

INCREMENT FORMATION IN THE OTOLITHS OF EMBRYOS, LARVAE, AND JUVENILES OF THE MUMMICHOG, *FUNDULUS HETEROCLITUS*¹

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ABSTRACT

The formation of otoliths and the effect of light cycles on increment formation were studied in embryos, larvae, and juvenile mummichogs, *Fundulus heteroclitus*. We found that increments in the sagitta of mummichogs were a reliable indicator of the daily age of the fish. Calcification of the sagitta was initiated in the core, after matrix formation, at stage 24 of embryological development. The sagitta was the first calcified tissue to develop and there were two or three increments formed before hatching. Daily increment formation in the sagitta was initiated by light and controlled by a 24-hour photoperiod. When embryos were subjected to a 24-hour dark or <24-hour (6L:6D) photoperiod, daily increment formation was disrupted. Laboratory experiments at 24°C and 30°C confirmed that there was one increment formed each day, which was independent of growth rate and which validated the age of fish in field collections. Wild populations reproduce in the intertidal zone, a physically stressed environment and, judging by the age, which was estimated from incremental data, reproduction is synchronized with tidal cycles.

Interpretation of increments in the hard tissues of fish has long been utilized as a method to estimate age composition of adult populations. Most of the interpretive emphasis has been placed on otoliths and scales. However, the process of age determination is not a simple one (Bagenal 1974). Otoliths are especially useful for determining the age of fishes, such as larval forms, which lack scales or have very small ones.

The otoliths of teleosts consist of deposits of calcium carbonate in the form of aragonite (Irie 1955; Degens et al. 1969). The morphology of these structures is so specific it can be used as a taxonomic character (Messieh 1972; Hecht 1978). Three structures (the sagitta, lapillus, and the asteriscus) are found in the membranous labyrinth of inner ear on each side of the brain cavity (Lowenstein 1971; Popper and Coombs 1980). The sagitta is often the largest and is most often used for age determinations and, unless otherwise stated, was the otolith used in the present study.

Pannella (1971, 1974) postulated that daily increments are found in otoliths of adult fishes, and Brothers et al. (1976) showed that such increments can indeed be found in otoliths of young fishes and be used for age estimation. Struhsaker and Uchiyama (1976) postulated that back calculation of daily increment data from otoliths could be used to age the nehu, a tropical marine fish, and Ralston (1976) obtained similar results with a tropical butterfly fish. Taubert and Coble (1977) did direct age observations of otoliths in juvenile freshwater fish and Barkman (1978) was equally successful with the young of a temperate estuarine species *Menidia menidia*. A more accurate daily journal is available in the otoliths of most young fishes than can be found in their scales, since scales are often absent in the early stages of development (Bagenal 1974), and scale metabolism is dynamic (Yamada and Watabe 1979).

The discovery of daily increments in otoliths increases the resolution and precision of age determination and promises to provide fishery biologists with new levels of information. The deposition of the increments in a rhythmic fashion could be a mark of a daily event, and possibly a measure of growth, but the full extent of the influence of external and internal factors on the formation of otolith increments has not been determined.

There is need of knowledge about the age com-

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position of larval fish populations, since this information can provide estimates of growth, mortality, and rates of survival (Gulland 1977). The highest mortality of fishes is during the growth period from larvae to juveniles (Hjort 1914; Tanaka 1972) and consequently, the survival and growth of larval fishes has a pronounced effect upon recruitment (Larkin 1978). It should be possible, by using otoliths for estimation of the age, to determine the growth rates and the age structure of larval fish populations.

Daily increments have been correlated with natural temperature cycles, light and food for freshwater species by Brothers (1978, 1980⁴). Taubert and Coble (1977) postulated that daily increments in otoliths of freshwater sunfish resulted from a 24-h diurnal light cycle that entrained an internal clock.

To utilize daily depositional increments of the otoliths in the analysis of fish population dynamics, it is important to understand the physiological mechanisms involved in the formation and growth of increments and otoliths. Age estimation requires knowledge of 1) age when increment formation begins; 2) factors which control the deposition of daily increments in the otoliths; and 3) length of time daily increments are formed without growth interruption. Information in these areas will make it possible to better understand age and growth in wild populations of fish.

An important area for research in the field of age and growth is the experimental study of the factors which influence the deposition of increments in otoliths. Brothers et al. (1976) showed that daily increments began to form at different ages in different species. Some species hatch with increments already formed, while others apparently do not form increments until later. Thus, it is necessary to study the formation of increments in each species and correlate increment formation with external factors before accurate age determinations can be made.

The mummichog, *Fundulus heteroclitus*, is an abundant estuarine fish and an important component of the estuarine ecosystem (Cain and Dean 1976; Valiela et al. 1977; Kneib and Stiven 1978; Merideth and Lotrich 1979). The biology of *Fundulus* is well-known and its embryology is well-defined (Armstrong and Child 1965).

The objectives of this study were to 1) delineate

the structure and formation of otoliths in the embryological and early larval stages of the mummichog, 2) determine the effect of photoperiod on increment deposition in embryonic and postlarval mummichog otoliths, 3) measure the effects of temperature on body growth and the deposition of increments in otoliths, and 4) test whether growth and age data can be obtained in wild populations of mummichogs by counting the increments in otoliths.

METHODS

Adult *F. heteroclitus* used as spawning stock were collected from North Inlet Estuary (lat. 32°20'N, long. 79°10'W) and North Edisto Estuary (lat. 32°26'N, long. 80°12'W), near Georgetown, S.C. Fertilized eggs were collected as previously described by Middaugh and Dean (1977). Only embryos which developed according to the criteria of Armstrong and Child (1965) were utilized in the embryological studies, and only larvae which hatched within 6 h of hatch induction were used in the growth studies. The embryo is the stage from fertilization to hatching; from hatching to yolk-sac absorption is the larval stage and the mummichog was considered a juvenile after yolk-sac absorption (Hubbs 1943).

The terms used to describe growth increments in otoliths are confused, as the increments in larvae are variously referred to as lamellae, rings, or layers. The term increment in this study refers to a unit formed by an unbroken incremental zone and a discontinuous zone after core formation (Fig. 1), Wild and Foreman (1979).

Newly hatched larvae were kept at 24°C and 30°C±1°C (Radtke and Dean 1979) and were fed brine shrimp, *Artemia nauplii*, ad libitum and maintained in L12:D12 with a daily change of water (30%) to determine the effect of the rate of growth on otolith size and increment number.

A daily sample of 10 larvae was collected for laboratory experiments from each group for the first 10 d, and every 5 d thereafter for 30 d. Standard lengths (SL) were measured on each larva and its otoliths were removed. Photomicrographs were made of each otolith for increment counts.

Juvenile mummichogs were collected from We Creek in North Edisto Estuary on 9 June 1977 (28°C, 29%). Each fish was weighed, measured for standard length (SL), and its otoliths extracted for increment counts from photographs. Statistical analyses of the data were done with

⁴E. B. Brothers, Section of Ecology and Systematics, Cornell University, Ithaca, NY 14850, pers. commun. October 1980.



FIGURE 1.—SEM of the sagitta from a 12-d-old *Fundulus heteroclitus*. I is the unbroken incremental zone, D is the discontinuous zone, and I+D=1 increment. Bar = 1 μ .

standard tests and models as described in Sokal and Rohlf (1969).

