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**Morphologic and Morphometric Analysis and Growth Rings Identification
of Otoliths: Sagitta, Asteriscus and Lapillus of Yellowfin Tuna
Thunnus albacares (Bonaterre, 1788) (Pisces: Scombridae)
in the Eastern Pacific**

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Abstract: A morphologic and morphometric analysis was conducted on three types of otoliths of yellowfin tuna *Thunnus albacares* (Bonaterre, 1788), as well as the identification of the growth rings in the sagittae and asterisci. The relationship among length, width and weight of the sagittae is expressed by the following equations: $y = 0.706x^{0.989}$ (rostrum-antirostrum), $y = 0.444x^{0.828}$ (rostrum-width) and $y = 0.0007x^{2.696}$ (rostrum-weight). In the case of the asterisci: $y = 0.860x^{0.694}$ (length-width) and $y = 0.0004x^{1.023}$ (length-weight). For the lapilli $y = 1.038x^{0.488}$ (length-width) and $y = 0.0004x^{1.077}$ (length-weight). The growth of these otoliths was related to the length of the fish. The average length was calculated for each of the six growth rings identified in the sagittae and the asterisci; the results are: 1 = 64.00 cm, 2 = 102.60 cm, 3 = 128.50 cm, 4 = 160.00 cm, 5 = 167.00 cm and 6 = 173.00 cm. Also the average length at each age was compared with those of yellowfin tuna of the tropical Atlantic and Pacific Oceans.

Key words: *Thunnus albacares*, yellowfin tuna, otoliths, sagitta, asteriscus, lapillus

INTRODUCTION

Yellowfin tuna *Thunnus albacares* (Bonaterre, 1788), is an epipelagic species distributed worldwide in tropical and subtropical seas (Chirichigno *et al.*, 1982; Collette and Nauen, 1983; Fischer *et al.*, 1995), between latitudes 40° North and South, in thermic ranges of 18 to 31°C.

The Mexican fleet works in the Eastern Pacific Ocean and the yellowfin tuna is the main resource with 60 to 65% of the capture in the region. During 2003, the Mexican tuna fleet obtained a record catch of 162,990 tons of yellowfin tuna (Dreyfus-León and Robles-Ruiz, 2006). In the Eastern Pacific Ocean, vessels of 13 countries fish tunas and the Mexican fleet is the most important in terms of the capacity and number of ships. This region provides approximately 18% of the total world catch. In México, the tuna is the second fishery in terms of catch volumes, after the sardine and second in economical value, after the shrimp. Of the eleven coastal states of the Mexican Pacific, Baja California, Sinaloa, Colima and Chiapas have the largest fleet and infrastructure and 90% of the catch is disembarked.

There are studies on their taxonomic description (Collette and Nauen, 1983; Fischer *et al.*, 1995) and on biological and fishery information (Dreyfus-León and Robles-Ruiz, 2006). Wild and Foreman (1980) and Uchiyama and Struhsaker (1981) made the identification of the growth rings of this species through the analysis of sagittae.

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The identification of the growth rings is commonly made through the analysis of the sagittae because it is the largest otolith and therefore easier to extract and to examine (Campana and Neilson, 1985; Stevensen and Campana, 1992; Mascareña-Osorio *et al.*, 2003; Francis *et al.*, 2005; Begg *et al.*, 2005; Berg *et al.*, 2005).

Some authors (Victor and Brothers, 1982; Brothers *et al.*, 1983; Solomon *et al.*, 1985; Suthers *et al.*, 1989; David *et al.*, 1994) have determined the daily growth increments in the lapilli of larvae and juvenile specimens. Barkman (1978), Bolz and Lough (1983, 1988), Lagardere (1989) and David *et al.* (1994) have used the asterisci with the same purpose.

However, there are no studies on age determination on for adult fish analyzing the rings of their asterisci and lapilli. Also the morphology and the morphometry of these otoliths have also not been studied.

This study is a morphologic and morphometric analysis of the otoliths: *Sagittae*, asterisci and lapilli of the yellowfin tuna *T. albacares*, as well as the identification of the growth rings in the sagittae and the asterisci, basic for the analysis of growth and consequently the population dynamics and its assessment.

MATERIALS AND METHODS

One hundred specimens of *Thunnus albacares* (from 50 to 190 cm fork length) were obtained during September and October 2000 from the cannery Marindustrias of Manzanillo, Colima. These samples belong to the populations from Eastern Pacific Ocean and were statistically significant by sample size analysis (Mendenhall, 1987; Daniels, 1991).

