

Ontogenetic and sexual variation in the *sagitta* otolith of *Menticirrhus americanus* (Teleostei; Sciaenidae) (Linnaeus, 1758) in a subtropical environment

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Abstract. This study aimed to verify variations in the form of the *sagitta* otolith of *Menticirrhus americanus* as to their ontogeny, sex and stage of such structure. Ontogenetically were found significant differences ($p < 0.05$) for all shape indices (aspect ratios, shape factor, rectangularity, ellipticity, relative surface of *sulcus acusticus*) and also in the wavelet of the otolith. The CVA presented a 98.6% correct reclassification of the otolith between interval class. Sexual variations were found in the wavelet and in the relative surface of *sulcus acusticus* index. Between adult females and young females, differences were not detected in the wavelet and rectangularity, but significant differences were found in all other shape indices. The CVA presented a 79.6% correct reclassification of the otolith of the sexes and stages of life sampled. Young females and adults females showed highest correct percentage of classification. The results of this study demonstrate for the first time the influence of the ontogenetic variation and sexual in the form of the otoliths.

Key-Words. Dimorphism; Otolith; Shape indices; Stage of life; Wavelet.

INTRODUCTION

Otoliths are calcium carbonate precipitated primarily as aragonite structures. Present in the inner ear in three pairs: *sagittae*, asteriscus and lapillus (Popper *et al.*, 2005). Their morphological patterns make them an important tool (Volpedo & Echeverría, 2000; Volpedo *et al.*, 2017). In this way, they are useful in studies of trophic ecology of ichthyophagous (Bustos *et al.*, 2012; Miotto *et al.*, 2017), in the identification of fish stocks through morphology and morphometry (Avigliano *et al.*, 2015; Zischke *et al.*, 2016), habitat use (Avigliano *et al.*, 2014; Carvalho *et al.*, 2017), connectivity between populations (Teimori *et al.*, 2012; Tanner *et al.*, 2013; Davoren & Halden, 2014) and age and growth (Vaz-dos-Santos & Rossi-Wongtschowski, 2007; Egbert & Rulifson, 2017).

Although otoliths have interspecific patterns among species, some morphological changes may occur influenced by various physiological or external factors (Volpedo & Echeverría, 1999).

Reproduction is one of the physiological factors that may influence the morphology of otoliths (Tombari *et al.*, 2005; Carvalho & Corrêa, 2014). Some species of the family Atheronipidae after the onset of the reproductive process develop a dorsal depression in the *sagitta* otolith (Tombari *et al.*, 2005; Carvalho & Corrêa, 2014). In addition to morphological differences between young and adult, it is also possible to identify variations in otolith growth rates before and after the first maturity (Carvalho *et al.*, 2015). Food availability may also differentiate otolith morphology among species (Gagliano & McCormick, 2004).

Also, in some species, the biomineralization of otoliths causes morphological and morphometric variations between the sexes (Légua *et al.*, 2013; Mille *et al.*, 2015; Bose *et al.*, 2016). Different sex growth rates may also influence the morphological and morphometric patterns of otoliths; this change was observed in *Micropogonias furnieri* (Bervian *et al.*, 2006) and *Anchoa tricolor* (Carvalho *et al.*, 2015) on the subtropical Southwestern Atlantic Ocean.

Pap. Avulsos Zool., 2020; v.60: e20206009

<http://doi.org/10.11606/1807-0205/2020.60.09>

<http://www.revistas.usp.br/paz>

<http://www.scielo.br/paz>

Edited by: Fernando César Paiva Dagosta

Received: 21/05/2019

Accepted: 16/12/2019

Published: 04/02/2020

ISSN On-Line: 1807-0205

ISSN Printed: 0031-1049

ISSN: 0000-0004-0384-1825



In addition to the physiological factors cited above, environmental factors may influence the morphology and morphometry of otoliths. The depth is related to alterations in the *sulcus acusticus* area allowing a greater sound perception (Torres *et al.*, 2000; Cruz & Lombarte, 2004). Salinity influences the otolith shape of *Odontesthes bonariensis* and it is possible to identify processes of environmental salinization or habitat change (Avigliano *et al.*, 2012; Avigliano *et al.*, 2014), the same process of habitat change was identified for *Anguilla anguilla* (Capoccioni *et al.*, 2011). Thermal amplitudes in water bodies also modify the shape of otoliths in populations exposed to this factor (Lombarte & Leonart, 1993; Légua *et al.*, 2013).

