AN OUTLINE OF THE GROWTH STUDY ON SKIPJACK TUNA (*KATSUWONUS PELAMIS*) IN THE WESTERN PACIFIC.

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ABSTRACT

The validation of daily increment formation in sagittal otoliths of skipjack tuna Katsuwonus pelamis and the estimation of growth trajectories in the western Pacific Ocean have been investigated. Our observations of skipjack otoliths by using a transmitted light microscope indicated the remarkable change in morphological structure of increments with life stages. We divided the otolith increments into 3 areas; the larval area, the juvenile-young area, and the young-adult area. The daily increments on the larval area were measured according to the reference of Radtke (1983). The daily increment formation on the juvenile-young area was verified by the temporal increase in widths of the marginal increments of juvenile specimens which were collected by a midwater trawl net in the tropical waters. The daily deposition rate of increments on the young-adult area was verified by the correspondence between the numbers of the deposited increments and the rearing days in the oxytetracycline marking experiments of young skipjack which were captured by hook and line in the coastal waters. On the basis of these results, we analyzed otolith daily increments of larva through adult specimens using by the otolith measurement system. The number and width of increments were measured and the relationship between age in days and body length was estimated. The aged data in 453 specimens of young through adult were fitted to the von Bertalanffy's growth curve; $L_t = 93.6 [1-\exp\{-0.43(t-0.49)\}]$*. Skipjack tuna in the western Pacific reaches 35 cm at 0.5 year, 45 cm at 1 year, and 65 cm at 2 years of age.*

INTRODUCTION

Age and growth of skipjack tuna has been determined mainly by three methods of analyses; including the ageing structure of hard parts such as vertebrae, dorsal spines, and otoliths, the temporal progression of length-frequency modes, and the direct estimate from releasing-recapturing data of tagged fish. In a lot of the past studies, however, these estimations did not always indicate the same age-length relationship. The minimum and maximum lengths at one-year old fish were 15 cm and 44 cm, respectively (Matsumoto *et al*. 1984). The reasons why such remarkable differences have been occurred were not known, so that the treatments of these various results have depended on each scientist. In the case of the Indian Ocean, Shabotiniets (1968) reported an estimate of skipjack age by using the first dorsal spine, however, there were not many information about growth of this species compared to the Pacific Ocean.

Since the discovery of daily otolith increments by Pannella (1971), the formation of daily ring has been verified in many species (Geffen 1992). Applications of the new technique contributed to the progress in age and growth studies. Several studies on tuna species have been also carried out (Foreman 1996, Itoh *et al.* 2000, Itoh and Tsuji 1996, Jenkins and Davis 1990, Lang *et al*. 1994, Scott *et al*. 1993, Stequert *et al*. 1996, Uchiyama and Struhsaker 1981). An experimental study of skipjack tuna showed that the otolith increments were not formed daily and the authors described that further research should be necessarily carried out (Wild and Foreman 1980, Wild *et al*. 1995). According to our observations of skipjack otoliths, the variation in morphological structure of increments with life stages resulted in an increase of the measurement difficulty (Tanabe *et al*. 2003). It should be considered for a source of error. Therefore, we thought the level of the resolution power of apparatus has a serious influence upon increment counts.

Increasing the reliability of the ageing technology and accumulating precise data for skipjack growth studies are primarily important. The main objectives of this study are the validation of daily increment formation for two different structure of otolith morphology with life stages and reliable estimation for skipjack growth in the western Pacific by using the relationship between age and body length data.

MATERIALS AND METHODS

1. DAILY INCREMENT FORMATION OF OTOLITHS

On the juvenile stage: the temporal analysis of marginal increment growth

Juvenile skipjack tuna for the otolith marginal increment analysis were collected by using a midwater trawl net TANSYU (Tanabe and Niu 1998) in the tropical western Pacific during November to December 1995 and February 1999. Various time sampling were carried out during the research cruises. Removed otoliths were embedded, polished, and measured number of increments from the core to the edge and each increment width by using an otolith-measurement system (ARP/W; Ratoc System Engineering, Tokyo, Japan). An index of completion of the marginal increment was calculated according to Gartner (1991) and the periodicity of a new increment formation was estimated (see Tanabe *et al*. 2003 for details).

