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Morphologic and Morphometric Analysis and Growth Rings Identification of Otoliths: Sagitta, Asteriscus and Lapillus of *Caranx caballus* (Pisces: Carangidae) in the Coast of Colima, Mexico

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Abstract: This study is a description and morphometric analysis of the otoliths sagittae, asterisci and lapilli of the green jack *Caranx caballus* (Günther, 1869) in the tropical Mexican Pacific. The relationship among length, width and weight of the sagittae is expressed by the following equations: $y = 0.935x^{0.832}$ (rostrum-antirostrum), $y = 0.272x^{1.139}$ (rostrum-width) and $y = 0.00007x^{2.524}$ (rostrum-weight). In the case of the asterisci: $y = 1.623x^{0.81}$ (length-width) and $y = 0.0005x^{1.657}$ (length-weight). For the lapilli $y = 1.061x^{0.874}$ (length-width) and $y = 0.0003x^{2.796}$ (length-weight). The growth of these otoliths was also related to the length of the fish. The average length was calculated for each of the four growth rings identified in the sagittae and the asterisci; the results are: 1 = 16.82 cm, 2 = 27.78 cm, 3 = 34.66 cm and 4 = 40.27 cm.

Key words: Sagitta, asteriscus, lapillus, otolith, *Caranx caballus*

Introduction

Analysis of the sagittae is commonly used to identify the growth rings, since 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; Wilhelm *et al*., 2005; Stransky *et al*., 2005; Popper *et al*., 2005).

Some authors (Victor, 1982; Brothers *et al*., 1983; Radke, 1984; Mugiya and Uchimura, 1989; Secor *et al*., 1989; David *et al*., 1994) have used the lapilli to determine the age of larvae and juvenile specimen by identifying the daily increments in growth. Barkman (1978), Bolz and Lough (1983; 1988), Lagardere (1989) and David *et al*. (1994) have used the asterisci with the same purpose.

There are no studies on age determination on periodical growth rings in asterisci and lapilli of mature fish. The morphology and the morphometry of these structures have not been studied either.

The green jack *Caranx caballus* (Günther, 1969) is a coastal pelagic species of commercial value in the artisanal fishery of the tropical Mexican Pacific. It is distributed from the coasts of Mexico to the north of Perú (Chirichigno *et al*., 1982; Fisher *et al*., 1995). An average of 93 tons is fished annnally in the state of Colima which is used for human consumption. There are studies on their taxonomic description (Chirichigno *et al*.,1982; Fisher *et al*.,1995; Castro-Aguirre *et al*., 1999) and on biological and fishery information (Cruz-Romero *et al*., 1993; Espino-bar, 2000; Espino-Bar *et al*., 2003; 2004).

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However, there are no data on the analysis of the growth rings in hard structures. The otoliths are adequate structures for this analysis, since the scales of this species are replaced during its growth. A morphologic and morphometric analysis of the otoliths: sagittae, asterisci and lapilli of the green jack *Caranx caballus*, as well as the identification of the growth rings in the sagittae and the asterisci was done.

Materials and Methods

Fifty specimens of this fish were obtained every month from the commercial catches of the artisanal fishing of Manzanillo, Colima (Mexican Pacific), between January and December 2001. The individuals were captured with pound net, beach seine, hand lines and gillnets, in order to obtain every length and age groups. The Standard Length (SL) and sex among other variables were registered for each organism.

To obtain the otoliths sagittae, asterisci and lapilli, a traverse cut in the ventral cranial cavity was made and the brain removed; the left and right semicircular canals were extracted. The otoliths were rinsed with water and stored dry in Eppendorf tubes labeled with number, date, standard length and sex.

The structure and microstructure of the otoliths were studied with a scanning electronic microscope, from the Institute of Physics of the Universidad Nacional Autónoma de México.

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 which have not been reported before.

Data on the length and the width were registered for each otolith (Fig. 4, 11a and 12) through their observation in a stereoscopic microscope with a graduated ocular lens. The weight of each otolith was also registered in a ultranalytic scale with a precision of a ten thousandth of a gram.

All measures were made on the right sided sagittae, asterisci and lapilli in order to eliminate possible differences due to variations between the otoliths in the same organism. The sample size was calculated with the formula described by Daniels (1991).

In the case of the sagittae the constants of the relationship 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). The relationships of all the measures of the three otoliths were also related to the standard length of the fish. The regressions were done by the least squares. To evaluate the relations a variance analysis (ANOVA) was carried out (Mandenhall, 1987; Zar, 1996). This last test was also applied to analyze the possible morphometric differences between the otoliths of males and females.

