

EFFECTS OF PHOTOPERIOD AND FEEDING ON DAILY GROWTH PATTERNS IN OTOLITHS OF JUVENILE *TILAPIA NILOTICA*

KUNIAKI TANAKA, YASUO MUGIYA, AND JURO YAMADA¹

ABSTRACT

Effects of varying photoperiods and feeding times on the formation of daily rings in otoliths of juvenile *Tilapia nilotica* were studied with a scanning electron microscope. Two alternate concentric zones, each different in structure, were distinguished in the acid-etched ground plane: one thin and appearing as a groove (discontinuous zone) and the other thick and with well-developed, needlelike crystals (incremental zone). The number of incremental zones showed a good correlation with the chronological age in days after hatching for at least 28 days. The otolith showed a daily rhythm of growth under a photoperiod of 24 hours (12 light-12 dark). Growth of incremental zones started a few hours after lights-on and stopped or slowed down within a few hours of the following lights-on, during which time the discontinuous zone seemed to be formed. When the light and dark cycle was reversed, the cycle of otolith growth began to change on the second day and accommodated to the new photocondition 6 days after the reversal. A change in length of light and dark phases (18L-6D or 6L-18D) or a shift of feeding time did not affect the time of the discontinuous and incremental zone formation.

Otoliths have been widely used for aging fishes along with other hard tissues such as scales and vertebrae. Determinations of ages of larvae and juvenile fish will yield information on their early life history which is important for analyses of their population dynamics. However, accurate age determinations in terms of months or days have not been possible for young fish up to 1-yr-old until Pannella (1971, 1974) showed the presence of daily rings in otoliths (sagittae) of some temperate as well as tropical species. Brothers et al. (1976), Struhsaker and Uchiyama (1976), Taubert and Coble (1977), Timola (1977), Barkman (1978), and Radtke and Dean (in press) have studied the use of such rings, or daily increments, for determining the age in days of some larval and adult fish in temperate or tropical species. Their results showed good correlations between the number of rings and the ages in days after hatching in larval fish. However, the number of rings was often greater than the age in days in larval fish and less in adult fish, and the difference seemed to vary by species. This shows that otolith daily rings may be formed in the embryonic stage in some fish (Brothers et al. 1976; Radtke and Dean in press) and that the increments may not be formed in old fish by cessation of growth under some environmental conditions (Taubert and Coble 1977).

In order to accurately interpret otolith daily rings for age readings, it is necessary to understand the mechanisms which induce formation of individual rings. Taubert and Coble (1977) suggested that a 24-h light-dark cycle is essential for the formation of the ring pattern in the otolith of *Tilapia mossambica*. The morphological studies by Degens et al. (1969) and Pannella (1971, 1974) showed that the ring pattern of fish otoliths is composed of increments of two alternate light and dark bands: a thick band of well-developed aragonite crystals with their long axis roughly perpendicular to the outer margin of the otoliths and a relatively thin band intersecting the aragonite crystals.

Our study was undertaken to verify the presence of a daily growth rhythm in the otolith of *T. nilotica* and to determine the time of a day at which a new increment is formed. We investigated the effects of various photoperiods and the feeding time on the zone formation cycle of the otolith.

MATERIALS AND METHODS

Juveniles of *T. nilotica* (12-24 mm standard length) were used. They were obtained by natural fertilization and rearing by eight females in our laboratory. Eggs hatched about 4 d after fertilization and the larvae remained in their mothers' mouths for about 10 d. Immediately after leaving the mothers' mouths, the juveniles were trans-

¹Laboratory of Physiology and Ecology, Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido 041, Japan.

ferred to aquaria and used in the experiments. Water temperature was maintained at $27 \pm 0.5^\circ \text{C}$.

Experiments under 24-h Photoperiod

The juveniles were transferred to four 60 l aerated aquaria, each kept under a different photoregime: 12L-12D (light phase, 0800-2000 h), 12L-12D (light phase, 2000-0800 h), 18L-6D (light phase, 0800-0200 h), and 6L-18D (light phase, 0800-1400 h). For lighting, a daylight fluorescent lamp (20 W) was fixed 15 cm above the water surface. In all groups, fish were fed to satiation on fish food pellets supplied throughout the 24-h photoperiods. After acclimation to each condition for at least 12 d, 6-10 fish were killed every 3 h and their otoliths removed for measurement of completion of the newest increment. In addition, 10 fish in the first group (12L-12D, light phase, 0800-2000 h) were killed 19 and 28 d after hatching to compare the number of otolith rings with the fish's age in days.