Removal, Preparation, and Inspection of Otoliths

Otoliths were removed from embryos, larvae, and juveniles with fine insect needles mounted on wood rods. The larvae are transparent and the otoliths are birefringent under polarized light, so it is possible to view the sagitta during the dissection. The sagittae were washed with distilled water, dried, and mounted on glass slides with Euparal⁵ mounting medium, and viewed with a compound light microscope.

Photomicrographs were made of each otolith for counts of increments and measurement of otolith diameters. (The I of the outside edge of the sagitta was considered as a portion of the last increment.) To make increment counts, the back of each photograph was marked and the photographs were shuffled. The counting process was performed three times, which gave three unbiased readings for each otolith. If two of the counts were identical, that value was accepted as the increment count for a particular otolith. In cases where all counts differed, the middle count was chosen unless all counts varied more than two increments from each other, in which case that otolith was disqualified and not used in the final tabulation. Sagitta were measured at the widest diameter on the photographs using a caliper calibrated on a photographed micrometer.

Sagittae viewed with light microscopy showed fine lines in the I that were concentric with the D; these fine lines have been referred to as "sub-units." In the otoliths of young mummichogs the so-called subunits could not be observed in decalcified sections with light microscopy or SEM (Fig. 1). The D and I compose an increment and are readily differentiated with light microscopy in *Fundulus sagittae* (Fig. 2).

Whole sagittae used for SEM studies were attached to viewing stubs in 5-min epoxy resin. The sagittae were ground to the core in the transverse plane on graded grinding stones, polished with diamond-polishing compound, and cleaned with 95% ethanol. The polished surface was decalcified with 7% EDTA (pH 7.4) (disodium ethylenediaminetertacetate) for 1 to 5 min. The specimens were coated with gold (150A) and observed with a SEM.

Embryological Formation of Otoliths

Fertilized eggs were kept in light 12 h and in the dark 12 h (L12:D12) at 24°C in hatching jars with recirculating seawater (30‰). A sample of 10 eggs was collected each day until hatching and viewed under polarized light (120×) to determine when calcification was initiated. The embryos were classified according to Armstrong and Child (1965) with the number of embryos with calcified sagitta noted in each stage.

Calcified sagittae were removed from the embryos and mounted for examination with light microscopy to determine the time of increment formation. Ten- and 14-d embryological sagittae were viewed using SEM to confirm the light microscope observations.

Effect of Light on Increment Formation in Embryos and Larvae

To determine the influence of light on increment formation, developing embryos and larvae were subject to the following conditions:

EMBRYOS:

Group ED24—Embryo-dark-24 h, fertilized in the dark, kept in constant darkness until sampled 3 d after hatching.

Group EL24—Embryo-light-24 h, fertilized in the light, kept in constant light until sampled 3 d after hatching.

Group ED24+L—Embryo-dark-24+L, fertilized in the dark, kept in constant darkness except for 1 min of light exposure 10 d after fertilization. Sampled 3 d after hatching.

Group EL12:D12—Embryo-light-12 h:dark-12 h, fertilized, placed in L12:D12 and sampled daily.

All groups were maintained at 24°C and the water (30‰) was changed daily. The water was changed in the ED24 group and ED24+L group by pouring the eggs onto a 505 μ mesh net mounted on the end of 10 cm plastic tubing. The eggs were then washed off the netting with a wash bottle and the entire exercise was performed in total darkness. Hatching in the ED24 group and ED24+L group was determined by touch, because embryos are hard and easily distinguished when they have hatched. A daily

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

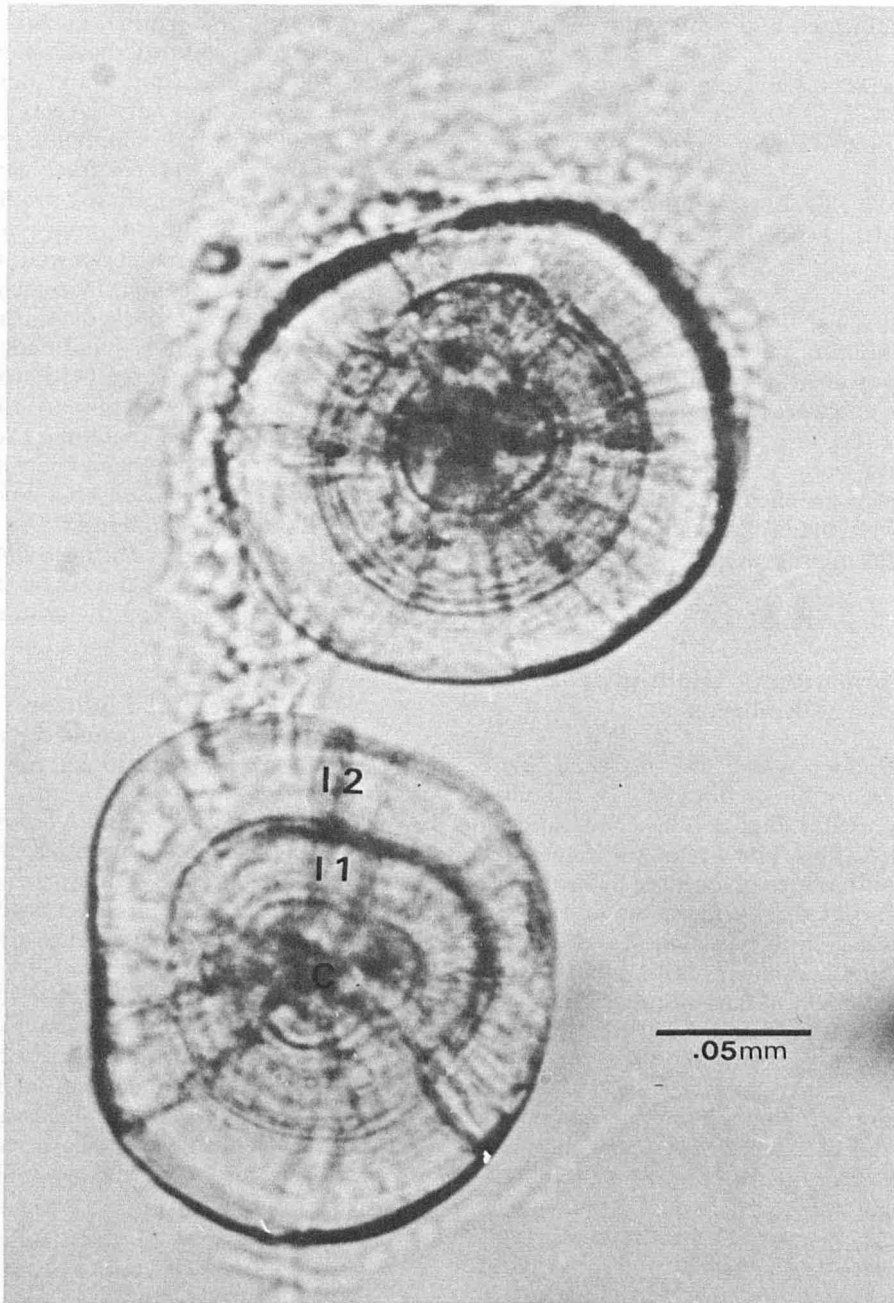


FIGURE 2.—Light micrograph of a *Fundulus heteroclitus* sagitta on the day of hatching. C is the core and I1 and I2 are increments formed after core formations but prior to hatching. Bar = 0.05 mm.

sample of three eggs was taken after day 14 to determine the events in otolith development.

Sagitta were removed from 10 larvae of each group according to the above schedule and photomicrographed.