Some methods can be used to describe the morphology and morphometry of otoliths (Lombarte & Tuset, 2015). Shape indexes contribute in understanding the morphometric relationships between fish and otoliths (Volpedo & Echeverría, 1999; Tuset *et al.*, 2003; Carvalho *et al.*, 2015). The evaluation of the contour of the otoliths can be done through different methods such as: polar coordinates (Lombarte & Tuset, 2015), landmarks (Monteiro *et al.*, 2005; Carvalho *et al.*, 2015), fourier harmonics (Libungan *et al.*, 2015; Bose *et al.*, 2016) and wavelets (Sadighzadeh *et al.*, 2014; Tuset *et al.*, 2015). According Sadighzadeh *et al.* (2012), the fourier harmonics present better results in phylogenetically distant species while wavelets are more efficient in the differentiation of nearby species and identify intraspecific variations.

The morphology and morphometry of the *sagitta* otolith of sciaenids has been described by several studies (Corrêa & Vianna, 1992; Volpedo & Echeverría, 1999, 2000; Waessle *et al.*, 2003; Siliprandi *et al.*, 2014). *Menticirrhus americanus* is a species belonging to the family Sciaenidae widely distributed in the Southwestern Atlantic Ocean between latitude 41°N and 51°S (Chao *et al.*, 2015). It is a demersal species with benthopagous habits (Froese & Paully, 2017; Haluch *et al.*, 2009). Previous studies made brief morphological and morphometric descriptions of the *sagitta* otolith of *Menticirrhus americanus* (Volpedo & Echeverría, 2000; Siliprandi *et al.*, 2014; Volpedo *et al.*, 2018). But these studies not testing the ontogenetic, sexual and life stage variations in the shape of the otolith. Due to this lack of knowledge in this study we will verify possible ontogenetic, sexual and life stage variations (young females and small adult females) in the shape of otolith *sagitta* de *Menticirrhus americanus*.

MATERIAL AND METHODS

Area of study and sampling

Bimonthly samplings were performed between August 2015 and February 2016 in two beaches. Initially, in an internal estuarine beach (P1) using gillnets of different mesh sizes (2.5, 4 and 5 cm) and was also used to obtain copies of fishing with hook. On the beach outside the estuary (P2), specimens of artisanal fishing using gillnets of different mesh sizes (6, 7 and 9 cm). Samplings were carried out at the mouth of an estuary and an adjacent

beach in the subtropical Southwestern Atlantic Ocean of Brazil (25°28'-25°36'S, 48°20'-48°28'W, Paranaguá Bay, Fig. 1), considered a transition region between tropical and temperate climate (Spalding *et al.*, 2007).

The specimens caught were identified, measured for total length and total weight (TL, in millimeters and TW, in grams, respectively) and separated into five interval classes (110-150; 160-200; 210-250; 260-300; 310-350 mm). *Sagitta* otoliths were extracted. Gonads were removed, the sex and maturity stage were determined by microscopic analysis according to the procedure described in Possamai & Fávoro (2015).

Morphometry of otolith

Only the right otolith was photographed, and the morphology of the internal face was classified according to Tuset *et al.* (2008). Measurements of the right otoliths were taken using the ImageJ program, namely: length (OL, greater longitudinal distance in mm) and height (OH, greater perpendicular distance in mm) and area (A) of the otolith, area (mm²) of the *sulcus acusticus* (AS) and otolith perimeter (P, in mm) (Fig. 2).

In order to verify the ontogenetic, sexual and in the stages of life variations, the following shape indices were applied as described: OL/TL and OH/OL% Aspect Ratios (Volpedo & Echeverría, 2003), Form Factor [FF = (4π*A) PS⁻¹], Rectangularity [Rc = A/(OL*OH)], Ellipticity [E = (OL-OH)/(OL+OH)] (Tuset *et al.*, 2003) and Relative surface of the *sulcus acusticus* [Rss = AS/A] (Lombarte, 1992).

Contour of otolith

The otolith contour reconstruction was done using the wavelets. Wavelets are the result of the expansion of a signal in a family of functions that represent the dilations and translations of a mother function (Mallat, 1991): $\Psi_s(x) = 1/s\Psi(\varphi/s)$, where: Ψ is the function with local support in a limited amplitude on the abscissa axis; φ is the lower pitch filter; s is the scale parameter. The wavelet analysis allows to identify morphological similarities and/or differences as well as at which position of the otolith they are found, since the functions are elaborated from 512 Cartesian coordinates projected at points equidistant along the otolith (Lombarte & Tuset, 2015). The wavelet acquisition was performed as described by Parisi-Baradad *et al.* (2010) in site AFORO (<http://isis.cmi-ma.csic.es/aforo/index.jsp>).