On and after young stage: the analysis in the marking otoliths of captive fish

Young skipjack tuna for rearing experiments were captured by using hook and line at coastal area of southern Kyushu on August 23, 2000 and August 24, 2001. Total captive fish of 137 individuals at 2000 and 328 individuals at 2001 were injected intramuscularly with oxytetracycline (OTC) and reared for one month in 2000 and 1.5 months in 2001 in a floating net cage of 8×8×7m. The injection dosage of OTC was about 2.8mg for 100g body weight (BW) according to the method of Wild and Foreman (1980). The fork length (FL) of each fish was measured and attached numbered tag on the back below the dorsal fin. Only 15 fish were tagged in 2000 because of lack of the experience. The second OTC injection was carried out for 50 individuals 31 days after beginning of the experiment in 2001. Small pelagic fish such as pilchard, sardine, and Antarctic krill were fed for the captive fish during the experimental periods.

After the experiment was finished, the FL and BW of survived fish were measured and sagittal otoliths were removed, cleaned, soaked in distilled water, and dried at ambient temperature. Each otolith facing the distal side up was mounted on a clear microscope slide with enamel resin and observed by a microscope (BX60-33; Olympus Optical, Tokyo, Japan) equipped with a fluorescent lighting. The fluorescent marking bands deposited by the OTC injection on otoliths were detected by an ultraviolet lighting with the WBV filters for 400-440 nm (Figure 1, left). Then an additional transmitted light was irradiated, the number of increments from the OTC marking to the edge of the rostrum which have been formed during the rearing period was counted (Figure 1, right). For otoliths of the twice OTC marking, the number of increments between the two fluorescent bands was counted. The periodicity of the increment formation was estimated by analyses of these data with number of the rearing days.

2. DETERMINATION OF AGE IN DAYS

Sampling

Larval skipjack tuna were collected by using the MOCNESS in 1996 and using a bongo net in 1997 both in the tropical western Pacific. Juvenile specimens were collected by using a midwater trawl net, TANSYU, during October to December 1994-1996 in the tropical western Pacific (Table 1). Young and adult skipjack were captured by fishing boats with gears of hook-and-line, pole-and-line, or purse seine in the near and offshore of Japan or in the tropical western Pacific (Table 2).

Otolith preparation and measurement of daily increments

Sagittal otoliths of larvae were removed, cleaned, and mounted on a clear microscope slide with enamel resin. Otoliths of juvenile (<60mmSL) were removed, cleaned, soaked with distilled water, dried, and embedded on a slide with enamel resin. The sagittal planes, the proximal and distal faces of the mounted otoliths were polished with lapping films. Otoliths from juvenile (>60mmSL), young to adult were removed, cleaned, soaked with distilled water, dried, and mounted on a slide. The distal face was dissolved with 10% HCl as for an etching procedure. After these preparations, the number of daily increments between the core and the edge was counted and the increment width was measured by using an otolith-measurement system (ARP/W; Ratoc System Engineering, Tokyo, Japan) for all of the individual otoliths (Figure 2).

RESULTS

1. DEPOSITION RATE OF INCREMENT FORMATION

Periodicity of the marginal increment deposition on the juvenile stage

Widths of the marginal increment grew regularly with the time of day in both the 1995 and 1999 samples. The index of the marginal increment completion increased from morning (averaged 40%), afternoon (66%), and evening (80%). Then a new increment of the otolith was completed in the night. These data show a diel periodicity of a new increment deposition in sagittal otoliths of juvenile skipjack tuna (see Tanabe *et al*. 2003 for details).

Periodicity of the experimental increment deposition on and after young stage

On the 2000 experiment, average numbers of increments (±SD) from the fluorescent OTC mark to the marginal increment were 29.77±0.98; ranging 27 to 32 in left otolith, 29.72±0.90; ranging 27 to 31 in right otolith (Figure 3). These number of increments formed during the experiment were statistically corresponded with the number of rearing days for captive skipjack ($p<0.05$). There was no significant difference in average number of increments between left and right otoliths.