LARVAE:

Developing embryos were maintained at 24°C in L12:D12 in hatching jars with running seawater (30%). Upon hatching, the larvae were di-

vided and subjected to the following conditions:

Group LaD24—Larvae-dark-24 h, constant darkness.

Group LaL24—Larvae-light-24 h, constant light.

Group LaL6:D6—Larvae-light-6 h:dark-6 h.

Group LaL12:D12—Larvae-light-12 h:dark-12 h.

All groups were fed newly hatched brine shrimp ad libitum and kept at 24°C with daily changes of water at 30%. Samples of 10 larvae from each group were taken at days 0, 6, 9, and 16 except the L12:D12 group, which was sampled daily. Sagitta were removed from each sample and photomicrographed. Scanning electron micrographs were made of samples for comparison with the light micrographs.

RESULTS

Formation of Otoliths in Embryos

The sagittae were the first tissues to calcify and were discernible on days 3 and 4 at embryonic developmental stages 24-28 (Armstrong and Child 1965). An amorphous mass was discernible in the labyrinth region of the larva before calcification was initiated. This mass, the core organic matrix, had a gellike consistency and could be dissected. Calcification was initiated in the core of the sagitta of 30% of the embryos on day 3 and 100% of the cores showed calcification by day 4 (Fig. 3). Increment formation began on day 12, and 20% had one increment. On day 13, 80% had one increment and 20% had two increments. On day 14, the day of hatch, 20% had one increment, 70% had two increments (Fig. 2), and 10% had three increments.

Calcification began with formation of crystals which extended to the edge of the core matrix. Histochemical analyses have shown that calcification begins in the core at the same time that the core becomes birefringent (J. Yamada⁶). Multiple spherules (Fig. 4a, b) are common in the calcified core but their origin and sequence of development is unknown. The newly formed sagittae had a mean diameter of 0.024 ± 0.004 mm. Calcification continued and additional crystals ex-

tended beyond the original boundary in an interlocking fashion until the diameter reached 0.048 ± 0.008 mm at day 9 and developmental stage 36. At this time only the core region could be observed, with no increments (Fig. 3). Two days later (on day 11 postfertilization), increment formation was initiated around the core, and the mean sagitta diameter had reached 0.074 ± 0.008 mm. When viewed with transmitted light, the concentric increments consisted of alternate narrow, dark discontinuous zones (D) and wider, lighter, incremental zones (I) (Fig. 3). The D intersected the I at right angles and were concentric with the core and outer surface of the otolith. Upon hatching at day 14, postfertilization, two or three increments were readily discernible as daily increments started forming 2-3 d before hatching.

Otoliths examined with the SEM confirmed the increment counts determined under transmitted light and showed the orientation of the crystals (Fig. 1).

Effect of Light on Increment Formation in Embryos and Larvae

The light cycle to which an embryo or larva was exposed had an effect on increment formation and hatching time. Embryos in the L12:D12 cycle had two or three increments prior to hatching and one increment per day after hatching (Table 1, Fig. 3). Embryos kept in L12:D12 hatched at 14 d while those exposed to other light cycles had longer incubation times and a difference in increment formation during incubation and after hatching was apparent in the other groups (Table 2). Embryos incubated in constant dark (ED24) had a delayed hatch, suppressed increment formation (Fig. 5), and a smaller otolith

TABLE 1.—Sagitta were from *Fundulus heteroclitus* embryos and larvae incubated on a L12:D12 cycle at 24°C (N = 10/d).

Age (days after hatching)	Increment count	Otolith diameter (mm)
0	2.75±0.5	0.128±0.011
1	4.00±0.0	0.150±0.015
2	4.50±0.5	0.158±0.015
3	5.50±0.5	0.180±0.140
4	6.20±1.1	0.187±0.011
5	7.20±1.0	0.203±0.003
6	8.50±1.1	0.220±0.111
7	9.50±0.6	0.240±0.110
8	10.60±0.8	0.255±0.042
9	12.00±1.0	0.268±0.058
10	12.60±0.9	0.280±0.018
15	17.50±1.2	0.350±0.120

⁶Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido, Japan, pers. commun.

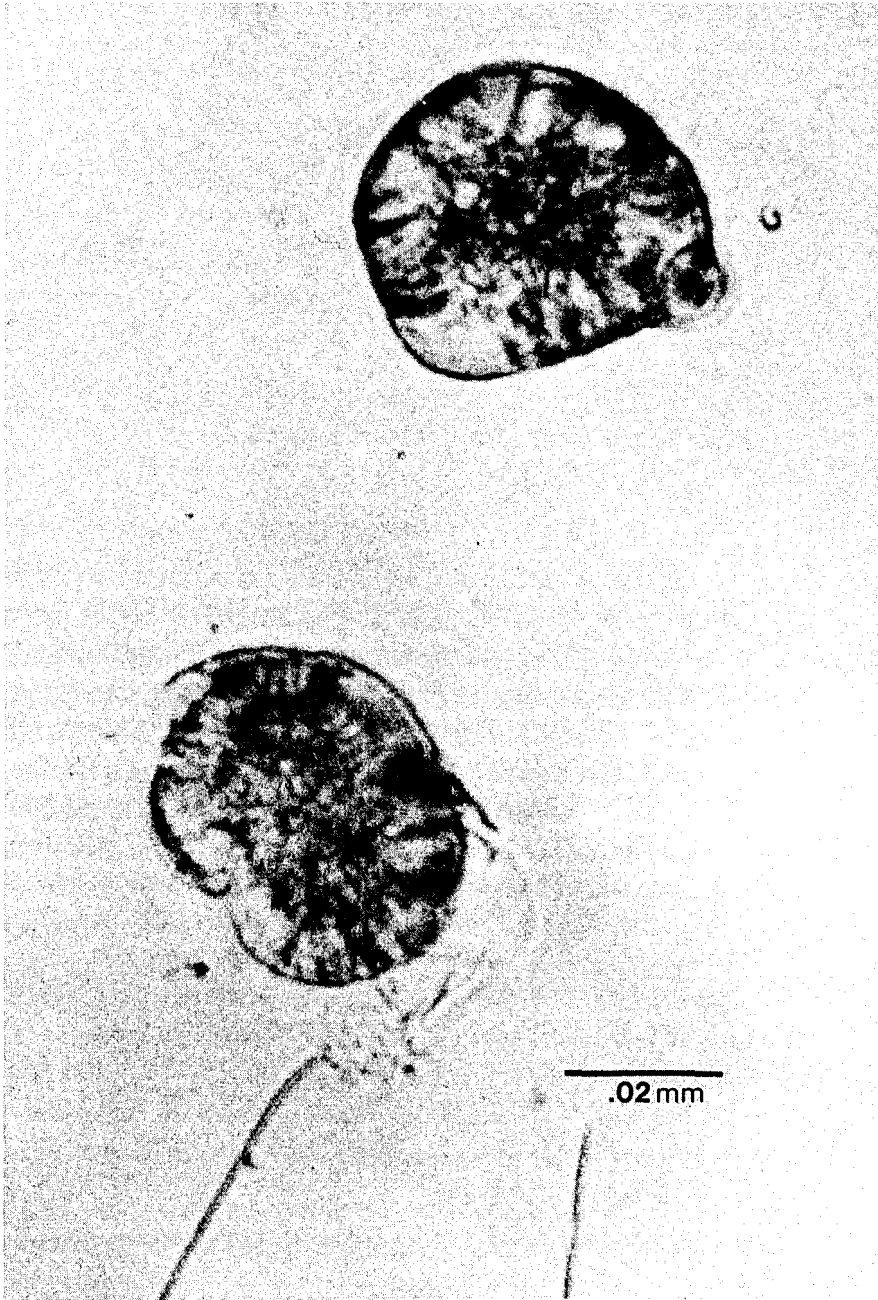


FIGURE 3.—Light micrograph of the core of the sagitta of *Fundulus heteroclitus* taken on day 10 of embryo formation. No increments have yet formed. Bar = 0.02 mm.

diameter (Table 2A, Fig. 5). The embryos incubated in constant light (EL24) hatched at 15 d postfertilization and showed 6.0 ± 0.67 increments when sampled 3 d after hatching (Table 2B). Constant light conditions did not significantly alter increment formation; the constant

light group (EL24) showed the same number of increments as in the EL12:D12 group at 3 d of age. Thus, the effect of light on embryonic increment formation and otolith diameters was the same for the EL24 and EL12:D12 groups.

