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Verification of Daily Growth Increment Formation in Saury Otoliths by Rearing Larvae from Hatching

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Abstract Naturally spawned eggs of the Pacific saury, *Cololabis saira*, were collected in the field and reared in a tank to examine daily periodicity of growth increment formation in the otolith. Larvae were 6.9 mm in knob length at hatching. Their otoliths (sagittae) were 31 μm in radius and had 3–6 faint concentric rings. They started feeding within two days and grew at a rate of 1.1 mm/day on average through larval and juvenile stages feeding on rotifers, *Artemia* nauplii, and artificial diets. Otolith growth increments showed a concentric pattern with a distance of 3.5–5.0 μm between two adjacent increments. The number of growth increments was almost equal to a known age in days plus 4 or 5. A regression line of number of increments (N) on known age in days (D) between 0–30 days after hatching was $N=4.81+1.01D$, which shows that one increment was deposited per day.

Age and growth of the Pacific saury, *Cololabis saira* (Brevoort) has long been controversial (Hatanaka, 1955; Hotta, 1960; Novikov, 1960; Sunada, 1974; Kim and Park, 1981), due to difficulties in interpreting marks on both scales and otoliths. Nishimura et al. (1985) first reported micro-increments in saury otoliths and suggested the possibility of age determination by these structures as in other fishes (Jones, 1986). Watanabe et al. (1988) drew growth curves of northeastern and northwestern Pacific saury based on the assumption that otolith micro-increments are deposited daily, and found that the western Pacific saury grows faster than its eastern counterpart. They concluded that the western saury becomes a 30 cm adult within one year. However, their conclusions needed to be supported by verification of the daily periodicity of increment formation. We collected fertilized eggs of saury in the field, incubated them in a tank, and raised hatched larvae in order to obtain larval and juvenile specimens of known ages. Thus, we could examine micro-increment formation in saury otoliths.

Materials and methods

Naturally spawned fertilized eggs of the Pacific saury, attached to drifting brown algae, *Sargussum horneri*, were collected in the morning off the Oshika Peninsula, Miyagi, Japan on the 23rd of June 1988. Surface temperature at the collection site was 14.7°C. Most of the embryos were at the morula stage. Judging from Yusa's (1960) description of saury embryonic development, they were assumed to have been spawned during the previous night.

The eggs were incubated in an open seawater system at temperatures ranging from 16 to 19°C. Hatching began on the 2nd of July and ended on the 4th. Most larvae hatched on the 3rd of July. We used this cohort to rear larvae of known age.

Hatched larvae were reared in a 500 *l* tank with aeration for 25 days after hatching, at which point the fish were transferred to a 1,000 *l* tank. We exchanged 100–300 *l* of the tank water on days 3, 5, 8, and 10 through 15. Then we changed to an open system with a flow rate of 1.2–3.5 *l*/min. Turnover rates of the water in the 500 *l* tank was 5–10 times per day. After transfer of the fish to the 1,000 *l* tank on the 25th day, the flow was 3 *l*/min for the first 5 days. We gradually increased the flow to 10 *l*/min on the 60th day. The turnover rate of the water in the tank was 5–17 times per day. The water in the tank flowed counter-clockwise. Water temperature during the rearing period was 18.5–24.1°C (Fig. 1). Dissolved oxygen concentration of the water ranged between 5.5 and 9.0 ppm during the experiment. The pH level stayed between 8.0–8.5. The illuminance at the water surface around noon ranged from 420 to 19,000 lux, depending on overhead weather conditions.