Of each size class 20 individuals were taken. The variables registered for each organism were Fork Length (FL), weight and sex.

The otoliths sagittae, asterisci and lapilli were obtained through a transverse section in the ventral cranial cavity, the brain was removed and the right and left semicircular canals were extracted. The otoliths were rinsed with water and stored dry in Eppendorf tubes labeled with number, date, fork length and sex.

Scanning photographs of the structure and microstructure of the otoliths were obtained with a scanning electronic microscope, from the Institute of Physics of the Universidad Nacional Autónoma de México (National University of Mexico).

Terminology of the glossary of Secor *et al.* (1992) was employed for the description of the labyrinth system and the sagittae. Similar terminology was applied to the description of the asterisci and lapilli which have not been reported before.

The otolith sample size was calculated with the formula described by Daniels (1991) and through the observation in a stereoscopic microscope with a graduated ocular lens; data on the length and the width for each otolith were recorded (Fig. 1). The weight of each otolith was also registered in an ultralytic scale with a precision of a ten thousand gram (0.0001 g). In order to eliminate possible differences due to variations between the otoliths in the same individual, all measurements were obtained from the right sagittae, asterisci and lapilli.

Constants of the relationships of the sagittae were calculated for Rostrum Length (RL), Antirostrum Length (AL), width (Wi) and weight (We). For the asterisci and lapilli the indexes of the constants of the relationship were obtained for length (L), width (Wi) and weight (We). Relationships between fork length of the fish and all the measurements of the three otoliths were also made. Regressions were done by the least squares. For the evaluation of the relationships and analysis of possible morphometric differences between the otoliths of males and females, analysis of variance (ANOVA) was carried out (Mendenhall, 1987; Zar, 1996).

Growth rings were identified, observing the sagittae and the asterisci under a stereoscope microscope with transmitted light and the average size of each ring was calculated.

RESULTS AND DISCUSSION

Description of the Sagitta

The anterior margin shows a well developed excisura major and a prominent antirostrum (Fig. 3). The posterior margin exhibits a blunt postrostrum without excisura minor and parastrostrum (Fig. 1, 2).

The dorsal margin is lightly curvilinear in the rostrum and the antirostrum. The ventral margin is rectilinear from the rostrum to the postrostrum (Fig. 1, 2).

The ventral margin presents regular denticles while the dorsal margin exhibits irregular denticles that disappear in some sections of the otolith as the fish grows.

The internal aspect of the sagitta is lightly convex with a smooth surface which curves widthwise forming a very wide and deep sulcus (Fig. 4) which increases in size from the center of the sagitta to the end of the postrostrum, without a difference between the ostium and the cauda, in the base of this sulcus is the acoustic channel composed by calcium carbonate crystals growing in an epitaxial sense (Fig. 5).

The external aspect of the sagitta is concave, its thickness increases longitudinally from the rostrum to the postrostrum. In the center of the external aspect, it is possible to observe the core (Fig. 6a) from which the growth bands begin to form, showing different growth patterns that can be of daily and seasonal periodicity (Fig. 6b). Through the identification of these bands, the age groups can be determined (Degens *et al.*, 1969; Pannella, 1971; Wild and Foreman, 1980; Uchiyama and Struhsaker, 1981; Gallardo-Cabello, 1986a, b; Gallardo-Cabello *et al.*, 2006).

The average width of the sagitta is 3.5 times the average length. There were no morphometric differences in the length of the left and the right sagitta $F_{0.05(1, 213) \rightarrow 880} = 0.022$. However, in the case of the sexes there were differences in the length of the sagitta: $F_{0.05(1, 200) \rightarrow 999} = 16.962$.