A 1-min light stimulus on day 10 of ED24+L

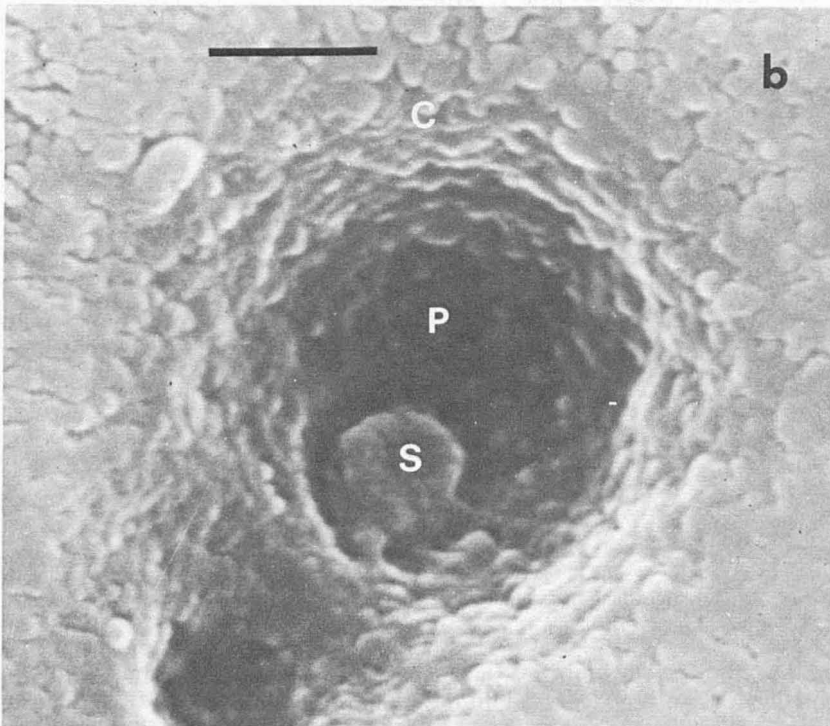
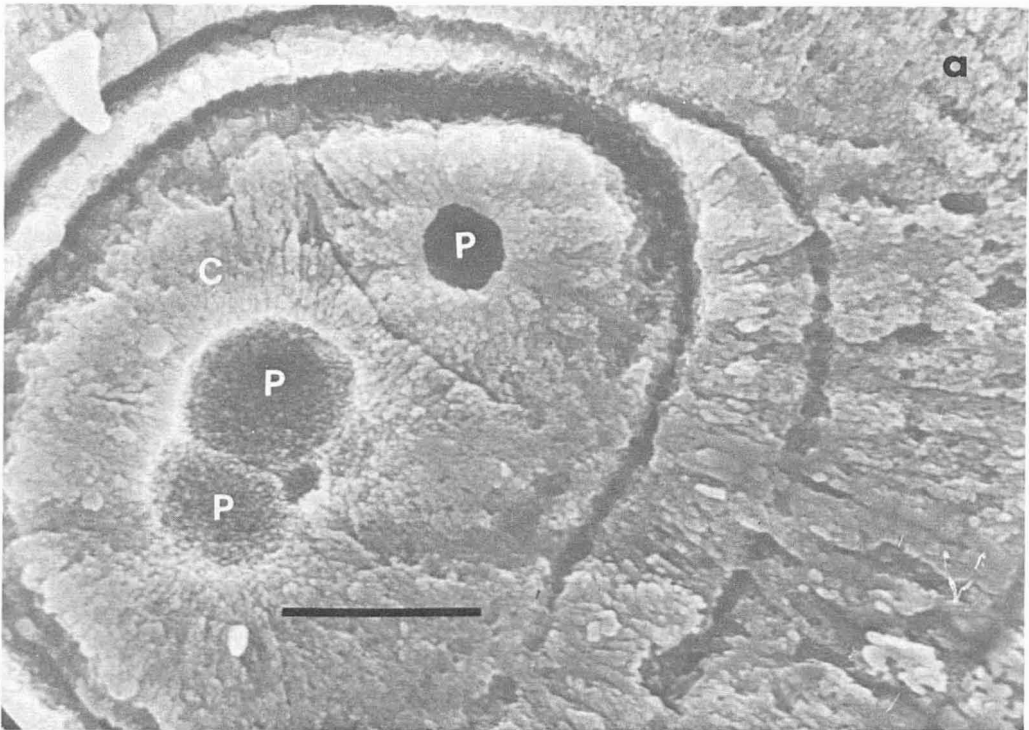


FIGURE 4.—a) SEM of the core of the sagitta of *Fundulus heteroclitus* showing the core (C) and the multiple primordia (P) surrounds the spherules. Bar = 1 μ . b) SEM showing a spherule (S) in the multiple primordia (P) of the core (C) or the sagitta. B = 0.5 μ .

resulted in increment formation (Table 2C) in embryos otherwise maintained in constant darkness. Increment counts for ED24+L were less

than those of the EL24 group and the EL12:D12 group at 3 d after hatching, but were very close to the increment counts found at day 2 of the EL12:D12 group.

The effect of light on larvae which were maintained under EL12:D12 during embryonic development and then transferred to constant darkness after hatching was not as evident as the effect of light was in the embryos maintained in constant darkness. Larvae raised in constant darkness (LaD24) showed a rapid addition of increments between day 0 and day 6 after hatching, but few increments formed after day 6 (Table 3). When the LaD24 data were compared with the data from the larvae hatched and raised

TABLE 2.—Effect of photoperiod and light stimuli on increment formation in sagittal otoliths of *Fundulus heteroclitus* embryos.

