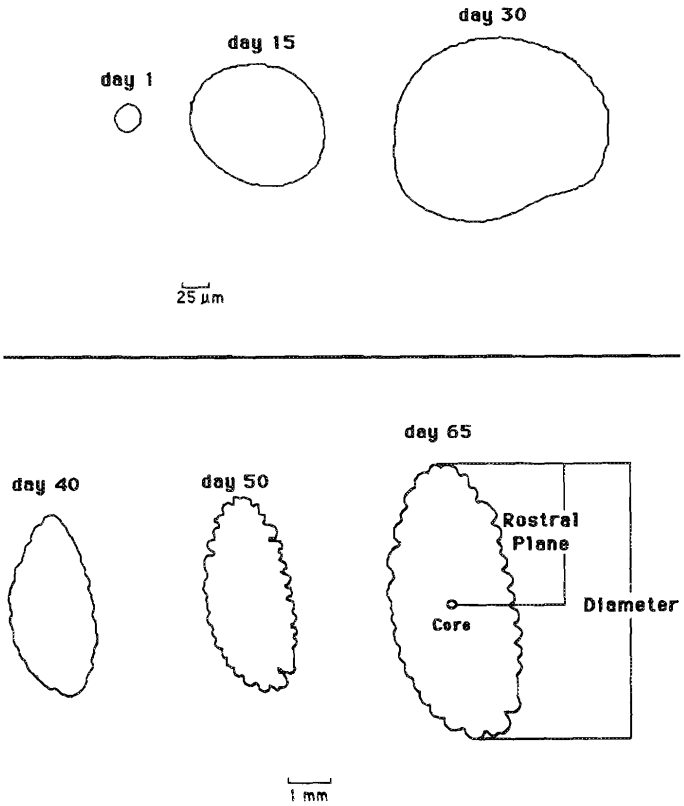

FIG. 1. Changes in shape of the sagitta of the Atlantic cod, *Gadus morhua*, as related to developmental time. Otoliths from newly hatched larvae through juvenile fish elongate along the rostrum followed by dentation of the edges. Otolith diameters were measured for the spherical otoliths. Lengths of the elongated sagittal otoliths of cod were taken along the longest plane within the otolith as shown, and incremental widths were obtained from the outermost increment in the rostral plane.

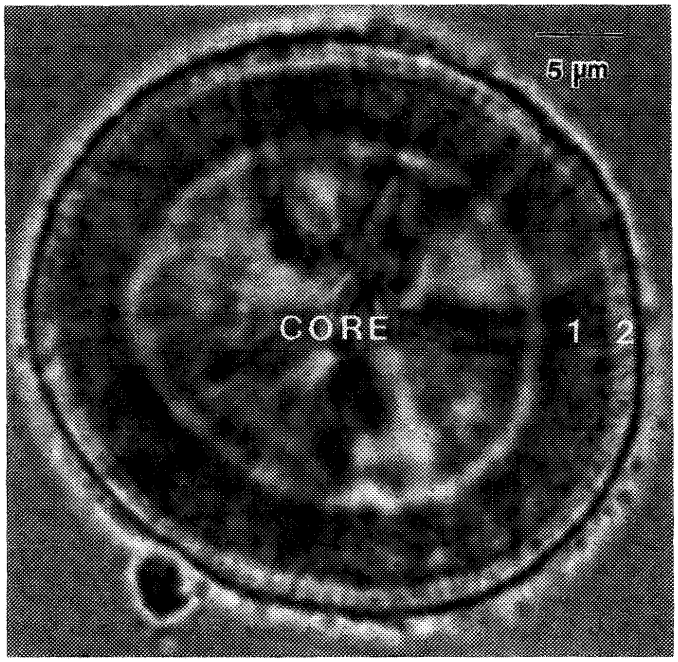

FIG. 2. A sagittal otolith from a 3-d-old cod larva displaying alternating light and dark zones, as viewed by light microscopy. The core of the otolith is not enumerated as an increment, since it was formed during embryological development.

from hatching through development into juvenile. A relationship between fish length and otolith size was determined and the use of otolith size to account for possible shrinkage is evaluated.

I used cod for these investigations because of its wide North Atlantic distribution (Dannevig 1933; Pinhorn 1969), its prominence in North Atlantic marine ecosystems, and worldwide interest in cod biology. Furthermore, cod have pelagic larvae which are subjected to a wide range of physical and nutritional conditions over a short time. The use of otoliths in the study of the effects of growth on survival and recruitment increases the understanding of life-history patterns in cod larvae and juveniles, and makes it possible to address important questions on how fish larvae function in the ocean.

Materials and Methods

Cod were reared at the Flødevigen Biological Station, Arendal, Norway. Fertilized eggs were obtained and incubated with a photoperiod of 12 h of light and 12 h of darkness. Only larvae which hatched within 24 h of each other were used. Data recorded included: development with respect to yolk absorption and gut differentiation, body growth, and total length. The eggs were incubated at water temperatures of 6, 8 and 10°C. Experiments lasted 4–6 mo.

Hatching times were noted and the fish were then fed ad libitum with plankton collected in the adjacent fjord. At each sampling, 10 fish from each temperature group were taken for otolith and growth measurements. Another 10 fish were sampled to study postmortem shrinkage, and 10 fish were preserved in 96% ethanol while still alive. Samples were taken daily for the first 15 d and weekly thereafter.

In the shrinkage experiments, the fish were removed from the incubation tanks while still alive, placed in a depression slide, and measured with an ocular micrometer to the nearest 0.01 mm. The fish were then allowed to die, and the time of death (based on cessation of heart movement) was recorded. All sacrificed fish were measured 15 min after death by exposure to air or alcohol. Otoliths were prepared for light microscopy, the sagittae were measured, and these measurements were correlated with the total length of the fish before death.