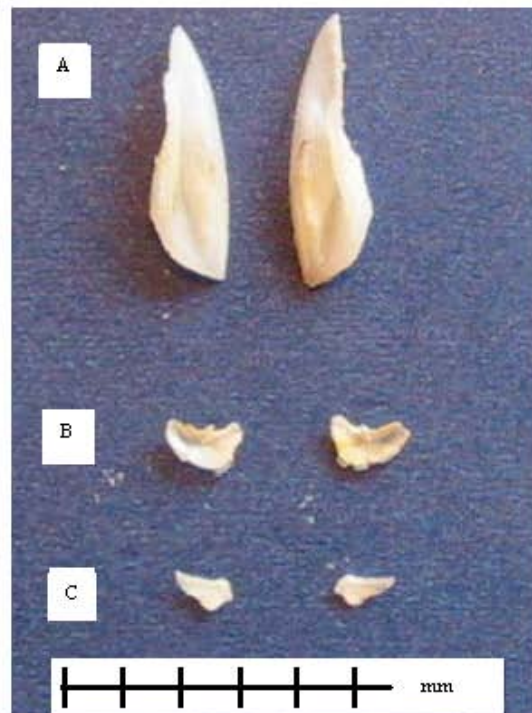


Fig 1: Sagittae (A), asterisci (B) and lapilli (C) of *T. albacares* showing its different characteristics in size and shape (increased 5 times)



Fig. 2: Sagittae selection of *T. albacares* (right sagitta, internal aspect) showing shape and size differences according size classes and age groups (increased 5 times)

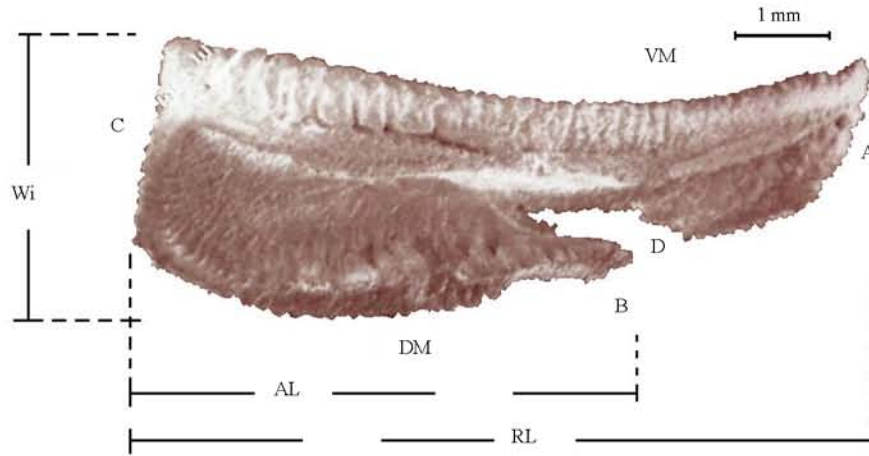


Fig. 3: Scanning photograph of the right sagitta, internal aspect of *T. albacares* showing its main characteristics: A = rostrum, B = antirostrum, C = postrostrum, D = excisura major, DM = dorsal margin, VM = ventral margin, AL = antirostrum length, RL = rostrum length and Wi = width

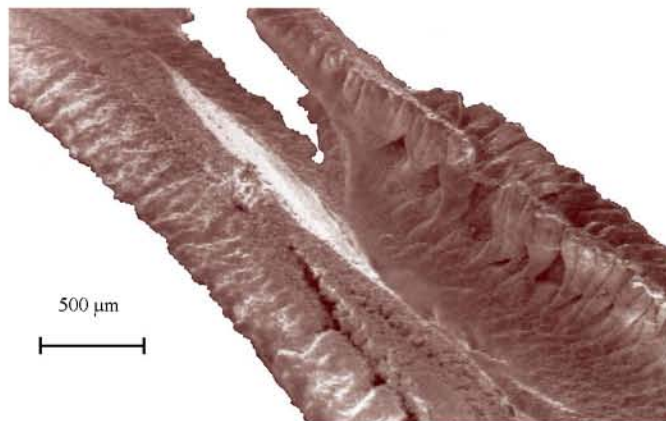


Fig. 4: Acoustic canal in the antirostrum section of the right sagitta (internal aspect) of *T. albacares*

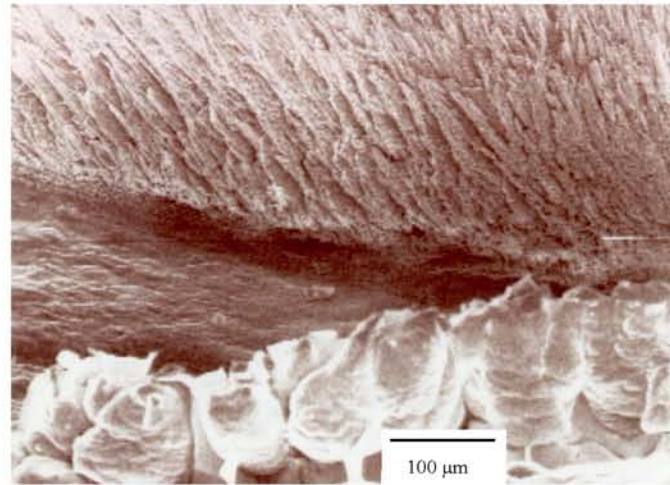


Fig. 5: Acoustic canal in the postrostrum section of the right sagitta (internal aspect) of *T. albacares*, showing aragonite crystals arrangement