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

Labyrinth System of C. caballus

In this species the membranous labyrinth has the shape of a sac where the semicircular canals end in a tubular way into a main cavity, i.e., the vestibular apparatus, which consists of a series of cameras wherein the utriculus contains the lapillus (Fig. 1), the sacculus contains the sagitta (Fig. 2) and the lagena contains the asteriscus (Fig. 3) (Secor *et al*., 1992; Lagler *et al*., 1962).

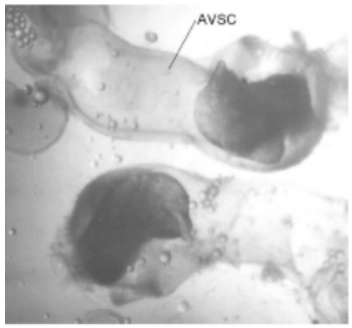

Fig. 1: Section of the right and left membranous labyrinths in an individual of *Caranx caballus* (30 cm of standard length), showing the lapilli contained in the utriculus and sections of the anterior vertical semicircular canal (AVSC) (increased 15 times)

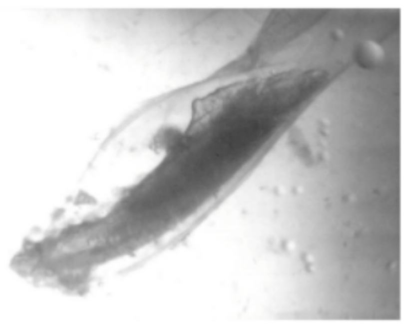

Fig. 2: Lateral side of the left sagitta in a specimen of *Caranx caballus* (35 cm of standard length) in the sacculus (increased 10 times)

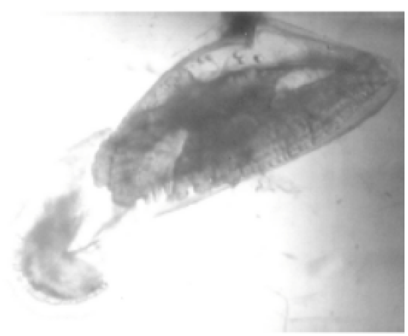

Fig. 3: Section of the left membranous labyrinth of an individual of *Caranx caballus* (25 cm of standard longitude) with the sagitta contained in the sacculus and the asteriscus inside the lagena (increased 10 times)

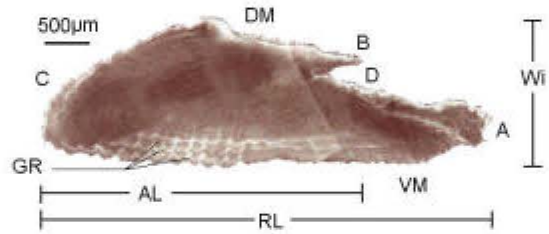

Fig. 4: Scanning photograph of the right sagitta, external aspect of *Caranx caballus* showing its main characteristics: A = rostrum, B = antirostrum, C = postrostrum, D = excisura major, DM = dorsal margin, VM = ventral margin, AL = antirostrum length, RL = rostrum length, Wi = width, GR = growth rings

Fig. 5: Selection of sagittae of *Caranx caballus* (right sagitta, internal aspect), showing the differences in shape and size according to the different classes of sizes and age groups (increased 2.6 times)

Fig. 6: Right and left sagittae (external aspect) of *Caranx caballus* of 22 cm of standard length, showing their morphologic differences (increased 11 times)

Each camera also contains sensorial nervous cells (neuromast cells) which support the otolith and are in contact with the macula through which the growth nutrients are deposited; the neuromasts transmit the stimuli to the brain by way of the eighth cranial nerve (Mugiya, 1964; 1966a; 1966b).

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

Otoliths (sagitta) are structures made of calcic carbonate (Lagler *et al*., 1962), in the form of aragonite (Hickling, 1931; Brandenberger and Schintz, 1945; Sasaki and Miyata, 1955; Carlstrom, 1963; Gallardo-Cabello, 1986b) and by otolina, a high weight molecular protein (Degens *et al*., 1969).

The sagitta is the largest otolith in *Caranx caballus*, reaching a total length of 5.23 mm; the lapillus measures 1.89 mm and the asteriscus 1.10 mm, in organisms of 20 cm of standard length.

