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DAILY GROWTH INCREMENTS IN OTOLITHS FROM LARVAL AND ADULT FISHES

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ABSTRACT

Daily growth increments have been found in otoliths of fish larvae. The daily nature of these layers was verified by examining larval fish of known age reared in the laboratory. A simple technique for observing these marks is described and can be used on otoliths from larvae and adults. This provides a convenient method for determining early growth in fishes and is particularly useful for fishes which do not lay down annual or seasonal rings.

The use of otoliths in age determination (by means of annual marks) is well known. The techniques used have been described by Williams and Bedford (1974) and Blacker (1974). Recently Pannella (1971) has suggested that daily marks may be formed in the sagittae (the otoliths used almost universally in age determinations) of some temperate species, while in 1974 Pannella claimed to have detected them in a number of tropical species. He also studied the temperate species—silver hake, *Merluccius bilinearis*; red hake, *Urophycis chuss*; Atlantic cod, *Gadus morhua*; and winter flounder, *Pseudopleuronectes americanus*—in greater detail in this latter paper. For some of these temperate species, particularly for the first, Pannella was able to show that there were fortnightly, monthly, and annual patterns. The annual marks detected in the conventional way were shown to contain about 365 daily units. Pannella used acetate replicas of ground otoliths which had been previously etched with HCl. Pannella's work appears to justify the following conclusions:

1. Daily increments[4] occur in certain temperate fish, e.g., *M. bilinearis*.
2. Periodic variations in increment thickness occur with fortnightly, monthly, and annual frequencies in this species.
3. Structural units that are similar to those shown to be daily in their occurrence in temperate species are also found in some tropical species.

Pannella (1974) was careful to explain that the marks present in otoliths of tropical fish that appeared to be annual on the basis of conventional criteria could be deceptive. He suggested that by analogy with temperate species, certain structures found in otoliths of tropical fish were also daily in occurrence. Although he found spawning marks, he did not find any seasonal or winter growth checks in the otoliths of tropical fish. In view of Pannella's expressed skepticism about the formation of annual marks and his tentative conclusions, further evidence is needed that daily increments occur in tropical fish. Furthermore, no one appears so far to have attempted to apply this method of age determination to larval fish, yet it is in this last area that the most accurate and useful results might be expected. Pannella (1974) commented on the great regularity of the presumably daily marks near the center of the otoliths of both tropical and temperate fish. In these portions of the otoliths, no superposition of more complex patterns (e.g., 14 day, 28 day) were found.

It is the object of this paper to show that 1) true daily increments are found in the otoliths of the larvae of several species, and that daily marks may be used to determine the ages of larval fish with great accuracy and precision, at least for approximately the first 100 days of life; and 2) in adults of fish from a variety of habitats, including tropical waters, daily increments may be proven to exist, and so to confirm Pannella's work.

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[4]The smallest visible concentric layers seen in an otolith.

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METHODS

Some material was examined with a Stereoscan[5] S4 scanning electron microscope (Cambridge Scientific Instruments Ltd.). These otoliths were prepared for viewing by embedding them in polyester resin, grinding and polishing them to the vertical mid-sagittal plane with a graded series of silicon carbide or aluminum oxide compounds (400, 600, and 900 grit), and finishing with 1-μm diamond paste. The polished surface was then etched with 0.1 N HCl before being rotary coated in a vacuum evaporator with 150 Å of gold-palladium alloy.

Both this technique and that of Pannella (1974) involve the use of equipment and materials that may be inaccessible in many countries. This is particularly true for those countries in which daily growth increments might prove to be especially helpful in stock assessment of commercial fish, so that an alternative practical method with minimal equipment was also used here and found to be successful.

Otoliths of adult fish were ground by hand on a glass plate covered with a water-silicon carbide powder mixture (400-600 grit). The final polish may be administered with diamond paste, but this step is not essential. The ground otolith was then examined in immersion oil. The grinding was done in the same plane as described by Pannella (1974). It is possible that storage in oil over a long period of time may reduce the resolution obtained when an otolith is examined. This appears to be particularly true for larval otoliths. The above technique is simple and requires only a good compound microscope. Magnifications used in this work ranged to 1,800×; at least 600× is required for general viewing.