Feeding Experiments

For experiments on effects of feeding we used two groups of juvenile fish of the same brood. Five days after leaving their mother's mouth, one group was fed for 3 h after each lights-on; and the other group was fed for 3 h before lights-off under 12L-12D cycle (light phase, 1200-2400 h). The experiment lasted 15 d. Five fish were killed from each group every 3 h during the last 2 d of the experiment and otoliths were examined for completeness of the newest increment.

Otolith Preparation for Scanning Electron Microscopy

Otoliths (sagittae) were removed from the fish under a dissecting microscope, washed in water, embedded in a few drops of epoxy resin (Bond E,² Konishi Co.), and placed on a glass microscope slide. After hardening, both otoliths from each fish were ground by hand with a whetstone to the mid-transverse plane, parallel to the long or short axis, and then polished with a compound whetting paste for sharpening microtome knives. The specimens were cleaned in xylene, etched with

0.5% HCl for about 20 s, and coated with gold in vacuum. The specimens were examined with a JSM-25 scanning electron microscope (SEM) at 15 kV for number of growth rings or for determination of completeness of the newest increment.

For the observation of the internal structure, otoliths were placed in a drop of water and broken into several pieces by a razor blade. The broken pieces were air-dried, coated with gold, and the nonetched fractured surface was observed with the SEM.

Measurement of Daily Growth Rhythm

In order to determine the exact hour when a new increment is formed in the otolith, we calculated the index of completion for current increment (C) by the following formula:

$$C = \frac{W_n}{W_{n-1}} \times 100$$

where W_n = width of current increment
 W_{n-1} = width of previous complete increment.

Measurements were made on SEM photographs of specimens collected every 3 h. Following the change of C in time sequence, one can estimate the time of completion or start of zone formation when C suddenly drops from approximately 100% to a lower level. The area of the measurement of C was at the distal surface near the anterior or the posterior edge of the otolith, where increments are thick, discontinuous, and easily observed.

RESULTS

The sagitta of a juvenile *T. nilotica* takes the form of an oval disc. It lies vertically in the sacculus of the inner ear with its long axis parallel to the anteroposterior axis of the fish. The inner surface of the otolith facing the macula region of the sacculus is somewhat concave, whereas the other surface is slightly convex.

When the otolith is ground parallel to its long or short axis down to the core region, or the growth center, and then etched, it exhibits a concentric ring pattern (Figure 1A), with each ring composed of two alternate layers, each different in structure. One layer usually measures 2-8 μm wide and has needlelike crystals of the aragonite form CaCO_3

²Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

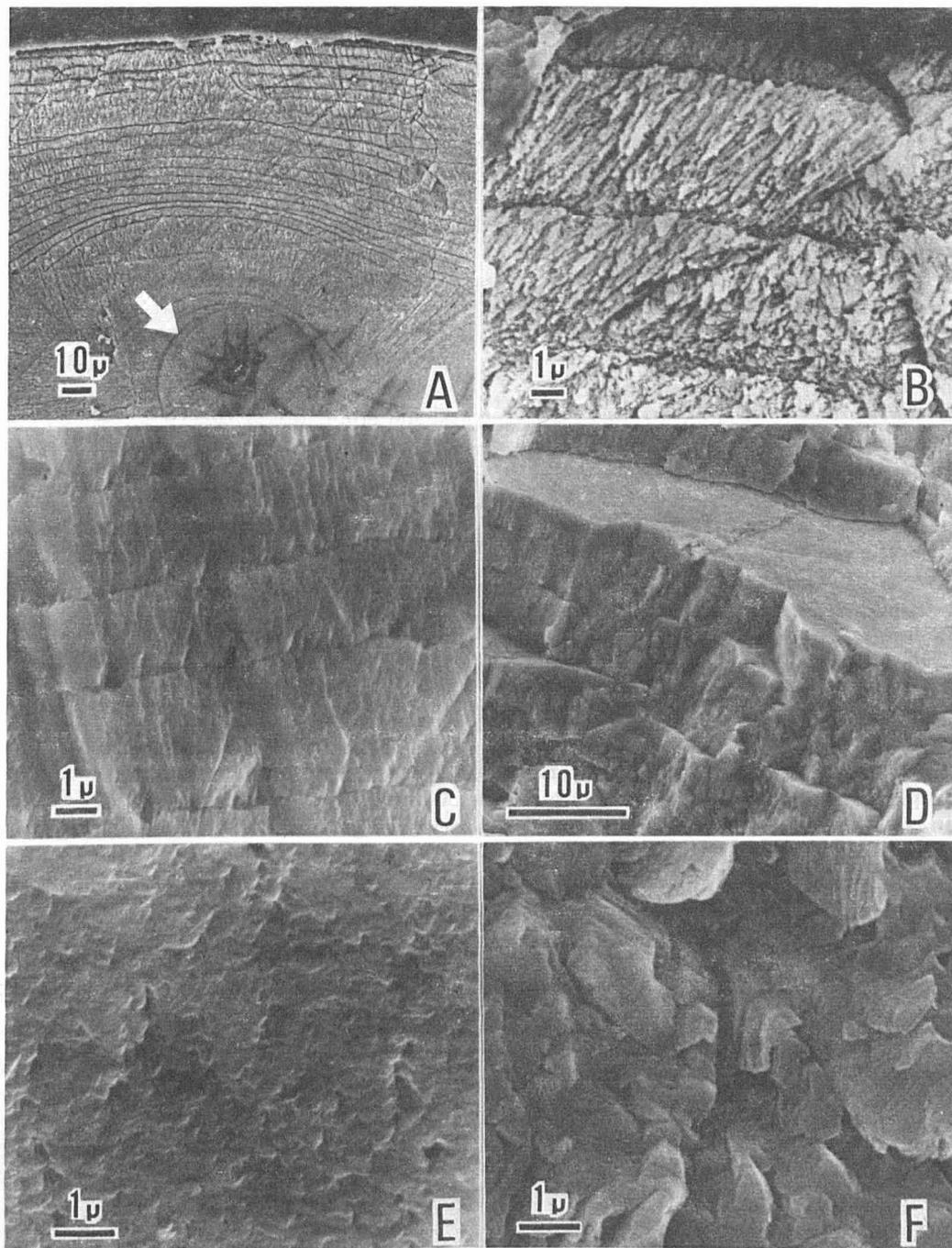


FIGURE 1.—Scanning electron microscope photographs of otoliths of juvenile *Tilapia nilotica*. A) Ground and etched otolith of a fish 28 d after hatching. The core region delimited by the innermost clear discontinuous zone (arrow) shows several vague, narrow bands. The center of the core region is deeply etched and has some spherules. B) Margin of a ground and etched otolith. The outermost incremental zone is not complete. C) Nonetched fracture plane of an otolith. The otolith is composed of a piling up of crystallized lamellae (incremental zone) with thin interstitial layers (discontinuous zone). D) Nonetched fracture plane of an otolith. The surface of a complete crystallized lamella at the boundary of the interstitial layer can be seen. E) Higher magnification of the surface of the crystallized lamella in D. F) Nonetched external surface of an intact otolith to show the growing surface of the outermost incremental zone.

with the long axis nearly perpendicular to the margin of the otolith, while the other is thin (about 0.2-0.5 μm wide) and appears as a concentric groove in slightly etched planes of the otolith (Figure 1B). Most of the grooves are not continuous around the whole area and fade out near the area along the long (anteroposterior) axis of the otolith. Usually, a few grooves in the area around the core region, which is delimited by the earliest clear circular groove, are weakly etched and set at wide intervals (Figure 1A).

On the other hand, examinations of nonetched fracture planes of the otolith revealed several thick crystallized lamellae piled up with narrow (<0.1 μm) interstitial layers between them (Figure 1C, D). The surface of a completed crystallized lamella that was fractured tangentially at the boundary to the interstice was observed to be flat and smooth (Figure 1D, E), while the growing surface of the otolith was rough with stacked crystals (Figure 1F). Therefore, we consider that the concentric grooves in acid-etched planes correspond to the interstitial layers where the growth of crystallized lamella was interrupted. The grooved zone is here defined as the discontinuous zone and the crystallized lamella as the incremental zone, both zones constituting a unit increment.