Description of the Otoliths of the Green Jack Caranx caballus

Description of the Sagitta

In most cases the anterior margin exhibits a prominent excisura major and a well developed antirostrum (Fig. 4). The shape of the rostrum varies considerably from one specimen to another (Fig. 5); furthermore the right and the left sagittae are morphologically different (Fig. 6). In many cases the rostrum grows toward the dorsal margin and fuses with the antirostrum (Fig. 7).

The posterior margin presents a rounded postrostrum which lacks an excisura minor and a pararostrum (Fig. 4).

The dorsal margin is rectilinear from the antirostrum to the center of the otolith, after which the dorsal margin descends with a marked inclination towards the posterior margin. The ventral margin is rectilinear from the rostrum to the posterior margin (Fig. 4).

The anterior, posterior, dorsal and ventral margins show regular denticles that spread irregularly and disappear in some sections of the otolith as the fish ages (Fig. 5).

The internal aspect of the otolith is convex, this feature increases with age; its surface is smooth and marked lengthwise by a deep sulcus (Fig. 8) which increases in width from the center of the otolith to the end of its posterior margin, without a clear difference between the ostium and the cauda (Fig. 9).

The base of the acoustic channel is made of calcic carbonate crystals which show mainly an epitaxial growth; albeit the orientation of the crystal axes show diverse orientation angles (Fig. 10).

The external aspect is concave; its thickness decreases abruptly in the longitudinal sense from the middle area to the anterior margin.

Figure 7 shows the otolith core from which the growth bands begin to form as soon as the larvae hatch and the vitelin sack (egg yolk) has been absorbed (Brothers *et al*., 1976; Struhsaker and Uchiyama, 1976). The bands which form around the core show different daily and seasonal growth rate patterns; this allows the identification of growth rings through which age groups can be determined (Hickling, 1931; Degens *et al*., 1969; Dannevig, 1956; Kelly and Barker, 1961; Pannella, 1971; Gallardo-Cabello, 1986a, 1986b).

The average width of the sagitta is contained 2.86 times in the average length.

Description of the Asteriscus

The anterior margin of this otolith presents a marked projection which may be blunt or pointed; it divides the asteriscus in two parts, the dorsal side has a larger surface than the ventral side. The dorsal margin may show rectilinear sections and curves from the anterior to the posterior margin or else it may be completely curvilinear. The ventral margin descends with a marked curvature from the anterior to the posterior margin (Fig. 11a).

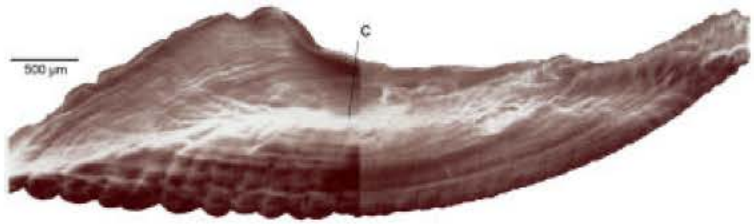

Fig. 7: **Scanning photograph of the right sagitta, external aspect of** *Caranx caballus* **showing the core (C) and the growth rings**

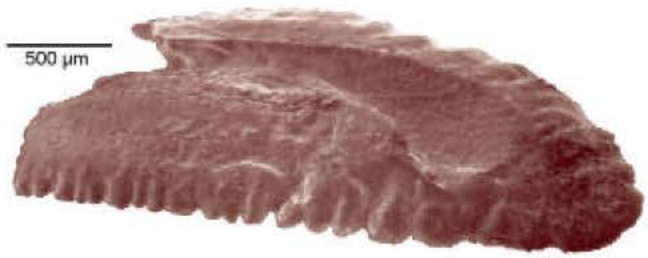

Fig. 8: **Acoustic canal in the internal aspect of the right sagitta of** *Caranx caballus*

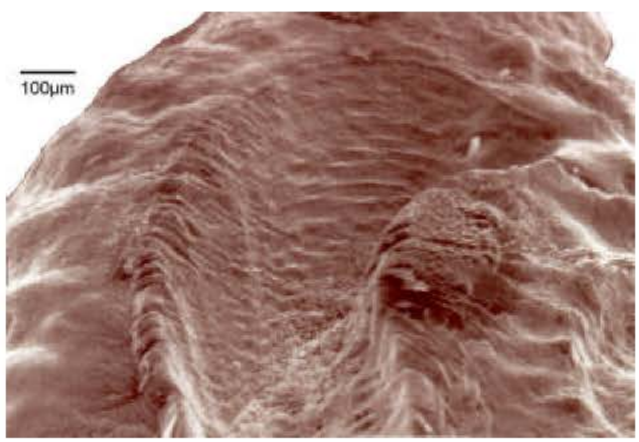

Fig. 9: **Scanning photograph of the right sagitta, internal aspect of** *Caranx caballus* **showing the acoustic canal at the postrostrum**

