Rearing Method and Daily Otolith Ring of Japanese Sardine Larvae

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Japanese sardine larvae Sardinops melanostictus hatched out from field-collected eggs, were successfully reared for 30 days with the survival rate of 13% of initial egg number. The relation between rearing days (D) and number of sagittal otolith rings (N) was expressed by the linear regression of N=0.99D-1.88 based on the 31 reared larvae, and the slope is not significantly different from 1 (p>0.05). It is proved that ring on the otolith is formed daily. The first ring is formed mainly 3 (2-5) days after hatching which coincides with the first feeding. Since Pannella suggested that thin growth layer in otolith of larval fish is formed daily,1,2) the daily increments of otolith have been verified in many species and used in various ecological studies in the field.3,4) In anchovy, the daily rings were proved in northern anchovy Engraulis mordax5) and Japanese anchovy Engraulis japonicus.6) In sardine, Butler and Mendiola assumed that daily ring formation in otolith begin 5 days after hatching without successful rearing experiment.7) This study aims to prove the formation of daily rings in larval otolith of Japanese sardine Sardinops melanostictus based on the rearing experiment.

Materials and Methods

Rearing Experiment

The larvae studied hatched out from field-collected eggs in the laboratory. The eggs were collected by a plankton net of 40 cm in mouth diameter from the surface waters in the Inoshiri harbor, Kochi Prefecture, Shikoku Island, on December 26-27, 1987. Polyethylene bag was used as cod end of the net and towing duration was limited within 5 min to prevent the damage of eggs. This experiment was done at Usa Marine Biological Institute, Kochi University. Eggs and larvae hatched were reared in 30 / black polyethylene tanks filled with filtered sea-water (<1 μm mesh) (Fig. 1). Water temperature were maintained about 18°C by water bath with heater and thermostat. Light cycle in this experiment was 13L:11D. Food supply started 2 days after hatching. The densities of food organisms were: rotifer ca. 20 ind./m/ 1st day, rotifer ca. 10 ind./m/ and wild zooplankton ca. 10 ind./m/ 2nd-5th days, wild zooplankton ca. 5-10 ind./m/ 6th-30th days. Wild zooplankton was collected with 40 μm mesh plankton net and filtered with 330 μm mesh net to avoid the large plankton. About 90% of wild zooplankton were composed of copepod nauplii and copepodites ranging mainly 50-150 μm in size. Bottom of tanks was cleaned every 4 or 5 days by siphone.

Daily Ring

Five to 10 larvae were sacrificed for the otolith observation every 5 days. Standard length (SL)
of larvae were measured to the nearest 0.1 mm, and they were washed by fresh water, and dried at 60°C for 24 h on small aluminium foil which was weighed in advance. Before notochord flexion stage, SL was defined as notochord length.

A pair of sagittal otoliths was removed from the dried specimen and mounted with Euparal on a microscopic slide. Counts of rings were made with a light microscope (LM) of 600 magnification. Innermost dark thin ring of about 6 μm in radius was defined as first ring. First and second rings were separated by most transparent space between rings under exactly adjusted focus only (Fig. 2a). Thereafter, remained dark rings were counted under intentional off-focus condition (Fig. 2b) reported as Tsukamoto and Kajihara. The rings were counted 5 times for each otolith and the count of the highest frequency was adopted as the number of rings for the otolith. When the otoliths of both sides were observed, counts of both sides were averaged. Otoliths of 5 individuals of 30 days-old larvae were observed with SEM, since the otoliths were too thick for LM observation (Fig. 2c).

Otoliths of 21 individuals of larvae were examined with both SEM and LM to verify the counts by light microscopy. The same otolith was observed with both methods using the larvae collected in Tosa Bay in December, 1986 and in the Kuroshio area in March, 1987. For the SEM observation, otoliths were removed from the Euparal mount by Euparal essence after LM observation, and mounted again in epoxy resin, cross-sectioned and polished to the nuclei. This polished otolith was etched in 1% HCl for 5 seconds and Au-coated.

