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Analysis of the Otoliths of Sagitta, Asteriscus and Lapillus of Pacific sierra *Scomberomorus sierra* (Pisces: Scombridae) in the coast of Colima México

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ABSTRACT

Age of fish is an important parameter for fisheries assessment; therefore, it was determined for Pacific sierra *Scomberomorus sierra* by morphological and morphometric analysis of otoliths sagitta, asteriscus and lapillus. Identification of growth rings was also done in the sagittae and asterisci; five growth rings were found: the first ring was formed when the fish measured 19.20 cm, the second at 35.14 cm, the third at 45.80 cm, the fourth at 56.20 cm and the fifth at 64.51 cm of total length. The relationship between length and width of the sagittae is expressed by the equations: $y = 0.582\ x^{0.778}$ (rostrum-antirostrum) and $y = 0.372\ x^{0.640}$ (rostrum-width). In the case of the asterisci: $y = 0.248\ x^{0.685}$ (length-width). For the lapilli $y = 0.488\ x^{0.760}$ (length-width). Growth of these otoliths was also related to the length of the fish. Differences between sexes were observed. Females presented larger sized otoliths than males from 10 to 40 cm of length; once the gonadic maturity begins, this growth diminishes and males present a larger growth in these structures.

Key words: Age determination, species dynamics, scombrid studies, ageing direct method

INTRODUCTION

Scomberomorus sierra is an epipelagic neritic species which forms schools close to the coast on the continental shelf. Its entire distribution area spans from South California to Peru including the Galapagos Islands. It feeds mainly on anchovies and sardines. Its major captures occur in Mexico. It is also captured by sport fisheries but is abundant enough to sustain commercial fishery (Aguirre-Villasenor *et al*., 2006).

Age determination of hard structures of fish (otoliths, scales, spines, vertebrae and opercula, etc.) is possible due to differences in feeding throughout the year and lifespan. These marks can be caused by differences in availability of food occurred during different seasons in the year and for dry and rainy periods (Gallardo-Cabello *et al*., 2007; Espino-Barr *et al*., 2008). These differences are shown in otoliths as bands of fast growth (higher deposition of calcium carbonate and otolina) and slow growth (lower deposition of these materials). These marks help identify growth rings,

considering in one year a ring of fast growth (opaque) and a slow growth (hyaline) if observed with transmitted light (Espino-Barr *et al*., 2006; Gallardo-Cabello *et al*., 2006). Other analysis with sagittal otoliths help differentiate stocks that can give information for fishery management (Ramirez-Perez *et al*., 2010).

In age determination the sagitta is usually used due to its larger size and easier removal (Wilhelm *et al*., 2005; Stransky *et al*., 2005; Popper *et al*., 2005). Some authors have determined the age through the lapilli in larvae and juvenile fish, identifying daily growth increments (Radke, 1984; Secor *et al*., 1989; David *et al*., 1994). Very few authors have studied asterisci with similar purposes (David *et al*., 1994) but none with *Scomberomorus sierra*.

Studies on age determination in asterisci growth rings, as well as morphometric and morphological analysis of sagittae, asterisci and lapilli have been made by Gallardo-Cabello *et al*. (2006), Espino-Barr *et al*. (2006) and Santana-Hernandez *et al*. (2008).

However, there are no studies on the structure, morphology and identification of growth rings in otoliths of Pacific sierra *Scomberomorus sierra*, therefore, the objective of this study is to analyze otoliths: sagitta, asteriscus and lapillus morphologically and morphometrically and identifying growth rings in the sagittae and asterisci.

MATERIAL AND METHODS

From January 2003 to March 2004, 464 organisms of *Scomberomorus sierra* were monthly taken directly *in situ* from the commercial captures of the coastal fishery in Manzanillo, Colima, México and taken to the lab, where Total Length (TL) and sex were registered for each organism. Individuals were captured with hand lines, troll and gillnet in order to obtain different lengths and age groups.

Otoliths, sagittae, asterisci and lapilli, were obtained from 127 individuals, through a transverse cut in the ventral cranial cavity, the brain was removed and the left and right semicircular canals were extracted. The otoliths were rinsed with water and stored dry in Eppendorf tubes labeled with number, date, total length and sex.

