Otoliths analysis of *Mugil curema* (Pisces: Mugilidae) in Cuyutlan Lagoon, Mexico

Análisis de otolitos de *Mugil curema* (Pisces: Mugilidae) de la Laguna de Cuyutlán, México

Espino-Barr, E.;[1*] Gallardo-Cabello, M.;[2] Cabral-Solís, E. G.;[1] Puente-Gómez, M.[1] y García-Boa, A.[1]

[1]Instituto Nacional de Pesca, Centro Regional de Investigación Pesquera
Playa Ventanas s/n, Manzanillo, Colima; México (C. P. 28200).
[2] Instituto de Ciencias del Mar y Limnología
Universidad Nacional Autónoma de México
Av. Ciudad Universitaria 3000, Col. Copilco
México, D. F.; México (C. P. 04360).
*Corresponding author: elespino@gmail.com

Abstract

Morphology, morphometry and growth rings of the otoliths: *sagitta*, *asteriscus* and *lapillus* of *Mugil curema* of Cuyutlan Lagoon, Colima, Mexico were analyzed, also the difference between right and left and sexes were studied. Samples were obtained from the commercial catch in the months of August to October 2007, August 2008 and July 2011. In all cases it was observed that the growth in otoliths is eccentric with respect to the core. Relations between total length of the fish and length and width of the otolith showed that this structure is useful in the growth description of fish. Six growth rings were identified in *saggitae* and *asterisci*: specimens with two rings measured 21.80 cm of total length (TL) (± 1.41 standard deviation), with three rings 22.90 (± 0.91), with four rings 25.30 (± 0.39), with five rings 28.30 (± 0.78), and with six rings 31.10 (± 2.22). Due to its thickness, these marks could not be seen in *lapilli*. During the immature stage of development, female otoliths have larger size, but once they reach sexual maturity, males' otoliths reach larger lengths.

Resumen

Se analizaron la morfología, la morfometría y los anillos de crecimiento de los otolitos: *sagitta*, *asteriscus* y *lapillus* de la lebrancha o liseta *Mugil curema* de la Laguna de Cuyutlán, Colima, México; asimismo, se analizaron las diferencias entre derecho e izquierdo y entre sexos. Los ejemplares fueron obtenidos de la captura comercial en los meses de agosto a octubre de 2007, agosto de 2008 y julio de 2011. En todos los casos se observó que el crecimiento de los otolitos es excéntrico con respecto al núcleo. Las relaciones entre la longitud total del pez y la longitud y ancho de los otolitos demostraron que esta estructura puede usarse para describir el crecimiento del pez. Se identificaron seis anillos de crecimiento en las *sagittae* y *asterisci*: especímenes con dos anillos midieron 21.80 cm de longitud total (TL) (± 1.41 desviación estándar), los de tres anillos 22.90 (± 0.91), con cuatro anillos midieron 25.30 (± 0.39), con cinco anillos 28.30 (± 0.78) y con seis anillos 31.10 cm (± 2.22). En los *lapilli*, debido a su gran grosor, no fue posible observar marcas de crecimiento. Durante la etapa inmadura, los otolitos de las hembras son de mayor ta-

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Key words:

Sagitta, *asteriscus*, *lapillus*, white mullet.

maño, pero una vez que alcanzan la madurez sexual, los otolitos de los machos alcanzan longitudes mayores.

Palabras clave

Sagitta, *asteriscus*, *lapillus*, liseta o lebrancha.

Introduction

One of the most important aspects in the study of fish population dynamics is the identification of growth rings on hard structures, because it allows us to evaluate age groups in a stock and analyze the growth pattern (Campana and Neilson, 1985; Mascareña-Osorio *et al*., 2003; Begg *et al*., 2005; Gallardo-Cabello *et al*., 2003; Sparre and Venema, 1995). Changes in size and forms of hard structures of fish and the individual itself validate their use. Growth marks observed on hard structures of fish, such as scales, otoliths, vertebrae, spines, and opercula, are formed periodically because of the rapid and slow growth, that depend on food availability in its surrounding (Francis *et al*., 2005; Berg *et al*., 2005; Stevensen and Campana, 1992; Ehrhardt, 1981). Landmarks can also be formed as a result of reproduction and migration (Joseph, 1962; Ehrhardt, 1981; Sparre and Venema, 1995; Espino-Barr *et al*., 2008).

Growth ring identification is carried out generally in scales, because they are easier to obtain. Secondly, otoliths are also used, mainly the *saggita* which is the larger otolith and easier to extract. Few authors have used *asterisci* and *lapilli* otoliths to observe growth rings, and only in fish larvae (Victor and Brothers, 1982; Brothers *et al*., 1983; Solomon *et al*., 1985; Suthers *et al*., 1989; Barkman, 1978; Bolz and Lough, 1983: 1988; Lagardere, 1989; David *et al*., 1994). Description of the three pairs of otoliths (*saggita*, *asteriscus* and *lapillus*) on adult fish have been made by Gallardo-Cabello *et al*. (2006), Espino-Barr *et al*. (2006), Santana-Hernández *et al*. (2008), Granados-Flores *et al*. (2010) and Gallardo-Cabello *et al*. (2011).

Mugil curema Valenciennes, 1836 is a popular and traditionally consumed fish on the coast of Mexican Pacific, where its production in recent years (1990-2009) has fluctuated between three and eight thousand tons annually. The production of white mullet (called in Mexico liseta or lebrancha) in the Mexican Pacific compared to the national is 3% (SAGARPA, 2010). Its commercial value is not high, which makes it popular; it is sold directly by fishers on the beach at $10.00 to $20.00 mexican pesos per kg ($1 to $2 USD). This activity is important in the coastal communities; it gives work to many people. Cuyutlan Lagoon in Colima has a yearly capture of *M. curema* of 7 to 24 tons, which represents around 0.50% of the Mexican Pacific production. This lagoon has one of the most important fisheries in the state of Colima, besides it is also a place where many marine species seek protection, as nursery areas or to reproduce, far away from predators.

Studies on stock dynamics, based on age and growth analysis, use models on maximum sustainable yield, simulation and prediction for the captures. These models are able to point out levels of extraction to avoid the catch of juvenile organisms that have not yet reproduced. These models are also helpful suggesting catch quotas and capture indexes, to obtain adult organisms that have, by their reproduction, provided the next generation of juvenile organisms, and therefore prevent overexploitation.

It is therefore that this paper describes the morphology and morphometry of otoliths and identifies growth rings of *M. curema*, studying *sagittae*, *asterisci* and *lapilli*.

Material and Methods

Specimens were obtained from the commercial fishery in the Cuyutlan Lagoon (103°57' and 104°19' W, 18°57' and 19°50' N). The fishing gears were gill-nets of 2.25, 2.50 and 3.00 inches mesh size (5.71, 6.35 and 7.62 cm). Samples were obtained in the months of August, September and October 2007, August 2008 and July 2011. Total length was measured to the nearest mm (from the snout tip to the caudal fin extreme for 144 organisms). Sample size was determined to ensure the number of specimens was significant to calculations (Daniel, 1991).