Otoliths from larvae were removed by teasing them from fresh specimens. Oven-dried material needed only to be moistened with a drop of water before otolith removal. The otoliths were manipulated and transferred to clean slides by picking them up on the end of a fine dissecting needle wetted with immersion oil. No additional preparation was necessary, and the otoliths were examined in immersion oil or after being permanently mounted under a cover slip in a quick-drying, neutral mounting medium. Ground sections from juveniles and adults may be similarly mounted with no apparent loss in clarity. Larval otoliths are thin enough that only optical sectioning (i.e., carefully focusing to the plane of maximum clarity) is necessary to make total increment counts.

Material from a variety of species was examined and larval material of known age was obtained by rearing eggs that had been fertilized in the laboratory (Lasker et al. 1970; Leong 1971). The chronological age from these fish was known and could be compared with the number of growth increments observed in their otoliths. Larvae of northern anchovy, *Engraulis mordax*, were kindly made available to us by John R. Hunter of the Southwest Fisheries Center, National Marine Fisheries Service, NOAA, at La Jolla, Calif.

RESULTS

Otoliths of 15 *E. mordax*, aged 6 days, were examined. The mean total length of the fish was 4.5 mm. The yolk-sac had been absorbed by the fifth day after hatching. Figure 1a shows the appearance of the otolith of one of these fish.

Only one or two daily increments were present, suggesting that daily growth increments appeared in the otoliths of *E. mordax* only after completion of yolk-sac absorption. In the laboratory, anchovy larvae were maintained in 14 h of light when feeding took place and 10 h of darkness when no feeding occurred (Lasker et al. 1970).

Table 1 shows the relation between chronological age and number of apparently daily increments for larvae of *E. mordax* aged 6 to 100 days. It is clear that there is an extremely close correspondence between the chronological age in days and the number of increments. Figure 1b is a micrograph showing the daily increments in an anchovy otolith from a larva 18 days old.

Additional data presently being collected on laboratory and wild-caught larvae indicates that there is some interaction between the rate of larval growth and the rate of increment formation which may complicate the interpretation of otolith age estimates.

Figure 2 shows the structure of adult anchovy otoliths with successively greater magnification of the scanning electron microscope. The darker areas in the photographs represent areas of the otolith that were more heavily etched because they contained a higher proportion of $CaCO_3$, while the lighter areas have relatively more organic material, probably otolin (see Degens et al. 1969). It is seen from Figure 2 that the smallest

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

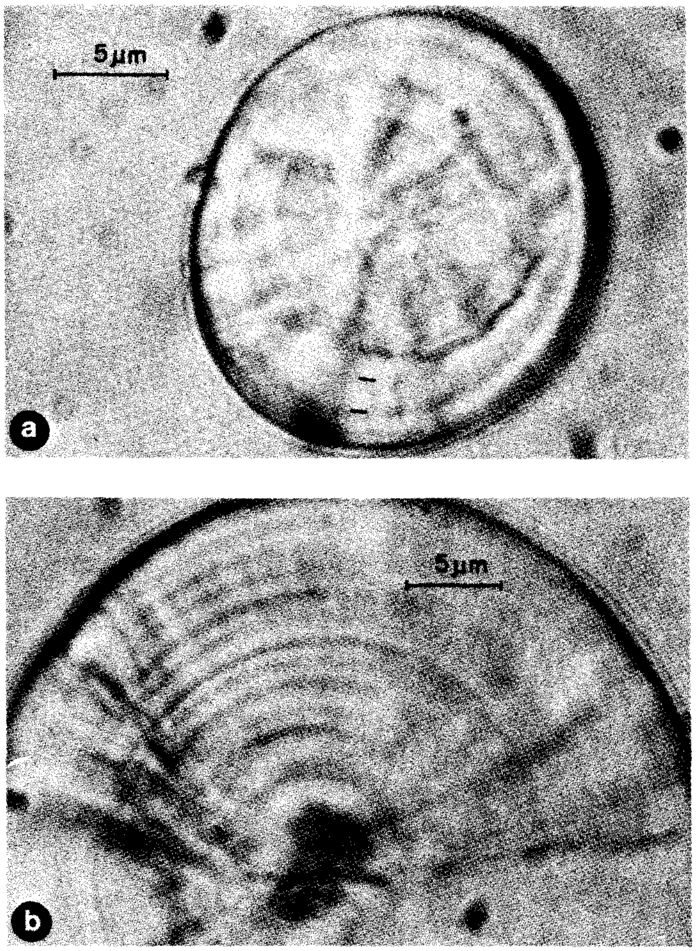

FIGURE 1.—Light microscope photographs of otoliths from laboratory-reared northern anchovy: a) 8-day-old larval otolith showing two daily growth rings; b) 18-day-old larval otolith showing 12 daily growth rings.