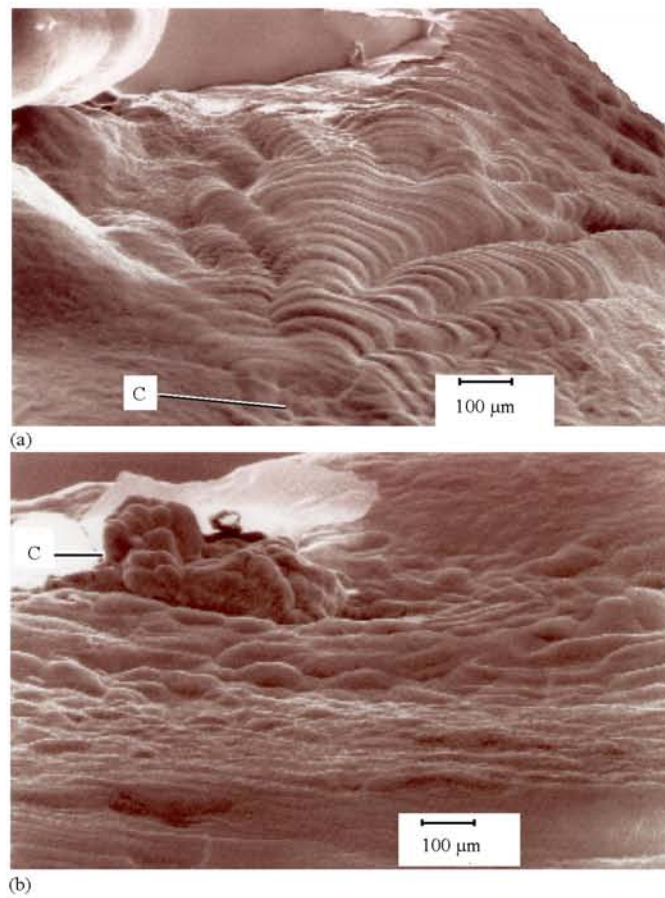


Fig. 6: Scanning photograph of the right sagitta, external aspect of *T. albacares* showing: (a) the core C and (b) the growth rings

Description of the Asteriscus

The anterior margin of the asteriscus shows a pointed protuberance which divides this otolith in two sections, the dorsal and ventral sides. The dorsal and ventral margins exhibit rectilinear and curve sections from the anterior to the posterior margin (Fig. 7).

The posterior margin is curvilinear and shows a sulcus along the dorsal and ventral margins which divides the asteriscus lengthwise in two sections, the internal posterior margin with a smaller ratio in the internal aspect of the asteriscus and the external posterior margin with a larger ratio in the external aspect of the otolith. The internal aspect of the asteriscus is in contact with neuromast cells.

The internal aspect of the asteriscus is concave; this characteristic increases as the fish grows. The external aspect is convex and presents all over its surface small denticles made by calcium carbonate crystals (Fig. 8).

The size of the asterisci does not vary from the right and the left side ($F_{0.05(1, 125)=3.918} = 0.047$), but between sexes the differences are significant: $F_{0.05(1, 90)=3.948} = 6.539$.

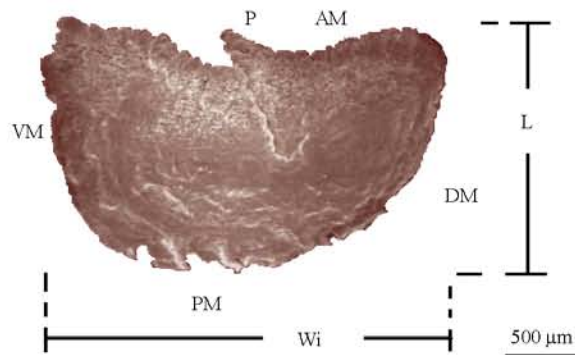


Fig. 7: Scanning photograph of the right asteriscus external aspect of *T. albacares* depicting its characteristics: AM = anterior margin, PM = posterior margin, P = projection, DM = dorsal margin, VM = ventral margin, L = length and Wi = width