Sex was determined *in visu* on fresh gonads of organisms taken to the laboratory the same day they were caught.

Otoliths: *sagittae*, *asterisci* and *lapilli* were obtained 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 analyzed with electronic microscope and the description of the system with a dissection microscope. The labyrinth system and the *sagittae* were described 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; 2011) 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 side of the three pairs.

Constants of the relationships of the *sagittae*, *asterisci* and *lapilli* were calculated for *rostrum* length (RL), and width (Wi). 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, using a potential relation, because the "*b*" parameter tends to one when there is a lineal correlation. 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) (Zar, 1996) was carried out. The same was made (ANOVA) between right and left *saggita*, *asteriscus* and *lapillus*.

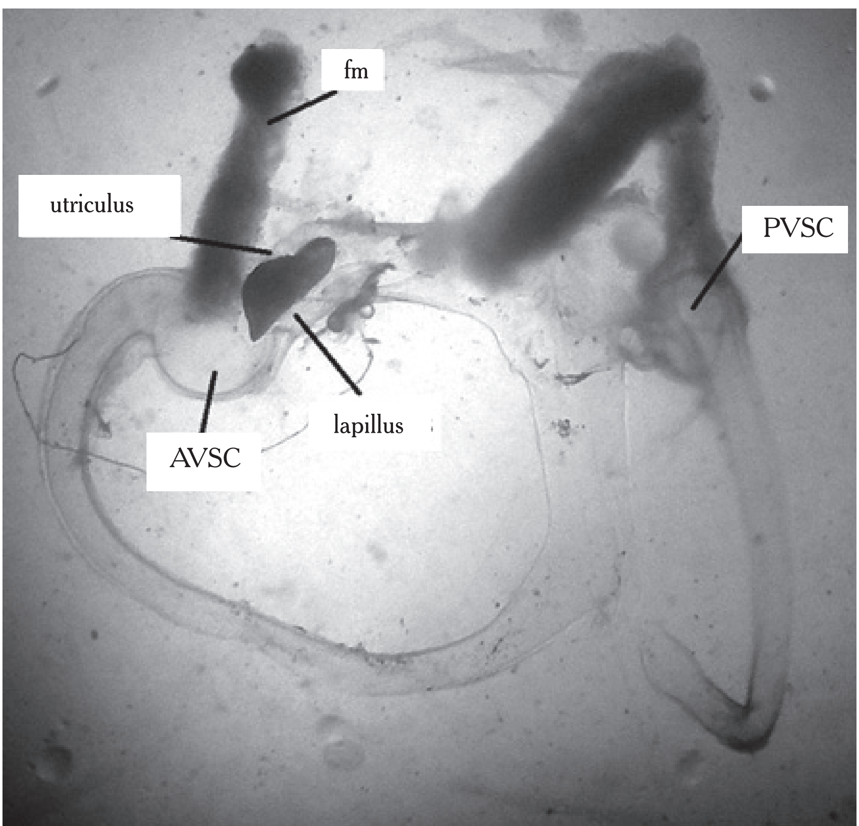
Identification and count of growth rings were done observing the *sagittae* and the *asterisci* in the stereoscopic microscope with transmitted light and the average length of the fish was calculated.

Results

Labyrinth system of Mugil curema

The membranous labyrinth presents a tubular system of semicircular canals called: anterior vertical canal (AVSC), posterior vertical canal (PVSC) (figure 1) and horizontal canal. These canals form chambers containing otoliths, the *lapillus* is included in the *utriculus* (figure 1), the *sagitta* in the *sacculus* and the *asteriscus* is included in the *lagena* (figure 2). The otoliths are immersed in their chambers in a liquid named endolymph (Lagler *et al.*, 1962).

Figure 1
Lapillus contained in *utriculus* of an individual of *Mugil curema* (25 cm of total length) (magnified 6.96 times).



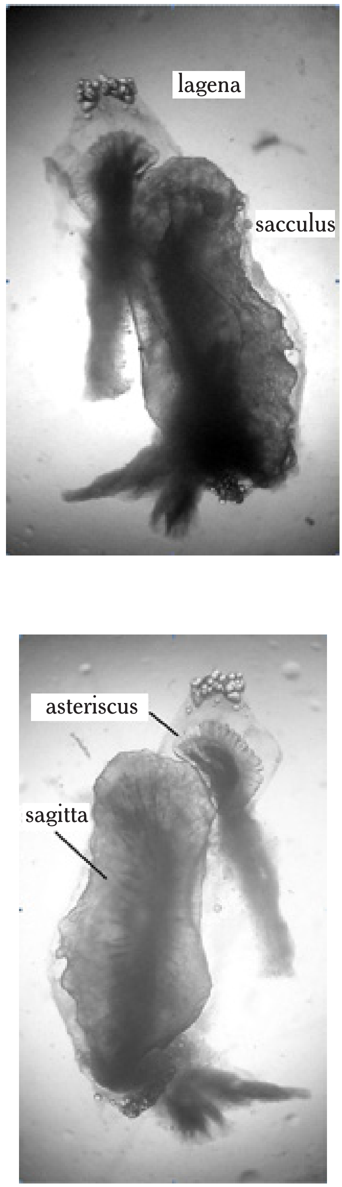
AVSC = anterior vertical semicircular canal; PVSC = posterior vertical semicircular canal; m= fragments of macula.

Figure 2

Sagitta in *sacculus* and *asteriscus* in *lagena* of the membranous labyrinth in an individual of *Mugil curema* (30 cm total length) (magnified 4.84 times).

a)

b)



a) Internal side showing the macula running along the acoustic canal of the *sagittal* and the *asteriscus* with the macula in its acoustic canal; b) external aspect.

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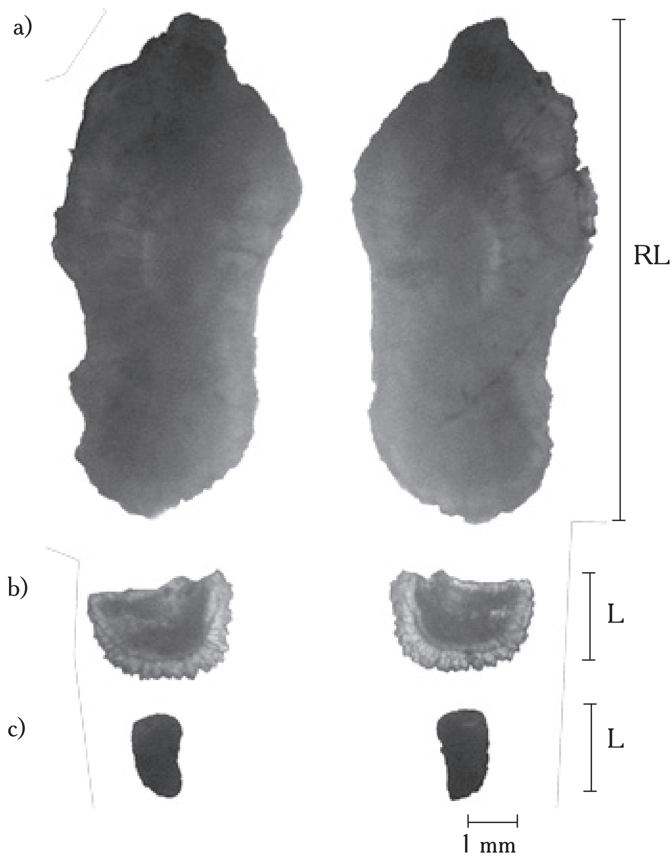
The otoliths are in contact with the acoustic macula that enters in the acoustic canal of the *sagitta* and the *sulcus* of the *asteriscus* and the *lapillus*.