The structure of otoliths was studied with a dissection microscope. A description of the labyrinth system and the sagittae was made with the terminology of the glossary of Secor *et al*. (1992). The same concepts were applied to the description of the asterisci and lapilli according to Gallardo-Cabello *et al*. (2006) and Espino-Barr *et al*. (2006).

Data on length and width were registered for each otolith through their observation in a stereoscopic microscope with a graduated ocular lens. Measures were taken on the right and left aspect for the three pairs.

The otolith sample size was calculated with the formula described by Daniel (1991). Constants of the relationships of the sagittae were calculated for Rostrum Length (RL), Antirostrum Length (AL) and Width (W). For the asterisci and lapilli the indexes of the constants of the relationship were obtained for Length (L) and Width (W). The relationships between the total length of the fish and all the measures of the three otoliths were also recorded. Regressions were done by the least squares.

For the evaluation of the relations and the analysis of the possible morphometric differences between the otoliths of males and females, a one way variance analysis (ANOVA) was carried out (Zar, 1996).

The identification of growth rings was done observing the sagittae and the asterisci in the stereoscopic microscope with transmitted light and the average size of each ring was calculated.

RESULTS AND DISCUSSION

The descriptions made here are original for *S. sierra*, there are no other studies to compare these results and discuss them.

Labyrinth system of *Scomberomorus sierra*. The membranous labyrinth consists of tubular structures called the semicircular canals which are three: anterior vertical canal, posterior vertical canal (Fig. 1) and horizontal canal. Each canal ends at both sides in chambers containing otoliths; the sacculus contains the sagitta (Fig. 4), the lagena contains the asteriscus (Fig. 3) and the utriculus contains the lapillus (Fig. 1). In each chamber the otoliths are inmersed in a liquid called endolymph (Lagler *et al*., 1962).

Each of the chambers with the otoliths is in contact with nerve cells called neuromasts which connect with the otoliths through the acoustic macula that enters in the acoustic canal of the sagitta and in the sulcus of the asteriscus and lapillus (Fig. 2). This connection makes nutrient deposition possible which allows growth of the otolith (Fig. 3) (Gallardo-Cabello *et al*., 2006; Espino-Barr *et al*., 2006). It is through the acoustic macula that vibration of the otoliths impulses are transmitted to the brain via the eighth cranial nerve as has been described by Mugiya (1964) and Mugiya (1966a, b).

Sagittae and asterisci are responsible for the perception of sound, gravity and angular acceleration, whereas lapilli are responsible for balance (Holst *et al*., 1950; Lowenstein, 1957).

Sagittae are structures made of calcic carbonate (Lagler *et al*., 1962) in the form of aragonite (Hickling, 1931; Sasaki and Miyata, 1955; Carlstrom, 1963; Gallardo-Cabello, 1986) and a high molecular weight protein called otoline (Degens *et al*., 1969).

Sagitta is the largest otolith in *Scomberomorus sierra* reaching a total length of 7.07 mm while asteriscus measures 2.36 mm and lapillus 1.73 mm in specimens of 40 cm total length.

Description of the otoliths of Pacific sierra *Scomberomorus sierra*

Description of the sagitta: The anterior margin of the sagitta shows a prominent rostrum and a less developed antirostrum, separated by the excisura major (Fig. 5). As the fish ages the shape

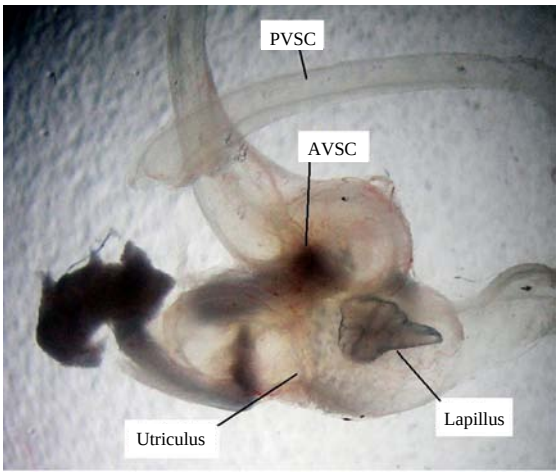

Fig. 1: Lapillus contained in the utriculus and section of the anterior vertical semicircular canal (AVSC) and the posterior vertical semicircular canal (PVSC) of the membranous labyrinth in an individual of *Scomberomorus sierra* (40 cm of total length) (increased 14.5 times)