Statistical analysis

The data (shape indices and wavelet) did not meet the assumptions of parametric analysis (Shapiro-Wilk; $p < 0.05$ and Bartlett, $p < 0.05$). In this way, the Kruskal-Wallis test was applied to check for differences in shape indices between class intervals and the Conover post-

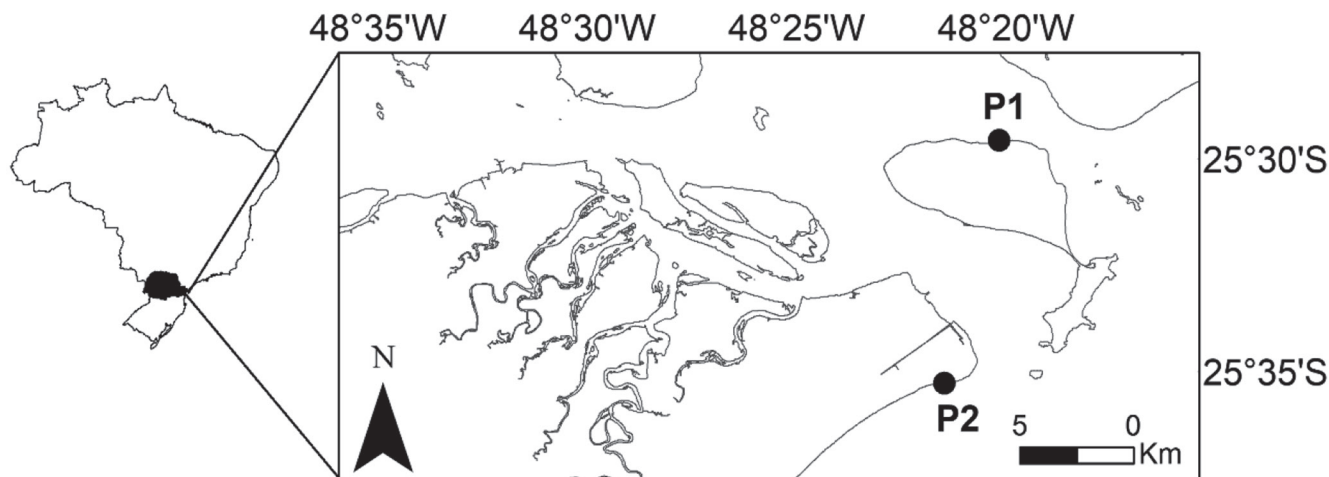


Figure 1. Sampling sites of *Menticirrhus americanus* in estuarine areas (P1) and a beach adjacent (P2) to the estuary in the Subtropical Southwestern Atlantic.

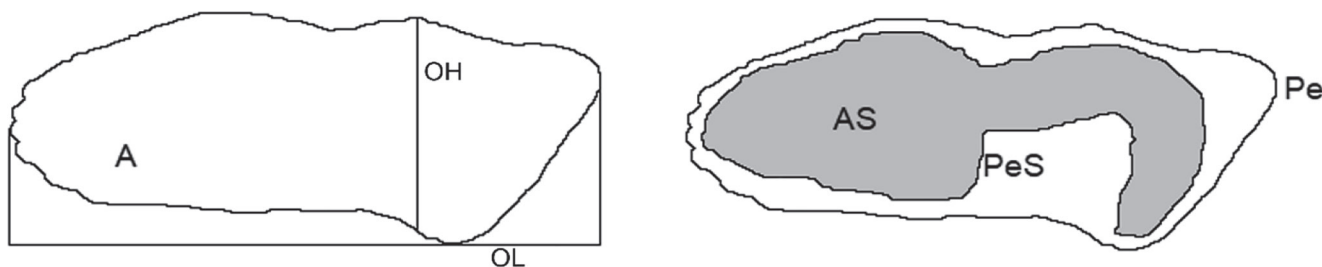


Figure 2. Scheme of linear morphometry applied to the right otolith of *Menticirrhus americanus* collected in a subtropical environment: OL = maximum longitudinal length, OH = maximum perpendicular height, A = area of the otolith and AS = area of the *sulcus acusticus*, Pe = perimeter of the otolith and PeS = perimeter of the *sulcus acusticus*.

hoc test was applied to identify between which class intervals the indices varied significantly (Pohlert, 2016).

In order to test the sexual differences and in the life stages (young and young females) among the indexes of form were selected specimens collected exclusively in P1. Specimens of similar lengths of females were selected (n = 30; TL mean = 230 ± 12 mm) and adults males (n = 30; TL mean = 222 ± 16 mm), young females (n = 29; TL mean = 144 ± 16 mm) and small young females (n = 29; TL mean = 175 ± 16 mm) this similar size selection between groups is to avoid the effect of allometry on the data. The Wilcoxon Mann-Whitney test was used to test possible variations in the shape indices between males and females and between young females and small young females.

The Permutational Analysis of Variance (PERMANOVA) was used to identify variations in the contour between

class intervals. The Bonferroni test was used to identify between which intervals the interactions were significant (p < 0.005). A principal component analysis (PCA) was used to verify the shape variation between the class intervals of the otoliths obtained. Later, with the main components (PCs), a canonical variable analysis (CVA) was performed to verify the percentage of correct reclassification of otoliths between class intervals (Linde *et al.*, 2004).