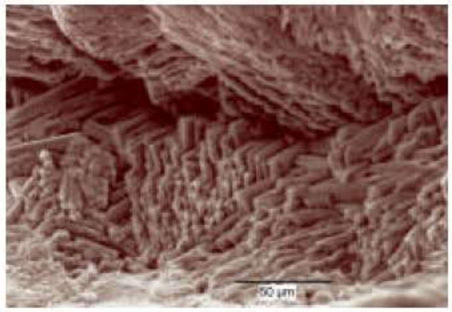

Fig. 10: Scanning photograph of the right sagitta, internal aspect of *Caranx caballus* showing the calcic carbonate crystals in the base of the acoustic canal

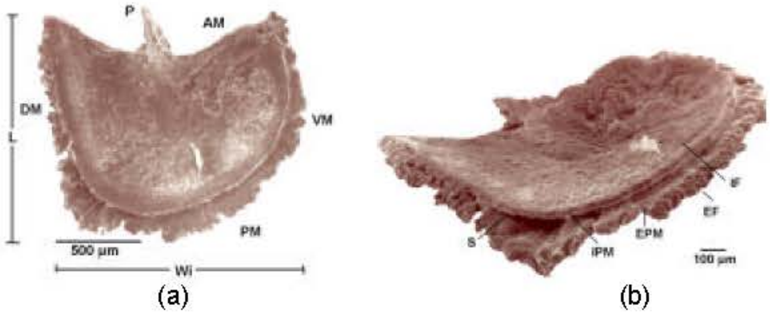

Fig. 11: Scanning photograph of the left asteriscus, internal aspect of *Caranx caballus*, a) ventral view, and b) lateral view, depicting its characteristics: AM = anterior margin, PM = posterior margin, P = projection, DM = dorsal margin, VM = ventral margin, L = length, Wi = width, IF = internal aspect, EF = external aspect, S = sulcus, IPM = internal posterior margin and EPM = external posterior margin

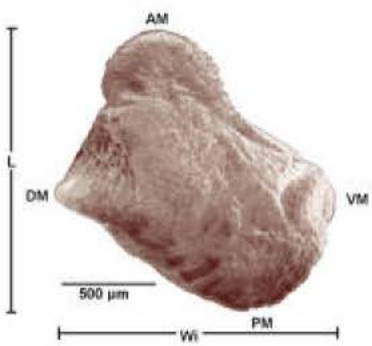

Fig. 12 Scanning photograph of the left lapillus, external aspect of *Caranx caballus* showing its main characteristics: AM = anterior margin, PM = posterior margin, DM = dorsal margin, VM = ventral margin, L = length and Wi = width

The posterior margin is curved; it exhibits a "sulcus" along the dorsal and ventral margins which divides the asteriscus lengthwise in two bodies, the one with a smaller radio is the internal aspect of the otolith (internal posterior margin) and the one with a larger radio exhibits the external aspect of the asteriscus (external posterior margin) (Fig. 11b). The internal aspect of the asteriscus is in contact with the neuromast cells.

The internal aspect of the asteriscus is concave, a feature which becomes more pronounced with age; its posterior area is smooth. The external aspect is convex and its posterior margin presents small denticles whose small crystals are placed in an epitaxial form (Fig. 11b).

The form of the asterisci varies from an organism to another, but they are not as apparent between the right and the left side as in the case of the sagittae.

Description of the Lapillus

Figure 12 shows that the anterior margin of this otolith is constituted by a spherical structure oriented toward the front part of the fish with an inclination of approximately 15°. The anterior margin is prolonged on the dorsal side towards a pointed region and a blunt region toward the ventral margin.

The dorsal and the ventral margins descend toward the middle part of the otolith and form a fan-like structure, which is the posterior margin. The anterior margin is wider and projects downwards toward the dorsal and ventral margins.

The internal aspect of the lapillus is concave, a feature which increases with age and shows radios that divide the otolith in several lobes. The external face is convex and the dorsal and ventral margins show smooth and rough serrated sections. In the posterior region, the margins show denticles formed by small crystals disposed in epitaxial form.