Sagittae were too large for whole mounts in juveniles, so they were ground from the proximal and distal sides using a size-graded series of grinding compounds. Transverse sections at the focal level appeared to give the most complete picture of the increments found in the otolith and these were chosen for routine use. Such sections are described by Steffensen (1980). In preparation for light microscopy, otoliths were mounted on glass slides using a mounting medium having a similar refractive index as that of glass, and covered with a glass cover slip.

In preparation for internal structural analyses, otoliths from larvae and juveniles were mounted whole on SEM viewing stubs using 5-min epoxy resin. The otoliths were ground with 600 grit sand paper and polished with 0.3 µm alumina polish. The polished surfaces were then decalcified with 7% EDTA at pH 8 for 5–15 min. Following decalcification, the samples were coated with gold and observed in a SEM.

Larval otoliths and otolith increments were measured under a light microscope using an ocular micrometer. Larval otoliths

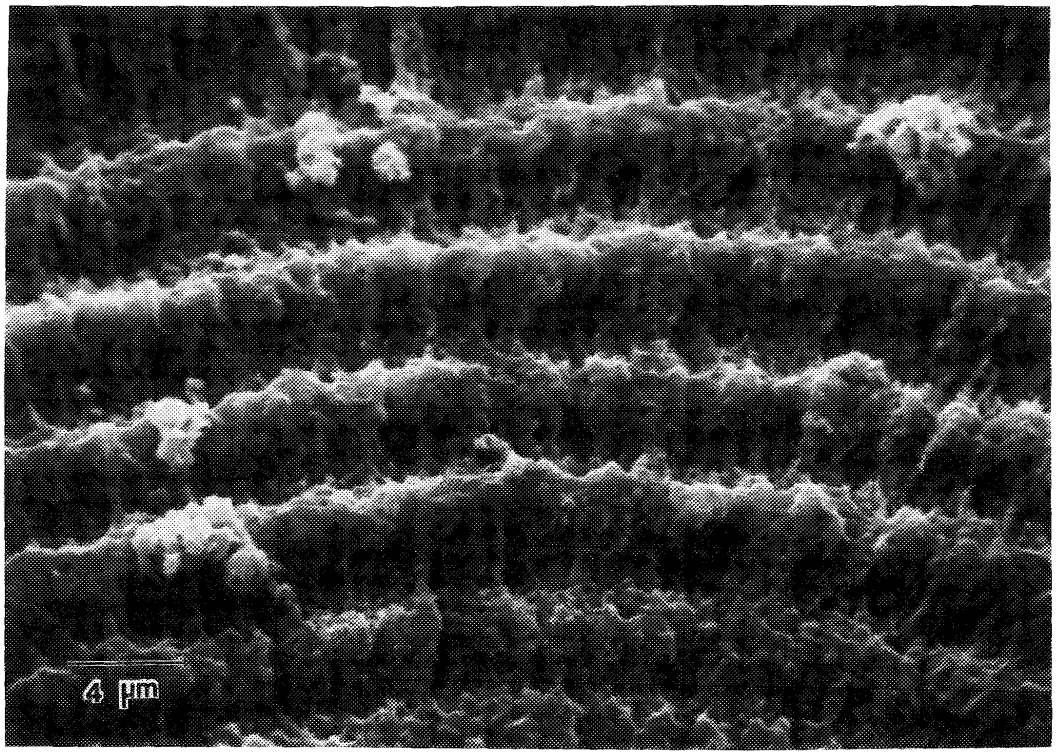

FIG. 3. Scanning electron micrograph of the microstructural components of cod sagitta demonstrating protein deposition which produced distinct increments. These increments were deposited daily.

FIG. 4. Scanning electron micrograph of a sagitta from a 37-d-old cod larva with increments (arrow) which began formation at the time of hatching. Faint increments are observed inside the check.