A principal component analysis (PCA) was also performed to identify sexual variations and life stages in wavelets obtained from selected otoliths. The CVA to verify the percentage of correct reclassification of the otoliths between the sexes and between young females and young adults with com PCs. The Bonferroni test was used to verify between which parameters these interactions were significant (p < 0.05). Statistical analyses were performed using the R software and Past (Hammer *et al.*, 2001).

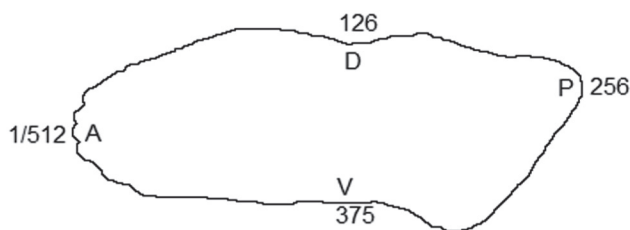


Figure 3. Otolith contour using 512 equidistant point in the otolith the *Menticirrhus americanus* para a in a subtropical environment: D = dorsal region of otolith, V = ventral region of otolith, A = anterior region of otolith and P = posterior region of otolith.

RESULTS

A total of 291 specimens of *M. americanus* (TL range: 120 and 345 mm; TW range: 15.33 and 291 g) were analyzed, which were distributed in five length class intervals to describe the ontogeny (Fig. 4). Were used for sexual variation a total 60 specimens of which 30 females (TL mean = 230 ± 12 mm) and 30 males (TL mean = 222 ± 16 mm). Also, were used for life stage

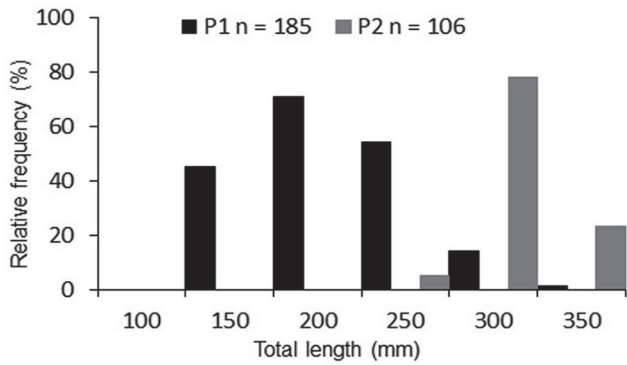


Figure 4. Frequency distribution of the total length (mm) of the *Menticirrhus americanus* in two environments in the Subtropical Southwestern Atlantic.

variations 29 young females (TL mean = 144 ± 16 mm) and 29 small young females (TL mean = 175 ± 16 mm).

The morphological classification of the otolith showed that some characteristics were constant along the ontogeny such as: absent rostrum and excisura, pseudo-ostial type of *sulcus acusticus*, the anterior region was round, the posterior region was peak and the external face had calcareous concretions at all class intervals (Fig. 5).

The length and height of the otolith and the ellipticity and SRS indices presented a positive and direct relationship with TL (Figs. 6A, B, G and H). In contrast, the inverse was observed between TL and the OL/TL aspect ratio, OH/OL% aspect ratio and the form factor

Table 1. Values of probability obtained through the Conover post-hoc test applied to Bonferroni correction between the class intervals and the morphometric indices and the length and height of the *sagitta* otolith of *Menticirrhus americanus* from a subtropical Southwestern Atlantic. Values $p < 0.05$ and $p < 0.001$ were considered significant.

Class interval (mm)	110-150	160-200	210-250	260-300	310-350
160-200	< 0.0001				
210-250	< 0.0001	> 0.05			
260-300	< 0.0001	< 0.0001	< 0.05		
310-350	< 0.0001	> 0.05	> 0.05	> 0.05	

(Figs. 6C, D and E). The rectangularity index showed a tendency to increase only for the intervals 110-150 and 160-200 mm, and this upward trend was not found in the upper intervals (Fig. 6F).

The Kruskal-Wallis test between the morphometric parameters (OL and OH) and all shape indices between the class intervals indicated significant differences ($p < 0.001$), suggesting ontogenetic variations along the growth of *M. americanus*. The Bonferroni correction of the Conover post-hoc test interactions are shown in Table 1.

In the Table 2, the morphometric parameters of the fish and otoliths analyzed together with the otolith shape indices, separated by sex and life stage. Due to size selection the averages of Table 2 are similar for both males and females and young females and small adult females.

