**Daily increment formation in otoliths of juvenile skipjack tuna
*Katsuwonus pelamis***

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ABSTRACT: In the present study, we verified daily increment formation within sagittal otoliths of juvenile skipjack tuna *Katsuwonus pelamis* by analysis of diel changes in marginal increment width. Specimens were collected by midwater trawl in the tropical western Pacific from November to December 1995 and in February 1999. The sagittal plane of the otolith was embedded in enamel resin and polished with lapping films. The otolith-measuring system was equipped with a light microscope and was used for marginal increment analysis. The index of completion of the marginal increment increased with time of day, approximately 40% at 07:00–10:00 h, 66% at 13:00–16:00 h and 80% at 19:00–23:00 h. Therefore, growth of the marginal increment progressed from morning to evening and was completed during the night. We conclude that increments are formed daily in juvenile skipjack otoliths and these increments have a width of 15–40 µm.

KEY WORDS: daily increment, juvenile, marginal growth, otolith, skipjack tuna.

INTRODUCTION

Clarification of the age–growth relationship of marine fish is basic information for the assessment and management of fishery resources. Studies on the growth of marine fish using daily otolith increments have rapidly developed in recent years and have contributed to the progress in the assessment of fishery resources.^{1–8} Utilization of a precise and reliable aging structure is necessary for age determination; however, the formation of daily growth increments in otoliths should be verified for age and growth estimates.

Three reports have examined the periodicity of increment formation in otoliths of larval or adult skipjack tuna *Katsuwonus pelamis*.^{9–11} Radtke⁹ validated daily periodicity in increment deposition during the first 5 days after hatching in larval skipjack tuna. Uchiyama and Struhsaker¹⁰ studied increment formation in adult skipjack by counting growth increments that were formed during a normal feeding period with check marks that were deposited during a starvation period. However, the

authors described the daily increment validation results as inconclusive. They examined only four individuals in the experiment and the number of increments was larger than the expected days in all fish examined. The authors pointed out the necessity for further experiments performed under precise conditions. Wild *et al.*¹¹ also studied the periodicity of increment deposition in adult skipjack tuna. The otoliths of experimental fish were marked with oxytetracycline at the time of release and the number of increments that were formed during the duration between mark and recapture were counted. Results indicated a deposition rate of 0.7 increments per day. The authors hypothesized that increments were not deposited daily due to: (i) resolution difficulties in counting increments, which results in an underestimation of the actual number of days; and (ii) physiological factors, such as the maturation of females, which negatively affects increment deposition.

Daily increment formation is commonly recognized in the otoliths of many marine species, but previous studies indicate a non-daily deposition rate in adult skipjack otoliths.

Our observations of skipjack otoliths indicate that increments formed during the juvenile stage are obviously different in morphological structure in comparison with the larval stage, as illustrated by Radtke.⁹ Increment width increases and the structure was more complicated. Given the diffi-

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culties in enumerating post-larval increments in otoliths, we have conducted a precise examination of increment formation in otoliths of juvenile skipjack. This study is a part of an age and growth study of skipjack tuna in the western Pacific Ocean. In the present study, we measured the marginal growth of sagittal otoliths of juvenile skipjack tuna collected at various times of days and verified the formation of daily growth increments.

MATERIALS AND METHODS

Skipjack specimens

Juvenile skipjack tuna were collected in the tropical western Pacific from November to December 1995 and February 1999 (Fig. 1). A midwater trawl net was used for sampling skipjack.¹² Maximum towing speed and the mouth opening of the net were approximately 5 knots and 20×20 m, respectively. Trawling activities were conducted from 06:00 to 00:00 h. Specimens were immediately preserved in 80% ethanol on the research vessel and then taken to the laboratory. During 1995, trawling operations were conducted four times a day at 20 sampling stations and a total of 122 juvenile skipjack tuna from four locations (seven tows) were used in the analysis (Table 1). During 1999, trawling operations were conducted for 2 days at only one location and a total of 72 juveniles from eight tows were used. Specimens used in the marginal increment analysis were selected randomly from the individual collections.

Otolith analysis

Standard length (SL) of the juvenile skipjack tuna was measured to the nearest 0.1 mm and the sagittal otoliths were removed under a dissecting microscope. Fibrous tissues were removed with sharpened needles and the otoliths were cleaned. Otoliths were soaked in distilled water on a slide

Fig. 1 Sampling locations of juvenile skipjack tuna used for the otolith marginal increment analysis in 1995 (●) and 1999 (▲) cruises.