	Sagitta diameter (mm) ($\bar{X} \pm SD$)	Increment numbers ($\bar{X} \pm SD$)
A) Embryos were incubated in total darkness and sagitta were removed 3 d after hatching. (ED24)	0.071±0.008	0.6±0.77
B) Embryos were incubated in constant light and sagitta were removed 3 d after hatching. (EL24)	0.177±0.013	6.0±0.67
C) Embryos were incubated in constant darkness with 1 min of light at day 10 of development. Sagitta were removed 3 d after hatching. N = 10 in all groups. (ED24+L)	0.146±0.012	4.7±0.48

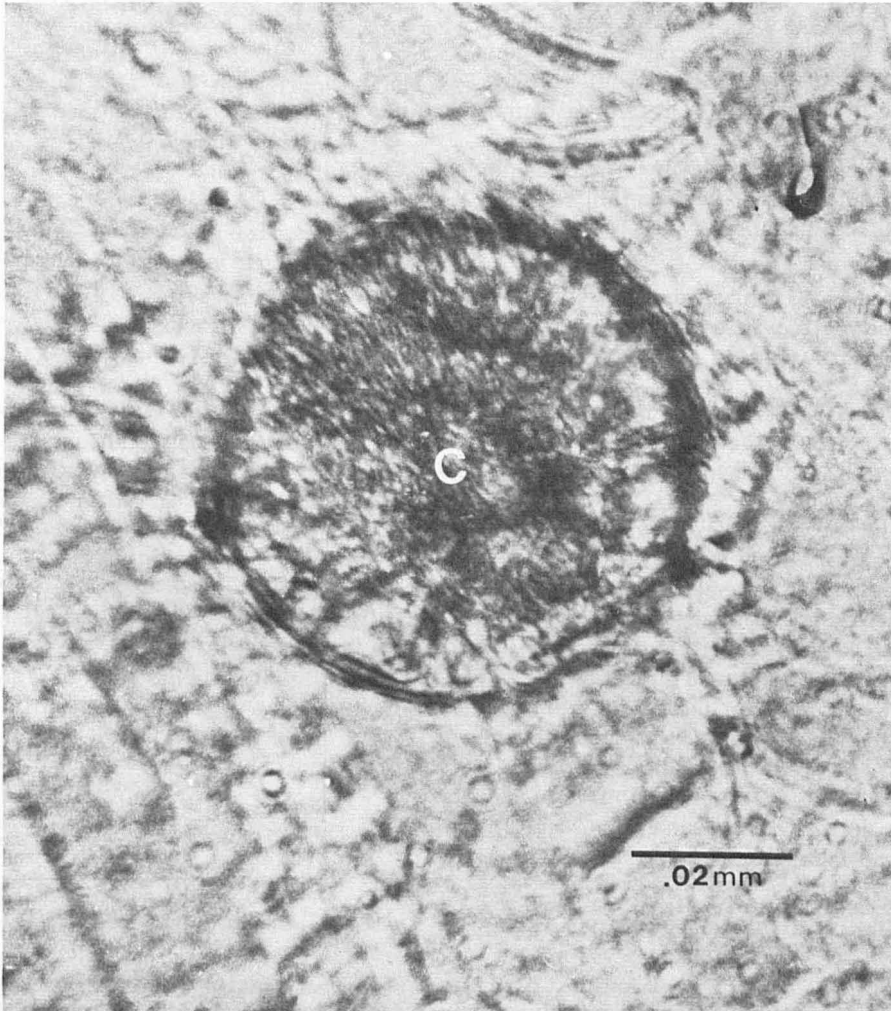


FIGURE 5.—Light micrograph of the sagitta from a newly hatched larvae incubated for the total embryonic period in total darkness. Core formation is present but no increments have formed. Bar = 0.02 mm.

TABLE 3.—Otoliths are from *Fundulus heteroclitus* larvae. Embryos were incubated on a L12:D12 cycle and the larvae transferred to constant darkness at 24°C immediately after hatching.

Age	Increment count	Otolith diameter (mm)
0	2.75±0.5	0.128±0.011
6	13.89±1.69	0.181±0.010
9	15.22±0.83	0.190±0.010
16	15.60±2.01	0.204±0.015

in EL12:D12 (Table 1), sagitta of LaD24 had reduced increment numbers after day 6 and sagitta diameters in experimental fish were smaller than those found in the control (LaL12:D12).

Some groups that had increment formation (LaD24 and LaL6:D6) during the first 6 d had increments formed after day 6 that were unclear and it was difficult to differentiate the D and I in the outer areas. However, the LaL12:D12 group showed distinct increments beyond day 6.

The ED24 group larvae were sluggish upon hatching as were the ED24±L group. The larvae appeared to be normal in every other fashion except that the yolk sacs were notably smaller than the 12L:12D group.

Effect of Temperature and Body Growth on Otolith Formation in Larvae

An increase in temperature caused an increase in the growth rate in the larvae (Fig. 6a). The 30°C group grew significantly faster than the 24°C group ($P < 0.05$).

The 30°C larvae, also formed otoliths (Fig. 6b) which were significantly larger ($P < 0.05$) in diameters than those in fish held at 24°C. However, the difference in growth rates had no effect on the increment counts from either group (Fig. 6c). Both showed daily increment formation in their otoliths but the faster growing otoliths had wider daily increments, which accounted for the increased diameter measurements. When the otolith diameter data were pooled and compared with length data, the relationship was highly correlated ($r = 0.95$; Fig. 7).

Estimation of Age of Wild Fish

It is difficult to gain any insight into the age structure of the wild population from the length-frequency histograms, e.g., larvae collected 9 June 1977 had a standard length-frequency

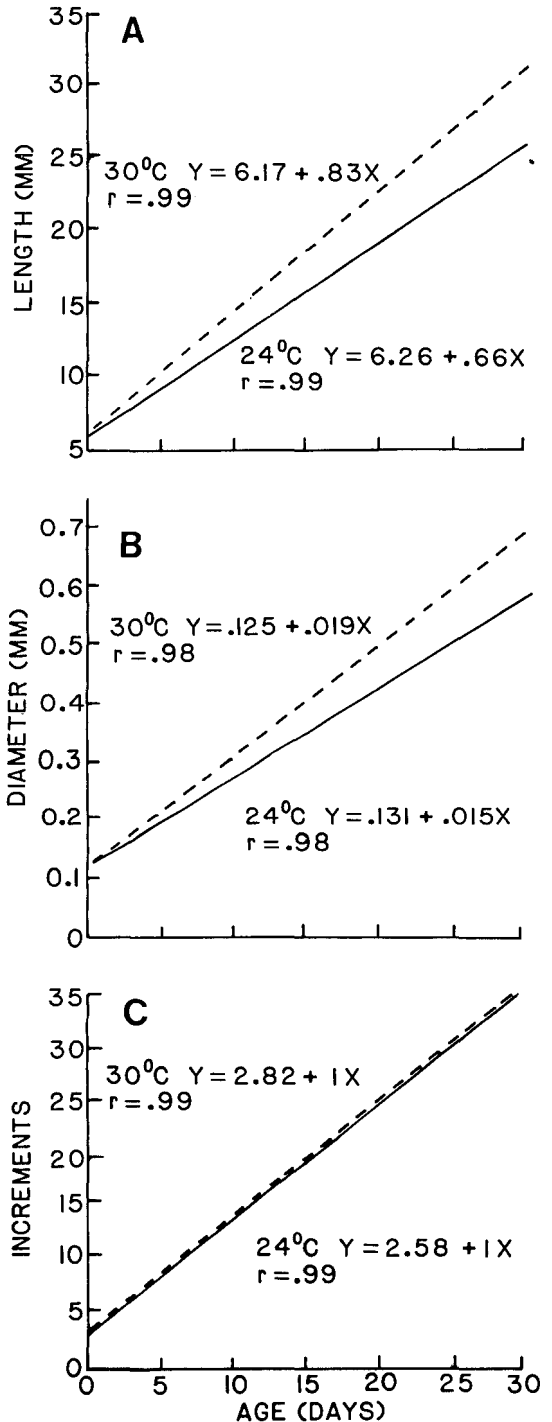


FIGURE 6.—A regression plot of A) standard length (SL), B) the diameter of the sagitta, and C) the numbers of increments of the sagitta of *Fundulus heteroclitus* reared at 24°C and 30°C plotted against age of the fish.