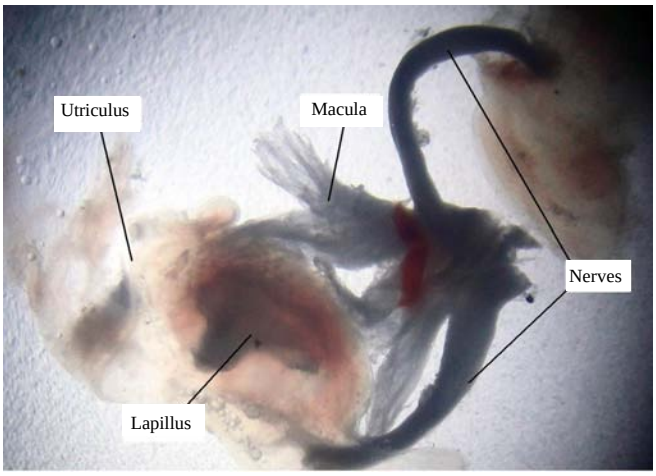

Fig. 2: Section of the membranous labyrinth showing the macula on the lapillus and the utriculus camera in a specimen of 40 cm of total length (increased 18 times)

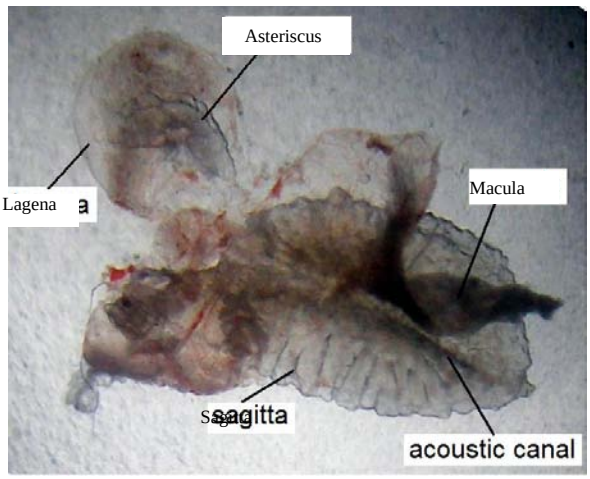

Fig. 3: Section of the membranous labyrinth showing the macula in the acoustic canal of the sagitta and the asteriscus in the lagena, in an individual of *Scomberomorus sierra* of 40 cm total length (increased 14 times)

of the rostrum varies and lengthens (Fig. 6). Its form varies between specimens; right and left sagittae in the same individual can be totally different.

The anterior section of the sagitta shows a rounded postrostrum without excisura minor and therefore no pararostrum (Fig. 5).

The dorsal margin has a rectilinear profile extending from the antirostrum to the postrostrum. The ventral side is also rectilinear up to the middle part of the otolith; beyond this point, it descends towards the postrostrum showing some irregularities such as indentations (Fig. 6).

The anterior, posterior and ventral borders have unevenly distributed denticles which disappear in certain sections of the otolith, as in the antirostrum (Fig. 7).

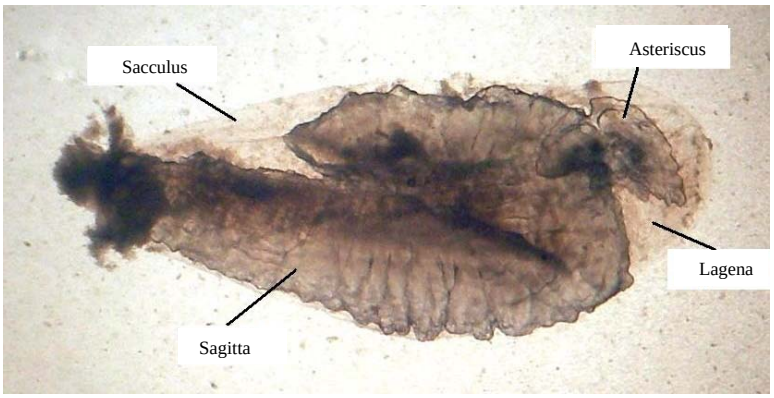

Fig. 4: Sagitta contained in the sacculus and the asterisci in the lagena of the membranous labyrinth in an individual of *Scomberomorus sierra* (40 cm total length) (increased 15 times)

