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Daily Growth Increments in the Larval Otolith of the Japanese Eel, *Anguilla japonica*

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Abstract Early formation of otolith was studied on artificially hatched larvae of the Japanese eel, *Anguilla japonica*. Newly hatched larvae had a pair of sagittae which were flat and subelliptical with 8.3 μm in mean diameter. The diameter of the sagitta increased linearly with age. No growth increments were observed in the sagitta at hatching, while larvae which were 2, 4 and 6 days old had on average 2.1, 3.6 and 6.0 increments, respectively. The number of the increments (Y) and the age in days after hatching (X) showed a close linear relationship ($Y=0.96X+0.06$, $r=0.913$, $n=40$), suggesting daily deposition of sagittal increments. In 95% of the field-caught elvers of this species, a distinct dark ring (check) with the diameter of 6–12 μm was found around the nucleus of the sagitta. This seems to be a "hatch check" deposited at hatching, since its diameter roughly agreed with that of the sagitta in the newly-hatched larvae. Possibly, the number of the increments outside the hatch check represents the age of the fish in days.

The aging technique by daily growth increments in fish otolith has been developed and applied to the ecological examination of field-caught larvae (Struhsaker and Uchiyama, 1976; Taubelt and Coble, 1977; Barkman et al., 1981; Laroche et al., 1982; Radtke and Dean, 1982; Wilson and Larkin, 1980; Victor, 1982, 1983; Tsukamoto et al., 1987). Although accurate aging of individual fish can improve the estimation of population growth rates, age-specific growth, individual variation in growth and survival, the studies which validate the deposition rate of otolith increments are not so many (Campana and Neilson, 1985; Jones, 1986). Indeed, the results of many studies carry the explicit assumption that the otolith increments are formed daily.

In the Japanese eel, *Anguilla japonica* Temminck et Schlegel, a full-scale validation of the otolith growth increments has not been established, although a few studies have been carried out on the otolith microstructure (Tabeta et al., 1987).

The object of this study is to establish the aging method of the Japanese eel by determining the timing of the initial increment formation and the deposition rate of increments in the artificially hatched larvae.

Materials and methods

Larvae and elvers. Larvae of *A. japonica* were obtained through artificial maturation of parent fish in April 1986. Males were reared from the elver stage in a 4.5t tank at the Chiba Prefectural Inlandwater Fisheries Experimental Station. A female (922 g in BW, 78.6 cm in TL) was caught in the Marsh Inba, Chiba Prefecture, in October 1985, and kept in a 6t tank (16°C). They were matured with repeated injection of pituitary homogenate of the Amur bass (*Hypophthalmichthys molitrix*), LH-RH hormone (Lab. Protein) and Gonatropin (Teikoku Zoki Co. Ltd.). After ovulation of the female, eggs were inseminated by wet method (Chiba Prefectural Inlandwater Fisheries Experimental Station, 1986).

Table 1. Size and number of the specimens of *Anguilla japonica* examined in the study. LM, transmitted light microscope; SEM, scanning electron microscope. Parentheses indicate the number of fish used in the SEM studies.

Fish	N	TL (mm)		Method of otolith observation
		Mean	SD	
Larva				
0 day old	16	2.0	0.17	LM
2 day old	17	4.7	0.70	LM
4 day old	5	4.5	1.01	LM
6 day old	2	4.6	1.70	LM
Elver	59 (6)	58.0	2.37	LM & SEM

Eggs were kept in a glass tank (45×30×30 cm) containing seawater at the temperature of 23°C under constant dim light. They hatched 2 days after fertilization and the larvae survived 6 days without food supply. They were sampled every other day until 6 days after hatching. They were frozen for 4 months, and then transferred to 95% ethanol. The number of the fish examined in the study is listed in Table 1.

Fifty-nine elvers which were caught by a set net in Lake Hamana, Shizuoka Prefecture, were fixed in 95% ethanol in January 7, 1987, and their otoliths were also examined (Table 1).

Otolith preparation and observation. Total length of larvae was measured to the nearest 0.1 mm under a binocular microscope. After measurement, the fish were dried on a glass microscope slide, and mounted with a drop of Euparal (Chroma-Gesellschaft) and coverslip. Otoliths were observed under the light microscope (Nikon Optiphoto, 1,560×). The shape and microstructure of the otolith were traced with camera lucida, and its diameter was measured to the nearest 0.1 μm. A growth increment is a bipartite structure consisting of incremental and discontinuous zones (Mugiya et al., 1981). The incremental zone appears as a broad, translucent band, while the discontinuous zone is relatively narrow and opaque in the sagitta of this species. The number of increments in the sagitta was counted.

As for the elvers, a pair of the sagittae was removed under the binocular microscope after body length measurement. The otoliths were embedded in a few drops of epoxy resin (Konishi Bond co. Ltd.), and placed on a glass microscope slide. A sagittal section of the otolith was ground with an electric grinder (Sum Flex Co. Ltd.) with rubber stone. It was further polished with 2,000 to 8,000 grit oil-proof emery paper. For light microscope examination, the polished otolith was mounted with a coverslip and Euparal. A central part of the otolith was observed with transmitted light at the magnification of 1,560× using oil immersion objective lens (100×).