We fed larvae with rotifers as an initial food. On

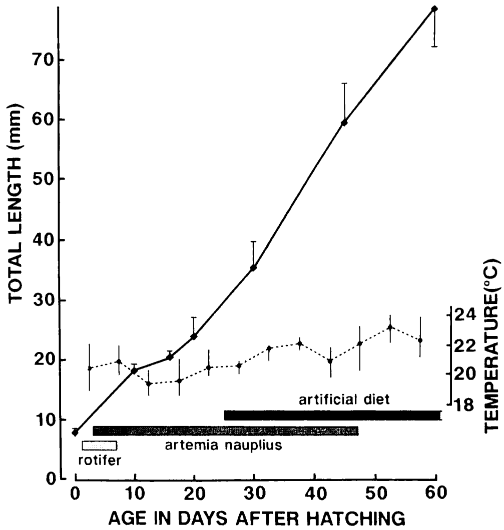

Fig. 1. Growth curve of reared Pacific saury. Diamonds show mean total lengths with standard deviations indicated by vertical bars. Dots show average water temperature for 5 day intervals with ranges in those periods given by vertical bars. Feeding regime is indicated by shaded columns.

the day after hatching, we added 360,000 rotifers to the 500*l* tank. On the 2nd and the 3rd days we added 620,000 and 1,130,000 rotifers, respectively. Average prey concentration before we gave additional rotifers in the morning was 1,000 ind./*l*. We stopped adding rotifers after the 4th day, but the prey maintained a concentration of 10,000 ind./*l* by proliferation until 7 days after hatching. On the 4th day after hatching, we added 97,500 *Artemia* nauplii to the 500*l* tank. The nauplii were added to the tank once a day in the morning. We gradually increased the number of nauplii added, reaching over 1,000,000 by day 13. An artificial dry diet for juvenile red sea bream, *Pagrus major*, No. 2 (Nippon Nosankogyo) was fed 6–10 times a day from day 25. The No. 3 diet for red sea bream was used from day 34. Total diet fed per day was 1 g on day 25, 5 g on day 26, 20 g on day 27, and 30 g up to day 44, 40–45 g up to day 47. A diet for juvenile ayu, *Plecoglossus altivelis*, (Nippon Nosankogyo) was added from day 48.

Saury specimens were sampled on days 0, 1, 2, 5, 10, 20, and 30 after hatching. They were anesthetized by weak MS-222 solution, measured for total length (TL) and knob length (KnL, the distance from the tip of the lower jaw to the posterior end of the muscular knob at the base of the caudal peduncle), and preserved in 80% ethanol. Sagittae were dissected from the preserved specimens under a microscope equipped with a polarizing filter. Membranes and muci were removed using sharpened needles and the otoliths were cleaned in a weak hypoclorite solution. They were then rinsed in a distilled water, dried, embedded in an enamel resin, and observed under a light microscope.

Results

Saury larvae were 7.9 mm TL (6.9 mm KnL) at hatching and had a small amount of yolk which was covered with pigmentation. They swam constantly at the surface of the water and began feeding within two days. Larvae staying at the bottom of the tank were usually not successful in feeding and eventually died. Larvae staying at the surface fed actively on rotifers and *Artemia* nauplii. Yolk absorption was completed 3–5 days after hatching. Live total and knob lengths of reared saury at different ages are summarized in Table 1. They grew at 1.0 mm/day from hatching to 10 days old, whereupon the growth rate decreased to 0.4 mm/day for about a week. It subsequently increased and became higher than 1 mm/day after 20 days. The rate between day-30 and day-60 was as high as 1.4 mm/day.

Table 1. Total and knob lengths of saury at increasing age. *1, mean±standard deviation.

Age in days	No. samples	Live total length (mm)	Live knob length (mm)	Average growth rate (mm total length/day)
0	20	7.9±0.4*1	6.9±1.5*1	
10	10	18.2±1.1	—	1.0
16	6	20.7±0.9	18.8±0.8	0.4
20	10	23.8±3.3	21.8±3.0	0.8
30	10	35.7±4.1	32.4±3.7	1.2
45	10	59.6±6.4	57.9±2.2	1.6
60	10	78.7±6.6	72.9±6.5	1.3