The core region, defined by the earliest clear discontinuous zone, was about 40 μm in diameter and had several, usually five or more, narrow unclear rings (Figures 1A, 2A). Ten or more

spherules of crystals, about 1.5 μm in diameter, were aggregated at the center of the core region. Some of them had the relatively finely organized core surrounded by coarse granules along the margin (Figure 2B). The peripheral region of the aggregate of the spherules was often deeply etched and appeared as a hole.

Correlation between the Number of Otolith Rings and Age in Days after Hatching

The number of rings (incremental zones) was counted from preparations of otoliths from fish 19 and 28 d after hatching. In this case, unclear and narrow rings in the core region were not counted because they seemed to be formed during the embryonic stage. Sixteen otoliths from 8 fish 19 d after hatching and 14 otoliths from 7 fish 28 d after hatching showed 18.89 ± 0.23 and 27.12 ± 0.27 (mean \pm SE) rings, respectively. The observed close correlations between the number of rings and the age in chronological days indicated that the ring formation proceeded on a daily basis until at least 28 d after hatching.

Formation of Otolith Rings Under a 12L-12D Photoperiod

When fish were acclimated to a 12L-12D photoperiod of lights-on at 0800 h and lights-off at 2000

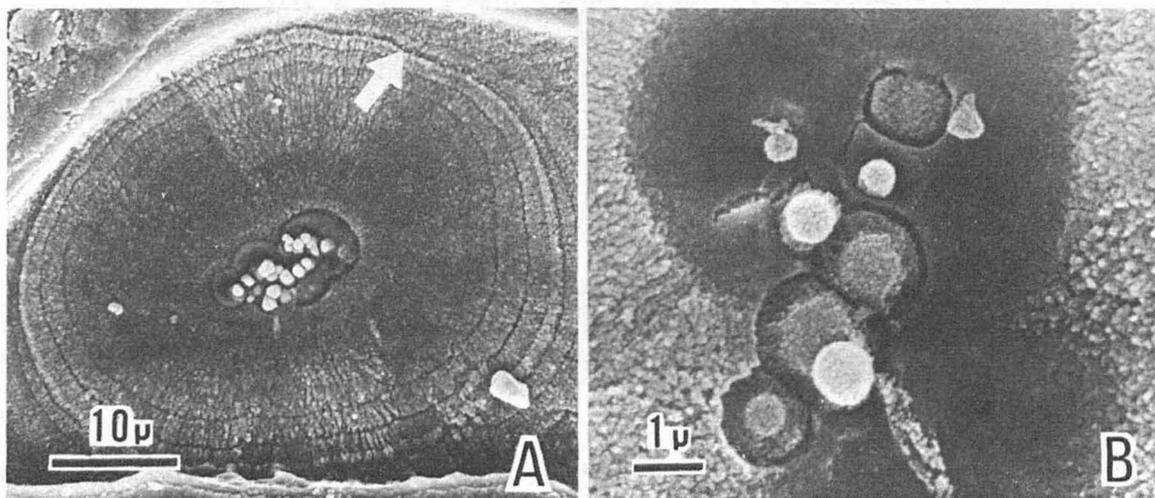


FIGURE 2.—Scanning electron microscope photographs of otoliths of *Tilapia nilotica* 1 d after hatching. A) Core region encircled by the first clear discontinuous zone (arrow). An aggregate of a number of small crystallized spherules is visible in the center. B) Crystallized spherules in the center area of the core region.

h, the otolith showed two phases, growth and rest, in a 24-h period (Figure 3A). The growth phase started between 0900 h and 1200 h and continued until 0500-0800 h next morning. The growth seemed to stop or slow down about the time of lights-on, resulting in the formation of a discontinuous zone. After several hours, the otolith started the next cycle of growth. This growth pattern occurred regularly for a consecutive 3-d period (Figure 3A). Accordingly, one incremental and one discontinuous zone were formed in the otolith in 24 h. When the photoperiod was reversed to lights-on at 2000 h and lights-off at 0800 h, the time of zone formation was also shifted about 12 h (Figure 3B), i.e., the growth phase started at sometime between 2100 h and 2400 h and lasted until about 2100 h. It should be noted that the formation of the incremental zone started a few hours after lights-on in both the experiments.