The largest otolith is the *sagitta* reaching a total length of 14.32 mm, while *asteriscus* measures 2.87 mm and *lapillus* 2.33 mm in individuals of 25 cm total length (figure 3).

Figure 3
Left and right otoliths of *Mugil curema*: a) *sagittae*, b) *asterisci* and c) *lapilli*.



RL = *rostrum* length, L= length. Note: magnified 2.58 times.

Description of the sagitta

The anterior margin of the *sagitta* shows a rostrum in the form of a bump, without excisura major (notch in the margin of the fish otolith separating the *rostrum* and *antirostrum*) and therefore no *antirostrum*. As the fish ages the shape of the *rostrum* varies (figure 4).

Figure 4
Different shapes and sizes of right *sagittae* of *Mugil curema*.

Note: magnified 3.2 times.

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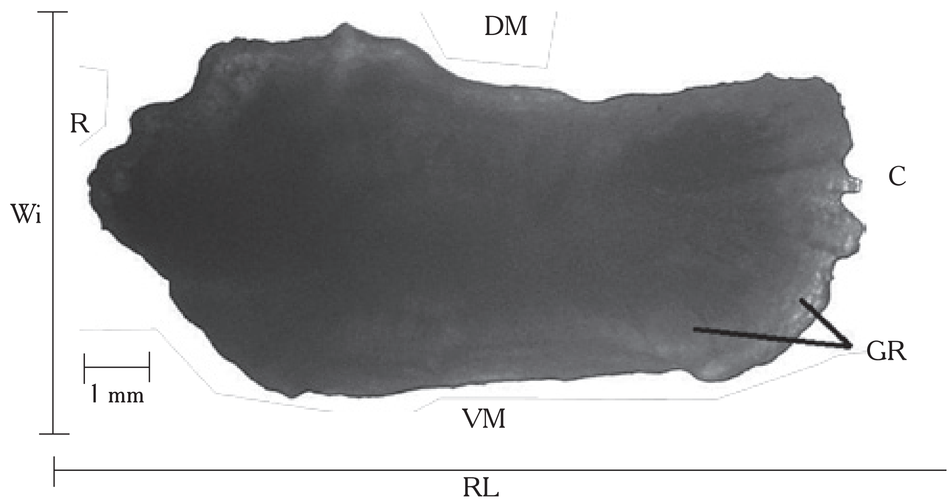
Its form varies between specimens and also in right and left *sagittae* of the same individual. The posterior section of the *sagitta* shows a rounded *postrostrum* without excisura minor (notch in the margin of the fish otolith separating the *postrostrum* and *pararostrum*) and therefore no *pararostrum*. The dorsal margin has round *rostrum* and *postrostrum* with a rectilinear profile in the middle section of the otolith. The ventral side shows irregularities, similar to big denticles, which are more apparent in the *rostrum* section (figure 5).

Figure 5
Left *sagitta*, external side of *Mugil curema* of 26 cm TL.



RL= *Rostrum* length; C= *postrostrum*; DM = dorsal margin; VM = ventral margin; Wi = width; GR= zone where growth rings are seen (magnified 8.92x).

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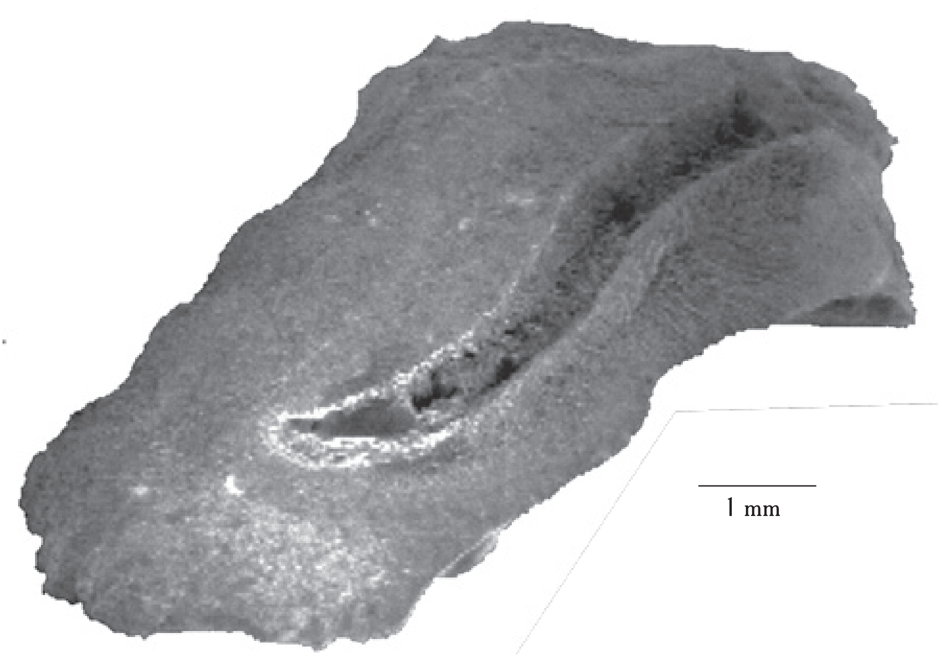
The internal side of the *sagitta* is convex, a feature which increases with age, its surface is smooth and it is traversed completely by the acoustic channel (figure 6) with the same width from the anterior to the posterior part of the otolith, there is no a differentiation between *ostium* and *cauda*.

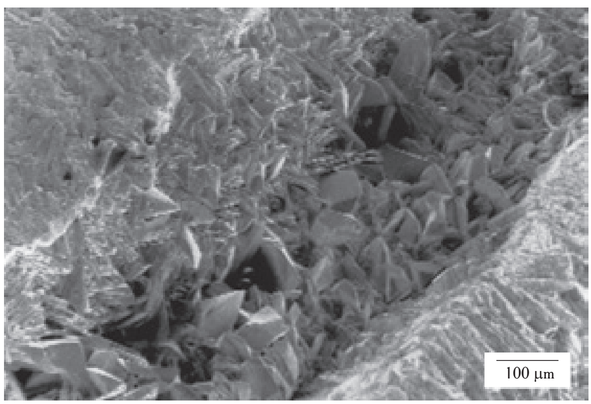
Figure 6
Scanning photograph of the internal side of the right *sagitta* of *M. curema* showing the acoustic channel.