TABLE 1.—Chronological age (days from hatching) and numbers of growth increments in otoliths of northern anchovy.

Number of fish	Chronological age in days	Chronological age less 5 days	Mean number of increments	Range
15	6	1	1	0- 2
10	8	3	3	2- 4
10	12	7	7	4- 8
10	15	10	10	8- 11
7	16	11	10	9- 11
5	18	13	13	12- 15
7	20	15	15	14- 16
8	24	19	18	16- 19
9	25	20	20	18- 21
3	26	21	21	18- 23
4	94	89	97	95-100

cyclical units are 1 to 2 μm thick in this part of the anchovy otolith and that they do not appear to contain any smaller units. It is these units that are counted and appear in the data in Table 1. The daily increment would therefore appear to be the smallest unit of growth that is formed at the supra-molecular level and, as such, is in principle the most natural unit to use for age estimation.

Fertilized eggs of the California grunion, *Leuresthes tenuis*, were obtained and reared in the laboratory. The larvae were maintained in a natural light cycle at 17° to 20°C with food (*Artemia* nauplii) continuously available. Larvae were sacrificed at intervals and their otoliths were examined. Table 2 shows the results obtained and Figure 3 shows a photograph of a grunion otolith.

Table 2 shows that there is a close relation between the number of growth increments and the chronological age of the larvae. Although the agreement between age and daily increments is not as good as it is for the anchovy, the results are still very good. Table 2 also shows that in *L. tenuis*, daily increments appear at hatching, rather than at yolk absorption. Prehatching marks also occur, although they were not tallied in Table 2. Clearly the exact timing of the initiation of daily increment formation varies from