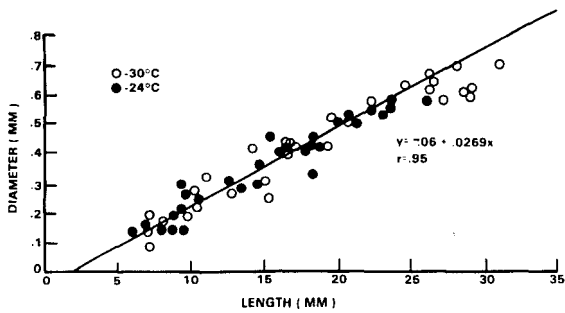


FIGURE 7.—A regression plot of the diameters of all of the 24° and 30° *Fundulus heteroclitus* sagitta plotted against the standard length of the fish.

mode of 23 mm (Fig. 8a). However, otolith increment-frequency histograms of the sample enabled us to differentiate cohorts (Fig. 8b).

A statistical analysis of the data from the field population showed that the relationship between the length of the fish and otolith diameter was linear ($y = 0.01 + 0.027x, r = 0.90$). The relationship of increment number and otolith diameter was curvilinear ($y = 0.7601 - 0.0217x + 0.000rx^2, r = 0.92$). Thus, the diameter of the otolith increased as the fish grew; the width of the increment was wider in younger, smaller fish than in older, larger fish; and the number of increments increased as the length of the fish increased.

When the time of hatching was estimated, using increment counts (Fig. 9a), groups were found that correlated with the occurrence of new and full moons. We observed that the increments tended to be more distinct in larvae collected from the field than in laboratory-reared larvae. When ages were adjusted for the two or three prehatching increments, the relationship was even more obvious (Fig. 9b). Incremental data indicated that the fish collected hatched at the new and full moon spring tides.

DISCUSSION

Embryological Formation of Otoliths

Otoliths (sagittae) are the first calcified tissues to form in developing *F. heteroclitus* embryos, and although they are prominent and easily observed features that have been presented in numerous developmental studies, their formation is not discussed. Long and Ballard (1976) clearly showed otoliths that formed at stage 20 in

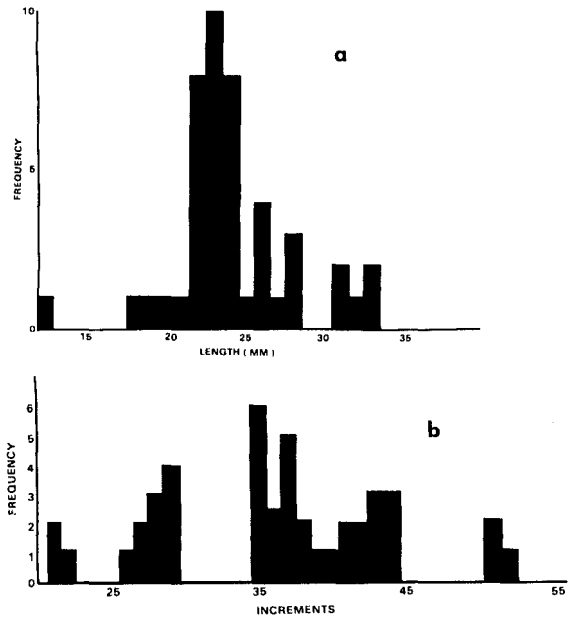


FIGURE 8.—a) A length-frequency histogram of all fish collected in the sample. b) A histogram showing the frequency of the increment number of the sagitta of same fish as in 8a.

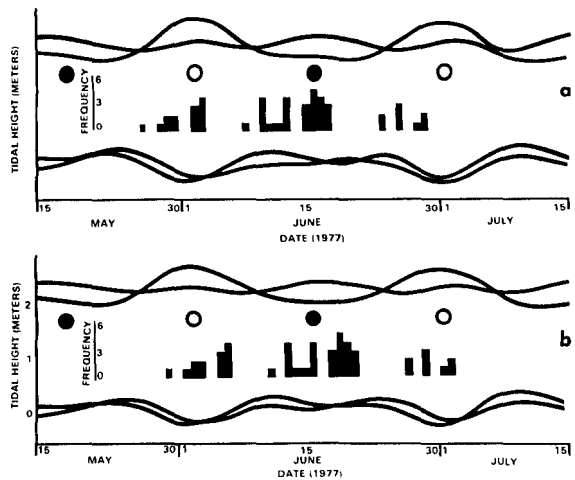


FIGURE 9.—*Fundulus heteroclitus* larvae collected on 9 June 1977. a) Estimated hatching dates are determined from numbers of increments in the sagitta; b) estimated hatching times are adjusted for the two increments formed prior to hatching. Also represented are diurnal high tides (upper lines) and low tides (lower lines) and lunar phase (open circles = full moon, closed circles = new moon).

embryos of the white sucker, and Armstrong and Child (1965) showed otoliths in mummichog embryos at stage 23 with calcification at stage 24,

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which agreed with this study, but their ontogeny is not well known. The importance and functional nature of the early otolith calcification has not yet been determined.

Two or three increments were easily visible in the mummichog otolith at the time of hatching. Accurate age determination of field samples could be affected until the number of increments formed at the time of hatching is considered. Brothers et al. (1976) studied increment formation in several fish species and found that the California grunion, *Leuresthes tenuis*, had two increments at hatching. Some species, such as the northern anchovy, *Engraulis mordax*, had no increment formation until the time of yolk-sac absorption, 6 d after hatching (Methot and Kramer 1979). Taubert and Coble (1977) found that three species of *Lepomis* began increment formation at swim up. Scott (1973) studied the otolith structure in larvae of the northern sand lance, *Ammodytes dubius*, and suggested that otoliths first formed in the postlarvae at a mean total length of 2.4 cm. However, his interpretation was a result of back calculations, not direct observations of otoliths from known age or larval stages of the fish.

We have found that multiple spherules in the core of the sagitta, followed by numerous increments, are formed prior to hatching in the Asiatic salmon or masou, *Oncorhynchus masou*; chum salmon, *O. keta*; pink salmon, *O. gorbuscha*; Arctic char, *Salvelinus alpinus*; brook trout, *S. fontinalis*; rainbow trout, *Salmo gairdneri*; and the sculpin, *Cottus nozawa*. The juveniles of the live bearing guppy, *Lebistes reticulatus*, and mosquitofish, *Gambusia affinis*, form a large number of increments prior to being spawned (Radtke and Dean unpubl. data). Mummichogs, California grunion, and the Atlantic silverside, *Menidia menidia*, have tidally correlated incubation periods of about 10 to 14 d and the salmonids incubation period can exceed 50 d. In contrast, the northern anchovy and spot, *Leiostomus xanthurus*, have short incubation periods of <2 d. This indicates that embryos which have longer incubation periods and large yolk sacs may form several increments before hatching, while embryos that have short incubation periods might not start increment formation until hatching or after yolk-sac absorption (Brothers et al. 1976; Methot and Kramer 1979). Much work remains to be done on a range of species before we can attempt to interpret the functional significance of increment formation in embryos.

The increments observed in otoliths in this and other studies (Pannella 1971; Brothers et al. 1976; Struhsaker and Uchiyama 1976; Taubert and Coble 1977; Barkman 1978; Methot and Kramer 1979) appear to be indicators of daily biological events. Rhythmic physiological activities, such as the occurrence of rhythmic mineral deposition in coral (Wells 1963), crayfish gastroliths (Scudamore 1947), and marine bivalves (Clark 1968; Pannella and MacClintock 1968), are controlled to a large extent by environmental changes synchronized to the diurnal astronomical cycle.