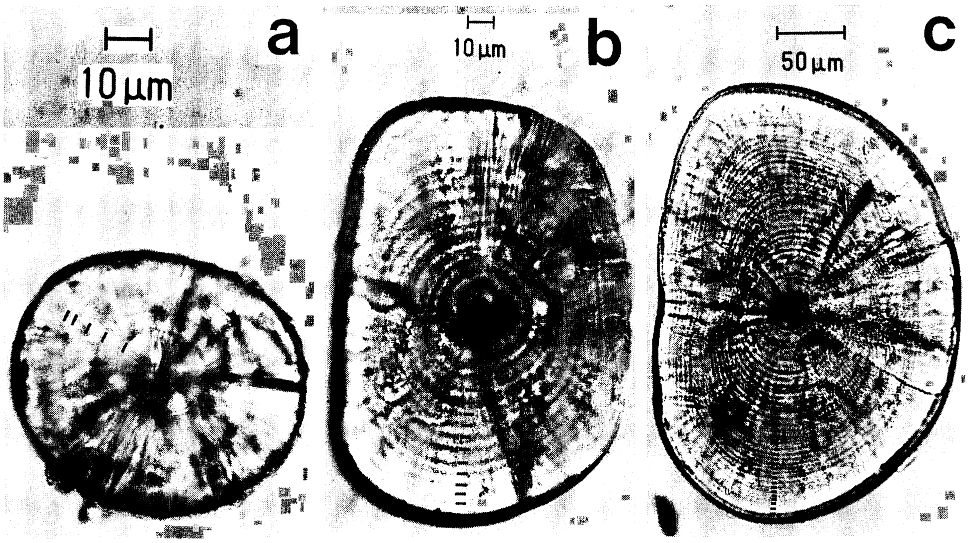

Fig. 2. Sagittae of reared saury of known ages. a. Larva on the day of hatching with 5 increments. b. Larva 10 days after hatching with 13 increments. c. Juvenile 30 days after hatching with 34 increments.

At around 30 days of age, juvenile sauries stayed at the middle or the bottom of the tank in the morning before the first feeding of the day. When we fed them, they came up to the surface and showed active feeding behavior. More than 50% of the juveniles showed this behavior by 40 days of age. At this point they formed a dense school when they were frightened by something approaching the tank. They started swimming constantly in an anti-current direction and tended to jumped out of the water. Some in fact jumped over the top of the tank, about 10 cm above the water surface.

Water temperature of the tank, feeding regime, and the growth curve of the reared saury are shown in Fig. 1. The fish reached 79 mm TL (73 mm KnL) in two months. Though we did not record survival rate, at some 2 months of age (70 mm KnL) about 300 juveniles survived out of 3,000–4,000 hatched larvae.

Otoliths of the saury larvae at hatching were marble-shaped and about 31 μm in radius (Fig. 2a). They had 3 to 5 faint concentric increments of irregular width at hatching. Otoliths of 10 day-old larvae were oval and had 15.4 increments on average (Fig. 2b). The increments were somewhat diffused and wide, and not as distinct as in otoliths from field-caught specimens. Otoliths of 30 day-old juveniles were 162.8 μm in radius and had 35.3 increments (Fig. 2c). The average increment width in the first 30 days after hatching was 4.2 μm. The increments were not clear between the 15th and 20th numbering from the nucleus (Fig. 2c), which correspond to the period of low fish growth rate.

Knob length, otolith radius, and number of increments at different ages are summarized in Table 2. Known age in days versus the number of increments

Table 2. Preserved knob length, otolith radius, and number of growth increments at increasing age. *1, mean ± standard deviation.

Age in days	No. fish examined	Preserved knob length (mm)	Otolith radius (μm)	No. increments
0	7	6.0±0.4*1	31.0± 3.5*1	4.3±0.8*1
1	1	6.1	24.0	5.0
2	5	7.6±0.3	39.8± 2.6	7.2±0.8
5	6	8.3±0.6	49.8± 5.1	10.2±0.8
10	10	15.3±0.8	84.3± 6.9	15.4±0.8
20	9	20.2±2.8	113.1± 8.4	24.3±1.6
30	10	30.7±3.7	162.8±14.2	35.3±1.1