On the 2001 experiment, average numbers of increments (±SD), which have been deposited between the two fluorescent bands, were 31.96±0.66; ranging 30 to 34 in left otolith, 32.11±0.65; ranging 31 to 33 in right otolith (Figure 4). There were no significant differences between numbers of increments and days and also between left and right otoliths ($p<0.05$). Another result from the linear relationship between numbers of the deposited increments and rearing days of 10, 20, 32, and 47 was corresponded to the others (Figure 5). The equations were:

$I = 0.9845D$ (left)

$I = 0.9979D$ (right)

where I is number of the deposited increment and D is number of rearing days. Both slopes of the equations were significantly corresponded to 1.0 and were also no difference between left and right.

All of these data strongly suggested that the formation of otolith increments in young skipjack has been occurred on a daily basis.

2. RELATIONSHIP BETWEEN NUMBER OF INCREMENTS AND SIZE OF SKIPJACK

The relationships in the skipjack growth study between number of daily deposited increments and body length of the specimens were estimated during the life stages of larva to juvenile and young to adult (Figure 6, 7). The daily growth rate of larva, which means the first stage of the life history, was estimated 0.55 mm/day for 12 individual's data of 3.3 to 7.6 mmSL. After the metamorphosis which means the change of life stage from larva to juvenile during 10 to 11 mmSL at 10th to 12th days after hatching, the growth rate until 100 mmSL rapidly increased approximately 3 mm/day. After the second change of life from juvenile to young at 100 mmSL, the fastest growth rate of 4-5 mm/day in the life history of this species occurred during 100 to 200 mmSL. Then the growth rate gradually decreased after the period during the life stages from young to adult. The aged data in 453 specimens of larger than 18 cmFL were fitted to the growth curve of von Bertalanffy. The equation was calculated as

$$L_t = 93.6\,[1 - \exp\{-0.43\,(t - 0.49)\}]$$

and the lengths at age of skipjack tuna in the western Pacific were estimated approximately 35 cm at 0.5 year, 45 cm at 1 year, and 65 cm at 2 years old.

3. RELATIONSHIP BETWEEN OTOLITH RADIUS AND BODY LENGTH

The radius of sagittal otolith increased in direct proportion to growth of the body (Figure 8). The relationship between the otolith radius and body length indicated a gentle curve nearly like a linear line. These otolith data about the lengths from the core to each number of increments could be useful for the trace of the growth trajectory for individuals. We are carrying out the data analysis for an examination of comparison in the growth trajectories between different areas such as the near-shore Japan and the tropical waters of the western Pacific (Figure 9).

DISCUSSION

Validation of daily increments in skipjack tuna

In the life history of skipjack tuna, the morphological features of otolith increments complicatedly change with the growth process of life stages from larva just after hatching to adult. Our observations with a transmitted light microscopy indicated that the first change appeared at the metamorphosis stage from larva to juvenile at 10 to 12 mm and the second change occurred at the young stage of 20 to 30 cm. According to the results, we divided the otolith increments of skipjack tuna into three areas as follows; the larval area, the juvenile-young area, the young-adult area. In the case of the species which have complicated and changeable otolith increments such as skipjack tuna, the deposition rate of an increment should be validated for each life stage in order to carry out a precise age determination.

Radtke (1983) examined the daily increment formation of larval skipjack until 5th day after hatching by using a rearing method. In this study of the incremental formation, the daily deposition rate in the next juvenile-young area has been verified by results of the marginal increment analysis that indicated a daily periodicity of an outermost increment growth in relation to time of day. We have been also verified the daily deposition rate in the last young-adult area by results of the correspondence between numbers of the deposited increments after the OTC marking and the rearing days. Wild *et al*. (1995) examined the deposition rate in otoliths of adult skipjack in the eastern Pacific by the OTC-injection marking, tag-recapture experiment; however, the average rate was significantly less than 1 increment a day. They described that couple of reasons why the daily increment formation could not be verified were technical problems such as the mechanical or artificial errors in the measurement operation and biological problems such as the growth interruption occurred by reproductions of females or other physiological unfavorable conditions. After their experiment, remarkable advantages in measurement technology for the otolith micro-increment observation such as a high-resolution 3-CCD camera have been developed. Several laboratories in Japan could use such a high-performance measurement system for otolith increment analysis in recent years. We consider that one of the reasons why we could verify daily increment deposition should be an introduction of these technical advantages.