For scanning electron microscope (SEM) examination, the otolith which was ground and polished on the sagittal plane was etched with a 0.1 N HCl solution for 10–20 seconds. Then they were mounted on SEM stubs, and spattered with gold/palladium. Simultaneously, the otoliths ground on the frontal or transverse plane were prepared for SEM examination to compare with that ground on the sagittal plane. There were no significant differences in size and morphological feature of the otolith microstructure among the three grinding planes. A central part around the nucleus was examined to compare with the light microscopical observation.

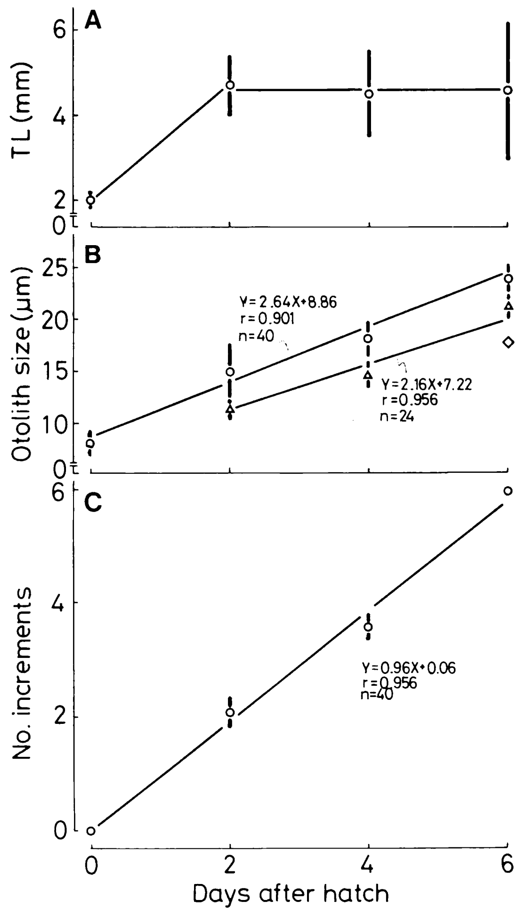

Fig. 1. Relationships between the age in days after hatching and the total length (A), diameter of three kinds of otoliths (B) and the number of increments (C) in *Anguilla japonica*. Circle, sagitta; triangle, lapillus; square, asteriscus.

Results

Growth of larvae. Larvae of *A. japonica* hatched at 2.0±0.17 mm (mean±SD) in TL. The body length increased until 2 days after hatching and then became constant at 4.6 mm in TL (Fig. 1). The mouth opened 4 days after hatching and

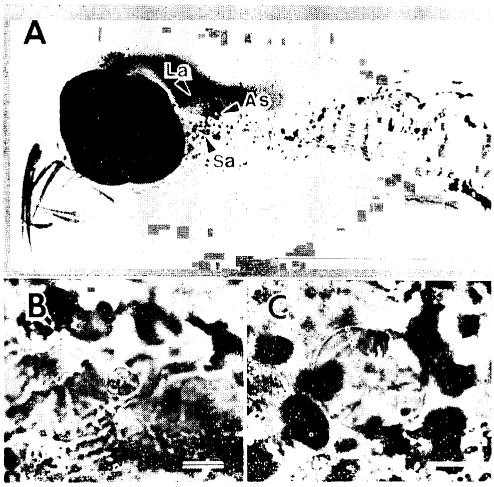

Fig. 2. Larval otoliths of *Anguilla japonica*. A: Position of three pairs of otoliths. Sa, La and As indicate sagitta, lapillus and asteriscus, respectively. B: Sagitta at hatching. C: Sagitta of 6-day-old larvae. Dots show each increment. Scales indicate 1 mm in A and 10 μm in B and C.

the yolk was almost absorbed when the larvae were 5 days old.

Morphology of the otolith. The sagitta was already formed at hatching (Fig. 2). It was flat and subelliptical with a diameter of 8.3 μm; the nucleus was positioned in the center of the otolith. The diameter of the otolith increased linearly with age in days (Fig. 1). The lapillus and asteriscus appeared 2 and 6 days after hatching, respectively. The lapillus was also flat and elliptical with the nucleus in the center. The diameter of the lapillus was 11.7±0.81 μm (mean±SD) at 2 days after hatching and increased linearly with age in days (Fig. 1). The diameter of the asteriscus in 6-day-old larvae was 18.0±1.10 μm, while those of the sagitta and lapillus in the same age were 24.0±1.48 μm and 21.2±1.20 μm, respectively. (Figs. 1, 2).

Number of the Increments. The sagitta at hatching had no increments (Fig. 2), although 1 or 2 faint layers were observed in some specimens (19%). The mean(±SD) increment counts in 2-, 4- and 6-day-old larvae were 2.1±0.72, 3.6±0.72 and 6.0±0 (Fig. 1), respectively, showing a highly significant linear relationship between the number of the increments (Y) and the days after hatching (X):

Fig. 3. Central part of the sagitta in the elver of *Anguilla japonica*. A, transmitted light microscope; B, SEM. Arrows indicate "hatch check." Scales, 10 μm.

$$Y=0.96X+0.06,\ r=0.956\ (p<0.01),\ n=40.$$

The slope of the regression did not differ significantly from 1.0 ($p>0.5$, F-test).