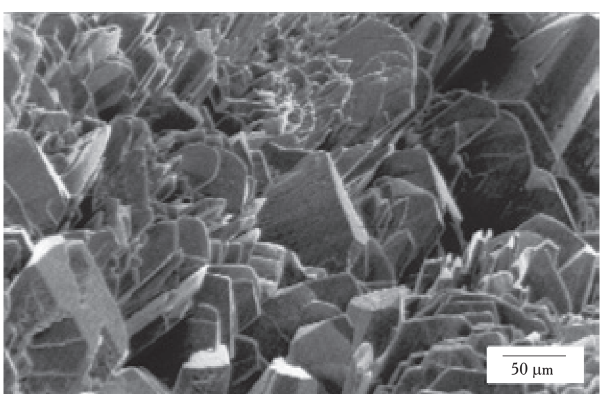
The *sulcus* shows in its base different growth patterns of calcium carbonate crystals which are arranged epitaxially, that could increase the impulse transmission through the acoustic macula. Figure 7a shows the acoustic canal, where the difference of patterns are more apparent between the base and the walls. Figure 7 b is a detail of the base of the acoustic canal of sagitta.

Figure 7
Scanning photograph on the base of the acoustic canal, in the right *sagitta* internal side of *M. curema*, showing the calcium carbonate crystals.

a)



b)



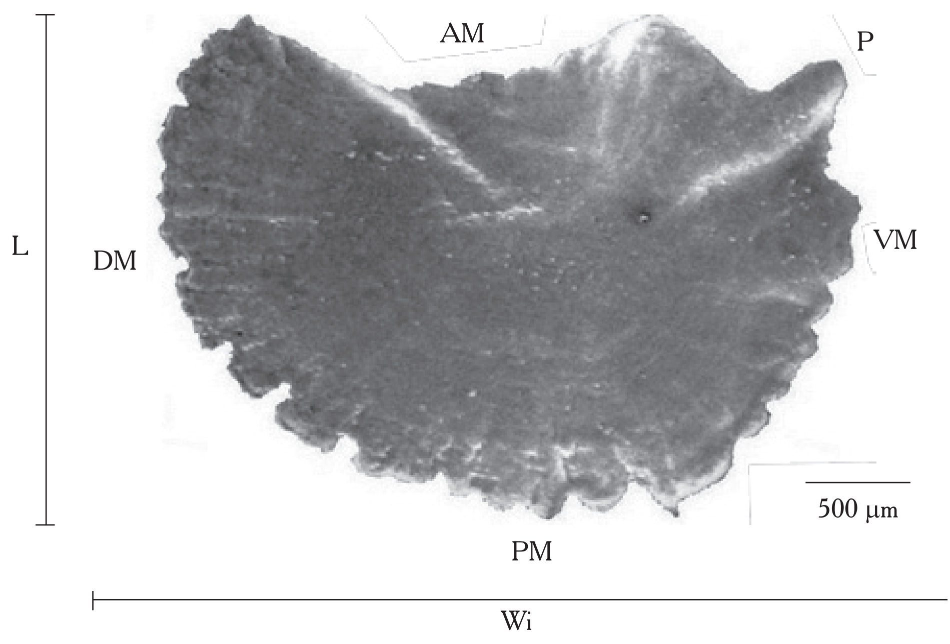
The external side of the *sagitta* is concave without variations in its thickness. Average width of the *sagitta* is 2.24 times its average length.

Even though there is visual difference in the shapes and details of individual *sagitta*, there was no statistical difference between the length of the left and the right *sagittae* ($F'_{0.05\ (2,\ 120=3.92)} = 0.056$, $p>0.05$). Also, there were no statistical difference between males and females *sagittae* shapes ($F'_{0.05\ (2,\ 95=3.941)} = 0.670$, $p>0.05$).

Description of the asteriscus

The anterior margin shows a blunt projection which divides the otolith between two parts: dorsal part with a larger surface than the ventral part. The anterior margin shows some sections that can be rectilinear from the dorsal to the ventral margins (figure 8).

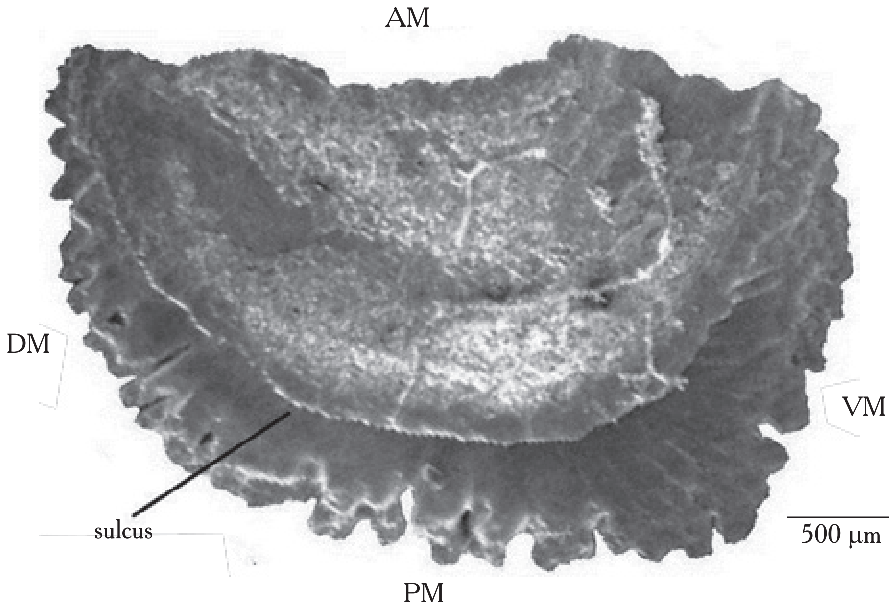
Figure 8
Scanning photograph of the left *asteriscus*, external side of *M. curema*.



AM= Anterior margin; PM= posterior margin; P= projection; DM= dorsal margin; VM= ventral margin; L= length, Wi= width.

The posterior margin is rounded presenting interruptions in the form of notches. The posterior margin presents grooves all around the dorsal and ventral margins which divide the otolith in two parts. The first part side has larger sized radius in the external aspect (posterior external margin) (figure 9).

Figure 9
Scanning photograph of the right *asteriscus*, internal side of *M. curema*.

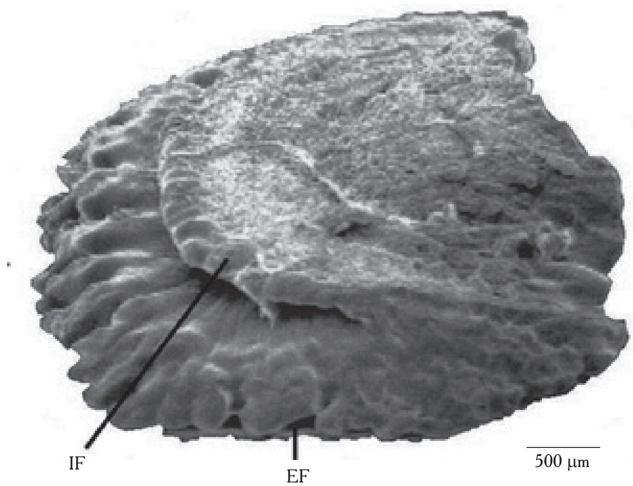


AM = Anterior margin; PM = posterior margin; DM = dorsal margin; VM = ventral margin.