The external and internal surfaces of the lapillus exhibit a large number of crystal growth patterns, with different shapes and sizes.

Morphometric Analysis of the Green Jack Caranx caballus

The calculated sample sizes are: for sagitta, 21 individuals; asteriscus, 22 and lapillus, 15. There were no morphometric differences between the otoliths of male and female specimens.

Growth of the Sagitta

Results are shown in Table 1. The relationship between length and width of the sagitta is expressed by the value of exponent $b = 1.139$ which correspond to a positive alometric growth (Table 2). The determination index of the relationship of the two series of data is $R^2 = 0.80$, with a $F = 289$ of the ANOVA, which indicates that the sagitta grows more in width than lengthwise. Also, the allometric relationship between the length of the rostrum and of the antirostrum is expressed in the negative allometric growth index $b = 0.832$, with the highest values of R^2 and F obtained for this analysis, i.e., 0.912 and 744, respectively. This shows that among the sizes and age groups, the length of the antirostrum decreases until it disappears and remains as a protuberance (Fig. 4 and 5).

The slope of the relationship between the length and weight of the sagitta is of $b = 2.524$ ($R^2 = 0.88$ and $F = 505$) which indicates a negative allometric growth wherein the deposit of materials in the sagitta diminishes as the fish increases its length and age; this phenomenon is probably related

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

Classes (cm)	RL (mm)	AL (mm)	Wi (mm)	We (mg)
10	3.59	2.59	1.07	1.41
15	4.47	3.17	1.43	2.64
20	5.23	3.66	1.75	4.12
25	5.90	4.09	2.04	5.82
30	6.51	4.47	2.32	7.71
35	7.08	4.83	2.59	9.79
40	7.61	5.16	2.85	12.04
45	8.11	5.47	3.09	14.44
50	8.58	5.76	3.33	17.00
55	9.04	6.04	3.56	19.70
60	9.47	6.31	3.79	22.54

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

Rostrum	a	b	n	R^2	F
Antirostrum	0.935	0.832	74	0.912	744
Width	0.272	1.139	74	0.800	289
Weight	0.00007	2.524	74	0.880	505

Table 3: Relationships between the Standard Length of the fish (SL) and the measures of the sagitta

SL	a	b	n	R^2	F
Rostrum	1.034	0.541	74	0.825	344
Antirostrum	0.828	0.496	78	0.838	392
Width	0.212	0.704	78	0.863	479
Weight	0.00004	1.547	74	0.915	776

to the beginning of gonadic maturation where metabolic products are oriented to the formation of sexual features and fatty acids storage (Gallardo-Cabello, 1986a). These findings indicate that in different size classes and ages of fishes, the growth of otoliths is eccentric, i.e., the postrostrum grows more than the rostrum and antirostrum; likewise, the dorsal margin grows more than the ventral margin; finally, the deposit of material is higher in the internal than in the external aspect.

The relationship between the fish length and the length, width and weight of the sagitta is shown in the Table 3. The highest value of the allometric index related to the standard length of the fish corresponds to the width, b = 0.704; this means that there is a proportionality between the increase of the width of the sagitta and of the fish. This structure is suitable to describe the growth in length of the organism. The values of the determination index R^2 and ANOVA (F) show a high correlation between the analyzed structures in every case. The values of the allometric growth indexes are lower than 1 due to the difficulty to relate very small structures (in millimeters) with values of the corporal length of the fish (in centimeters).

Growth of the Asteriscus

The results of this are shown in Table 4. The relationship between the length and the width of the asteriscus (Fig. 11) is described by the allometric index b = 0.810 (R^2 = 0.289 and F = 30), which is a negative allometric growth wherein the increment in length is higher than in width owing to the fact that the dorsal margin grows proportionally more than the surface of the otolith.

The relationship between length and weight of the asteriscus shows a negative allometric growth index of b = 1.657 (R^2 = 0.138 and F = 12), a small value which describes the thinness of this structure with a large surface and a small volume (Table 5).