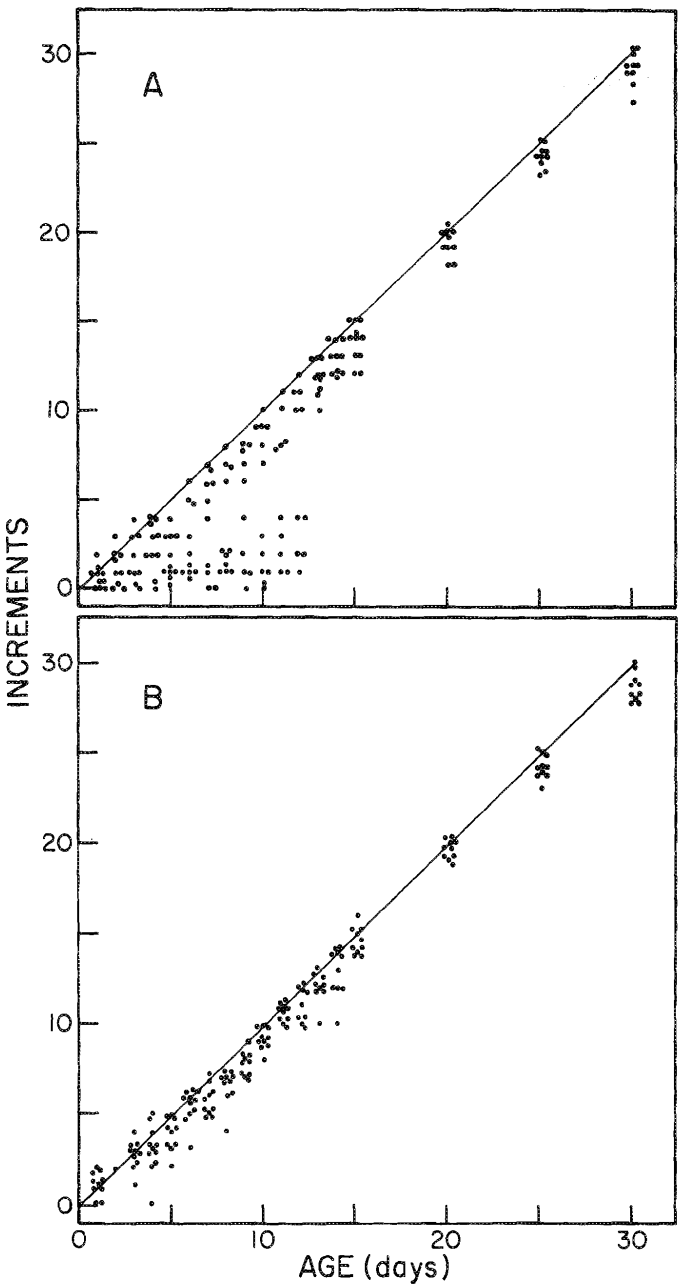

FIG. 5. The relationship between otolith (sagitta) increments and age (days) in cod larvae. (A) Larvae from culture 1 were sampled at random and all individuals were viewed. A large mortality took place at day 12; (B) Larvae from culture 2. Only actively swimming individuals with food in their guts were examined for daily increments.

are nearly spherical and thus appear circular. A simple measurement of the longest length was found to be acceptable for describing their growth. When the sagitta began to show signs of differentiation into other planes, the otoliths were measured along their longest anterior/posterior axis. A diagram of measurements taken is shown in Fig. 1. Each increment is composed of a discontinuous and an incremental zone (Tanaka et al. 1981; Radtke and Dean 1982), which together were counted as one increment. In some fish one or two faint pre-hatching increments were observed by SEM in the core area. These were not counted, and neither was the core counted. The increment appearing closest to the core was usually more prominent than the outer increments and was chosen as the starting point for counting and measuring increment widths. To count increments, the otoliths were presented to the author in a random and undisclosed sequence with respect to age. Three independent readings were performed by the author on each otolith. The counts did not vary significantly and the median was accepted. Approximately 5% of the otoliths were discarded due to abnormal shape, breakage during handling, or difficulty in reading. Both the left and right otoliths of the larval fish were counted, but no significant differences were observed. Thus, the mean for the two otoliths were used to estimate age.

In addition, one cohort of hatchlings was not fed and kept at 6°C. After 10 d all larvae were still robust and demonstrated healthy behavior. These fish were then sacrificed and examined for increment formation

Only the right otolith for each juvenile cod (20 d or older) was counted and measured. Otolith shapes and structural properties were analyzed with a computer digitizer. Counts of composite zones, consisting of one incremental and one discontinuous zone, were made. All sections on juvenile fish were found to be readable. Increment width measurements were made on the outermost increments of sagittae examined in the rostral plane. All counts and measurements were made with a light microscope. Scanning electron microscope (SEM) measurements and counts were done on a subsample, usually one otolith per group of fish, to compare to the light microscope results.

Results

The sagittae and lapilli of developing cod were the first tissues to calcify, as determined by polarized light examination. Calcification of these otoliths occurred just before eye pigmentation during embryogenesis. The asterisci were not formed at the time of hatching, and did not become apparent until the fish reached about 6 mm in length, or about 20 d of age. At hatching, the lapilli were slightly larger than the sagittae, but this size difference was reversed within a few days. Diameters averaged 26.8 ± 1.7 μm at hatching for lapilli, and sagittae measured 4.0 μm smaller.

From the otolith morphology it was possible to distinguish three stages in sagittal growth (Fig. 1). The first stage (spherical stage) occurred in larvae of approximately 20 d or younger, and was characterized by nearly spherical sagittae. Thereafter, otolith shape began to elongate and a sulcus became apparent (oblong stage). At 50–60 d of age a third phase in otolith growth (crenulated stage) could be distinguished, and sagittae became distinctly crenulated.

Spherical Stage

There were distinct increments of microstructure originating at the core region. When otoliths were examined under transmitted light, the increments appeared as alternate light and dark areas (Fig. 2). Under the SEM microincrements could be enumerated and measured from the rugose surface of prepared otoliths (Fig. 3). The fertilized eggs reared at 10,8, and 6°C hatched after 10, 12, and 14 d of incubation, respectively. In a majority of larval otoliths, a prominent increment was found close to the core (Fig. 4), corresponding to hatching. Outside the core the matrix was arranged in concentric, undulating bundles with growth increments beginning to form the day after hatching.

Increments examined using light microscopy appeared to form on a daily basis. Otoliths from laboratory-reared larvae had less distinct increments than those from wild larvae. When