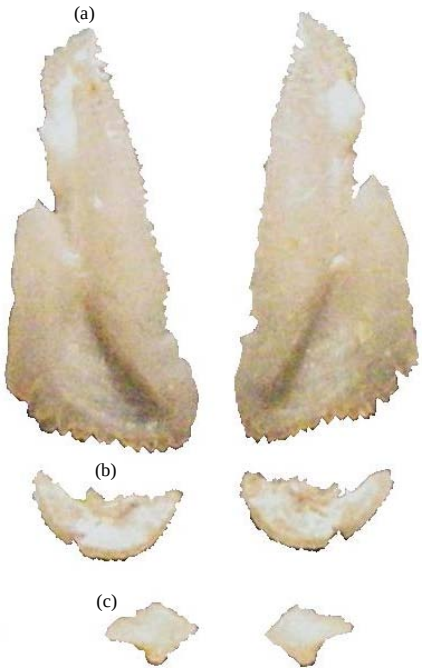

Fig. 5: Three pairs of otoliths: (a) sagittae, (b) asterisci and (c) lapilli of *Scomberomorus sierra* (increased 9.8 times)

The internal aspect of the otolith is convex, a feature which increases with age; its surface is smooth and it is traversed in its entirety by the acoustic channel (Fig. 6) which increases in width from the anterior to the posterior part of the otolith; it differs in the cauda and the ostium (Fig. 7).

The external aspect of the sagitta is concave, slightly thicker in the middle of the otolith than the postrostrum. Growth rings go around the sagitta which are easier to observe from the middle part of the otolith to the postrostrum (Fig. 8). Average width of the sagitta is 2.55 times its average length.

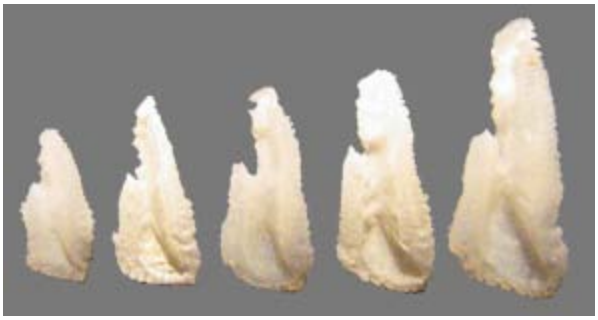

Fig. 6: Selection of sagittae of Scomberomorus sierra internal aspect, left sagittae, showing the differences in form and size according to the different classes of sizes and age groups (increased 4.8 times)

Fig. 7: Microscopic photograph of the internal aspect of the right sagitta of *S. sierra*, showing the acoustic canal (increased 18.3 times)

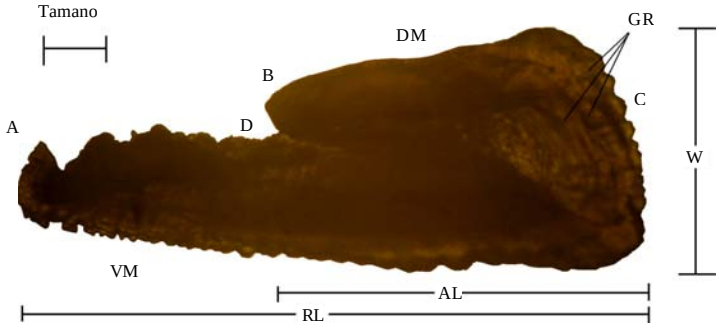

Fig. 8: Microscope photograph of the left sagitta, external aspect of *Scomberomorus sierra* showing its main characteristics : A = Rostrum, B = Antirostrum, C = Postrostrum, D = Excisura major, DM = Dorsal margin, VM = Ventral margin, AL = Antirostrum length, RL = Rostrum length, W = Width, GR = Growth rings (increased 18.3 times)

Description of the asteriscus: The shape of the asterisci varies between specimens but there are no differences between right and left, as in the sagittae (Fig. 5b).