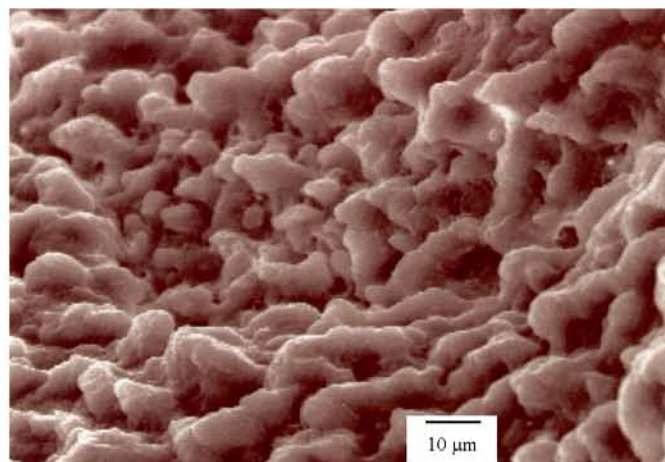


Fig. 8: Scanning photograph of the right asteriscus external aspect showing the calcium carbonate crystals disposition in its surface

Description of the Lapillus

The anterior margin of this otolith is constituted by an irregular structure of strong formed denticles of calcium carbonate crystals, oriented toward the fish front part with an angle of 45° (Fig. 9, 10). The anterior margin is prolonged in the dorsal margin towards the posterior margin forming a wider surface in relation to the ventral margin. The dorsal and the ventral margins descend towards the rear of the otolith and form a triangular structure, whose base is the posterior margin.

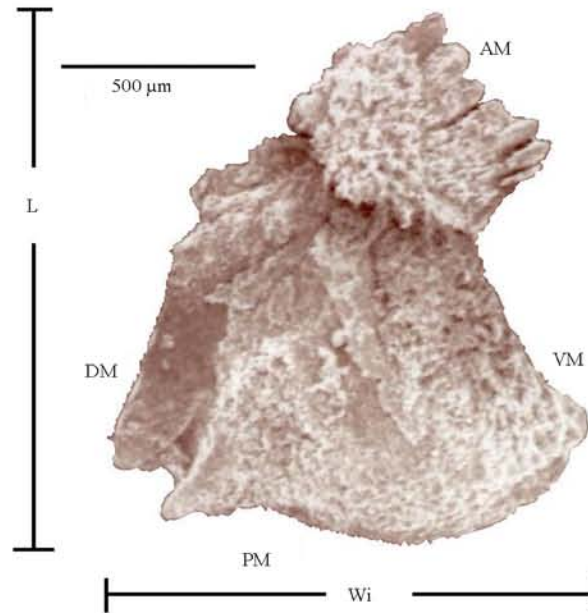


Fig. 9: Scanning photograph of the external aspect of the left lapillus of *T. albacares*, showing its main characteristics: AM = anterior margin, PM = posterior margin, DM = dorsal margin, VM = ventral margin, L = length and Wi = width

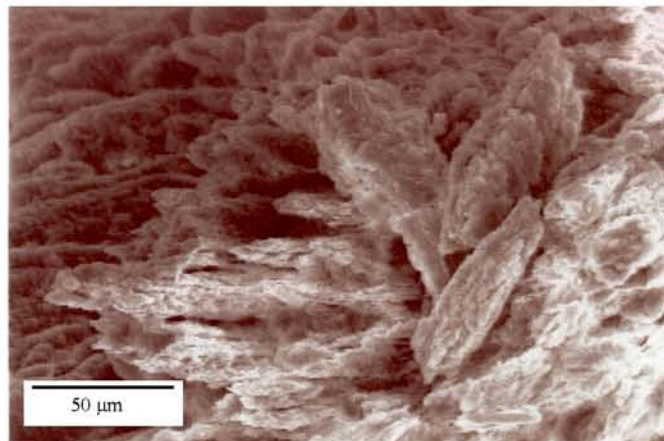


Fig. 10: Scanning photograph of the anterior margin of the left lapillus, external aspect of *T. albacares*, depicting the aragonite crystals arrangement

The dorsal and ventral margins show smooth and rough serrated sections.