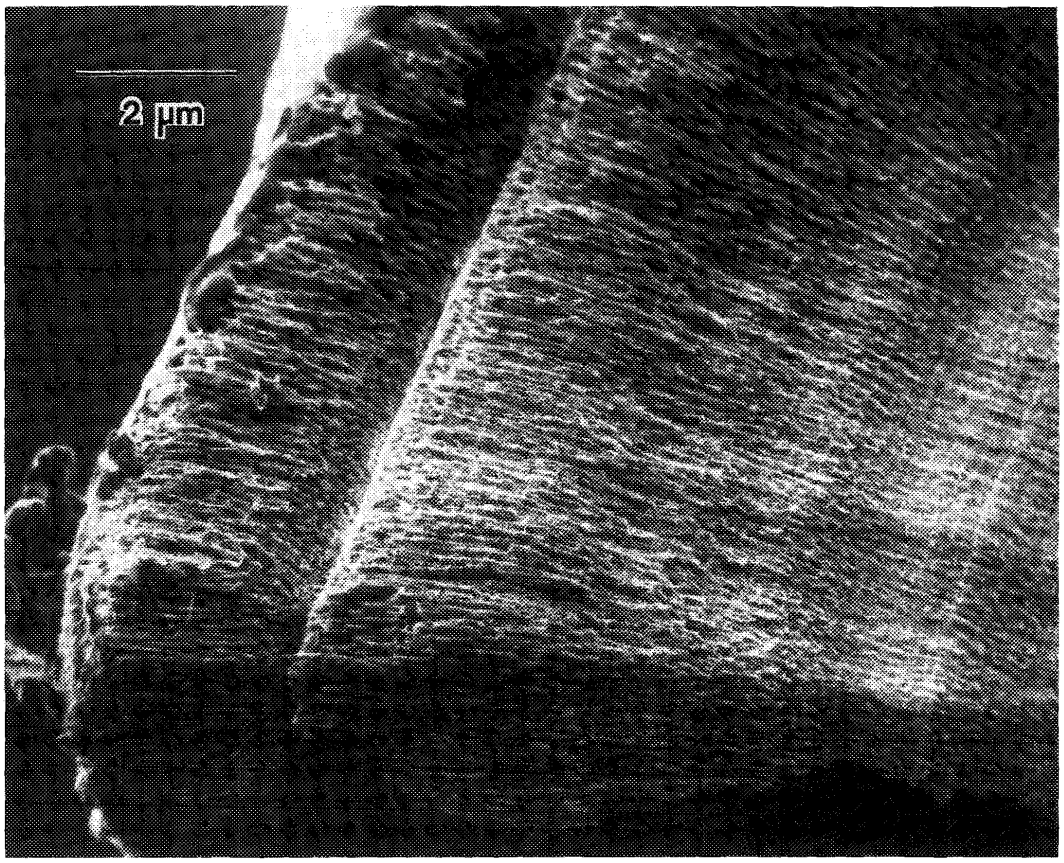

FIG. 6. Scanning electron micrograph of a sagitta from a 7-d-old cod showing a distinct incremental check corresponding to the time of yolk-sac absorption and first feeding. The ridges are the protein matrices of each increment after decalcification.

increments in the sagittae of a random sample of larval cod were viewed, their frequency approximated one per day (Fig. 5A), except for the first 12 d after hatching. For actively swimming and feeding larvae the relationship was closer to one increment per day (Fig. 5B). The state of health or physical condition of the larvae examined from culture experiments appeared to affect increment counts. In moribund larvae, increments seemed to cease forming and this could confound the interpretation of counts if these larvae were examined mixed with healthy ones. The present study suggests that with experience, non-competent larvae could be distinguished solely on the appearance of their otoliths.

The yolk-sac phase ranged from 4 d at 10°C to 12 d at 6°C. Most otoliths demonstrated a definite transition zone at the end of yolk-sac absorption (Fig. 6). After yolk-sac absorption, growth was slow for 7–20 d at all three temperatures. The highest mortality occurred during this phase. This transition to active feeding may not be successful in many individuals hence the high mortality rate. The onset of feeding in larvae produced heavier, more distinct increments. Faint daily increments, which were often difficult to interpret, were observed in otoliths of larvae that had been starved for up to 10 d. They could easily be misinterpreted if not observed with the necessary caution.

The sagitta and lapillus grew slowly during the first 15–20 d, and otolith growth was related to larval growth (Fig. 7B and 8A). Increment widths were narrow during this period of slow growth, and the widest increments were observed at the highest temperature. Sagitta diameter was found to be a good predictor for larval length, and during the early growth phase, the relationship between the size of the otolith and the size of the fish was best fitted by a quadratic curve (Fig. 8A).

Oblong and Crenulated stages

Cod sagittae changed as they grew, from spherical to elongated to crenulated over a 65-d period (Fig. 1). The proximal surface of each sagitta was oriented toward the central axis of the fish. The convex surface was demarcated by a shallow sulcus, the depth of which increased with growth. The sagittae have a rounded rostrum with an undifferentiated antirostrum (nomenclature from Hecht 1978). The margins of the sagittae began dentation at a TL of 50 mm, and there was an increase in dentation with growth. No distinct excisural notches were present. The sagittae were robust, with a shape distinguishable from other gadoid species. These changes in shape are the result of peripheral primordia which make it possible for different shapes to be formed. However, the primary primordia were still present in the otoliths and could be traced from the core area to the edge of the otolith for accurate increment counts. Changes in otolith shape are reflected in the relationship between fish and otolith lengths, as the fish reaches metamorphosis and begins a benthic lifestyle. In addition to the changes in otolith shape, a transitional area was visible at the time of settlement, at 80–90 d of age.

Daily increments were documented through the first 60 d. Some variation in individual growth occurred, but daily incre-