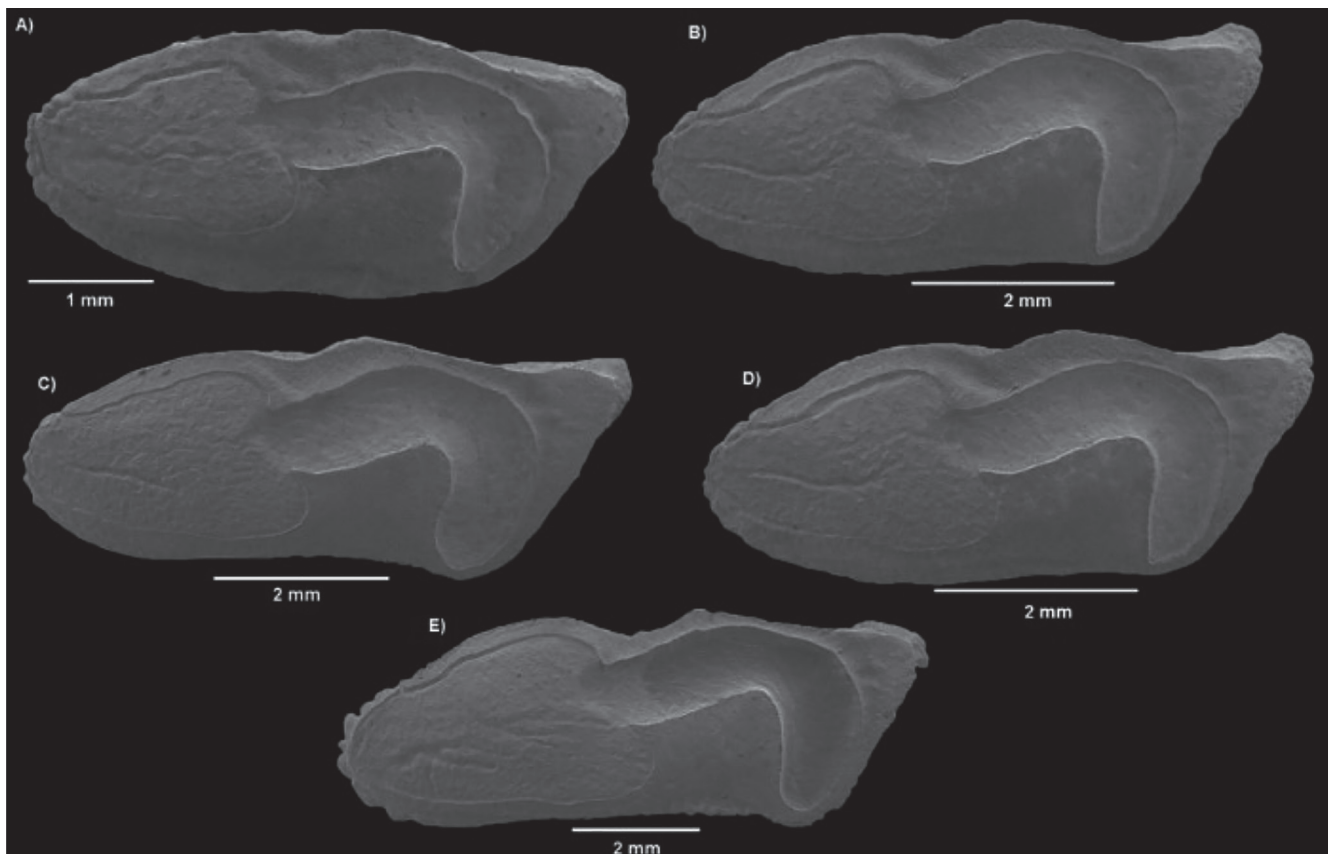


Figure 5. Micrograph of the inner face of the right *sagitta* otolith of *Menticirrhus americanus* throughout its ontogenetic development: (A) 132 mm interval 110-150 magnification 52x, (B) 169 mm interval 160-200 magnification 42x, (C) 214 mm interval 210-250 magnification 36x, (D) 285 mm interval 260-300 magnification 42x and (E) 333 mm interval 310-350 magnification 26x.

The Wilcoxon Mann-Whitney test evidence significant differences ($W = 545$, $p = 0.0002$) only for SRS between males and females. For small adult females and young females, the Wilcoxon Mann-Whitney test among all otolith parameters was significant ($p < 0.005$), except for

the rectangularity ($W = 546$, $p = 0.09$) and SRS ($W = 530$, $p = 0.15$) indices.