Results

Rearing Experiment

A total of 460 eggs were collected on December 26 and 27, 1987, and their developmental stages were early (Aaa to Ac). The eggs started to hatch in the morning 2 days after collection, and all the eggs hatched out by the evening of the same day. Daily age of this day was defined as 0. The first feeding was observed in 3 days-old larvae.

Increases of SL and body weight (BW) during the first 30 days were shown in Fig. 3a, b. Average growth rates of SL and BW for the first 15 days were 0.67 mm/day and 0.06 mg/day, respectively. The rates of the next 15 days were 0.58 mm/day and 0.67 mg/day. Fifty eight larvae from 460 eggs survived until 30 days after hatching, i.e. percent survival during this experiment was about 13%. The 30 days-old larvae have following morphological characters: 1) origin of dorsal fin base located in front of the median point of body, 2) digestive tract almost integrated into body cavity, 3) melanophores appearing on body sides. Based on these characters, 30 days-old larvae can be considered to reach just before the juvenile period. After this stage the rearing seemed to become much easier than in larval stage, since larvae began to feed actively.

Daily Ring

Fig. 2a, b shows the sagittal otolith of 10 days-old larvae with 8 rings. Five (or 6) larvae were examined every 5 days from 5 to 30 days after hatching and a total of 31 larvae were examined. Otoliths from both sides were examined with LM for 23 specimens. Only one side was examined for 3 specimens because of 1 malformation and 2 breaks in preparation. Visibility of rings on otoliths were same as those of field-collected larvae.

Fig. 2. Photomicrograph of sagittal otolith of Japanese sardine larva with light microscope (A and B) and SEM (C). Bar indicates 10 μm: A, otolith of 10 days-old larva with 8 rings in exactly adjusted focus condition. Arrow indicates space between the first and second rings, B, the same otolith as above in intentional off-focus condition, C, otolith of 30 days-old larva with 28 rings.
Fig. 3. Growth of Japanese sardine larvae. The numbers beside the symbols indicate numbers of larvae averaged. Vertical bars indicate standard deviation: A, increases of mean SL (mm), B, increases of mean BW (mg dry weight).

Subdaily ring was not examined under intentional off-focus condition in this experiment. Differences in numbers of rings between otolith from both sides were 0 in 15 larvae, 1 in 6 larvae and 2 in 2 larvae. Based on this result, it is concluded that there is no significant difference in numbers of rings between otoliths form both sides ($p > 0.05$).

The relationship between number of rings and its rearing days after hatching is shown in Fig. 4 based on the 31 reared larvae. The linear regression,

$$N = 0.99D - 1.88$$

was obtained by the least square method, where $N$ and $D$ were numbers of rings and days after hatching, respectively. The slope of the regression line shows no significant difference from 1.00 ($p > 0.05$). This proves that formation of rings on otoliths of Japanese sardine larvae is daily.

Comparison between the number of rings read with LM and SEM was shown in Fig. 5. The values subtracting the SEM count from LM counts ranged from 1 to −4, and 80% of the values were within ±1. The regression line

$$S = 0.94L + 0.61$$

was obtained, where $S$ and $L$ were SEM and LM reads, respectively. The slope of this regression shows no significant difference from 1.00 ($p > 0.05$).

**Discussion**

Based on the relationship between rearing days and number of rings on the otolith of sacrificed larvae, it is estimated that 6 individuals of larvae formed the first ring on 2 days-old, 21 larvae on 3 days-old, 3 on 4 days-old, and 1 on 5 days-old. Therefore 87% larvae in this study formed first
ring when 2 or 3 days-old. Their mean and the intercept of regression is not significantly different from \(-2\) \((p>0.05)\), indicating that the first ring formation coincides with the first feeding. The similar coincidence has been reported also on northern anchovy\(^5\) and Japanese anchovy.\(^6\) No detection of subdaily ring was indicate good rearing condition and efficacy of intentional off-focus condition on rings counting with LM.

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References