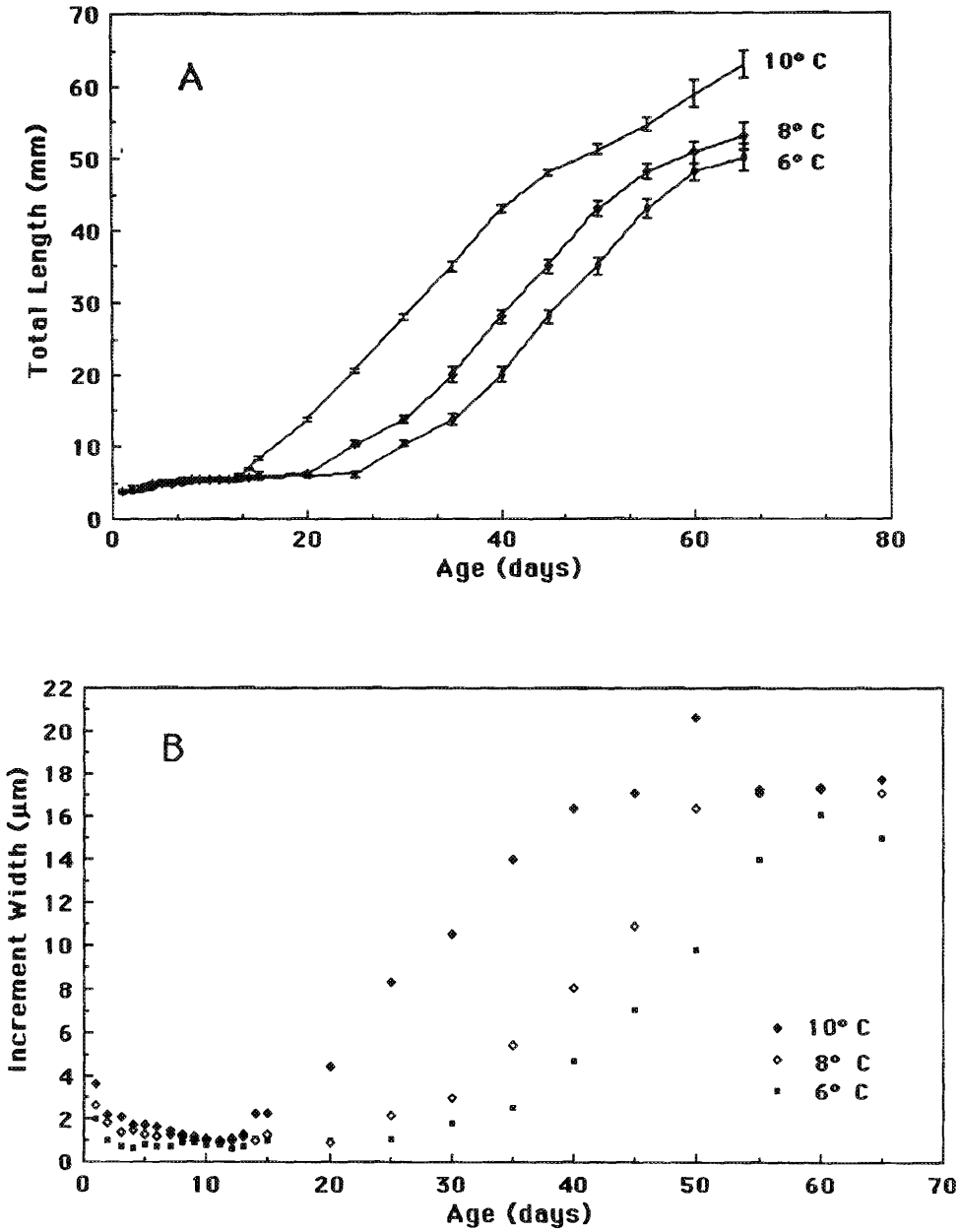

FIG. 7. Temperature related differences in growth and increment width for cod. (A) Changes in total length associated with age in days of cod reared at three different temperatures. Data presented for each point are the means of measurements from 10 fish ± one standard deviation; (B) Width of the outermost increments in cod sagittae as related to age.

ment deposition did not vary significantly. In a comparison between number of increments to age in days, the slope of a straight line fitted to the data was not significantly different from unity ($P > 0.05$). An average of 30 increments were found in the sagittae taken from juveniles with a mean age of 31 d. After the initial slow growth phase, otoliths and juveniles grew very rapidly (Fig. 7), and sagittae increment width increased.

The oblong stage was characterized by nearly linear increase in total fish length at all three temperatures (Fig. 7). The highest growth rates were observed between fish lengths of 6.8 and 47.5 mm at all three temperatures. This period of rapid growth occurred between 13 and 45 d at 10°C, 20 and 55 d at 8°C, and 25 and 60 d at 6°C. Growth rates were 1.22, 1.22, and 1.33 mm/d at 6, 8, and 10°C, respectively. The approximately 10% difference in growth rates among these three temperatures is small compared to the temperature effect on length of the yolk-sac stage and subsequent initiation of feeding.

Total juvenile length was a linear function of otolith diameter for the oblong and crenulated stages (Fig. 8B). This relationship is different from the one observed for the younger larvae (Fig. 8A). Over the entire growth period there were two inflection points (marked by arrows) which differentiate the otolith size–body size relationship into stanzas. At the inflections there appears to be a stalling in otolith growth, and major axis growth may be diverted into accessory primordia. Over the entire growth period the combined data fitted a third degree polynom-