The anterior margin shows a blunt projection which divides this otolith in two parts: dorsal part with a larger surface than the ventral part (Fig. 9). The anterior margin has sections that can be rectilinear from the dorsal to the ventral margins (Fig. 10).

The posterior margin is curved; in some cases it presents interruptions in the form of notches (Fig. 5) or larger irregularities (Fig. 10). The posterior margin presents grooves all around the dorsal and ventral margin which divide the otolith in two parts. The first side has a larger sized radius in the external aspect (posterior external margin). The smaller radius in the internal aspect of the otolith (posterior internal margin) is in contact with the acoustic macula (Fig. 10).

The external aspect of the asteriscus is convex, a feature which increases as the fish ages; the internal aspect is concave. Both aspects of the otolith present small indentations (Fig. 9a, b). Its average width is 2.56 times its average length.

Description of the lapillus: The anterior margin of the otolith is spherical and it is oriented toward the front of the fish. Dorsal and ventral margins descend towards the central part of the otolith, in a fan-shaped structure. The ventral margin is rounded and the dorsal edge of this structure is notably larger and is projected towards the posterior edge (Fig. 11).

The inner surface is concave, a feature which increases with age. It shows several radios which divide the otolith in several lobes (Fig. 11a). The sulcus is found in the posterior border, where it comes in contact with the acoustic macula which extends along the dorsal and ventral margins. The outer aspect is convex; its dorsal margin shows large indentations which become smaller at the ventral margin (Fig. 11b).

The anterior margin of the external aspect shows a large number of calcium carbonate crystals arranged epitaxially which show different growth patterns in shape and size (Fig. 11b). Average width of the lapillus is 1.34 times its average length.

Morphometric analysis of the pacific sierra *Scomberomorus sierra*: The calculated sample size for sagitta, asteriscus and lapillus was done in 36 individuals. No significant morphometric differences were found between the right and left otoliths; however, there were morphometric differences between the otoliths of male and female specimens.

Growth of the sagitta: Table 1 shows the relation between rostrum, antirostrum and width of sagitta and the length classes of fish, for the species and sexes. Growth of the rostrum and width of sagitta are larger in females during the first stages, that is, lengths 10 to 40 cm; thereafter growth rate decreases and it is higher in males' sagittae. This phenomenon may be related to the beginning of gonad maturation and reproduction. During this maturation, metabolism of calcium and protein are oriented to egg formation and fatty acids storage. In females it reaches a higher ratio, therefore growth of otoliths decreases more markedly in females than in males (Gallardo-Cabello, 1986).

The relationship between length and width of Sagitta is expressed by the exponent value $b = 0.64$ which is a negative allometric growth (Table 2). Determination index of this relation is $R^2 = 0.753$ with a value for ANOVA's $F = 381.613$, indicating that the sagitta tends to lengthen as the fish ages. Similar results are found in the relations of females and males, females having a higher determination index $R^2 = 0.836$. On the other hand, the allometric relation between the

Fig. 9: Microscopic photograph of the asterisci of *Scomberomorus sierra*: (a) left external aspect and (b) right internal aspect; S = sulcus, IPM = internal posterior margin and EPM = external posterior margin (increased 43 times)

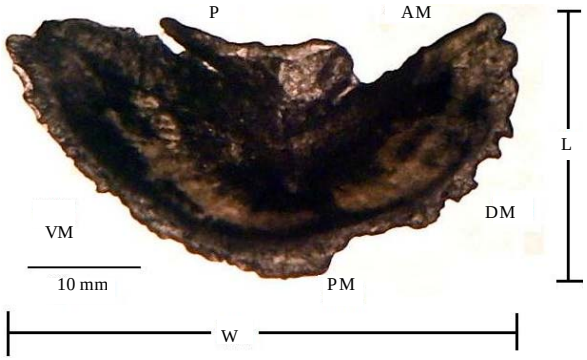

Fig. 10: Microscopic photograph of the right asteriscus, external aspect of *Scomberomorus sierra* ventral view, depicting its characteristics: AM = anterior margin, PM = posterior margin, P = projection, DM = dorsal margin, VM = ventral margin, L = length, Wi = width, (increased 43 times)