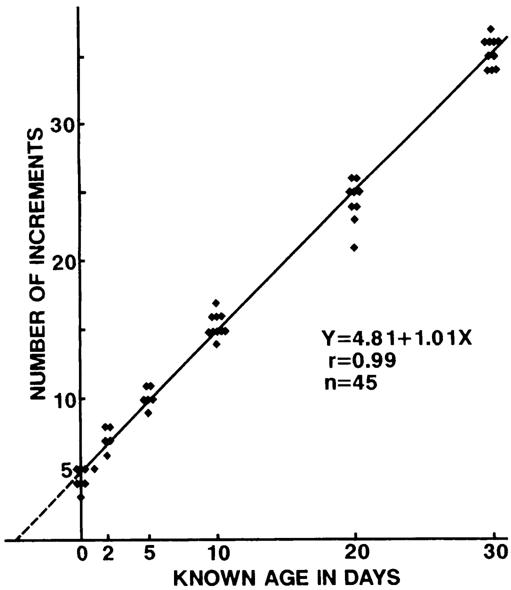

Fig. 3. Linear relationship between known age in days and the number of otolith growth increments.

is presented in Fig. 3. The equation of the regression line was,

Number of increments
$$=4.81+1.01\times(\text{known age in days})$$

Discussion

Rearing Pacific saury in a tank was first attempted in Tokushima Prefectural Fisheries Experimental Station, Japan, in 1968 (Joh and Tonogai, personal communication). Larvae were 7.0 mm in knob length on hatching and grew at a rate of 0.95 mm/day to 50 days of age. Rotifers and *Artemia* nauplii were used for food during the rearing experiment. In this early experiment the juvenile sauries became lean after 45 days of age. Feeding *Artemia* nauplii only might not be sufficient for juvenile sauries. In our experiment, we used artificial diets from day 25 and obtained a growth rate as high as 1.4 mm/day.

The growth curve of reared saury presented in this paper was close to that of wild saury drawn by Watanabe et al. (1988). The growth rate of wild saury is assumed to be as fast as that achieved in this study, at least for larval and early juvenile stages to 70 mm in knob length.

The present experiment has demonstrated that one micro-increment is deposited on the otolith per day in the Pacific saury. This supports the growth curve for wild saury reported by Watanabe et al. (1988), and their conclusion that the western Pacific saury become 30 cm adults within one year. Even so, daily ring deposition in late juvenile and adult stages should also be demonstrated for complete confirmation.

In the regression equation, the figure 4.81 suggests that the first increment is formed 4 or 5 days before hatching provided that the embryonic increments are formed daily. Thus, formation of the first increment coincides with the eye pigmentation stage in embryonic development, as reported by Yusa (1960). In wild specimens, Watanabe et al. (1988) found a distinct ring about 27 μm in radius, and 4 or 5 faint increments between the nucleus and the distinct ring. This ring is supposedly a check formed at hatching. When determining the age of natural specimens, we count all the otolith rings and subtract 5 from each count.

The decrease in growth rate 10 days after hatching might be due to low water temperatures as seen in Fig. 1. The low growth rate seemed to induce indistinct increment formation rather than narrowing the increment width in this experiment.

The Pacific saury spawns almost year-round in an extensive area in the northwestern Pacific (Watanabe and Lo, 1988). Growth and survival rates of young saury could differ between areas and seasons. Year-to-year changes of dominant hatching cohorts in adult stocks may constitute the major factor in year-to-year fluctuations in the stock level and fish-size composition of exploited sauries. Otolith analyses of the hatching cohorts over some years would give us essential information on population dynamics of the western Pacific saury.

Acknowledgments

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サンマのふ化仔魚飼育による耳石日輪形成の実証

渡辺良朗・久慈康支

サンマ (*Cololabis saira*) の天然受精卵を実験室内でふ化させ，シオミズツボワムシ，アルテミア幼生，および配合飼料を与えて水温 18–24℃ で飼育した．仔稚魚の平均成長速度は 1.1 mm/日で，60 日間の飼育で全長 79 mm に成長した．日齢既知の仔稚魚について日齢と耳石輪紋数との関係を求めた．回帰式は　輪紋数=4.81+1.01×日齢　となり，サンマの耳石輪紋が 1 日に 1 本形成される日輪であること，4-5 本の輪紋が胚期に形成されることが確認された．

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