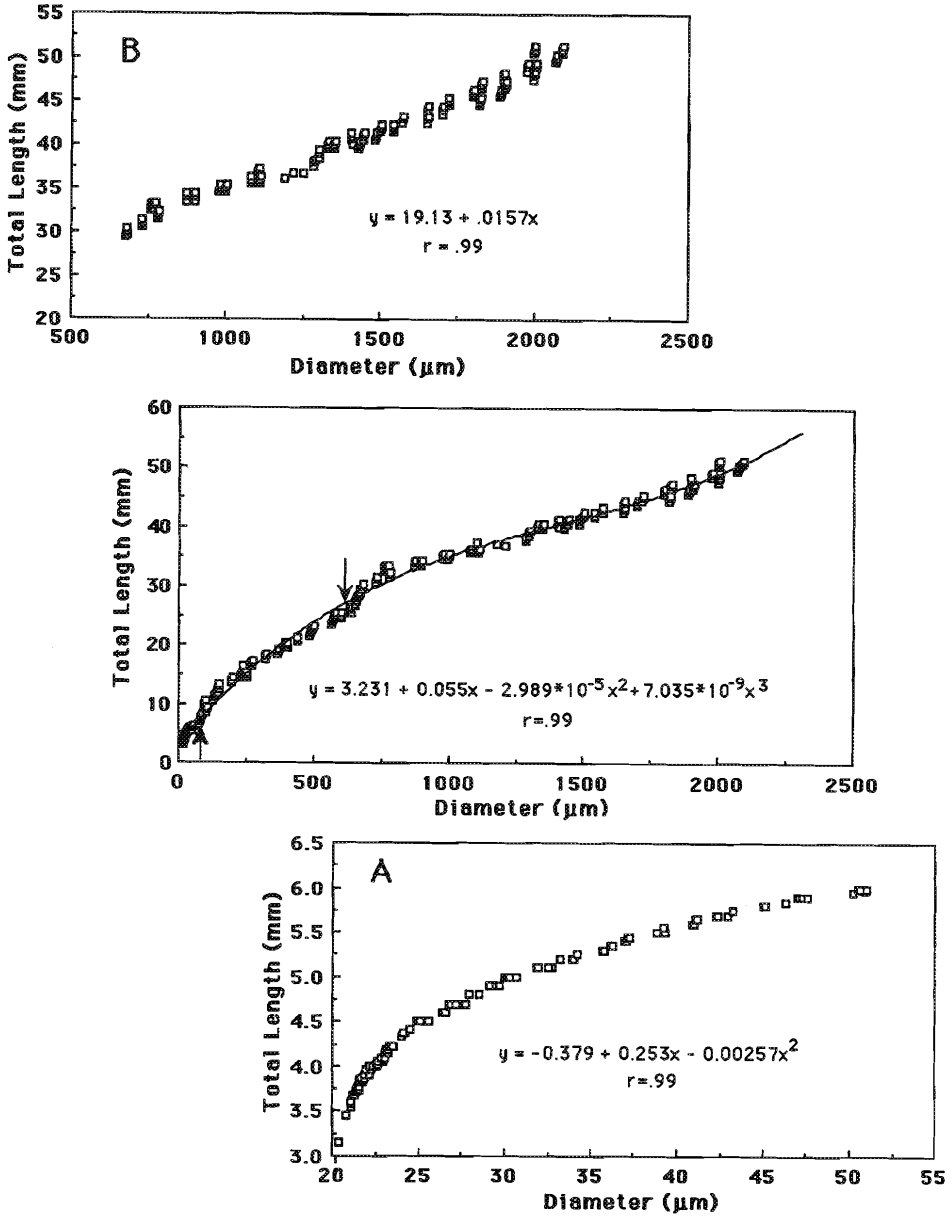

FIG. 8. The relationship between otolith size (diameter) and total length of live larvae in the cod. The figure can be broken into two growth stanzas, A and B, showing growth for the smallest (<6.5 mm) and the larger (>25 mm) larvae, respectively. (A) Demonstrates growth best described by a quadratic equation and shows that there is a rapid increase of growth with a slight leveling of otolith dimensions as the fish grows; (B) Demonstrates that the relationship between total length and otolith diameter is linear during the oblong and crenulated stages.

ial with a correlation coefficient of 0.99 (Fig. 8, middle). Once the relationship between body size and otolith size is established, it may then be used to correct for shrinkage caused by handling. However, caution is necessary at the inflections, where the cubic polynomial is a poor fit.

Body Shrinkage of Cod Larvae

Newly hatched larvae shrank from 30 to 40% within 15 min of death and over 40% overall. Shrinkage appeared to be related to the size of the fish (Fig. 9A and B, Table 1), and no statistical differences were found between fish raised at different temperatures ($P > 0.05$). Shrinkage was greatest in fish between 3.5 and 6 mm in length. Percent shrinkage, y, was related to fish total length, x, by the equation $y = 43.969e^{-0.04x}$ ($r^2 = 0.99$, Table 1). Shrinkage was almost negligible (about 1% for newly hatched larvae, and as low as 0.2% for older fish) in cod that were placed into ethanol directly after death. The shrinkage phenomenon may create problems with plankton net collections when portions of the collection have been dead for 15 min while other portions are still alive upon preservation.

Discussion

Cod growth and development rates can be determined through the analysis of otolith microincrements, which can be counted and measured with a light microscope or a SEM. Light micros-