To see how the time of incremental zone formation shifts following a sudden reversal of light and dark phases (12-h shift), the index of completion for current increment at 2000 h was traced through the reversal (Figure 4). Under the ordinary photoperiod before the change, the index had been maintained at about 50% and persisted in the first day after the shift to the new photoperiod. However, the index at 2000 h became variable in the second day, and then gradually changed to a level of about 100% by the sixth day.

Formation of Otolith Rings Under 18L-6D and 6L-18D Photoperiods

The daily growth pattern was found to be the same in the group acclimated to photoperiods of lights-on at 0800 h and lights-off at 0200 h (18L-

FIGURE 3.—Daily growth of *Tilapia nilotica* otoliths as represented by changes in the index of completion for current increment every 3 h for 72 h. The fish were maintained under two contrasting photoperiods: A) 12L-12D (light phase, 0800-2000 h); B) 12L-12D (light phase, 2000-0800 h). Each circle represents mean \pm SE for six fish. START = start of new incremental zone.

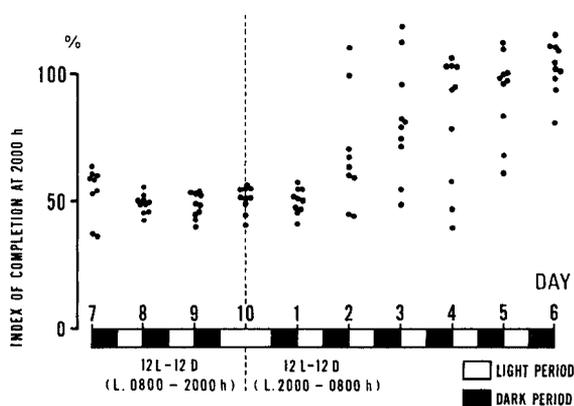
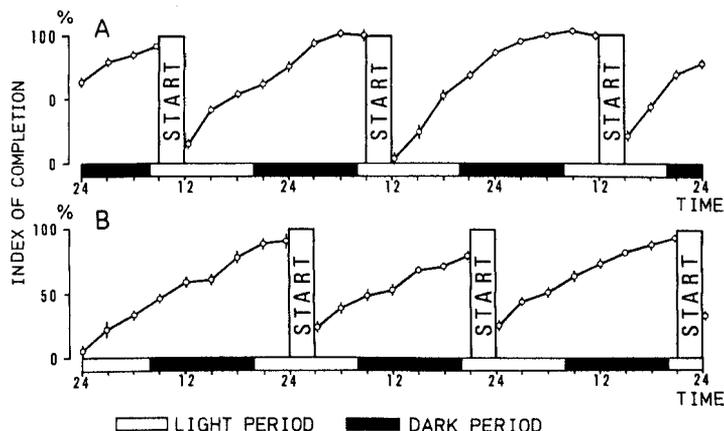


FIGURE 4.—Change in the index of completion for current increment at 2000 h in *Tilapia nilotica* otoliths when the fish were kept under a photoperiod of 12L-12D (light phase, 0800-2000 h) for 10 d and then transferred to the reversed light condition (light phase, 2000-0800 h). Each dot represents one otolith.

6D) and in the other with lights-off at 1400 h (6L-18D) despite the 12-h shift in the time of lights-off (Figure 5). The growth phase started about 3 h after lights-on and continued until about 0800 h the following morning. Thus, the discontinuous zones were formed sometime between 0800 h and 1100 h in both groups. It was also observed in both groups that the growth slowed down several hours before lights-on, as seen in 12L-12D experiments.

Feeding Time and the Formation of Otolith Rings

Two groups of fish were acclimated to different feeding schedules under the same photocondition (12L-12D, light phase, 1200-2400 h). One group

DISCUSSION

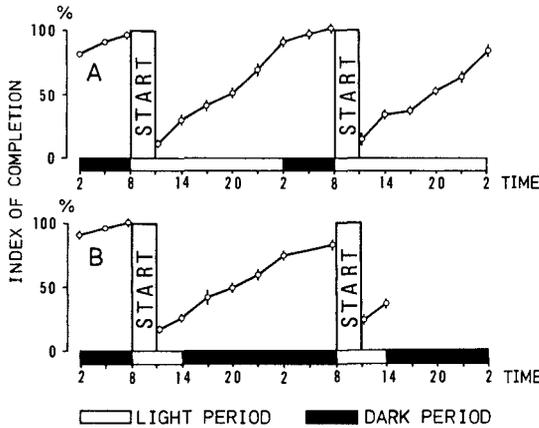


FIGURE 5.—Effects of long and short light periods on the daily growth of *Tilapia nilotica* otoliths. A) 18L-6D, light phase, 0800-0200 h; B) 6L-18D, light phase, 0800-1400 h. Each circle represents mean \pm SE for five or six fish. START = start of new incremental zone.