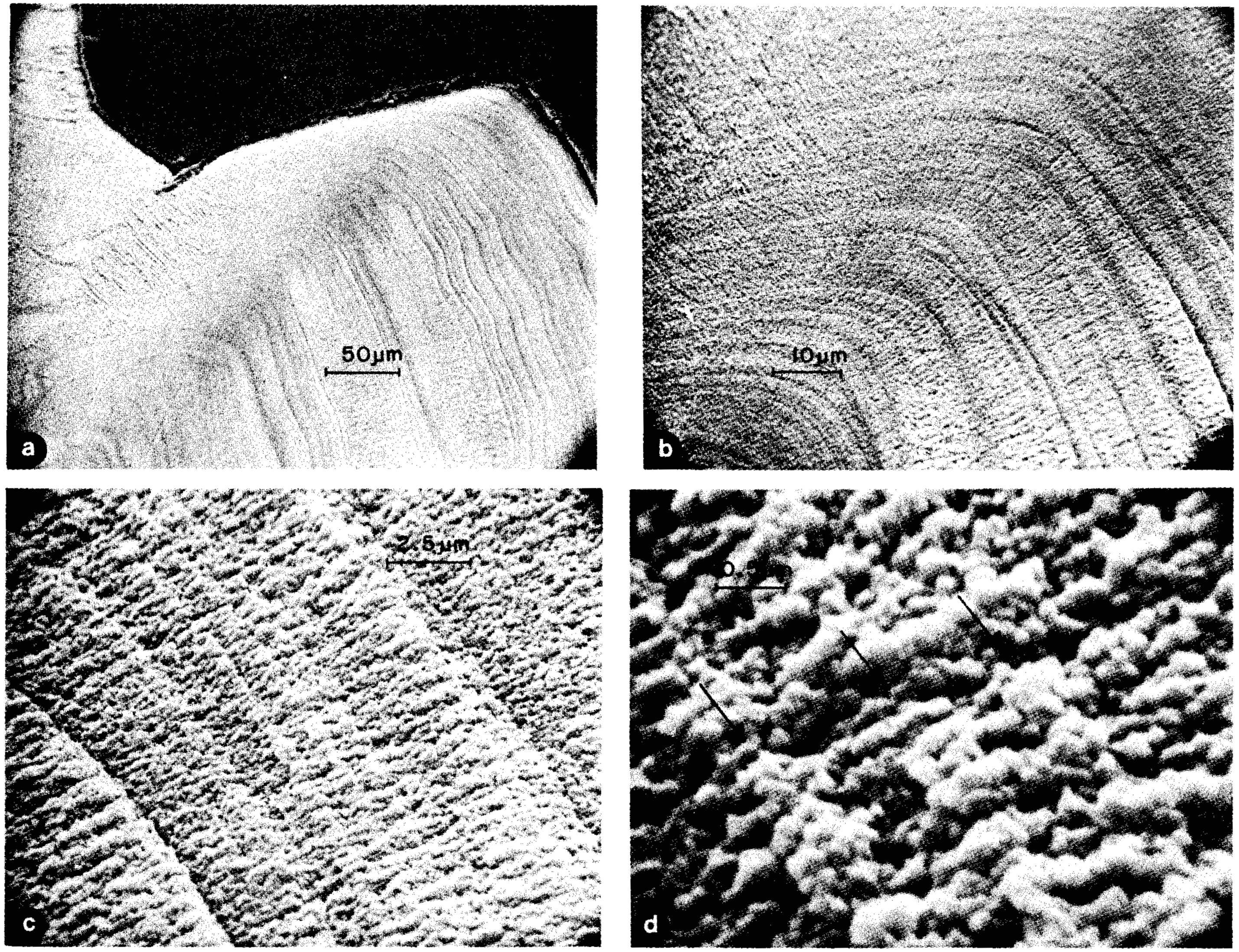

FIGURE 2.—Scanning electron microscope photographs of an otolith from a 2-yr-old northern anchovy. Successively higher magnifications are shown in a through d. At highest magnification, the distance between daily marks (indicated by arrows) is shown to be between 1 and 2 μm.

TABLE 2.—Chronological age and number of growth increments in the otoliths of the California grunion.

Number of fish	Chronological age in days	Mean number of increments	Range
2	0	2	1- 2
3	7	9	8-10
2	16	11	10-12
3	18	17	16-18
5	26	24	20-26

species to species and must be independently determined for each one.

Young striped bass, *Morone saxatilis*, were collected on 2 July 1974 in the Sacramento River delta (Tracy Pumping Station), Calif. These five fish measured 29 to 37 mm SL (standard length) and their otoliths had 62 to 120 observable increments; i.e., a sample of striped bass which should have been 2 to 4 mo old according to their known spawning season (Scofield 1931) were 2 to 4 mo old according to the presence of growth layers found in their otoliths. The spread in the age calculated from daily increments probably corresponds to a considerable spread in the dates when the fish examined were hatched.

Otoliths from two striped bass 135 and 142 mm SL were also examined. Published information on the growth rate of this species (Scofield 1931) indicates that striped bass of this size taken in July should be 14 to 16 mo old. The ages obtained by counting the presumed daily growth marks were 419 and 445 days respectively, i.e., 14 to 15 mo old.

Figure 4 shows the daily marks in an otolith of striped bass. Daily increments were fairly thick near the center, thinner in an intermediate area corresponding to the hyaline zone, and wider again near the edge. In one specimen the central

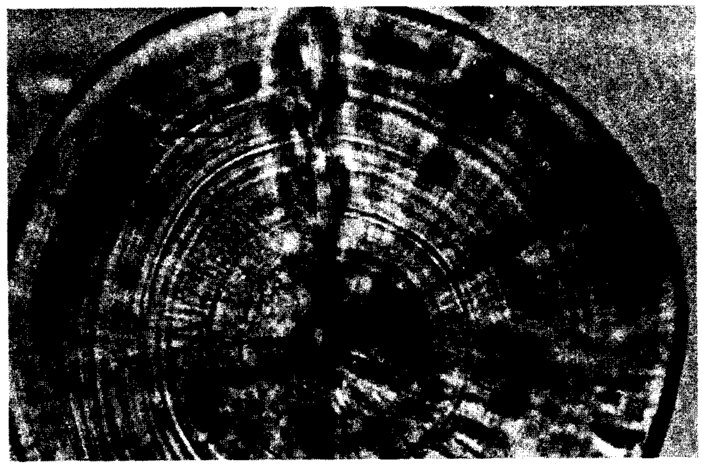

FIGURE 3.—Daily growth rings in an otolith of a California grunion larva. The larva was approximately 26 days old.