The internal aspect of the lapillus is concave, a feature that increases as the fish grows. The external aspect is convex.

The external and internal surfaces of the lapillus show a large number of calcium carbonate crystals with different growth patterns.

There were no differences between left and right lapillus ($F_{0.05(1, 52)=3.903} = 0.321$) and between males and females: $F_{0.05(1, 92)=3.946} = 1.791$.

Morphometric Analysis of the Otoliths of the Yellowfin Tuna *Thunnus albacares*

The calculated sample sizes were: for sagitta 15 individuals, asteriscus 30 and lapillus 59.

Growth of the Sagitta

Table 1 shows the measurements of the sagitta for different length classes. Table 2 expresses the relationship between length and width of the sagitta: the exponent $b = 0.828$ means a negative allometric growth; the determination index of $R^2 = 0.838$ and $F = 347$ of the ANOVA, indicate that the sagitta grows more in length than in width. The relationship between the length of the rostrum and of the antirostrum is expressed by the growth isometric index $b = 0.989$, the values of R^2 and F are 0.911 and 667, respectively, which means that the rostrum and antirostrum grow equally among the age groups (Fig. 2).

The slope of the relationship between the length and weight of the sagitta is $b = 2.606$ ($R^2 = 0.938$ and $F = 744$) which indicates a negative allometric growth, meaning that as the fish increases its length and age the deposit of protein and calcium carbonate decrease in the sagitta. When the individual grows, the metabolic products are probably oriented to the formation of sexual products and fatty acid storage instead of fish length growth (Gallardo-Cabello, 1986a). The growth of otoliths is eccentric in relation to the core, which means, that the postrostrum grows more than the rostrum and the antirostrum; likewise, the dorsal margin grows more than the ventral margin and finally, the deposit of material is higher in the internal than in the external aspect.

Table 1: Rostrum (RL) and antirostrum (AL) length, width (Wi) and weight (We) in different fork size classes of the sagitta of *T. albacares*

Length classes (cm)	RL (mm)	AL (mm)	Wi (mm)	We (mg)
50	7.7257	1.6966	0.4449	0.0000054
60	8.5952	1.8091	0.4593	0.0000056
70	9.4064	1.9100	0.4719	0.0000059
80	10.1706	2.0020	0.4830	0.0000061
90	10.8961	2.0868	0.4931	0.0000063
100	11.5888	2.1657	0.5022	0.0000065
110	12.2533	2.2396	0.5107	0.0000067
120	12.8932	2.3093	0.5185	0.0000069
130	13.5113	2.3753	0.5258	0.0000070
140	14.1099	2.4381	0.5327	0.0000072
150	14.6910	2.4981	0.5391	0.0000073
160	15.2563	2.5555	0.5452	0.0000075
170	15.8071	2.6107	0.5511	0.0000076
180	16.3446	2.6638	0.5566	0.0000077
190	16.8698	2.7150	0.5619	0.0000078

Table 2: Relationships between the rostrum and other measurements of the sagitta

Rostrum	a	b	n	R^2	F
Antirostrum	0.7057	0.9888	67	0.9112	667.10
Width	0.4439	0.8279	69	0.8382	347.15
Weight	0.00007	2.6055	51	0.9382	743.47

Table 3 shows the relationship between the fish length and the length, width and weight of the sagitta. The value of the allometric index, $b = 0.585$, relates the fork length of the fish with the rostrum, which means that there is a proportionality between the increase of the length of the sagitta and of the fish. The growth in length of this organism can be described by this structure. A high significance of the correlation between the analyzed structures was found by the high values of the determination index R^2 (0.932) and ANOVA ($F = 912$). When relating very small structures (in millimeters) with values of the corporal length of the fish (in centimeters), the allometric growth indices tend to be lower than 1.

Growth of the Asteriscus

Table 4 shows the measurements of the asteriscus. The relationship between length and width (Fig. 7) is expressed by the allometric index $b = 0.694$ ($R^2 = 0.656$ and $F = 111$), which is a negative allometric growth wherein the increment in length is higher to the width; it means that the dorsal margin grows in a higher proportion in relation to the otolith surface (Table 5).

The relationship between length and weight of the asteriscus is a negative allometric growth index of $b = 1.023$ ($R^2 = 0.246$ and $F = 14.7$), due to the characteristics of this structure which shows considerable thinness, a large surface and a small volume.

According to this, the asteriscus growth is eccentric with respect to the core, which means: that the anterior and dorsal margins grow more than the posterior and ventral margins.