On a basis of our results, we conclude that the age determination by using the daily increment analysis must be validated as a reliable ageing structure in skipjack tuna. Then we are able to estimate age and growth of this species more accurately than previous methods by applications of the daily increment counts.

Growth estimates by relationship between daily increments and body length

Various studies on age estimation of skipjack tuna were reported (Matsumoto *et al*. 1984). Results of the present study are approximately in agreement with the age estimation until 2 years old in the central Pacific by using otolith daily increment counts by Uchiyama and Struhsaker (1981). Their results showed that the daily growth rates of this species were 1.6 mm/day less than 27 cm, 0.8 mm/day between 27 and 71.4 cm, and 0.3 mm/day between 71.4 and 80.3 cm, indicating decrease of growth speed with advanced life stages. The body lengths at age were 44 cm at 1 year and 68 cm at 2 years old respectively. They described the difference in growth rate, indicating the slower growth in the eastern Pacific than that in the central Pacific.

In our study of the western Pacific, we are comparing the growth data between the seasonally migratory schools reached to the near-shore Japan waters and the schools captured in the tropical offshore waters. Our preliminary results suggesting a faster growth of the near-shore specimens are observed (Kayama *et al*. 2003, unpubbl. data). The comparable study on skipjack growth among local areas should be conducted with considering the migratory ecology, especially for their seasonal feeding or reproductive migrations and other ecological features in variable and uncertainty with stages of the life history. In addition, the individual data of the age estimation should be certified an agreement of technical or artificial procedures. We have to clarify sources of error in the otolith increment analysis (Neilson 1992).

In order to confirm the reliability of our results, we are analyzing data from other two ageing methods; the one is a tagging program for the direct growth estimates during releasing periods of recaptured fish and the other is the modal progression analysis of length-frequency data based on our regular research at a main landing port in Japan. All of the results from these analyses until the 2002 research support well the estimation from the otolith daily increment analysis in this study.

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Table 1. Data for larval and juvenile specimens used for the otolith daily increment analysis in the growth study of skipjack tuna Katsuwonus pelamis.

Years	Periods	Vessels	Locations	Nets	No. of samples	Size (mm SL)
1994	11.01-12.07	Omi	0-15˚N,130-160˚E	TANSYU-2	397	8.6-114.1
1995	10.29-12.07	Omi	0-20˚N,130-160˚E	TANSYU-2	399	8.6-57.7
1996	11.03-12.09	Omi	0-20˚N,130-160˚E	TANSYU-2	197	12.1-44.3
1996	11.10-12.01	Wakataka	0-20˚N,130-160˚E	MOCNESS	3	3.4-5.3
1997	4.20- 6.01	Shin-riasu	0-35˚N,130-150˚E	Bongo net	12	3.3-7.6

Table 2. Data for young and adult skipjack tuna specimens used for the otolith daily increment analysis.

Years	Periods	Locations	Fishing gears	No. of samples	Size (cm FL)
1997	8.27	37˚08'N,149˚30'E	Pole and line	13	29.8-35.5
1999	6.20-6.29	25-29˚N,127-130˚E	Midwater trawl net	68	8.9-17.1
1999	9.30	33˚28'N,136˚49'E	Hook and line	13	29.8-33.6
2000	7.30-9.27	34-40˚N,139-148˚E	Pole and line	91	26.5-45.2
2000	7.1-9.30	31-34˚N,130-136˚E	Hook and line	54	21.1-30.4
2000	9.15	00˚08'N,171˚56'E	Purse-seine	16	27.3-32.2
2001	4.5-9.24	26-39˚N,137-152˚E	Pole and line	125	24.9-55.3
2001	5.8-8.24	31-34˚N,130-139˚E	Hook and line	36	25.5-52.1
2002	3.15-4.29	02-05˚N,146-166˚E	Purse-seine	18	48.8-58.4
2002	1.31-2.3	12˚09'N,150˚56'E	Pole and line	6	65.8-71.0
2002	5.15-7.29	26-39˚N,149-153˚E	Pole and line	43	34.0-57.5