Check formation. A heavy dark check was observed around the nucleus of the sagitta in 95% elvers examined under the light microscope (Fig. 3). The diameter of this check ranged from 8.0 to 11.5 μm with a mean of 9.5±1.17 (SD) μm. The diameter of the check in the elver was slightly different from that of the otolith in the newly-hatched larva. The range of the former, however, includes that of the latter. Moreover, their difference was only 1.1 μm in the mean which was smaller than the mean width of one increment (2.64 μm) in the otolith of larvae. Therefore, we regard this heavy check in the elver as "hatch check". Increments were deposited from this check to the edge of the sagitta in all fish (Fig. 3). Inside this check, a pattern of 1 or 2 faint rings was observed in 62% elvers. On the other hand, the hatch check was not recognized on the otoliths of artificially hatched and reared larvae.

Under SEM, an increment in the sagitta of the elver was observed as a set of a discontinuous zone with a narrow and shallow groove, and a continuous zone with a wide, less-etched ridge. A deeply etched hole was observed at the centre of the otolith, and the hatch check was recognized as the deep circular groove surrounding the hole (Fig. 3).

Discussion

We confirmed the daily deposition of the otolith increments in the Japanese eel larvae which were 0–6 days old. This species seems to deposit the hatch check at the time of hatching, which can be observed as a prominent, dark ring under the light microscope. Therefore, we conclude that the number of the increments outside the hatch check represents the age in days.

Tabeta et al. (1987) examined the otolith rings on a longitudinal section of the Japanese eel by SEM, showing that the diameters of two specimens of 6-day-old larvae were 13 μm and 17 μm, respectively, while the diameters of the innermost distinguishable ring in 10 specimens of elvers were 18–24 μm which were larger than that of 6-day-old larvae. Therefore, they speculated that the initial ring of the otolith was not formed at hatching but at the transitional stage from preleptocephalus to leptocephalus. However, the otolith of 6-day-old larvae in our study with 24.0 ± 1.48 μm (mean$\pm$SD) in diameter and 6 increments was clearly larger in size and had more numerous increments than that of Tabeta et al. (1987). Under SEM examination in our study, the sagittal plane of the otolith in the elver presented a continuous deposition of increments outside the deeply etched groove, i.e. "hatch check", of 9.2 μm in mean diameter (Fig. 3B). A diameter of the innermost distinguishable ring was not different between longitudinal and sagittal planes. Thus, the otolith preparation in the study of Tabeta et al. (1987) might be treated through over or under-grinding.

In this experiment, the daily deposition of the otolith increments showed no direct relation with the body growth and yolk-absorption. Although the body length ceased to increase 2 days after hatching (Fig. 1) and the yolk-sac was absorbed 4–6 days after hatching, the otolith increased in size linearly with age and increments were deposited constantly until 6 days after hatching (Fig. 1).

One or two faint layers inside the hatch check do not seem to be daily increments, because Yamamoto et al. (1975a, b) suggested that the Japanese eel otolith is formed during 13 hours between the ear vesicle formation and hatching. The faint increments inside this check in the otolith would not represent the daily rhythm.

Concerning the timing of the initial incremental deposition, Radtke and Dean (1982) speculated that in species having slowly developing embryos it might occur before hatch, while in species having rapidly developing embryos, it might not occur until hatching or after yolk-sac absorption. The Japanese eel having short incubation time (38 hours) and the first increment deposition at hatching thus substantiate their suggestion.

Acknowledgments

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ウナギ仔魚の耳石日周輪形成

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ウナギ耳石の初期の輪紋形成過程を明らかにすることを目的として，孵化仔魚とシラスウナギの耳石の微細構造を検討した．3種の耳石の内，扁平石は孵化時に既に形成していた．耳石は扁平で楕円形をしており中心部に核が観察され，その直径は 8.3±1.02 μm であった．孵化後，耳石は日令と共に直線的に成長した．耳石上の輪紋は孵化時には認められなかったものの，2日後，4日後および6日後の魚の耳石には，それぞれ平均 2.1 本，3.6 本および 6.0 本認められた．輪紋数 (Y) と孵化後の日数 (X) の間には，次のような極めて相関の高い直線関係が得られた：$Y=0.96X+0.06$, $r=0.913$, $N=40$. 一方，95% のシラスウナギの耳石中心部において，直径 6-12 μm の極めて明瞭な輪紋 (check ring) が認められた．これは光学顕微鏡下では幅の広い暗帯として，電子顕微鏡下では深くエッチングされた輪紋として観察された．また，この輪紋は孵化時の耳石直径とほぼ等しく，本種は孵化輪を形成するものと考えられた．以上の結果より直径 6-12 μm の孵化輪の外側の輪紋数は，その魚の日令を示すことが明らかになった．

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