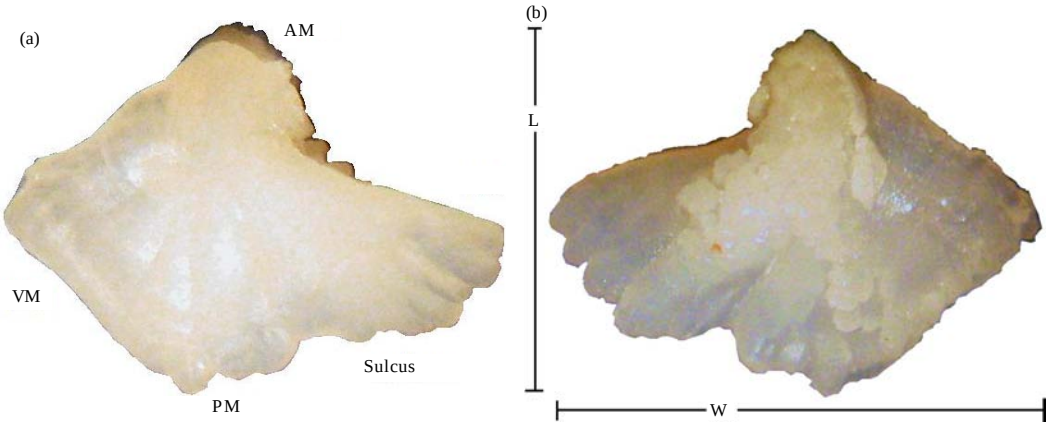

Fig. 11: Microscopic photograph of the left lapillus of *Scomberomorus sierra,* a) internal aspect, b) external aspect, showing its main characteristics: AM = anterior margin, PM = posterior margin, DM = dorsal margin, VM = ventral margin, L = length and W = width

Table 1: Measures of rostrum (R), antirostrum (AL) and width (W) at different size classes of the sagitta of *Scomberomorus sierra*

	Species			Females			Males		
Clases (cm)	R (mm)	AL (mm)	W (mm)	R (mm)	AL (mm)	W (mm)	R (mm)	AL (mm)	W (mm)
10	2.93	2.18	1.62	3.16	2.13	1.73	2.63	2.15	1.53
20	4.55	3.11	2.19	4.74	3.05	2.28	4.32	3.11	2.12
30	5.89	3.83	2.62	6.00	3.77	2.68	5.77	3.87	2.57
40	7.07	4.44	2.97	7.11	4.38	3.01	7.09	4.51	2.94
50	8.14	4.98	3.27	8.10	4.92	3.29	8.32	5.08	3.27
60	9.14	5.47	3.55	9.01	5.41	3.54	9.48	5.59	3.56
70	10.08	5.92	3.79	9.86	5.87	3.77	10.58	6.07	3.83
80	10.97	6.34	4.02	10.66	6.29	3.98	11.65	6.52	4.08
90	11.82	6.73	4.23	11.42	6.69	4.17	12.67	6.94	4.31

R = Rostrum, AL = Antirostrum, W = Width

Table 2: Relationship between the rostrum length and other measures of the sagitta

Rostrum length (mm)	Sex	a	b	n	R^2	F
Antirostrum	Both	0.582	0.773	127	0.791	472.627
	Females	0.589	0.859	64	0.877	440.396
	Males	0.575	0.711	63	0.702	144.04
Width	Both	0.372	0.64	127	0.753	381.613
	Females	0.376	0.645	64	0.836	316.396
	Males	0.364	0.608	63	0.65	113.359

a = Origin ordinate of relation, b = Slope of relation, n = No. of individuals, R^2 = Determination index of relation, F = Fischer index of relation

length of the rostrum and of antirostrum is represented by a negative allometric growth index b = 0.773 with a value of R^2 = 0.791 and F = 472.627 which shows that the length of antirostrum decreases as the fish ages tending to form a bulge (Fig. 6-8). A similar behavior is observed in the growth of sexes, females reaching a higher determination index value R^2 = 0.877. These results show that the growth of sagitta is eccentric to the core. This is a characteristic that increases with the age of organism, i.e., the postrostrum grows more than the rostrum and the antirostrum. The dorsal edge grows more than the ventral edge and a larger amount of material accumulates on the inner than the external aspect.