was fed during the first 3 h of light phases while the other was fed during the last 3 h of light phases, giving a 9-h difference in the beginning feeding time. However, no significant difference was found in the time of discontinuous zone formation in both groups (Figure 6). The zone was completed 1 or 2 h after lights-on in both groups, showing that the feeding time had no apparent effect on the formation of otolith rings under the 12L-12D photoperiod.

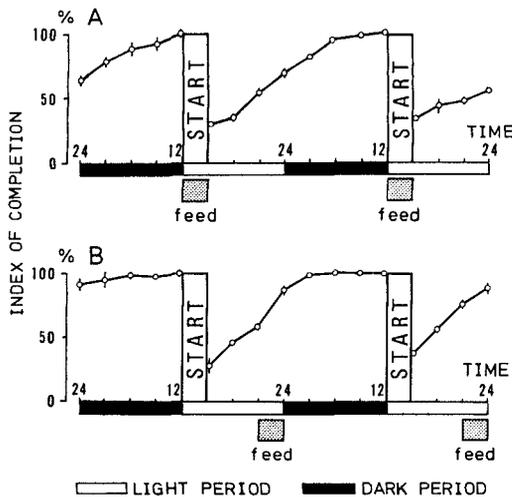


FIGURE 6.—Effects of feeding time on daily growth of *Tilapia nilotica* otoliths when the fish were reared under 12L-12D (light phase, 1200-2400 h). A) fed for 3 h after the onset of light phase; B) fed for 3 h before the end of light phase. Each circle represents mean \pm SE for five fish. START = start of new incremental zone.

The ground and etched plane of otoliths of *T. nilotica* exhibited a concentric ring pattern of thick incremental and thin discontinuous zones. In other teleost otoliths, a unit increment has been described as composed of light and dark bands in light microscope observations of replicas of ground and etched planes (Pannella 1971, 1974) and light microscope (Struhsaker and Uchiyama 1976; Taubert and Coble 1977; Barkman 1978) or SEM (Pannella 1974; Brothers et al. 1976; Timola 1977) observations of ground and etched specimens. The thickness of each band was variable but not as different as observed with the discontinuous and incremental zones in our results. This may be due to different ways of preparation and observation with different species of different ages. In particular, the thickness of discontinuous zones may vary depending on the concentration of acid and the etching time.

It has been considered that the band easily etched by acid contains more calcium and less organic materials (Brothers et al. 1976; Timola 1977). However, our observations of nonetched fracture planes in comparison with ground and etched planes indicated that acid effects thin interstitial layers between thick crystalline lamellae to form grooves, which are wider than the interstitial layers. This may be interpreted as caused by dissolution of calcium from the interstitial layers, and further, from the crystallized lamellae. The presence of organic materials intersecting growing crystals was reported in otoliths of various fishes (Degens et al. 1969; Pannella 1971). Dunkelberger et al. (1980) showed in a transmission electron microscope study of the *Fundulus* otolith that an interlamellar organic matrix interrupts each growth layer. Therefore, we consider that etching by acid discloses a layer containing more organic materials and less calcium and that acid infiltrated into the layer would affect crystalline layers at both sides. The discontinuous zones in the area along the anteroposterior axis of the otolith were not clear. Also, a few weakly etched discontinuous zones at wide intervals were found near the core region. This shows a possibility that clearness of a discontinuous zone reflects the growth rate of the otolith in relation to the ratio in amount of organic versus inorganic materials.

Results of our experiments in which the completeness of the outermost incremental zone was

measured every 3 h clearly showed that the ring is formed periodically every 24 h. This is supported with the observation that the number of rings (incremental zones), except those in the core region, is in good correlation with the chronological age in days after hatching of the fish. This also shows that the core region is formed during the embryonic stage. Several crystallized spherules found in the center of the core region indicate that the otolith rudiment is formed by fusion of many small primordia. Their presence is helpful to identify the plane that exactly passes through the center of the otolith.