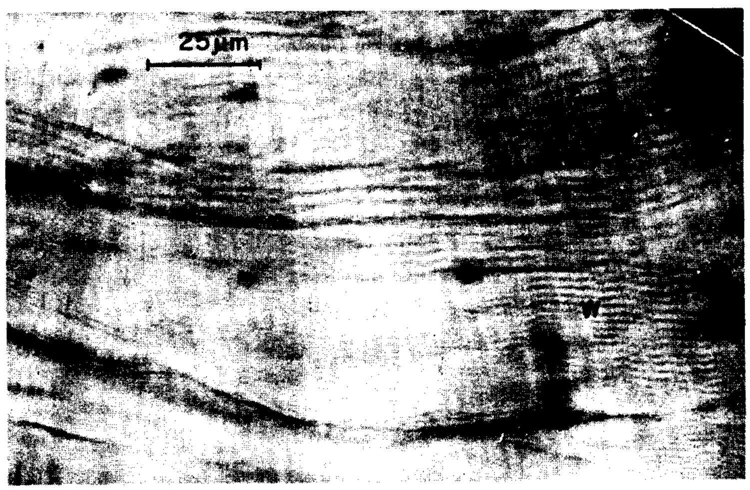

FIGURE 4.—Daily growth rings in a striped bass otolith. This fish was approximately 15 mo old. Differential growth can be seen in rings grown in adjacent seasons. F = fall; W = winter.

area contained 231 daily increments, the marginal area contained 120, and there were 94 thinner marks in the middle zone. Working backwards from the 2 July collection date, this indicated the slow growth zone occurred in December, January, and February. These figures correspond well with the known life cycle (Scofield 1931) which suggests a fast growth period in spring, summer, and fall (230 days, ≈ 8 mo), a short winter of slow growth (≈ 3 mo), and a spring and early summer (≈ 4 mo) of faster growth prior to capture.

Otoliths of postlarvae of the gobies *Clevelandia ios*, *Ilypnus gilberti*, and *Quietula y-cauda* were also examined. The fish were collected in Mission Bay, San Diego. The 2-mo larval period indicated in the otoliths agree with several independent estimates of the length of time between hatching and settlement (Brothers 1975).

Otoliths of two species of hake obtained from the Gulf of California were studied. Mathews (1975) has shown that annual marks (annuli) may be detected by means of the usual discrimination of hyaline and opaque zones in *Merluccius angustimanus* while in *Merluccius* sp. (Mathews in press) the same techniques have also been applied successfully. The ages of hake determined by means of annuli may be compared with age determined from counting the number of daily increments; these are identified by analogy with the structures shown to be daily in their incidence in anchovy, grunion, striped bass, and other fish and which appear to be the same as those shown by Pannella (1971) to be daily in *M. bilinearis* (Figures 5, 6). In most cases, direct total counts were not possible because increments were not equally visible over a complete nucleus to margin radius. For these otoliths measurements of incre-