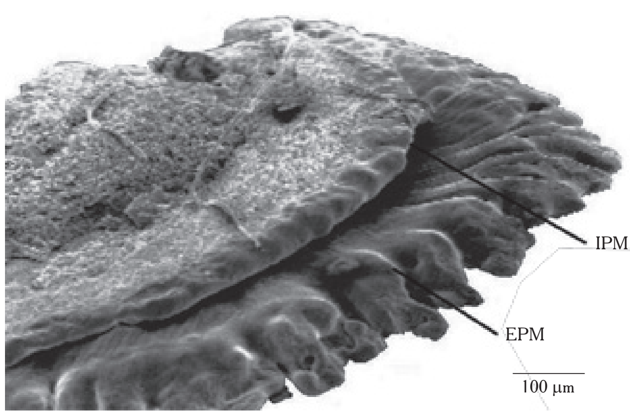
The smaller radius in the internal aspect of the otolith (posterior internal margin) is in contact with the acoustic macula (figure 10). The external side of the *asteriscus* is convex, a feature which increased as the fish ages, the internal side is concave.

Figure 10
Scanning photograph of the right *asteriscus*, internal side of *M. curema*, showing details of the sulcus.

a)



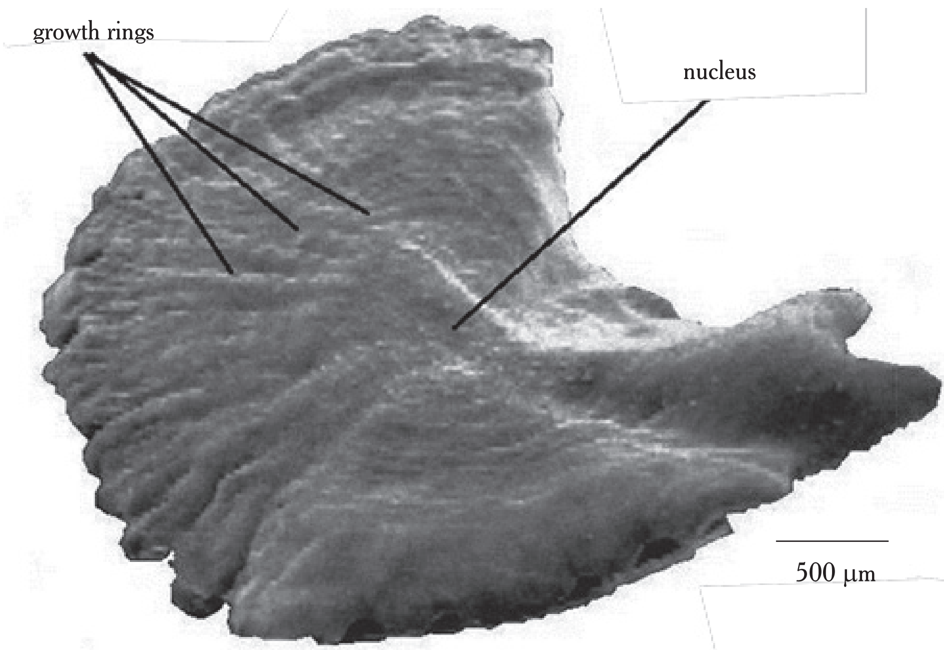
b)



a) IF = Internal aspect and EF = external aspect; b) IPM = internal posterior margin and EPM= external posterior margin.

Growth rings can be observed in the external side of the *asteriscus* (figure 11). Its average length is 0.69 times its average width. At this moment their periodicity cannot be confirmed, but these are rings formed by the aggregations of materials deposited during growth periods.

Figure 11
Scanning photograph of the left *asteriscus*, external side of *M. curema* showing growth rings.



The shape of the *asterisci* varies between specimens and between right and left, as in the *sagittae* but there were no statistical difference in their length ($F'_{0.05\ (2,\ 95=3.941)}$ = 0.256, $p>0.05$). Also between males and females, no statistical difference was found ($F'_{0.05\ (2,\ 94=3.942)}$ = 0.278, $p>0.05$).

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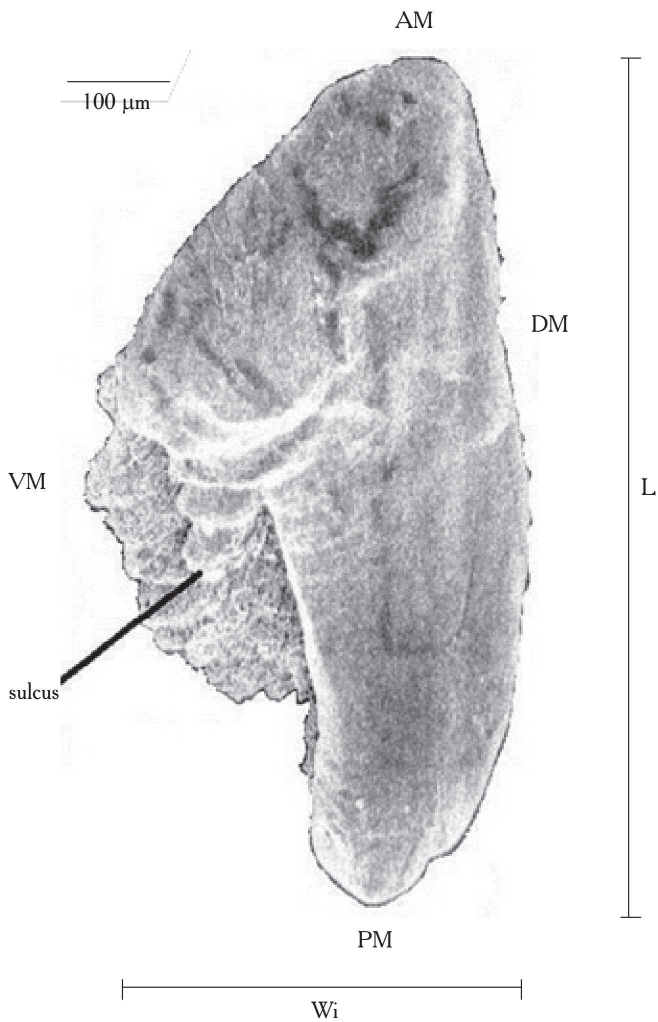
Description of the lapillus

The anterior margin of the otolith is rounded and it is oriented toward the front of the fish. Ventral margin descend towards the central part of the otolith and it is shorter than dorsal margin, which is more enlarged and projected towards the posterior edge (figure 12).

Figure 12
Scanning photography of the right *lapillus* external side of *M. curema*.



AM = Anterior margin; PM = posterior margin; DM = dorsal margin; VM = ventral margin; L = length and Wi = width.