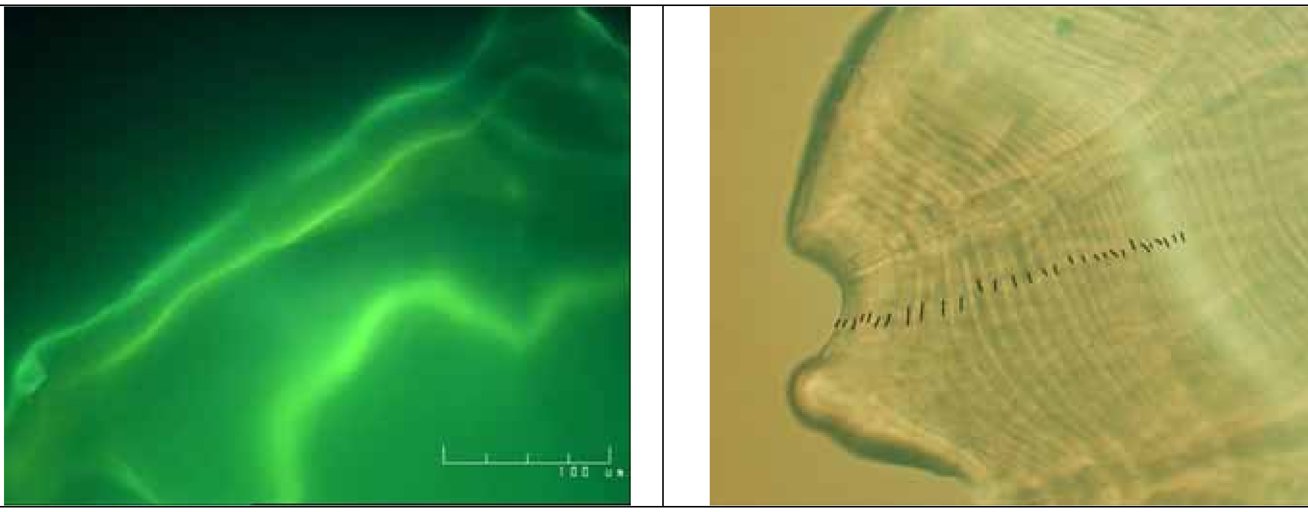

Figure 1. Micrographs of the fluorescent observation in sagittal otoliths of young skipjack tuna marked with oxytetracycline (OTC). (Left) The yellow-green 2 bands of twice OTC injection at the 2001 rearing experiment, observed only with the ultraviolet (UV) lighting. The outermost band indicates the edge of the rostrum. (Right) The increments deposited during the 2000 rearing experiment (thin black lines) and the fluorescent band of once OTC injection (a thick white line), observed by the UV and normal transmitted lightings.