Table 1 Data for juvenile skipjack tuna specimens used for marginal increment analysis of sagittal otoliths

Date	Time (h)	Location		No. juvenile otoliths used	SL (mm)	
		Latitude	Longitude		Minimum	Maximum
1995						
4 November	14 : 20	5°59'N	137°17'E	14	18.6	25.5
25 November	22 : 30	1°56'N	154°02'E	28	18.6	39.8
26 November	08 : 35	1°57'N	154°03'E	19	16.8	28.6
27 November	13 : 05	4°54'N	154°56'E	10	12.9	21.0
27 November	20 : 00	4°54'N	154°55'E	11	17.5	28.8
3 December	20 : 45	11°00'N	155°00'E	19	13.5	29.8
4 December	07 : 05	11°00'N	154°59'E	21	12.9	32.8
1999						
6 February	14 : 10	15°04'S	173°00'E	10	20.8	32.7
6 February	15 : 35	15°00'S	173°00'E	8	25.6	30.9
6 February	20 : 25	15°03'S	173°00'E	11	23.0	34.2
7 February	08 : 00	14°59'S	173°00'E	6	20.8	34.3
7 February	09 : 25	15°03'S	173°00'E	10	24.2	31.3
7 February	13 : 05	15°00'S	173°00'E	8	24.7	32.2
7 February	19 : 05	15°00'S	173°00'E	10	23.2	32.2
7 February	21 : 55	15°00'S	172°59'E	9	26.2	33.2

glass and then dried at ambient temperature. An otolith was embedded in enamel resin (Overcoat NA; Shiseido, Tokyo, Japan) on a clear microscope slide. The mounted otolith was allowed to set for 48 h; thereafter, the proximal side of the otolith was polished with lapping films at grit sizes of 9, 3 and 1 μm . The enamel resin was dissolved with a few drops of solvent and the otolith was turned over. After the otolith was mounted again, the distal side was also polished with the same procedure. During the polishing process, the appearance of increments was observed under a light microscope (LM). The polishing procedure was finished when the increments between the core and the edge along the growth axis could be observed. The polished surface was covered with enamel resin to protect it from dust.

The number of increments between the core and the edge was counted and increment widths were measured using an otolith-measurement system (ARP/W; Ratoc System Engineering, Tokyo, Japan). The otolith radius was measured as the maximum length between the core and the posterior edge. The system was composed of an LM (BX60-33; Olympus Optical, Tokyo, Japan), charge-coupled device (CCD)-camera (CS-580; Olympus Optical) and a personal computer. The magnification of the LM was 500 $\times$ or 1000 $\times$.

An index of completion of the marginal increment was calculated with the following equation:¹³

$$C = W_n / ((W_{n-1} + W_{n-2}) / 2) \times 100$$

where W_n is width of the marginal increment and W_{n-1} and W_{n-2} are widths of the previous complete increments that exist prior to the marginal increment.

We estimated the periodicity of increment formation by changes in the index of completion during the time of day.

RESULTS

Otolith microstructure

A growth increment consisted of an optically transparent zone and dense zone, when observed under a LM with transmitted light (Fig. 2). These two bands were formed alternately and were deposited regularly. They were equivalent to the accretion zone and the discontinuous zone, respectively.¹⁴ The core existed in the central region of the sagittal plane and was observed as a dark structure. The initial fifth to seventh increments were usually deposited in a round shape and the width of each increment was narrow (2–4 μm). The width of the fifth to 10th increments gradually increased from 5

Fig. 2 Transmitted light micrograph of a sagittal otolith from a 29.8 mm standard length juvenile skipjack *Katsuwonus pelamis*. The number of daily increments counted with the otolith measurement system was 15. (a) The discontinuous zone (arrowhead) of the fifth to 14th increments, which illustrates the outer edge of a daily increment. (b) Subdaily increments existed within the 12th to 14th daily increments (arrowhead) and can disappear when slightly changing the focus of the microscope.

to 15 μm and the increments became oval in shape, which was caused by an extension along the rostrum–post-rostrum axis. Increment width ranged from 15 to 40 μm along the growth axis after the 10th increment.

Subdaily increments existed in all otoliths examined. The discrimination of daily increments from subdaily increments was performed by observing the clearness of the increment; that is, daily increments have a distinctly dark discontinuous zone, whereas subdaily increments disappeared when slightly changing the focus of the LM (Fig. 2). The number of subdaily increments per daily increment increased with an increase in daily increment

width. Fewer than five subdaily increments were usually observed from the fifth to 10th daily increments and five to eight subdaily increments existed after the 10th daily increment. The periodicity of subdaily increment deposition was not estimated in the present study.