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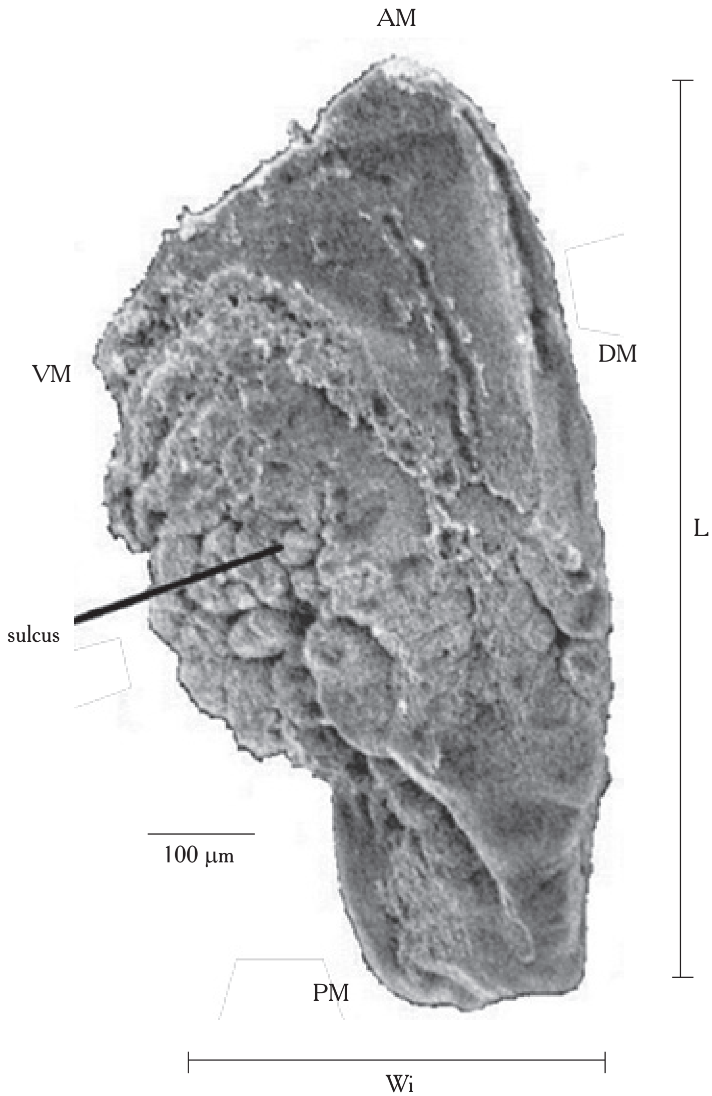
The inner surface is concave, a feature which increase with age. The *sulcus* is found in the posterior border, where it comes in contact with the acoustic macula which extends along the dorsal and ventral margins (figure 13).

Figure 13
Scanning photograph of the left *lapillus*, internal side of *M. curema.*

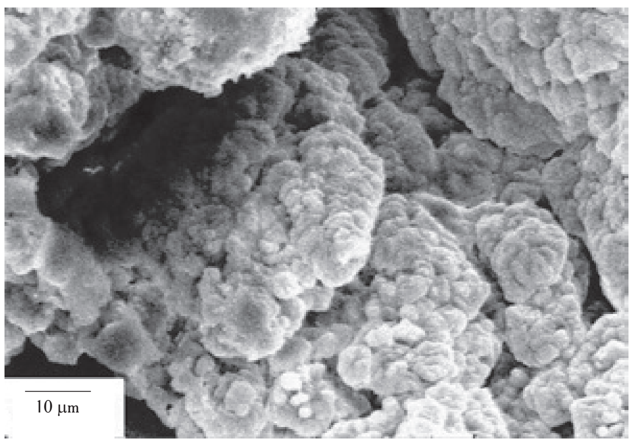


AM = Anterior margin; PM = posterior margin; DM = dorsal margin; VM = ventral margin; L = length and Wi = width.

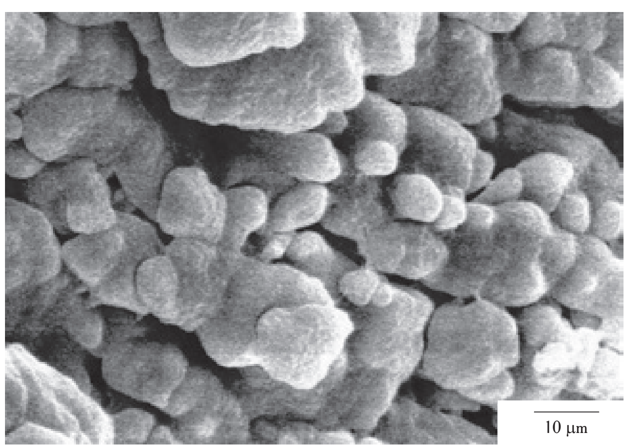
The *sulcus* shows a large number of calcium carbonate crystals arranged epitaxially with different growth patterns in shape and size that could increase the impulse transmission to the eighth cranial nerve. Figure 14a shows a close up of the *sulcus* with different patterns at the base and the walls. Figure 14b is a close up of the rounded cusps of the *sulcus* formation.

Figure 14
Scanning photograph of the left *lapillus*, internal side of *M. curema*, showing the calcium carbonate crystals in the base of the *sulcus*.

a)



b)



The outer aspect is convex, its dorsal margin shows a smooth surface. Average width of the *lapillus* is 0.47 times its average length.

As in the other two otoliths, visual details were noticed in *lapilli*, although statistical differences could not be found between the length of right and left ($F'_{0.05\ (2,\ 125=3.917)}$ = 0.699, p>0.05), and between males and females ($F'_{0.05\ (2,\ 125=3.917)}$ = 0.499, p>0.05).

Morphometric analysis of otoliths of Mugil curema

To analyze the morphometric relationships of otoliths, 152 organisms were obtained randomly from the commercial fishery were taken to the lab where otoliths were obtained. Specimen sizes ranged from 20 to 33.60 cm of total length (TL), an average of 24.30 cm (± 2.92 standard deviation); 100 females, 43 males and 9 juvenile organisms that could not be sexed. Sample size determined a minimum of 113 sagittae, 172 asteriscus and 26 lapillus (α = 0.05), meaning a higher variation in asteriscus.

Growth of the sagitta

Table 1 shows the calculated average of the rostrum length (total otolith length) and width of *sagitta* by length classes of fish and sexes. Growth of the *rostrum* and width of *sagitta* are smaller in males during lengths 5 to 20 cm; thereafter 25 to 45 cm growth rate decreases in females and it is higher in males' *sagittae*, ANCOVA's result is $F_{(3,138)}$ = 1.54.

Table 1
Calculated measures of rostrum (RL) and width (Wi) at different size classes of the *sagitta* of *Mugil curema*.

Classes	Both sexes		Females		Males	
(cm)	RL (mm)	Wi (mm)	RL (mm)	Wi (mm)	RL (mm)	Wi (mm)
5	7.19	3.19	7.35	3.25	5.73	2.73
10	9.68	4.30	9.79	4.34	8.59	3.95
15	11.51	5.13	11.58	5.14	10.88	4.91
20	13.02	5.81	13.04	5.80	12.86	5.73
25	14.32	6.40	14.30	6.37	14.65	6.46
30	15.49	6.92	15.42	6.88	16.29	7.13
35	16.54	7.40	16.44	7.34	17.82	7.74
40	17.52	7.84	17.38	7.76	19.27	8.31
45	18.42	8.25	18.24	8.15	20.64	8.86

RL=*Rostrum* length; Wi=width.