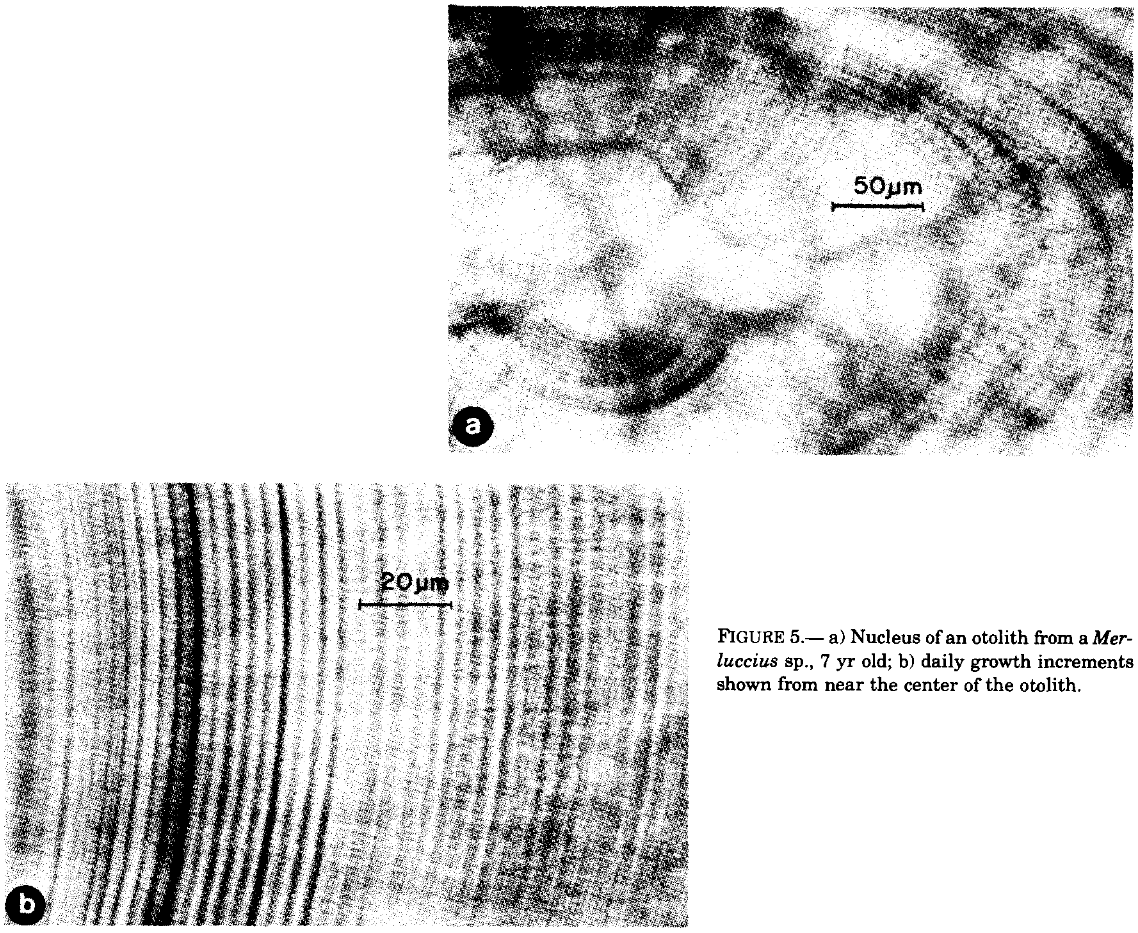

FIGURE 5.— a) Nucleus of an otolith from a *Merluccius* sp., 7 yr old; b) daily growth increments shown from near the center of the otolith.

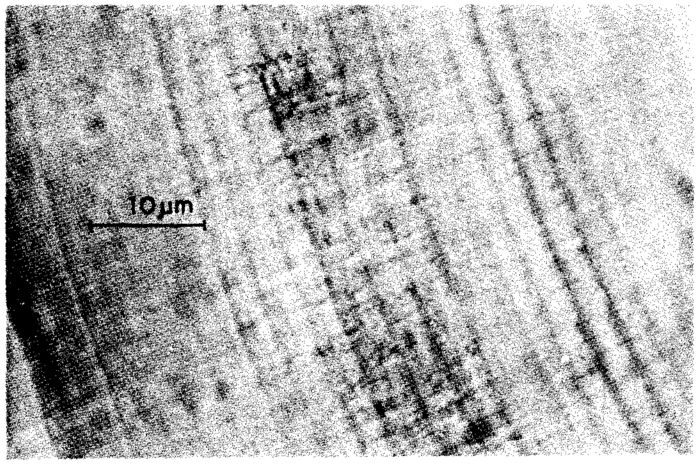

FIGURE 6.—Daily growth increments from the otolith of *Merluccius angustimanus*. Note radial fibers crossing the growth layers.

ment width were made at five or more locations along a radius and then total counts were calculated by extrapolation. No larval or very young hake were available for examination.

For *Merluccius* sp., data were available for 22 specimens aged 1 to 7 yr from the annuli present in their otoliths. Figure 7 shows the graph of age by annuli against age by daily increments for this species. The correlation coefficient was 0.91 (20 df, $P >> 0.001$). The slope of the regression line was 1.14 (99% confidence limits [C.L.], 0.81-1.46). This is not significantly different from the value of 1.00 expected if age by years and by days were to yield identical values.