Reconstruction of the contour of the otoliths along the ontogeny showed a high variability in the contour between the different intervals (Fig. 8). From PERMANOVA,

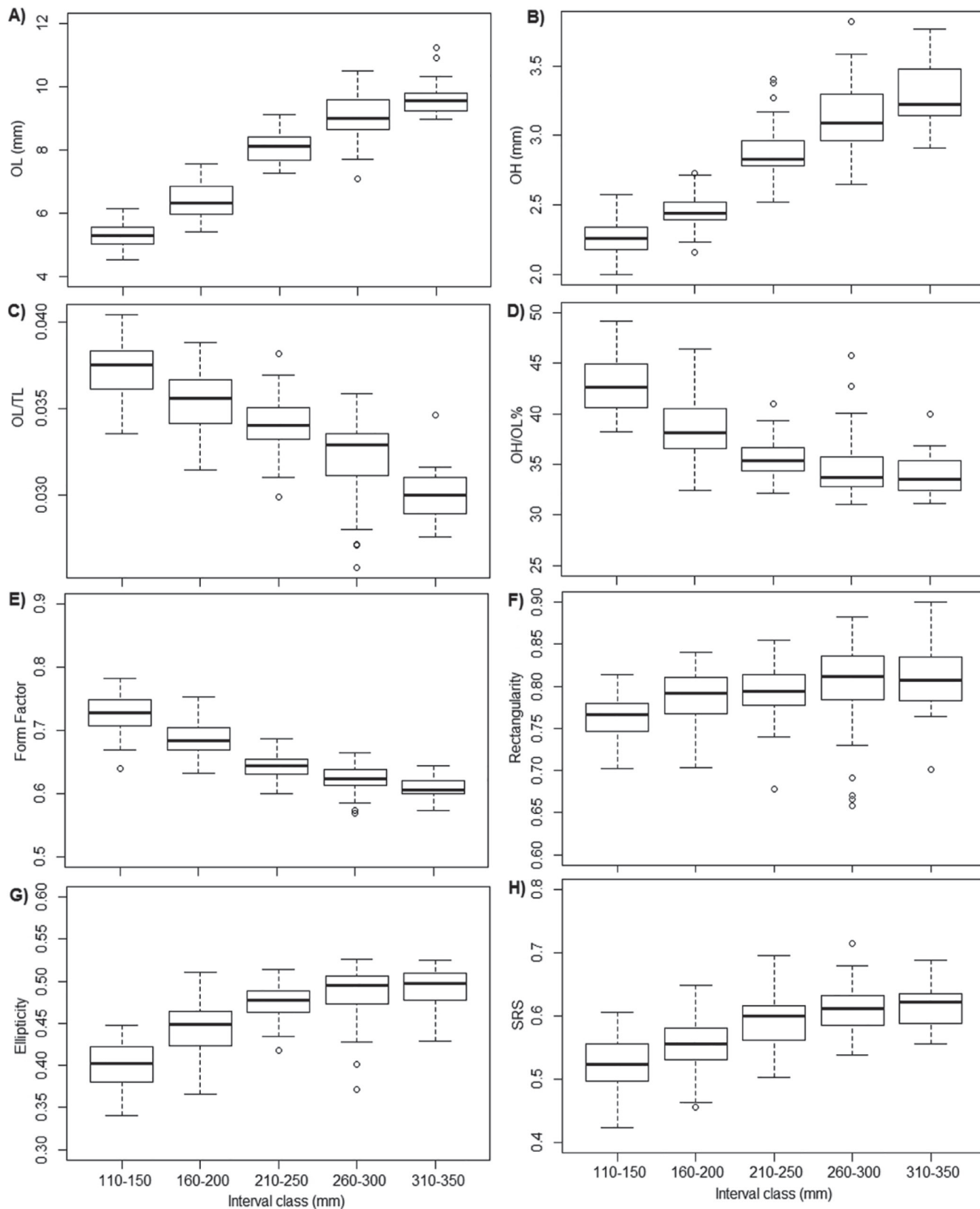


Figure 6. Box plot (mean and confidence interval) of morphometric parameters and morphometric indices of the otolith *sagitta* of *Menticirrhus americanus* per class interval: (A) otolith length (OL), (B) otolith height (OH), (C) OL/TL aspect ratio, (D) OH/OL% aspect ratio, (E) form factor, (F) rectangularity, (G) ellipticity and (H) surface of the *sulcus acusticus* (SRS). Circles indicate outliers.

Table 2. Morphometric parameters and shape indices (mean ± standard deviation) of *Menticirrhus americanus* from a subtropical environment were: males (M, n = 30), females (F, n = 30), young females (Y, n = 29) and small females (A, n = 29).