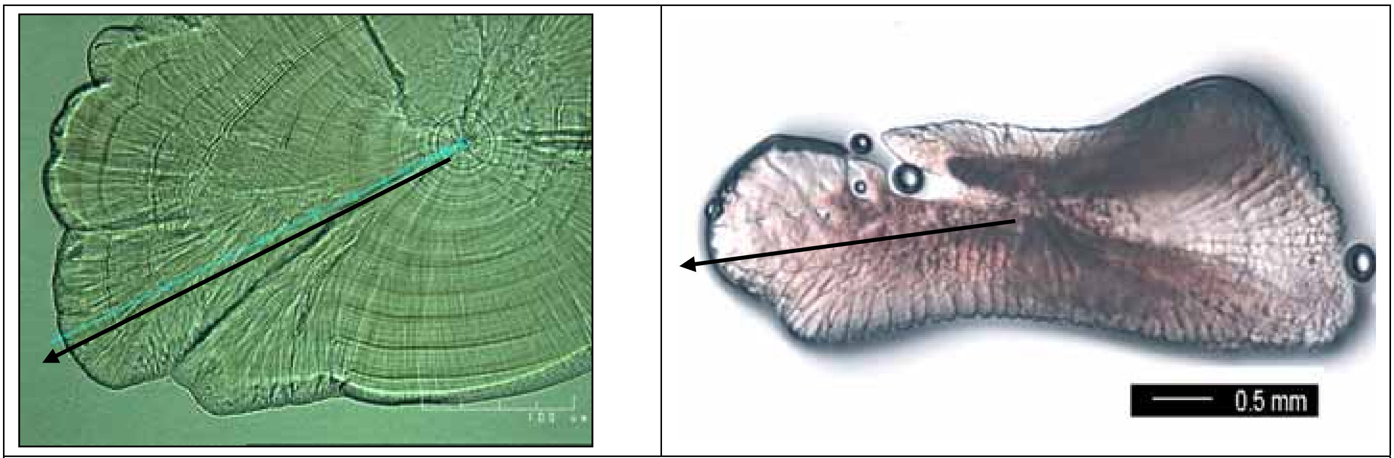

Figure 2. Micrographs of the observation for the daily increments by using the otolith measurement system (ARP/W; Ratoc System Engineering, Tokyo, Japan). (Left) A juvenile otolith of 29.8 mmSL. (Right) An adult otolith of 50.7 cmFL. Arrows indicate the measurement lines for counting increments on the sagittal plane of otoliths.

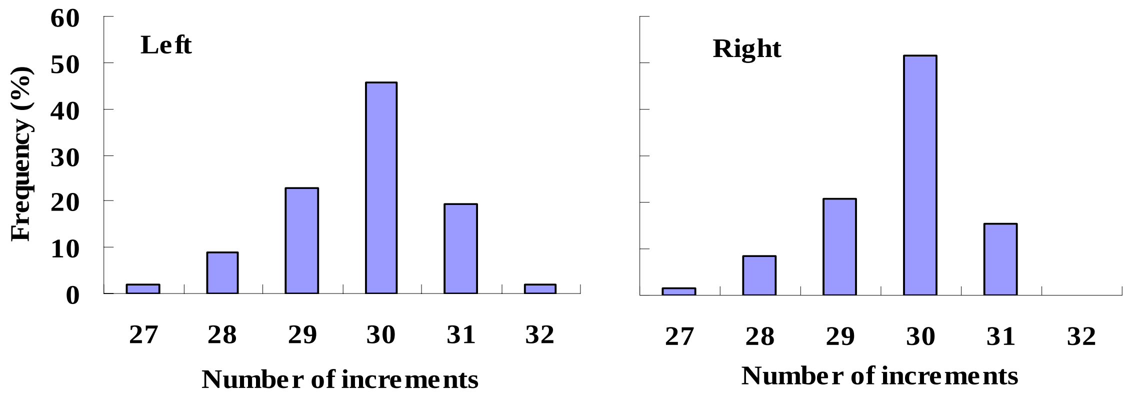

Figure 3: Frequency distribution of number of the increments deposited after the oxytetracycline injection during rearing period (30 days) of the 2000 experiment in sagittal otoliths of young skipjack tuna.