The rate of otolith growth, judged from a change of the completeness of current increment, decreased or stopped for at least a few hours before and/or after the beginning of a light period in all the experiments. This may indicate that the growth of aragonite crystals at the margin of the otolith stopped or slowed down during this period of a day. On the other hand, growth of the otolith was apparent the rest of the time, showing that crystals deposited more rapidly. The structural difference observed between the surface of a complete crystalline layer and the outer surface of the growing otolith, flat and smooth in the former and rough with stacked crystals in the latter, also shows a difference in growth phases. These observations confirmed that in the fast growth phase an incremental zone is formed in the margin of the otolith and in the resting phase a discontinuous zone is formed. In relation to the structure of incremental and discontinuous zones discussed above, a cyclic deposition of organic materials or calcium or both seems to occur according to a daily photoperiod. Recently, Mugiya et al. (1981) reported that ^{45}Ca uptake by goldfish otoliths slowed down or stopped at sunrise and resumed in 3 h.

When a light and dark cycle was suddenly reversed, it took at least 6 d for the rhythm of the otolith growth to be adapted to the new photocondition. This indicates that otolith growth is primarily controlled by an endogenous rhythm synchronized with the environmental photoperiod. Taubert and Coble (1977) also presented a hypothesis that the formation of otolith rings is controlled by an internal, diurnal clock when fish are exposed to a 24-h photoperiod.

It was clearly shown that the otolith resumes its growth a few hours after lights-on, leaving a new discontinuous zone behind it. This pattern of growth was not affected by the change of the lengths of light and dark phases such as 18L-6D

and 6L-18D. This shows that the stimulus of lights-on entrains the rhythm of otolith growth to the photoperiods and thus is important for the formation of the otolith rings. The importance of this stimulus as a cue for the ring formation is supported by the experiment in which light and dark phases were reversed, where changes in phase of otolith growth started after the first stimulus of lights-on, but not lights-off, in the reversed cycle (Figure 4).

Since *T. nilotica*, as a typical diurnal fish, begins to feed immediately after lights-on, feeding was also expected to be one of the factors which control the daily rhythm of otolith growth. However, a difference of 6 h in daily feeding did not affect the phase of the rhythm. Therefore, feeding time is not critical for the formation of otolith rings when the fish is exposed to a 24-h photoperiod. Taubert and Coble (1977) also showed that the feeding cycle of 24 h did not cause the formation of daily rings on the otolith of *T. mossambica* held under constant light.

ACKNOWLEDGMENTS

We thank Norimitsu Watabe and John M. Dean, University of South Carolina, for their discussion and advice in preparing the manuscript. This work was supported by a fund from the Japan Society for the Promotion of Science awarded for a cooperative research under the Japan-United States Cooperative Science Program.

LITERATURE CITED

- 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., C. P. MATHEWS, AND R. LASKER.
1976. Daily growth increments in otoliths from larval and adult fishes. Fish. Bull., U.S. 74:1-8.
- DEGENS, E. T., W. G. DEUSER, AND R. L. HAEDRICH.
1969. Molecular structure and composition of fish otoliths. Mar. Biol. (Berl.) 2:105-113.
- DUNKELBERGER, D. G., J. M. DEAN, AND N. WATABE.
1980. The ultrastructure of the otolithic membrane and otolith in the juvenile mummichog, *Fundulus heteroclitus*. J. Morphol. 163:367-377.
- 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), International symposium of the aging of fish, p. 28-39. Unwin Brothers, Surrey, Engl.
- RADTKE, R. L., AND J. M. DEAN.
In press. Increment formation in the otoliths of embryos, larvae, and juveniles of the mummichog, *Fundulus heteroclitus*. Fish. Bull., U.S.
- STRUHSAKER, P., AND J. H. UCHIYAMA.
1976. Age and growth of the nehu, *Stolephorus purpureus* Pisces: Engraulidae, from the Hawaiian Islands as indicated by daily growth increments of sagittae. Fish. Bull., U.S. 74:9-17.
- 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.
- TIMOLA, O.
1977. Scanning electron microscope studies on the growth patterns of smelt, *Osmerus eperlanus* (L.), otoliths. Aquilo Ser Zool. 17:57-60.