The relationship between fish length and otolith length, its width and weight is shown in Table 6. The most accurate relationship is between the fish length and asteriscus length according to R^2 and F . Proportionality between the growth of the asteriscus and of the fish, is showed by the values of the allometric index 0.541 and 0.408, which indicates that it is possible to determine the age groups through these structures.

Table 3: Relationships between fork length of the fish (FL) and measurements of the sagitta

FL	a	b	n	R^2	F
Rostrum	0.78350	0.5850	69	0.9316	911.96
Antirostrum	0.49550	0.6020	72	0.9031	652.56
Width	0.34220	0.4965	76	0.8610	458.57
Weight	0.00002	1.6248	51	0.9588	1139.89

Table 4: Relationships between the fork length of *T. albacares* and the length, width and weight of the asteriscus

Length classes (cm)	Length (mm)	Width (mm)	Weight (mg)
50	2.4102	0.4490	0.0000635
60	2.6599	0.4674	0.0000649
70	2.8910	0.4836	0.0000662
80	3.1075	0.4981	0.0000673
90	3.3118	0.5112	0.0000683
100	3.5059	0.5232	0.0000692
110	3.6912	0.5343	0.0000701
120	3.8690	0.5446	0.0000708
130	4.0401	0.5544	0.0000715
140	4.2052	0.5635	0.0000722
150	4.3651	0.5721	0.0000728
160	4.5200	0.5803	0.0000734
170	4.6706	0.5881	0.0000740
180	4.8172	0.5956	0.0000745
190	4.9601	0.6027	0.0000750

Table 5: Relationships between the length and other measurements of the asteriscus

FL	a	b	n	R^2	F
Width	0.8597	0.6939	60	0.6563	110.74
Weight	0.0004	1.0230	47	0.2458	14.66

Growth of the Lapillus

Results of the relations of the lapillus are shown in Table 7 (Fig. 9). There is a high tendency to negative allometric growth index between the length and width of the lapillus (Table 8): $b = 0.488$ ($R^2 = 0.232$ and $F = 18.1$) which indicates that as the length increases the structure curves and apparently the width diminishes.

The relationship between the length and the weight of the lapillus (Table 8) also shows a negative allometric growth, due to the thinness of the structure, a large surface and a small volume ($b = 1.08$, $R^2 = 0.120$ and $F = 5.73$).

The analyses indicate that the growth of the lapillus is eccentric with respect to the core; it grows more at the anterior and dorsal margin than at the posterior and ventral, margin. In the anterior and the posterior margins a larger amount of material is settled for which reason its thickness decreases considerably as fish grow.

Table 9 shows the relationships between fish length and the length, width and weight of the lapillus. The high value of R^2 and F (0.610 and 96.6, respectively) and the slope $b = 0.362$ show a high proportionality of the growth of the lapillus and that of the fish, which makes this otolith adequate for age determination.

Identification of the Growth Rings

One hundred percent of the sagittae otoliths showed defined growth rings. The area where the growth rings can be observed more clearly is from the middle half of the dorsal margin to the postrostrum of the otolith (Fig. 3). Six age groups were identified through the analysis of the growth rings in the otolith sagittae (Table 10).

Table 6: Relationships between the fork length of the fish (FL) and measurements of the asteriscus

FL	a	b	n	R^2	F
Length	0.2908	0.5406	60	0.8529	336.26
Width	0.3136	0.4080	61	0.6601	114.60
Weight	0.0001	0.5677	49	0.2065	12.23

Table 7: Relationship between fork length of *T. albacares* and the length, width and weight of the lapillus

Length classes (cm)	Length (mm)	Width (mm)	Weight (mg)
50	2.4198	0.7313	0.000173
60	2.5850	0.7430	0.000175
70	2.7334	0.7531	0.000176
80	2.8689	0.7619	0.000177
90	2.9939	0.7698	0.000178
100	3.1104	0.7769	0.000178
110	3.2196	0.7834	0.000179
120	3.3227	0.7893	0.000180
130	3.4205	0.7949	0.000180
140	3.5135	0.8000	0.000181
150	3.6024	0.8049	0.000181
160	3.6876	0.8094	0.000182
170	3.7695	0.8137	0.000182
180	3.8484	0.8178	0.000182
190	3.9245	0.8217	0.000183