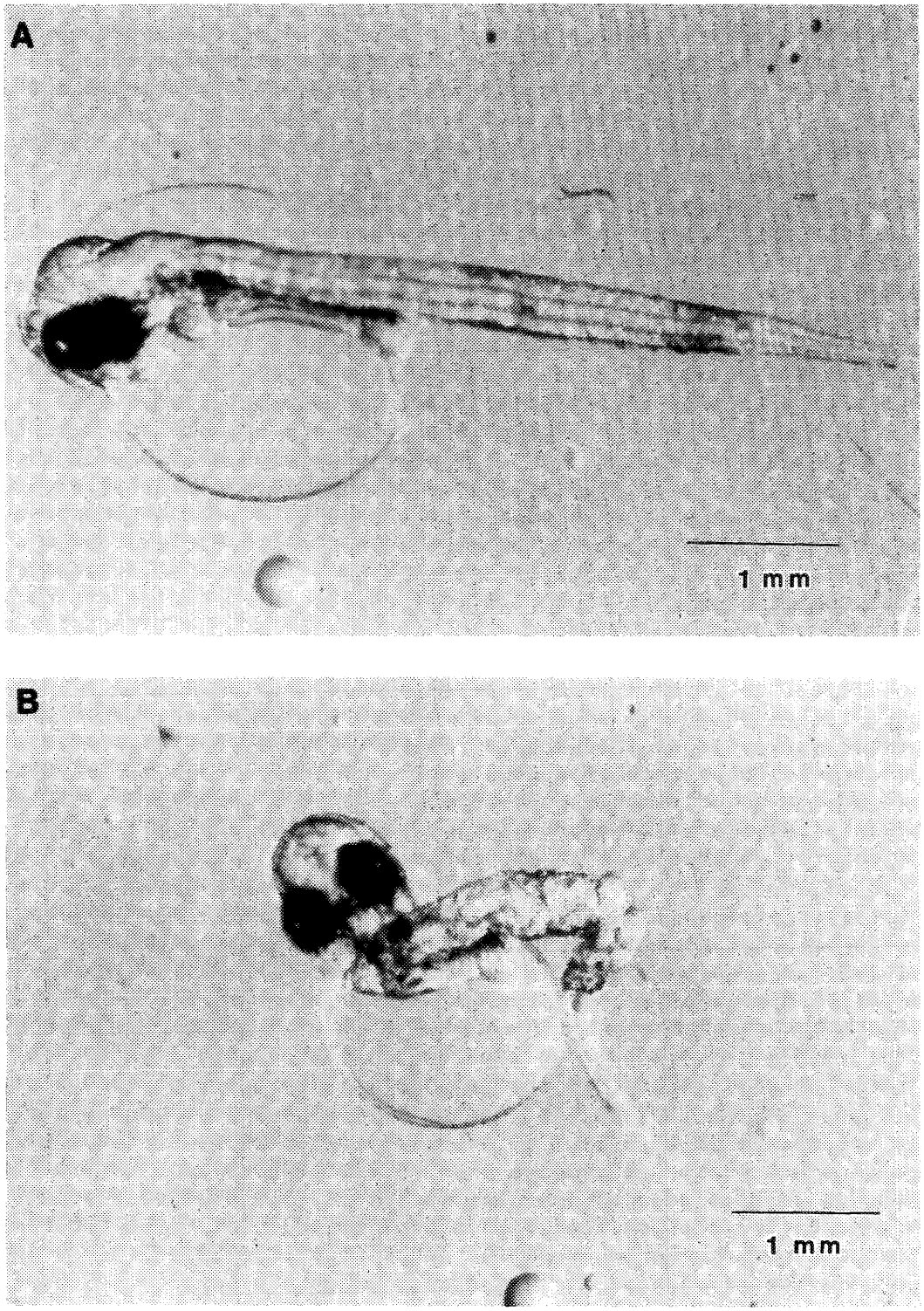

FIG. 9. Shrinkage of young cod larvae associated with death. (A) Young larva which is still alive demonstrates no shrinkage. All lengths taken in the present experiments were from live fish; (B) The same larva 15 min after cessation of heart activity. There is a rapid contraction of the body, and measurements for shrinkage were taken from these larvae once the tails had been extended again.

copy is relatively simple and can be used routinely for large numbers of samples. In this study this technique was used successfully for all routine counts. Under light microscopy, transmitted light is refracted at obtuse angles at the otolith edge due to its curved surface. As a result microincrements may be difficult to distinguish, when faint boundaries occur. When using SEM, visual artifacts do not occur, and increments, which are not easily discerned with a light microscope, can be seen clearly (Campana and Neilson 1985). Otolith increment width measurements are more precise when digitized from SEM photo-

TABLE 1. Shrinkage of the Atlantic cod, *Gadus morhua*, as caused by death processes, and its relationship to fish size.

TL (mm)	% shrinkage	SD
3.618	40.15	2.6
3.784	39.35	3.9
4.140	39.35	4.0
4.402	30.05	4.6
4.710	39.05	3.3
4.776	38.75	3.8
4.916	38.0	2.1
5.182	37.25	3.8
5.322	37.25	3.8
5.488	36.60	2.8
5.430	36.30	4.3
5.444	36.15	3.9
5.472	35.70	4.8
5.570	35.55	5.1
5.612	34.95	5.2
6.084	33.40	6.9
10.0	27.0	7.0
13.75	25.3	3.2
20.0	20.6	5.9
28.0	18.3	3.2
35.0	15.0	3.6
43.25	11.3	3.0
48.0	8.0	3.7
50.0	6.0	5.1

graphs or video-taped images, than when measured with a light microscope. SEM techniques have been used to confirm otolith structure (Dunkelberger et al. 1980; Watabe et al. 1982) and to compare increment counts to those obtained by light microscopy (e.g. Radtke and Dean 1982). However, extensive use of SEM for field surveys is impractical due to the cost and preparation time required. Confirmation of light microscope counts by SEM is highly desirable.

Otoliths form early in cod larvae, but I found no increments before hatching. The lack of increments in the initial stages of otolith formation suggests that the deposition of growth material takes place at a steady rate. Increment formation began at hatching and was continuous thereafter. The formation of growth increments in otoliths is probably due to differential rates of production and/or deposition of growth material, which is reflected in differences in density of otolith deposits. The highest density of organic matrix occurs when deposition is resumed after a resting phase, during the active growth phase, which occurs at dawn (Tanaka et al. 1981). This layer also corresponds to the interlamellar organic matrix as shown by Dunkelberger et al. (1980), Tanaka et al. (1981) and Radtke and Dean (1982). These authors demonstrated that discontinuous zones in otoliths contain more organic matrix than incremental layers.

Three major landmarks were observed in the present work, the core check at hatching, the check marking yolk-sac absorption, and the transition zone at metamorphosis. Bolz and Lough (1983, 1988) observed check rings at hatching and yolk-sac absorption in field-caught Atlantic cod, but could not discern the settling mark. Little is known of the physiological processes responsible for check formation. Thus, it is not clear when checks would be expected to form. A source of error in ageing larval fish is associated with the date of formation of the first increment. The date of first increment formation has been reported from before hatching (as in salmon, Neilson and Geen 1982, 1985; and in *Fundulus heteroclitus*, Radtke and Dean 1982), to the time of first feeding (as in anchovies, Brothers et al. 1976). In the absence of data concerning the age of first increment production, many researchers have assumed that it coincided with an event of biological significance in the life history, such as hatching. Assumption of an incorrect event could be responsible for the poor correspondence between observed and expected increment counts reported in some studies (particularly for young fishes) (Jones 1986).

It appears that duration of the yolk-sac stage and subsequent adjustment period to active feeding are temperature-dependent, as evidenced by differences demonstrated by larvae raised at 6 and 10°C. Yolk-sac absorption in field-captured Atlantic cod on Georges Bank was observed to occur at 2–8 d of age (Bolz and Lough 1983), which suggests that these larvae experienced, on the average, higher temperatures than the larvae in the present study, where the yolk phase lasted from 4 to 12 d at 10 and 6°C, respectively. The present laboratory experiments demonstrated that faster development rates occurred at higher temperatures. However, microincrements were still deposited daily in both larvae and juveniles. This daily deposition occurred at all three temperatures and in both starved and fed animals, in spite of differences in development rates.