It was difficult to observe increments in otoliths from larger juveniles because of the increasing thickness of the frontal plane. The maximum fish size that we could resolve increments using this method was 60 mm SL (S Kayama, unpubl. data, 2001). The visibility of growth increments differed markedly between individual otoliths.

Diel change in the index of completion of the marginal increment

The index of completion of the marginal increment changed with the time of day in both the 1995 and 1999 samples (Fig. 3). The index increased from morning, afternoon and evening. The index averaged 40% (range 15.4–74.7%) in the morning (07:00–10:00 h), 66% (range 30.9–107.0%) in the afternoon (13:00–16:00 h) and 80%

(range 82.6–110.6%) in the evening (19:00–23:00 h). These changes in the growth of the marginal increment from morning, afternoon and evening were highly significant ($P < 0.001$, t -test) and those from afternoon to evening were also significant ($P < 0.05$).

A portion of samples collected in 1995 included one individual at 20:00 h and two individuals at 22:30 h that already had completed the marginal increment and had the next increment beginning to form.

Number of increments and relation to SL

The size of juvenile skipjack tuna ranged from 12.9 to 39.8 mm SL and the number of daily increments observed in the present study ranged from 12 to 19. The mode of daily increments was 14 in 1995 and 17 in 1999. A positive linear relationship between the number of daily increments and SL was evident (Fig. 4). The daily growth rate or slope of the linear regression line was 2.8 mm/day in 1995 and 1.7 mm/day in 1999.

Fig. 3 Diel change in the index of completion of the marginal increment within otoliths of juvenile skipjack *Katsuwonus pelamis*.

Fig. 4 Relationship between standard length (mm) and the number of increments within otoliths of juvenile skipjack *Katsuwonus pelamis*.

DISCUSSION

We conclude that daily growth increments are formed in the otoliths of juvenile skipjack tuna based on marginal increment analysis with time. In physiological and biochemical studies, a daily increment is formed by two different zones: one is an accretion zone that is predominantly aragonitic calcium carbonate and the other is a discontinuous zone composed of an organic matrix.¹⁴ Daily increment formation in otoliths has occurred with experimental photoperiodicity in a laboratory environment.¹⁵ In the daytime, from morning to afternoon, the activity of calcium carbonate deposition is high and results in the formation of an accretion zone. After sunset, the deposition of an organic matrix increases and results in the formation of a discontinuous zone.¹⁶ In the present study of juvenile skipjack tuna, the accretion zone, which is relatively wide, is deposited from early morning to afternoon, whereas the deposition of the discontinuous zone, which is relatively narrow, occurs during the evening of

Fig. 5 Relationship between the number of daily increments and distance from the core in a sagittal otolith from a 29.8 mm standard length juvenile skipjack *Katsuwonus pelamis*.

each day. Thus, a new marginal increment is formed during the night. This diel progression of the marginal increment formation was similar between the 1995 and 1999 samples. Our study only presents evidence for daily increment deposition in the otoliths of juvenile skipjack, but does not address the apparent non-daily (approximately 0.7) increment formation in otoliths of adult skipjack tuna.¹¹

The first daily increment was formed during the day after hatching (3 days after fertilization);⁹ therefore, the number of daily increments estimated in the present study could be used as an indication of age and growth of juvenile skipjack. The maximum daily growth rate of juvenile skipjack was approximately 3 mm. The age of all specimens analyzed in the present study was less than 20 days after hatching, indicating rapid growth in the early life stage. The piscivorous food habit of skipjack tuna is revealed within the larval stage because the main prey organisms are fish larvae.^{17–20} We consider that this feeding habit contributes to the rapid growth during the early life stage of skipjack tuna. An extremely high growth rate was also reported in the case of Spanish mackerel *Scomberomorus niphonius*, which would be related to piscivory and early development of an adult-type digestive system.²¹

Variability in the index of completion of the marginal increment was large. Individual variation in the marginal increment formation among the same specimens was also reported for larval southern bluefin tuna *Thunnus maccoyii*,²² although there is no information about the marginal growth in otoliths of other tuna species during early life history.

The increment width of sagittal otoliths in juvenile skipjack tuna increased from the core to the edge, especially after the 10th daily increment (Fig. 5). A few individuals had an index of marginal increment formation that was greater than 100%. These data occur because of the increasing width of the marginal increment compared with that of the previous increment. The number of subdaily increments also increased with increasing increment width.

There is much information about the growth of skipjack tuna from previous studies; however, there are differences in results among scientists that may be primarily due to the use of different analytical methods.²³ Therefore, a more reliable method for growth analysis of skipjack tuna should be established. The verification of daily increment formation within otoliths of juvenile skipjack contributes to estimating age and growth in this species, although additional studies of larval and adult stages are also necessary.

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