The only examination of the effect of endogenous daily biological rhythms on fish otoliths was by Taubert and Coble (1977), who studied the effect of environmental factors on daily increment formation of *Tilapia mossambica* larvae hatched in constant light. Their different experimental groups all showed increment formation but it was not always daily. They found normal increment formation in all experimental groups with a 24-h periodicity and any other cycle other than 24-h period disrupted increment formation. Since daily cycles are known to occur in blood chemistry of fish (Garcia and Meier 1973), those daily chemical changes could be reflected in the daily increments of the otoliths. Mugiya (1966) found monthly changes in total and diffusible calcium in the endolymph of the semicircular canals of the rainbow trout and the flatfish, *Kareius bicoloratus*, and he related his finding to the formation of the opaque and translucent zones found in adult otoliths. Daily changes in the calcium metabolism of the fish also occur (Mugiya et al. 1980) which are reflected in the formation of the I and D.

Daily increments were formed in *F. heteroclitus* larvae kept in a L12:D12 cycle, but were absent when the developing embryos were kept in constant darkness (Fig. 5). Light had a definite effect on increment formation, as embryos kept in constant light showed increment formation and otolith diameters that were comparable with the L12:D12 group. An insight into this discrepancy was gained in the analysis of the group which initiated increment formation after a light stimulus on day 10 after fertilization. The possibility that light is a synchronizing stimulus at the cellular level was demonstrated by Pitten-

drigh and Bruce (1957), who showed that a light stimulus synchronized emergence in fruit flies. More study is necessary to determine the timing of light needed for increment formation as well as the quantity and quality of light necessary. Whether the control of increment formation is an endogenous or exogenous rhythm (Harker 1957) is beyond the scope of these experiments. But the experiment on increment initiation in the dark group with 1 min of light exposure on day 10 indicated that light can act as a synchronizing stimulus, similar to that observed by Pittendrigh and Bruce (1957). Mugiya et al. (1980) found that D formation was initiated when light interrupted a photo period of 12L:12D or longer light period, but they did not determine the minimum dark period necessary for formation of the D or the free running period for the D and I.

When *F. heteroclitus* larvae were hatched in L12:D12 and then placed in light regimes other than a 24-h photoperiod, the increment formation became aphasic in each group and increment formation occurred at a slower rate. The "biological clock" of this group seemed to be out of phase under photoperiods other than those with a 24-h periodicity. A great deal of very exciting work is necessary to resolve these fundamental questions on increment control.

Effects of Temperature and Body Growth on Otolith Formation in Larvae

Under the various experimental conditions employed in this study, daily otolith increments formed regardless of body growth or otolith growth rate (Fig. 6a, b, c), so it was possible to determine age and daily growth rates of individual larvae which lived under different environmental conditions. Although *F. heteroclitus* larvae grew faster at 30°C than at 24°C, the number of increments was still directly related to chronological age. This documents the reliability of otolith increments for the age estimation of mummichog larvae. It has been demonstrated that daily increments exist in several other species of fish (Pannella 1971, 1974; Brothers et al. 1976; Struhsaker and Uchiyama 1976; Ralston 1976; Taubert and Coble 1977) and the relationship between increment counts and fish and otolith size was shown for the Atlantic silversides (Barkman 1978). In this study, otolith diameter increased with increased body length and increments formed on a daily basis with wider increments

found in younger fish than older fish. This is consistent with the fact that younger fish are growing faster, and although the relationship is non-linear, it is predictable and these results are consistent with those of Methot and Kramer (1979).

Estimation of Age of Wild Fish

Daily increments observed in field samples were easier to interpret than increments found in laboratory-reared larvae. We were not able to make age estimations of field collections of mummichogs from length-frequency histograms, but it was possible to determine the age and growth rate of individual larvae from increment counts.

Ralston (1976) and Struhsaker and Uchiyama (1976) determined growth rates of the milletseed butterfly fish, *Chaetodon miliaris*, and the nehu, *Stolephorus purpureus*, respectively, and found that the growth, as represented in incremental units in the otolith, was nearly linear. Similar results were obtained by Barkman (1978) for Atlantic silversides and Methot and Kramer (1979). Our results are consistent with theirs: that increment formation is independent of growth rate but is age dependent; thus growth rates can be estimated for individual larval fish.

Analysis of the age structure of samples of wild larval mummichogs showed that larvae hatched on or near the time of full and new moons. This is corroborated by observations on the reproductive biology of *F. heteroclitus* by Taylor et al. (1977, 1979) and DiMichele and Taylor (1978), New Zealand white bait, *Galaxias maculatus*, by McDowell (1968), and Atlantic silversides by Middaugh (1981). Eggs of the California grunion, an intertidal spawner, have been found to hatch during spring tides (Clark 1925) and have otolith increments at hatching (Brothers et al. 1976). An analysis of age structure of wild populations of mummichog larvae, as determined from their otoliths showed that South Carolina mummichogs spawn from March to mid-August and have a lunar spawning periodicity during that season. Analysis of otolith increments enabled us to differentiate individual fish in the wild population of the same size but of different ages.

Photoperiod is a critical factor in increment formation, but other factors such as diurnal migratory behavior, rhythmic feeding, temperature, respiration, and tidal rhythms might also

play significant roles. Even though the control and/or mechanism of daily increment formation in larval fish is not fully understood, the increments are a powerful tool for analysis of individual growth and age determination of very young fish.