The relationship between RL and Wi of *sagitta* is expressed by the exponent value b = 0.683, which is a negative allometric growth (table 2) indicating that the *sagitta* tends to lengthen as the fish ages. Similar results are found in the relations of females b = 0.658 and males b = 0.687. These results show that the growth of *sagitta* is eccentric to the core. This is a characteristic which increases with age, *i.e.* the *postrostrum* grows more than the *rostrum*. The dorsal edge grows more than the ventral edge, and a larger amount of material accumulates on the inner than the external side.

Table 2
Relationship between *rostrum* length and width of *sagitta*.

	a	b	n	r^2	F
Both sexes	1.039	0.683	152	0.40	93.21
Females	1.115	0.658	100	0.36	54.95
Males	1.015	0.687	43	0.45	32.94

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 RL and Wi 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 = 0.583, smaller values are found for the species and females, b= 0.428 and b= 0.414, respectively. Similarly, the relation between fish length and *rostrum* width for males has a value of b= 0.536 (0.419 for females and 0.433 for species). In all cases, the values show that there is a direct proportionality between *sagitta* length, width 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.

Table 3
Relationship between the total length of the fish and the measures of *sagitta*.

		a	b	n	r^2	F
RL	Both	3.612	0.428	144	0.64	249.97
	Females	3.773	0.414	100	0.63	166.77
	Males	2.243	0.583	43	0.71	101.98
Wi	Both	1.587	0.433	152	0.57	190.81
	Females	1.654	0.419	100	0.55	125.67
	Males	1.1506	0.536	43	0.57	54.32

a= Origin ordinate of relation; b= slope of relation; n= number of individuals; r^2= determination index of relation; F= Fischer index of relation.

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, although not statistically.

Table 4
Calculated measures of length (L) and width (Wi) of *asteriscus* at different length classes of *Mugil curema*.

Length classes	Both sexes		Females		Males	
(cm)	L (mm)	Wi (mm)	L (mm)	Wi (mm)	L (mm)	Wi (mm)
5	1.36	1.88	1.63	1.86	0.81	1.71
10	1.88	2.64	2.08	2.63	1.42	2.53
15	2.27	3.23	2.40	3.21	1.96	3.18
20	2.59	3.73	2.65	3.70	2.48	3.74
25	2.87	4.16	2.87	4.14	2.96	4.24
30	3.13	4.56	3.05	4.53	3.43	4.70
35	3.36	4.92	3.22	4.89	3.88	5.13
40	3.57	5.25	3.38	5.22	4.33	5.53
45	3.78	5.57	3.52	5.54	4.76	5.91

The relationship between the length and width of the otolith (table 5) is described by the allometric index b = 0.468 ($r^2 = 0.374$) and (F = 55.598) with similar values for males and females, the latter having a higher value of the allometric index of 0.497. Although r^2 has a very low value, F is high, which indicates that the relation is significant. The low values of r^2 are a result of sampling the commercial catches which have fish sizes from certain length on, between 20 and 40 cm. These results show a negative allometric growth in which the increase in width is greater than length, so, *asteriscus* growth is eccentric to the core; and its anterior border grows more than the posterior border and the dorsal margin grows more than the ventral margin.

Table 5
Relationship between the length and width of the *asteriscus*.

	a	b	n	r^2	F
Both sexes	2.534	0.468	95	0.374	55.598
Females	2.459	0.497	66	0.368	37.205
Males	2.650	0.423	29	0.389	17.213

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 shows the relationship between fish total length and length and width of *asteriscus*. The allometric index value closest to one is for males b = 0.805 (statistically different from one, negative allometry), for fish total length and *asteriscus* length. Values of the Anova (F) show that the correlation between the structures analyzed in each case, with a tendency to a direct proportionality between fish length and *asteriscus* length are significant. The relation values are low because the sizes of the organisms are those obtained in commercial fishery and organisms smaller than 20 cm are missing.

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

		a	b	n	R^2	F
Length	Both	0.643	0.465	98	0.31	43.32
	Females	0.932	0.349	68	0.21	17.12
	Males	0.222	0.805	29	0.59	38.38
Width	Both	0.846	0.495	95	0.64	165.50
	Females	0.838	0.496	66	0.67	129.29
	Males	0.688	0.565	29	0.63	46.37

a= Origin ordinate of relation; b= slope of relation; n= number of individuals; R^2= determination index of relation; F= Fischer index of relation.

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 25 mm in length.

Table 7
Calculated measures of length (L, mm) and width (Wi, mm) of *lapillus* at different length classes of *Mugil curema*.

Length	Both sexes		Females		Males	
classes	L (mm)	Wi (mm)	L (mm)	Wi (mm)	L (mm)	Wi (mm)
5	1.06	0.62	0.99	0.80	1.04	0.36
10	1.48	0.79	1.43	1.04	1.49	0.67
15	1.81	0.92	1.76	1.21	1.84	0.96
20	2.09	1.02	2.05	1.35	2.14	1.24
25	2.33	1.11	2.31	1.46	2.41	1.51
30	2.54	1.19	2.54	1.56	2.65	1.78
35	2.74	1.26	2.75	1.66	2.87	2.04
40	2.93	1.32	2.95	1.74	3.08	2.30
45	3.11	1.38	3.14	1.82	3.27	2.56

The relationship between the length and width of the *lapillus* (table 8) shows that the higher value of the allometric index was found for females with a value of $b = 0.916$, smaller indexes were found for the species and males: $b= 0.869$ and $b= 0.613$, respectively. These values represent a negative allometric growth, in which *lapillus* grows more lengthwise than in width. *Lapillus* growth is eccentric to the core, which means, the anterior and ventral margins show a higher deposition of growth materials than the posterior and dorsal margins.

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Table 8
Relationship between length and width of *lapillus*.

Otolith length	a	b	n	r^2	F
Both sexes	0.874	0.613	129	0.34	66.06
Females	0.566	0.916	95	0.34	48.53
Males	0.689	0.869	33	0.39	19.66

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 *lapillus* is shown in table 9. The higher value of the allometric index for fish total length and *lapillus* width was found for males with a value of b = 0.891 which suggests a trend toward direct proportionality between fish length and *lapillus*. This proportionality validates the use of otolith to evaluate the growth of white mullet *M. curema*.

Table 9
Relationship between fish length and other measures of *lapillus*.

	Sex	a	b	n	R^2	F
Length	Both	0.479	0.491	129	0.44	98.05
	Females	0.427	0.524	95	0.47	82.70
	Males	0.447	0.523	33	0.39	20.18
Width	Both	0.343	0.365	129	0.31	56.41
	Females	0.440	0.373	95	0.26	31.96
	Males	0.086	0.891	33	0.59	44.13

Identification of growth rings

Otoliths of 60 specimens were observed with stereoscopic microscope to find growth rings. These were observed in an area from the middle of the otolith to the *posrostrum* on the dorsal margin. The rings on asteriscus were observed on the external side.