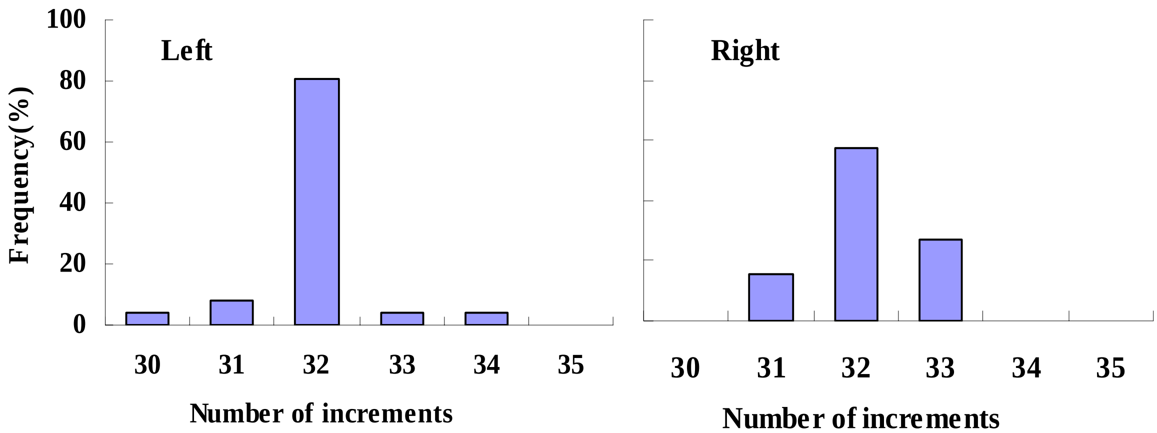

Figure 4. Frequency distribution of number of the increments deposited between the twice oxytetracycline injection during rearing period (32 days) of the 2001 experiment in sagittal otoliths of young skipjack tuna.

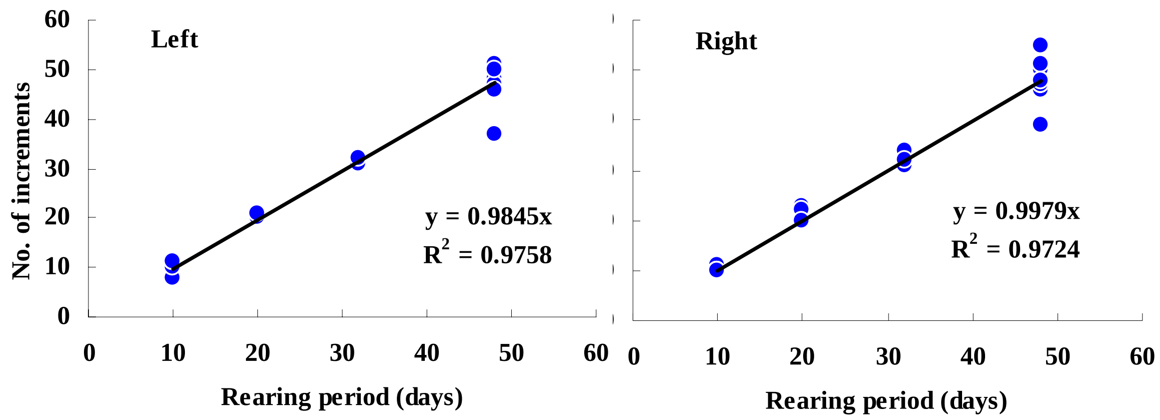

Figure 5. Relationship between numbers of the increments deposited after the oxytetracycline injection on the sagittal otoliths of young skipjack tuna and the rearing period (days) in the 2001 experiment.

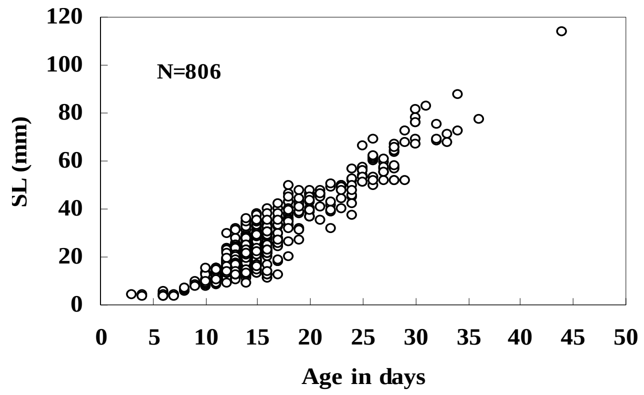

Figure 6. Relationship between estimated age in days from the daily increment counts and body length (SL) of larval and juvenile skipjack tuna collected during the 1994-1997 research cruises.

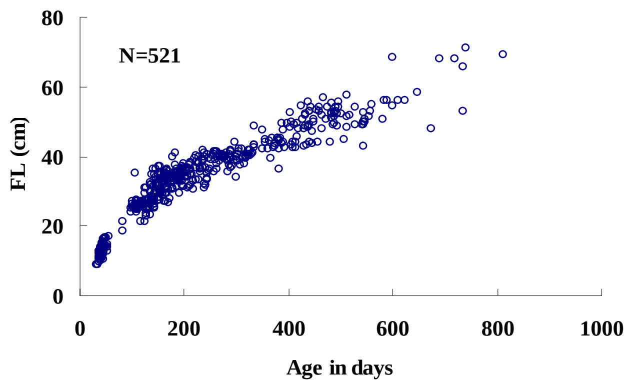

Figure 7: Relationship between estimated age in days from the daily increment counts and fork length (FL) of young and adult skipjack tuna captured during the 1997-2002 research.