Sex	TL (mm)	OL (mm)	OH (mm)	OL/TL	OH/OL%	Form Factor	Rectangularity	Ellipticity	SRS
M	222 ± 16	7.5 ± 0.5	2.7 ± 0.2	0.03 ± 0.002	36.0 ± 0.01	0.6 ± 0.02	0.8 ± 0.02	0.4 ± 0.02	0.6 ± 0.04
F	230 ± 12	7.9 ± 0.4	2.8 ± 0.1	0.03 ± 0.001	35.2 ± 0.01	0.6 ± 0.01	0.8 ± 0.02	0.5 ± 0.01	0.6 ± 0.03
Y	144 ± 16	5.3 ± 0.5	2.3 ± 0.1	0.04 ± 0.002	43.1 ± 0.02	0.7 ± 0.03	0.8 ± 0.02	0.4 ± 0.03	0.5 ± 0.04
A	176 ± 15	6.2 ± 0.5	2.4 ± 0.1	0.04 ± 0.002	39.2 ± 3.0	0.7 ± 0.03	0.8 ± 0.03	0.4 ± 0.03	0.5 ± 0.04

Table 3. Values of probability obtained through the PERMANOVA with the Bonferroni correction applied in the contour of the *Menticirrhus americanus* otoliths among the class intervals. Values $p < 0.05$ and $p < 0.001$ were considered significant.

Class intervals (mm)	110-150	160-200	210-250	260-300	310-350
160-200	< 0.001				
210-250	< 0.001	< 0.001			
260-300	< 0.001	< 0.001	< 0.001		
310-350	< 0.001	< 0.001	< 0.05	< 0.001	

significant differences were detected between the contours of the class intervals ($F = 3.32$; $p < 0.0001$). The Bonferroni test identified significant variation between all interactions (Table 3).

From the Fig. 7, there is a high variability of contour of the otoliths throughout their ontogeny. The otoliths distributed along PC1 tend to be rounded to elongated, being represented by the smaller intervals in the negative values of PC1 (interval class 110-150 and 160-200) and in the positive values of PC1 are distributed more elliptical otoliths represented by the range 260-300 with pointed posterior region. In PC2, there are the elongated, higher otoliths represented by the intervals 210-250 and 310-350 with a rounded posterior region (Fig. 7).

The CVA presented 98.6% of correct reclassification of the otoliths among the class intervals sampled (Table 4).

Table 4. Correct reclassification of the contour of the otolith *Menticirrhus americanus* among the class intervals obtained through the analysis of canonical variables (CVA). The number in parentheses corresponds to the frequency of reclassification.

Class intervals (mm)	110-150	160-200	210-250	260-300	310-350
110-150	30 (100)	0	0	0	0
160-200	0	30 (100)	0	0	0
210-250	2 (6)	0	28 (94)	0	0
260-300	0	0	0	30 (100)	0
310-350	0	0	0	0	19 (100)
Total	32	30	28	30	19

The contour of the otoliths of young and adult females shows a variability in the wavelet 1, wavelet 4, wavelet 5 and wavelet 6 (Fig. 9). The wavelets of males and females are distinguished mainly in the wavelet 4 (Fig. 10). With PERMANOVA, significant differences were identified between the contours of the sexes and life stages ($F = 4.53$; $p = 0.0031$). The Bonferroni test identified significant variation between the sexes only (Table 5).

The PCA of contour of otoliths between sexes and stages of life demonstrates a high variability of shape of otoliths. Females have peak posterior region (Figs. 11A, C, D) and males have a more rounded posterior region (Fig. 11B). Young females and small young females present more rounded otoliths (Figs. 11C, D).