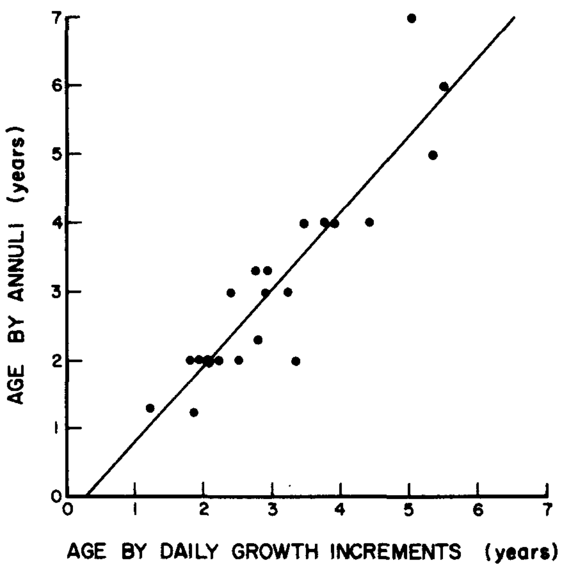

FIGURE 7.—Graph of age-by-annuli against age-by-daily-growth-rings in the otoliths of *Merluccius* sp. The encircled point represents two points at the same position.

Data from seven specimens of *M. angustimanus* were available and they varied in age from only 1 to 2 yr. Given the much narrower ranges and the smaller sample, the results obtained were acceptable: $r = 0.74$ ($0.05 > P > 0.01$) and the slope of the line was 1.25 (99% C.L., 0.24-2.25); i.e., the slope was significantly different from zero, but not from 1.0.

The precision of estimates of age obtained for *M. angustimanus* was not very good, with deviations of up to 0.5 yr being obtained; however, for *Merluccius* sp. a somewhat narrower range was usual, with some values differing by 0.1 yr or less. Extreme variations occurred with fish aged 7 to 13 yr, where errors of up to 2 to 3 yr could be obtained where daily counts were made.

The average widths of the daily bands found in the hake otoliths were 3 to 4 μm, with wider and narrower bands appearing sometimes in apparently weekly, fortnightly, and monthly units. The incidence of these units has not been examined in detail and requires further study, but preliminary work suggests that the basic unit used in age estimates should be the daily unit; the higher order units may be of great ecological interest, but should probably not be used in aging these hake: Only daily increments occur with the necessary consistency and regularity.

In addition to the species mentioned above, apparently daily marks have been found in a wide variety of other fish, e.g., in *Tilapia zilli, T. nilotica*, and *Clarias mossambicus* from Lake Victoria (examined by E. B. B. and C. P. M.; specimens kindly collected by John Rinne and Dr.

Peretti of the East African Freshwater Fisheries Research Organization, Jinja, East Africa), and the following species examined by one of the authors (E.B.B.): in the deep living Pacific rattail *Coryphaenoides acrolepis* (58 cm SL; 10 to 11 yr); in the myctophids *Stenobrachius leucopsarus, Tarletonbeania crenularis,* and *Triphoturus mexicanus*; in the freshwater fish *Cottus asper* and *Salmo gairdneri*; in the tropical marine fish *Chromis atrilobata* and *Apogon retrosella*; in adults of the gobies *Clevelandia ios* and *Gillichthys mirabilis*, where clear growth checks also occur, so that daily marks alone would lead to distinct underestimates of age; and in four species of rapidly growing tropical and temperate tunas. Statoliths from the squid *Loligo opalescens* (both wild caught adults and laboratory-reared juveniles) also show what appear to be growth layers analogous to those in fish otoliths. The appearance of growth interruptions in a number of species, e.g., the rockfish (genus *Sebastes*), either as winter checks, spawning checks, or apparently dispersed more evenly throughout the year, may impose a severe limitation upon the use of daily marks to age these fish. The technique seems best suited to larvae, juveniles, fast-growing species, and tropical species.

It is clear from our work that some difficulties must be overcome before age estimation by means of daily rings can become a standard tool in fisheries biology. However, it is also clear that

1. Daily rings may be used to estimate the ages of larvae of some species up to 100 days old with very great precision and that they probably can be used for fish up to 1 yr of age, perhaps with a smaller degree of precision. Struhsaker and Uchiyama (1976) show similar results with the tropical engraulid *Stolephorus purpureus.*

2. Daily marks may be used as a means of accurate age determination for at least some species of fish up to 6 yr old.

3. Daily marks may be used for age determination of at least some tropical fish. Pannella's (1974) suggestion that daily increments might be used in tropical fish as a means of age estimation is almost certainly true, and should be applicable to most species.

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