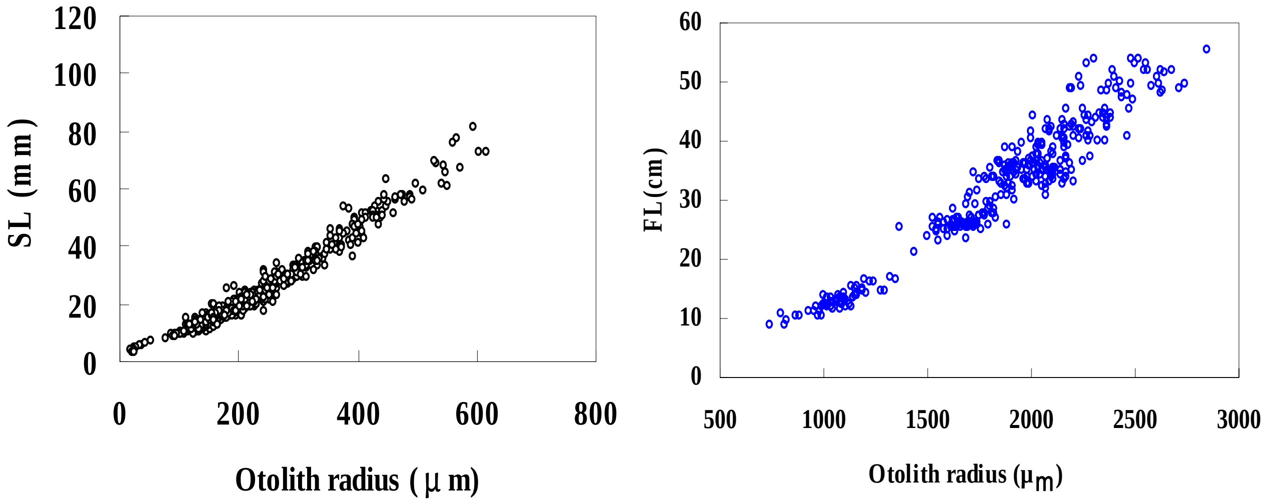

Figure 8. Relationship between the radius in sagittal otoliths of skipjack tuna and body length, (Left) during larva to juvenile period and (Right) during young to adult period of skipjack tuna.

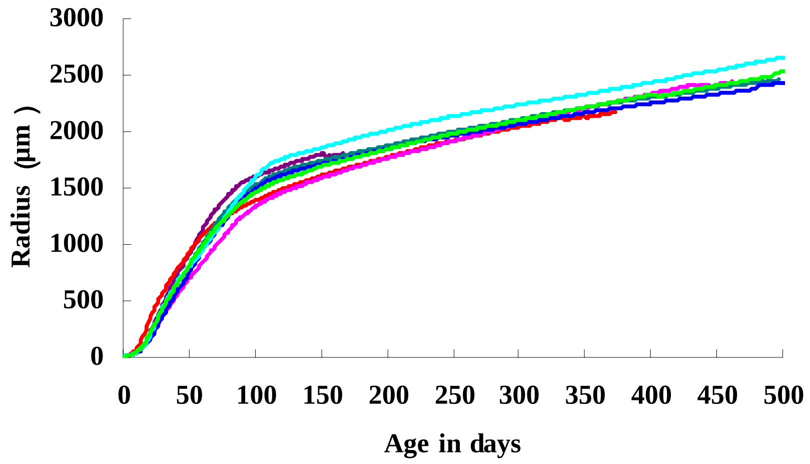

Figure 9. The traces on the growth of sagittal otoliths in skipjack tuna estimated by the radius at each daily increment. These lines are able to use for the research of growth trajectories in the life history of skipjack tuna. (During the period after hatch out to the captured day of each specimen)