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LITERATURE CITED

- ARMSTRONG, P. B., AND J. S. CHILD.
1965. Stages in the normal development of *Fundulus heteroclitus*. Biol. Bull. (Woods Hole) 128:143-168.
- BAGENAL, T. B. (editor).
1974. The proceedings of an international symposium on the ageing of fish. Unwin Brothers, Surrey, Engl., 234 p.
- BARKMAN, R. C.
1978. The use of otolith growth rings to age young Atlantic silversides, *Menidia menidia*. Trans. Am. Fish. Soc. 107:790-792.
- BROTHERS, E. B.
1978. Exogenous factors and the formation of daily and subdaily growth increments in fish otoliths. (Abstr.) Am. Zool. 18:631.
- BROTHERS, E. B., C. P. MATHEWS, AND R. LASKER.
1976. Daily growth increments in otoliths from larval and adult fishes. Fish. Bull., U.S. 74:1-8.
- CAIN, R. L., AND J. M. DEAN.
1976. Annual occurrence, abundance and diversity of fish in a South Carolina intertidal creek. Mar. Biol. (Berl.) 36:369-379.
- CLARK, F. N.
1925. The life history of *Leuresthes tenuis*, an atherine fish with tide controlled spawning habits. Calif. Dep. Fish Game, Fish Bull. 10, 51 p.
- CLARK, G. R., II.
1968. Mollusk shell: Daily growth lines. Science (Wash., D.C.) 161:800-802.
- DEGENS, E. T., W. G. DEUSER, AND R. L. HAEDRICH.
1969. Molecular structure and composition of fish otoliths. Mar. Biol. (Berl.) 2:105-113.
- DI MICHELE, L., AND M. H. TAYLOR.
1978. Environmental influences on the hatching of *Fundulus heteroclitus*. (Abstr.) Physiologist 21(4):29.
- GARCIA, L. E., AND A. H. MEIER.
1973. Daily rhythms in concentration of plasma cortisol in male and female gulf killifish, *Fundulus grandis*. Biol. Bull. (Woods Hole) 144:471-479.
- GULLAND, J. A. (editor).
1977. Fish population dynamics. Wiley, Lond., 372 p.
- HECHT, T.
1978. A descriptive systematic study of the otoliths of the neopterygian marine fishes of South Africa. Part I. Introduction. Trans. R. Soc. S. Afr. 43:191-197.
- HJORT, J.
1914. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapp. P.-P.-V. Réun. Cons. Int. Explor. Mer 20:5-38.
- HUBBS, C. L.
1943. Terminology of early stages of fishes. Copeia 1943:260.
- IRIE, T.
1955. The crystal texture of the otolith of a marine teleost, *Pseudosciaena*. J. Fac. Fish. Anim. Hus. Hiroshima Univ. 1:1-13.
- KNIEB, R. T., AND A. E. STIVEN.
1978. Growth, reproduction and feeding of *Fundulus heteroclitus* on a North Carolina salt marsh. J. Exp. Mar. Biol. Ecol. 31:121-140.
- LARKIN, P. A.
1978. Fisheries management—an essay for ecologists. Annu. Rev. Ecol. Syst. 9:57-73.
- LONG, W. L., AND W. W. BALLARD.
1976. Normal embryonic stages of the white sucker, *Catostomus commersoni*. Copeia 1976:342-351.
- LOWENSTEIN, O.
1971. The labyrinth. In W. S. Hoar and D. J. Randall (editors), Fish physiology, Vol. 5, p. 207-240. Acad. Press, N.Y.
- MCDOWELL, R. M.
1968. *Galaxias maculatus* (Jenyns), the New Zealand whitebait. N.Z. Mar. Dep. Fish Res. Div. Bull., New Ser. 2, p. 1-84.
- MERIDETH, W. H., AND V. A. LOTRICH.
1979. Production dynamics of a tidal creek population of *Fundulus heteroclitus* (Linnaeus). Estuarine Coastal Mar. Sci. 8:99-118.
- MESSIEH, S. N.
1972. Use of otoliths in identifying herring stocks in the southern Gulf of St. Lawrence and adjacent waters. J. Fish. Res. Board Can. 29:1113-1118.
- METHOT, R. D., JR., AND D. KRAMER.
1979. Growth of northern anchovy, *Engraulis mordax*, larvae in the sea. Fish. Bull., U.S. 77:413-423.
- MIDDAUGH, D. P.
1981. Reproductive ecology and spawning periodicity of the Atlantic silverside, *Menidia menidia*. Copeia 1981:766-775.
- MIDDAUGH, D. P., AND J. M. DEAN.
1977. Comparative sensitivity of eggs, larvae, and adults of the estuarine teleosts, *Fundulus heteroclitus* and *Menidia menidia* to cadmium. Bull. Environ. Contam. Toxicol. 17:645-652.
- MUGIYA, Y.
1966. Calcification of fish and shell-fish—VI. Seasonal change in calcium and magnesium concentration of the otolith fluid in some fish, with special reference to the zone formation of their otolith. Bull. Jpn. Soc. Sci. Fish. 32:549-557.
- MUGIYA, Y., N. WATABE, J. YAMADA, J. M. DEAN, D. G. DUNKELBERGER, AND M. SHIMIZU.
1981. Diurnal rhythm in otolith formation in the goldfish, *Carassius auratus*. Comp. Biochem. Physiol. 68A:659-662.
- PANNELLA, G.
1971. Fish otoliths: Daily growth layers and periodical patterns. Science (Wash., D.C.) 173:1124-1127.
1974. Otolith growth patterns: an aid in age determination in temperate and tropical fishes. In T. B. Bagenal

- (editor), The proceedings of an international symposium on the ageing of fish, p. 28-39. Unwin Brothers, Surrey, Engl.
- PANNELLA, G., AND C. MACCLINTOCK.
1968. Biological and environmental rhythms reflected in molluscan shell growth. *Paleontol. Soc. Mem.* 2:64-80.
- PITTENDRIGH, C. S., AND V. G. BRUCE.
1957. An oscillator model for biological clocks. In D. Rudnich (editor), *Rhythmic and synthetic processes in growth*, p. 75-109. Princeton Univ. Press, Princeton.
- POPPER, A. N., AND S. COOMBS.
1980. Auditor mechanisms in teleost fishes. *Am. Sci.* 68:429-440.
- RADTKE, R. L., AND J. M. DEAN.
1979. Feeding, conversion efficiencies and growth of larval mummichogs, *Fundulus heteroclitus*. *Mar. Biol. (Berl.)* 55:231-237.
- RALSTON, S.
1976. Age determination of a tropical reef butterflyfish utilizing daily growth rings of otoliths. *Fish. Bull., U.S.* 74:990-994.
- SCOTT, J. S.
1973. Otolith structure and growth in northern sand lance, *Ammodytes dubius*, from the Scotian shelf. *Int. Comm. Northwest Atl. Fish. Res. Bull.* 10:107-116.
- SCUDAMORE, H. H.
1947. The influence of the sinus glands upon molting and associated changes in the crayfish. *Physiol. Zool.* 20:187-208.
- SOKAL, R., AND F. ROHLF.
1969. *Biometry: The principles and practice of statistics in biological research*. Freeman, San Franc., 776 p.
- STRUHSAKER, P., AND J. H. UCHIYAMA.
1976. Age and growth of the nehu, *Stolephorus purpurus* (Pisces: Engraulidae), from the Hawaiian Islands as indicated by daily growth increments of sagittae. *Fish. Bull., U.S.* 74:9-17.
- TANAKA, M.
1972. Studies on the structure and function of the digestive system in teleost larvae—V. Epithelial changes in the posterior-gut and protein ingestion. [In Jpn., Engl. summ.] *Jpn. J. Ichthyol.* 19:172-180.
- TAUBERT, B. D., AND D. W. COBLE.
1977. Daily rings in otoliths of three species of *Lepomis* and *Tilapia mossambica*. *J. Fish. Res. Board Can.* 34:332-340.
- TAYLOR, M. H., L. DIMICHELE, AND G. J. LEACH.
1977. Egg stranding in the life cycle of the mummichog, *Fundulus heteroclitus*. *Copeia* 1977:397-399.
- TAYLOR, M. H., G. J. LEACH, L. DIMICHELE, W. M. LEVITAN, AND W. F. JACOB.
1979. Lunar spawning cycle in the mummichog, *Fundulus heteroclitus* (Pisces: Cyprinodontidae). *Copeia* 1979:291-297.
- VALIELA, I., J. E. WRIGHT, J. M. TEAL, AND S. B. VOLKMANN.
1977. Growth, production and energy transformations in the salt-marsh killifish *Fundulus heteroclitus*. *Mar. Biol. (Berl.)* 40:135-144.
- WELLS, J. W.
1963. Coral growth and geochronometry. *Nature (Lond.)* 197:948-950.
- WILD, A., AND T. J. FOREMAN.
1980. The relationship between otolith increments and time for yellowfin and skipjack tuna marked with tetracycline. [In Engl. and Span.] *Inter-Am. Trop. Tuna Comm. Bull.* 17:509-560.
- YAMADA, J., AND N. WATABE.
1979. Studies on fish scale formation and resorption. I. Fine structure and calcification of the scales in *Fundulus heteroclitus* (Athereniformes: Cyprinodontidae). *J. Morphol.* 159:49-66.