The relationship between fish length and length and width of sagitta is shown in Table 3. The higher value of the allometric index relating fish length to rostrum's length for males has a value of b = 0.716; similar values are found for the species and females, showing that there is a direct proportionality between sagitta length and fish length; this structure is adequate to describe the growth of the organism. High values of the Anova (F) show a strong correlation between the structures analyzed in each case. Values of allometric growth rates are below one, due to the difficulty of relating very small structures (in millimeters) with values of total fish length, expressed in centimeters.

Growth of the asteriscus: The relationship between fish length and length and width of asteriscus is shown in Table 4. Similar to sagitta, growth of asteriscus is higher in females during the early stages preceding gonadal maturity; once it starts, at 40 cm total length as mentioned by Nava-Ortega (2008), its growth decreases and is higher in males.

Table 3: Relationship between the total length of the fish (TL) and the measures of the sagitta

Total length (cm)	Sex	a	b	n	R^2	F
Rostrum	Both	0.068	0.634	127	0.784	452.64
	Females	0.082	0.585	64	0.808	266.539
	Males	0.051	0.716	63	0.746	178.949
Antirostrum	Both	0.067	0.513	127	0.691	279.058
	Females	0.064	0.521	64	0.789	232.506
	Males	0.063	0.533	63	0.574	82.103
Width	Both	0.059	0.438	127	0.719	320.306
	Females	0.069	0.401	64	0.795	240.642
	Males	0.051	0.473	63	0.618	98.885

a = Origin ordinate of relation, b = Slope of relation, n = No. of individuals, R^2 = Determination index of relation, F = Fischer index of relation

Table 4: Measures of length (L) and width (W) of asteriscus at different length classes of *Somberomorus sierra*

	Species		Females		Males	
Classes (cm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)
10	0.89	0.45	0.93	0.51	0.79	0.37
20	1.45	0.64	1.47	0.68	1.38	0.59
30	1.93	0.79	1.93	0.82	1.91	0.77
40	2.36	0.92	2.33	0.93	2.42	0.92
50	2.77	1.03	2.71	1.02	2.89	1.07
60	3.14	1.14	3.06	1.11	3.35	1.20
70	3.50	1.23	3.39	1.19	3.80	1.33
80	3.85	1.32	3.70	1.26	4.23	1.45
90	4.18	1.40	4.01	1.32	4.65	1.57

The relationship between the length and width of the otolith (Table 5) is described by the allometric index b = 0.685 (R^2 = 0.588 and F = 178.53) with similar values for females and males, the latter having a higher value of the allometric index. These results correspond to a negative allometric growth in which the increase in length is greater than width, a phenomenon related to a higher growth of the dorsal margin than the ventral margin on the surface of asteriscus. Asteriscus growth is eccentric to the core; therefore its anterior border grows more than the posterior border.

Table 6 shows the relationship between fish total length and length of asteriscus. The allometric index value closest to one is of males b = 0.808, showing a trend to direct proportionality between fish and asteriscus length which allows age group determination based on the number of growth rings in asteriscus to be valid for the Pacific sierra.

Growth of the lapillus: Table 7 shows the relationship between fish length and length and width of the lapillus. As in the sagitta and asteriscus, the fastest growth of lapillus occurs in females at 40 mm in length. Once sexual maturity begins, growth slows down and males show faster growth of this structure.

The relationship between the length and width of the lapillus (Table 8) shows an index value b = 0.76 (R^2 = 0.623; F = 206.215), very similar in females and males; this represents a negative allometric growth, in which the lapillus grows more in length than in width. The anterior margin of this structure shows a higher deposition of growth materials at the posterior margin; the ventral margin and the dorsal margin have a similar growth of the lapillus is eccentric to the core.