Otolith size was found to be a good predictor for fish size, independent of temperature. Increment width increased with temperature in response to faster growth. Interpretation of increment width depends on the stability of the relationship between otolith size and fish size. The formation of accessory primordia in the otoliths of juvenile cod fish may confuse the interpretation of daily increment width, and it is important to measure increment widths using the same field of measurement both within an otolith and between specimens. However, used with caution increment width can be used to determine the history of the daily growth rates in an individual cod fish.

Rapid growth during the first months of life may be critical to survival (Houde 1987). Variation in recruitment may in part be explained by differences in developmental rates, which are affected by both temperature and food levels. Otolith increment widths and the position of check marks are indicative of growth and development rates, and could be used in recruitment studies. Jones (1985) found that differences in growth estimates between herring larvae hatched early in the season, and those hatched later could be demonstrated through use of otolith increments. Differences in overall mortality may be associated with these differences in growth rate (Methot 1983).

Wild cod larvae have an average growth rate of 0.3 mm per day during the first 2.5 mo (Bolz and Lough 1983, 1988), which is much lower than the overall growth rates of 0.7 to 0.9 mm per day observed during the first two months in the present experiment. This is not surprising, since the lab cod fish were fed ad libitum. The growth rates determined in the present study are in agreement with work by Steffensen (1980) on increments in juvenile Baltic cod. The fish in his collections were larger at a given age than those found in the 6°C group, but close in size to those in the 10°C group. For example, in his experiments, a 60 mm fish had only 46 increments. Gjøsaeter and Tilseth (1982), investigating growth rates in a North Sea cod, found slow growth in the small (< 10 mm standard length) larvae which is similar to the results in the present study.

Body Shrinkage of Cod Larvae

Lengths of larval fish are routinely measured during ichthyoplankton surveys to estimate larval age, growth and mortality, and occasionally are examined to calculate condition factors.

Whatever the purpose of the measurement, shrinkage of larvae during collection or fixation can result in many errors. Most ichthyoplankton is preserved in formalin, which results in various degrees of body shrinkage. Studies on the effects of formalin solutions (Blaxter 1971; Hay 1981, 1982; Lockwood and Daly 1975; Parker 1963; Rosenthal et al. 1978; Theilacker 1980; Tucker and Chester 1984) demonstrated that reduction in larval length caused by preservation can be as high as 22% (Baxter 1971; Schnack and Rosenthal 1978), and depends on the initial lengths of the specimens and duration of storage. It should be emphasized that generalized factors have not been easily extrapolated for use in correcting preserved lengths to live lengths for different species of fish larvae (Hay 1982; Schnack and Rosenthal 1978; Theilacker 1980).

I found no shrinkage in larvae placed directly into the ethanol. Reductions in larval lengths for specimens preserved in alcohol reported in previous studies (Radtke and Waiwood 1980; Theilacker 1980; Fowler and Smith 1983), were probably due to the handling associated with the preservation of specimens in these studies. The present results indicate that larval shrinkage occurred upon death. Maximum percent shrinkage was observed in the smallest larvae within 15 min of death. Fifteen minutes is a standard towing time for ichthyoplankton, and many larvae captured with plankton nets are killed in the net, and may have been dead for nearly 15 min by the time they are preserved. Thus, most shrinkage takes place while the fish is still within the net. This source of error is rarely considered in ichthyoplankton field studies, and it means that published larval lengths could be off by as much as 40%.

Accounting for larval shrinkage is important, and the use of otoliths may allow for correction for the effects of death and preservation. Whatever the degree of shrinkage, otolith size can be a reliable estimate of length (Templeman and Squires 1956); especially in larvae (Radtke and Dean 1982). Otoliths may deteriorate if preserved in formalin or in low concentrations or ethanol, but rarely do so if kept in 95% ethanol or pH-adjusted 95% ethanol. Consequently, if properly preserved, otoliths are unlikely to undergo postmortem shrinkage.

In a recent study of shrinkage in larvae of the bay anchovy, *Anchoa mitchilli*, Leak (1986) used the larval length–otolith diameter relationship determined in laboratory-reared larvae to study shrinkage in wild-caught larvae. The present study agrees with Leak's findings, but shows that there are different growth stanzas for cod otoliths which must be taken into account. The standard error (Table 2) is critical for the prediction of fish size from otolith size and it must be less than the percent shrinkage if the fish size–otolith size relationship is to be useful for shrinkage correction. The standard error for small larvae is $< 10\%$ and it would be appropriate to use otolith diameters to correct for the $> 30\%$ shrinkage. The greater variability in otolith size and the smaller degree of shrinkage among older fish may diminish the ability and need to correct for shrinkage. Otolith size–body size inflections occur for intermediate sized larvae, and this would limit the ability to correct for shrinkage. Still, by determining the fish size–otolith size relationship, it becomes possible to predict the size of net collected larvae from otolith diameter.

In conclusion, the structural composition of cod otoliths provides information about the calcification processes and life histories of individual fish. Otolith increment deposition remained daily, in spite of differences in developmental rates due to temperature. Daily, albeit faint, increments were observed in starved cod. Transition zones in the otolith occurred at hatching, yolk-sac absorption and settlement. Thus, microstructural analyses of otoliths allowed accurate ageing and the timing of life-history events. Otolith size was related to fish length, and daily growth patterns may be estimated from the width of individual microincrements. The otolith size–fish size relationship may also be useful in the correction of larval shrinkage. Interpretation of the life-history data recorded in otoliths requires characterization of otolith properties through laboratory experiments. Once the interpretation is clear, otoliths would be invaluable to population, ecological and evolutionary studies, and would supply a new level of knowledge to fisheries biologists.

TABLE 2. Parameters of the relationship between otolith diameter (x) and total body length (y).

Body size	a	(SE)	b	(SE)
< 7 mm	-5.5	0.0015	$6.9(\log x)$	0.0011
7–27 mm	2.3	0.0059	0.86	0.0051
>27 mm	19.1	0.0089	0.02	0.0082

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