Growth ring analysis in *sagittae* otoliths of the *Mugil curema* allowed the identification of seven age groups (table 10). The percentage of *sagittae* showing clearly defined growth rings was 100%. Growth rings are seen in an area from the middle of the otolith to the *posrostrum* on the dorsal margin; in this region the calcium carbonate is deposited faster (see figure 5).

Table 10
Number of the rings and average length (LT cm±S.D.) of *Mugil curema* in the *sagittae* and scales from several authors.

Number of rings	Otoliths *	n	Scales **	Otoliths ***
0			10.18	18.30
1	14.00 (± 0)	1	14.85	22.30
2	21.8 (± 1.41)	11	19.24	25.20
3	22.9 (± 0.91)	28	22.64	27.80
4	25.3 (± 0.39)	10	25.33	30.30
5	28.3 (± 0.78)	5	27.07	32.50
6	31.1 (± 2.22)	5		

S. D. Standard deviation.
*Present paper.
**Cabral-Solís (1999).
***Ibáñez-Aguirre (1995).

Thirty seven percent of *asterisci* showed the same number of rings as *sagittae*, in the remaining 63%, 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 (see figure 11).

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.

Discussion

Retrieving otoliths is not easy most of the time, they can brake easily, and therefore the number of items (n) is different for *sagittae*, *asterisci* and *lapilli*. The same happened to the semicircular canals and the labyrinth system. In this work, the description of such items was done based on bibliography. The acoustic macula transmits vibrations of the otoliths through the eighth cranial nerve to the brain (Mugiya, 1964; 1966a;b). This acoustic macula allows nutrient deposition and is responsible for the growth of otoliths (Gallardo-

Cabello *et al*., 2006; 2011; Espino-Barr *et al*., 2006). *Sagittae* and *asterisci* are related with the sound perception, gravity and angular acceleration. The third pair of otoliths, *lapilli*, is responsible for balance (Holst *et al*., 1950; Lowenstein, 1957).

The *sagittae* are made of calcium carbonate (Lagler *et al*., 1962) in the form of aragonite (Hickling, 1931; Sasaki and Miyata, 1955; Carlström, 1963; Gallardo-Cabello, 1986) and of a high molecular weight protein named otoline (Degens *et al*., 1969).

Right and left otoliths of *sagittae*, *asterisci* and *lapilli* can show morphologic differences in the same organism, but morphometric differences were not statistically significant.

In all studied otoliths, growth was higher in females than in males during the early stages preceding gonadal maturity; once it starts —when fish reach 25 cm total length (Cabral-Solís *et al*., 2010)— otolith growth was higher in males. This phenomenon may be also related to the beginning of gonad maturation and reproduction, when metabolism of calcium and protein are oriented to egg formation and fatty acids storage, and therefore, in females reach a higher ratio. Thus, growth of otoliths decreased more markedly in females than in males (Gallardo-Cabello, 1986; Gallardo-Cabello *et al*., 2011).

In the present paper it was possible to identify six growth rings on otoliths, compared with five observed by Cabral-Solís (1999) using scales (table 10), probably to the larger mesh sized gillnets used in this work. It is important to note that when the individuals grow, the *sagittae* becomes more curved, making it difficult to observe the last growth rings on the border of the older specimens (see figure 3). Ibáñez-Aguirre and Gallardo-Cabello (1986) also observed this phenomenon, being possible for these authors only the identification of five growth rings in the otoliths of *Mugil curema* from Tamiahua lagoon, Veracruz, Mexico.

Average length of growth rings obtained in the present paper were equal (growth ring 4) or very similar to those reported by Cabral-Solís (1999) working with scales from *M. curema* in Cuyutlan Lagoon. Average length for each growth ring determined on *sagittae* in *M. curema* from Tamiahua lagoon by Ibáñez-Aguirre (1995) was higher than those reported in the present paper (table 10). Also, the mean length from the nucleus to each fast growth band of the *sagittae* (table 11) shows the same phenomenon; values from *M. curema* in Tamiahua were higher to the obtained in the present study.

Table 11
Mean length (mm) from nucleus to each fast growth band of the *sagittae* of *M. curema*.

Number of rings	Present paper	Ibáñez-Aguirre and Gallardo-Cabello (1996)
1	0.8	1.25
2	1.2	2.05
3	1.5	2.81
4	1.7	3.47
5	1.9	3.76
6	2.1	

Average length and width of the nucleus of *sagittae* of *M. curema* from the Cuyutlan Lagoon were 11 and 3.50 mm, respectively. Width of the *sagittae* core of *M. curema* from Tamiahua Lagoon was 9 mm, which means 2.57 times bigger (Ibáñez-Aguirre and Gallardo-Cabello *et al.*, 1996). Taylor (1958; 1960) reported the fish growth as an indirect proportional relation index to latitude and temperature; temperature in Tamiahua Lagoon ranges from 10.3°C to 33°C, and in Cuyutlan Lagoon varies 17.5°C to 34.2°C (Gallardo-Cabello *et al.*, 2005). The temperature could partially explain the differences in fish size between Cuyutlan Lagoon and Tamiahua Lagoon.

Santana *et al.* (2009) carried out a study on micro- and macrostructure analysis of *sagittae* in *M. curema* from the coasts of Brazil. They analyzed daily growth increments in organisms from 1.3 to 15.7 cm of fork length finding five growth zones. Zones I to III corresponded to the first annual growth, meanwhile Zone IV indicated daily growth increment corresponding to the second annual growth ring. From Zone V on, it was not possible to observe daily increment rings, therefore, subsequent rings were considered annual periods.

In our case, we did not consider daily growth increments, or smaller than annually, because growth marks were not visible during the first sampled year and then, these disappeared after the second yearly ring, period related to spawning season. From this period, otolith growth decreases and calcium carbonate and otoline materials depositions were lower, and the growth ring pattern became irregular and difficult to observe. Also at this stage, interruptions sutures made it impossible to identify daily growth increments. This same phenomenon was observed for the daily growth increment and annual periodicity rings in *asterisci*, in which after the second growth mark, deposition of daily increments was diffuse.

We consider that studies on daily growth increments of otoliths, besides giving more information on growth ring identification and age determination, explain many life cycle phenomena of the organisms during their first life stages, that is, their larvae period, migration from planktonic media to pelagic, demersal and benthonic media and other changes that larvae go through, and also juvenile stages to adults before their recruitment to the area and fishing gears. These studies on otolith microstructure should be expanded, not only on sagittae, but also on asterisci and lapilli otoliths.

Conclusions

No morphometric differences were found between right and left otoliths of fish sides. There was statistical difference between sexes: in early years, otoliths of females were larger until their first reproductive maturity; then, males otoliths tend to reach larger sizes. In all cases otoliths growth is eccentric to the core.

In this paper, six growth rings in *sagittae* and *asterisci* were identified.

Average length and width of the core in the *sagittae* otolith of *M. curema* from Cuyutlan Lagoon, were 11 mm and 3.50 mm, respectively.

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