Table 5: Relationship between the length and width of the asteriscus

Otolith length	Species	a	b	n	R^2	F
Width	Both sexes	0.248	0.685	127	0.588	178.53
	Females	0.237	0.639	64	0.619	100.81
	Males	0.252	0.709	63	0.552	75.129

a = Origin ordinate of relation, b = Slope of relation, n = Number of individuals, R^2 = Determination index of relation, F = Fischer index of relation

Table 6: Relationship between the total length of the fish and the measures of the asteriscus

Total length	Species	a	b	n	R^2	F
Length	Both sexes	0.018	0.702	127	0.785	457.545
	Females	0.02	0.666	64	0.832	307.67
	Males	0.012	0.808	63	0.752	184.874
Width	Both	0.014	0.519	127	0.551	153.00
	Females	0.018	0.439	64	0.574	83.373
	Males	0.008	0.654	63	0.541	71.838

a = Origin ordinate of relation, b = Slope of relation, n = Number of individuals, R^2 = Determination index of relation, F = Fischer index of relation

Table 7: Measures of length (L, mm) and width (Wi, mm) of lapillus at different length classes of *Somberomorus sierra*

	Species		Females		Males	
Classes (cm)	L (mm)	W (mm)	L (mm)	W (mm)	L (mm)	W (mm)
10	0.88	0.69	0.95	0.71	0.78	0.61
20	1.23	0.95	1.29	0.95	1.16	0.90
30	1.50	1.13	1.54	1.12	1.47	1.13
40	1.73	1.29	1.75	1.27	1.73	1.32
50	1.93	1.42	1.92	1.39	1.97	1.49
60	2.11	1.54	2.08	1.50	2.19	1.65
70	2.28	1.65	2.23	1.60	2.40	1.80
80	2.44	1.75	2.37	1.69	2.59	1.94
90	2.58	1.85	2.49	1.77	2.77	2.07

Table 8: Relationship between the length of the fish and other measures of the lapillus

Otolith length	Species	a	b	n	R^2	F
Width	Both sexes	0.488	0.76	127	0.623	206.215
	Females	0.507	0.793	64	0.63	105.787
	Males	0.513	0.778	63	0.637	107.141

a = Origin ordinate of relation, b = Slope of relation, n = Number of individuals, R^2 = Determination index of relation, F = Fischer index of relation

The relationship between fish length and the length and width of the lapillus is shown in Table 9. Allometric index shows a higher value in males, b = 0.578 (R^2 = 0.574 and F = 82.089) which suggests a trend toward direct proportionality between fish length and lapillus that validates identification of growth rings in this structure of the Pacific sierra.

Identification of growth rings: Growth ring analysis in sagittae otoliths of the Pacific sierra allowed the identification of five age groups (Table 10). The percentage of sagittae showing clearly

Table 9: Relationship between the total length of the fish and measures of the lapillus

Total length	Species	a	b	n	R^2	F
Length	Both sexes	0.028	0.492	127	0.66	242.838
	Females	0.035	0.439	64	0.763	199.156
	Males	0.021	0.578	63	0.574	82.029
Width	Both	0.025	0.445	127	0.581	172.978
	Females	0.028	0.413	64	0.678	130.648
	Males	0.017	0.552	63	0.55	74.544

a = Origin ordinate of relation, b = Slope of relation, n = Number of individuals, R^2 = Determination index of relation, F = Fischer index of relation

Table 10: Number of the rings and average length (cm) of Scomberomorus sierra in the sagittae and asterisci

No. of rings	Total length (cm)
1	19.20
2	35.14
3	45.80
4	56.20
5	64.51

defined growth rings was 100%. Growth rings are seen very clearly in an area from the middle of the otolith to the posrostrum on the dorsal margin; in this region growth mater is deposited faster (Fig. 8).

Thirty five percent of asterisci showed the same number of rings as sagittae; in the remaining 65%, growth rings were not clearly identified. Growth rings in asterisci are shown on the inner side of the otolith as dark concentric lines which run from the ventral to the dorsal margin (Fig. 10).

In the case of lapilli, growth rings are very difficult to see due to the thickness of the structure which prevents the observation of growth rings by transmitted light transparency. However parts of the concentric rings were observed in the internal posterior margin, where they appear as dark lines that go from the ventral border to the dorsal border (Fig. 11).

Otoliths are structures that can be used to age *S. sierra*. Its size increases with age and growth of the specimen. There is statistical difference between sexes: in early years, females are larger, after their first reproductive maturity, males tend to reach larger sizes.

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