Table 8: Relationships between the length and other measurements of the lapillus

Length	a	b	n	R^2	F
Width	1.0379	0.4882	62	0.2317	18.10
Weight	0.0004	1.0769	44	0.1201	5.73

Table 9: Relationships between the fork length of the fish (FL) and measurements of the lapillus

FL	a	b	n	R^2	F
Length	0.5867	0.3622	64	0.6090	96.57
Width	0.5910	0.2410	63	0.2676	22.29
Weight	0.0002	0.4558	46	0.1120	5.55

Table 10: No. of rings and average fork length (cm) of *T. albacares* observed in different parts of the world (all data were calculated with the growth parameters, except the ones of this study)

No. of rings	Fork length (cm)						
	Present study	Maunder and Watters (2001)	Lessa and Duarte-Neto (2004)	Postel (1955)	Yabuta <i>et al.</i> (1960)	Baudin-Laurencin (1968)	Pauly (1978)
1	64.00	49.29	57.84	34.46	92.33	100.71	53.03
2	102.60	92.86	98.34	55.97	119.85	113.61	90.78
3	128.50	129.79	129.36	74.45	139.70	124.91	117.65
4	160.00	153.08	153.11	90.32	154.01	134.81	136.77
5	167.00	167.78	171.29	103.95	164.32	143.49	150.39
6	173.00	177.05	185.21	115.66	171.76	151.10	160.07

The asterisci showed a similar number of rings as the sagittae, only in 25% of the cases; it was not possible to identify growth rings in 75% of the otoliths.

It was not possible to identify growth rings in the lapilli, because of the thickness of the structure and the ornamentations of the surface of the external aspect, which avoids the observation of growth rings with transmitted light. Perhaps marks could be easily observed in very thin layered cuts of the lapilli. The irregular deposit of the material on the surface of the external aspect of the lapillus, as well as the high diversity in the orientation patterns of the crystal axes could be related with the piezoelectric capacity of the otoliths for the impulse transmission.

Future studies on the physiology of the otoliths will make the structure and the function of the asterisci and the lapilli more comprehensible.

Thunnus albacares reaches a furcal maximum length of 200 cm, although in the fishery the most common sizes are between 40 and 160 cm. The maximum registered weight is of 176 kg and in the fishery the most normal weight is of 20 kg (Compeán-Jiménez and Dreyfus-León, 1996).

Table 10 shows data obtained by means of the lecture of otoliths sagittae and asterisci. The number of age groups found was six. The observed data were compared with those recorded by other authors and countries, the average length were obtained by us by applying the von Bertalanffy equation for ages 1 to 6 years. The results show the enormous diversity of the mean length at the same age, partly because of the extensive geographical distribution of the yellowfin tuna in the tropical range of the Eastern Atlantic Ocean (Postel, 1955; Baudin-Laurencin, 1968), the Western Atlantic Ocean (Lessa and Duarte-Neto, 2004), Western Pacific (Yabuta *et al.*, 1960) the Indo-Pacific Ocean (Pauly, 1978) and the Eastern Pacific (Maunder and Watters, 2001 and present study). Likewise, there is a great variation en the structures used to identify the growth rings: scales (Yabuta *et al.*, 1960), dorsal fin spine (Lessa and Duarte-Neto, 2004) and otholiths (Maunder and Watters, 2001). Nevertheless, similar values in the average length of the age group of six years old can be observed in the area of the Eastern and Western Pacific: 173 cm (this study), 177.05 cm (Maunder and Watters, 2001) and 171.76 cm (Yabuta *et al.*, 1960). On the other hand, in the Atlantic Ocean larger variations are observed at age 6, with a minimum of 115.66 cm (Postel, 1955) and a maximum of 185.21 cm (Lessa and Duarte-Neto, 2004) and a medium value of 151.10 cm (Baudin-Laurencin, 1968).

CONCLUSIONS

The analysis between left and right otolith showed no morphometric difference, although there are apparent morphologic difference.

Growth is allometrically negative and eccentric in relation with the otolith core.

Sagittae is the best otolith to identify growth rings, from the middle half of the dorsal margin by transmitted light; 25% of asterisci could be read, but no rings were possible to identify in lapillus without process. The three structures have irregular deposits of calcium crystals, related to its physiological use.

Growth was related to the length of the fish, six age groups were identified: age 1 = 64.00, 2 = 102.60, 3 = 128.50, 4 = 160.00, 5 = 167.00 and 6 = 173.00 cm.

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