Table 4: Length (L), width (Wi) and weight (We) in different size classes of the asteriscus of *Caranx caballus*

Classes (cm)	L (mm)	Wi (mm)	We (mg)
10	0.79	1.02	0.16
15	0.96	1.35	0.30
20	1.10	1.64	0.48
25	1.23	1.91	0.69
30	1.34	2.16	0.92
35	1.45	2.40	1.18
40	1.54	2.63	1.46
45	1.63	2.85	1.76
50	1.72	3.06	2.08
55	1.80	3.26	2.43
60	1.88	3.46	2.79

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

Length	a	b	n	R^2	F
Width	1.623	0.810	75	0.289	30
Weight	0.0005	1.657	74	0.138	12

Table 6: Relationships between the standard length of the fish (SL) and the measures of the asteriscus

SL	a	b	n	R^2	F
Length	0.256	0.487	77	0.618	121
Width	0.213	0.681	75	0.541	86
Weight	0.000004	1.599	74	0.327	35

Table 7: Length (L), width (Wi) and weight (We) in different size classes of the lapillus of *Caranx caballus*

Classes (cm)	L (mm)	Wi (mm)	We (mg)
10	1.22	1.21	0.59
15	1.58	1.52	1.21
20	1.89	1.79	2.01
25	2.17	2.03	2.99
30	2.44	2.24	4.13
35	2.69	2.44	5.43
40	2.92	2.63	6.87
45	3.14	2.81	8.47
50	3.36	2.98	10.21
55	3.57	3.15	12.08
60	3.77	3.30	14.10

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

Length	a	b	n	R^2	F
Width	1.061	0.874	78	0.904	715
Weight	0.0003	2.796	77	0.890	609

According to this, the asteriscus growth is eccentric with respect to the core, since the anterior margin grows more than the posterior margin and the dorsal margin more than the ventral margin.

The relationship between the fish length and the otolith length, its width and weight are shown in Table 6). According to R^2 and F, the best relationship is between the fish length and the asteriscus length. The values of the allometric index 0.487 and 0.681, show a proportionality between the growth of the asteriscus and that of the fish, which makes it possible to determine the age groups through these structures.

Growth of the Lapillus

Table 7 shows the results of the relations of the lapillus (Fig. 12). There is a negative allometric growth index between the length and width of the lapillus (Table 8): b = 0.874 (R^2 = 0.904

Table 9: Relationships between the standard length of the fish (SL) and the measures of the lapillus

SL	a	b	n	R^2	F
Length	0.289	0.627	78	0.925	940
Width	0.335	0.559	78	0.869	505
Weight	0.00001	1.771	77	0.844	407

Table 10: Number of rings and average length (cm) of C. *caballus* observed in the sagittae and asterisci

Number of rings	Standard length (cm)
1	16.82
2	27.78
3	34.66
4	40.27

and F = 715) which indicates a larger growth in length of this structure in relation to its width. The analysis of the size classes and the age groups show that it grows more at the anterior margin than the rest of the otolith.

The relationship between the length and the weight of the lapillus (Table 8) shows a negative allometric growth, b = 2.796 (R^2 = 0.89 and F = 609); this suggests that the deposit of materials diminish as the fish ages and that calcium metabolism is directed to the spawning phenomena (Gallardo-Cabello, 1986a; 1986b).

The analyses also indicate that the growth of the lapillus is eccentric with respect to the core; it grows more at the anterior than at the posterior margin; more at the dorsal margin than at the ventral margin. A larger amount of material is deposited in the anterior than at the posterior margin for which reason its thickness diminishes substantially according to the sizes.

The relationships between fish length and the length, width and weight of the lapillus are shown in Table 9. The slope b = 0.627 together with the high value of R^2 and F (0.925 and 940, respectively) indicate a strong proportionality of the growth of the lapillus and that of the fish, which makes it possible to determine the age groups.

Identification of the Growth Rings

The analysis of the growth rings in the otolith sagittae allowed the identification of four groups (Table 10). The percentage of the sagittae otoliths which showed perfectly defined rings of growth was 100%. The growth rings can be observed with great clarity from the middle half to the dorsal margin and to the postrostrum of the otolith (Fig. 4); this area is where the deposit of the material takes place with more intensity.

The asterisci showed the same number of rings as the sagittae, in 25% of the cases; in the rest of the otoliths, growth rings were not identifiable.

In the case of the lapilli, it was not possible to identify growth rings, probably due to the thickness of the structure which prevents the observation of any mark with transmitted light and to the ornamentations of the surface of the external aspect, where there is not a regular disposition in the deposit of the material, but rather a great diversity of orientation patterns of the axes of the crystals; this is related to the piezoelectric capacity of the otoliths for the transmission of impulses.

Future studies on the physiology of the asterisci and the lapilli will aid to understand the structure and their function.

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