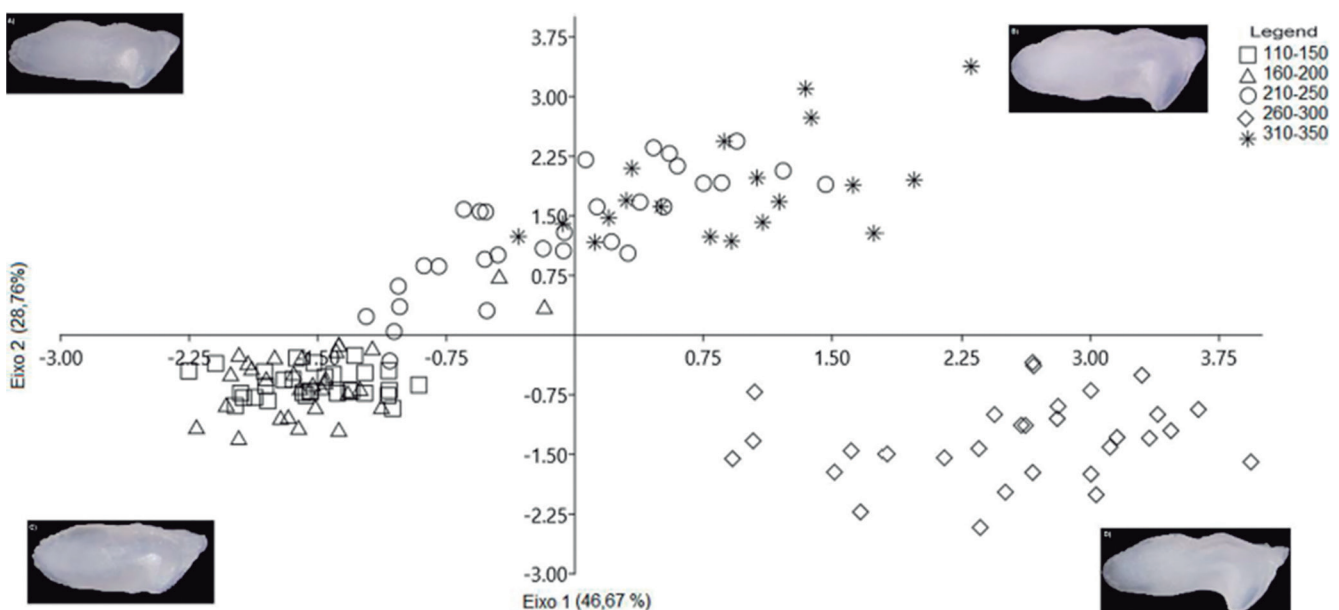


Figure 7. Scatterplot of the principal component analysis of the contour of the sagitta otolith of *Menticirrhus americanus* along its ontogeny sampled in two subtropical environments in the Southwestern Atlantic.

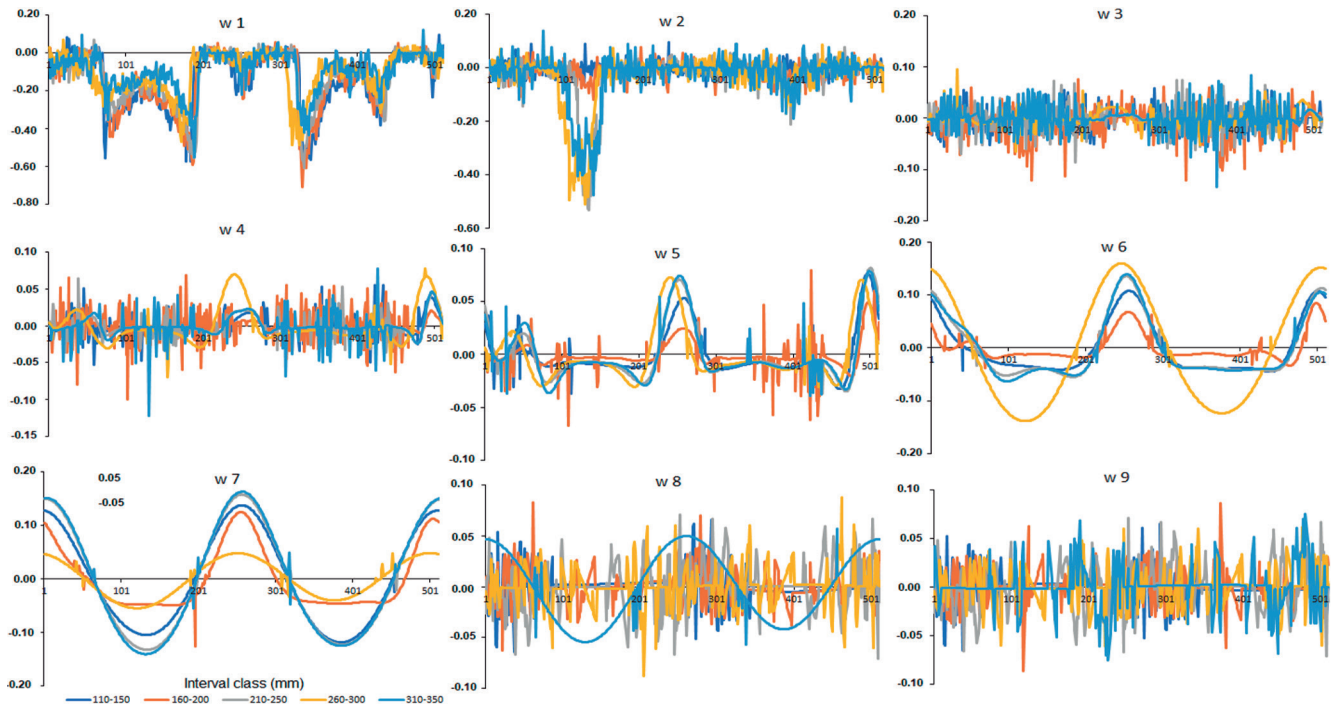


Figure 8. Decomposition of the contour of the *sagitta* otoliths throughout the ontogeny of *Menticirrhus americanus*, on the x-axis there are 512 Cartesian coordinates of the contour, y-axis, the means of the points. Interval class: dark blue 110-150 mm, orange 160-200 mm, grey 210-250 mm, yellow 260-300 mm and light blue 310-350 mm.

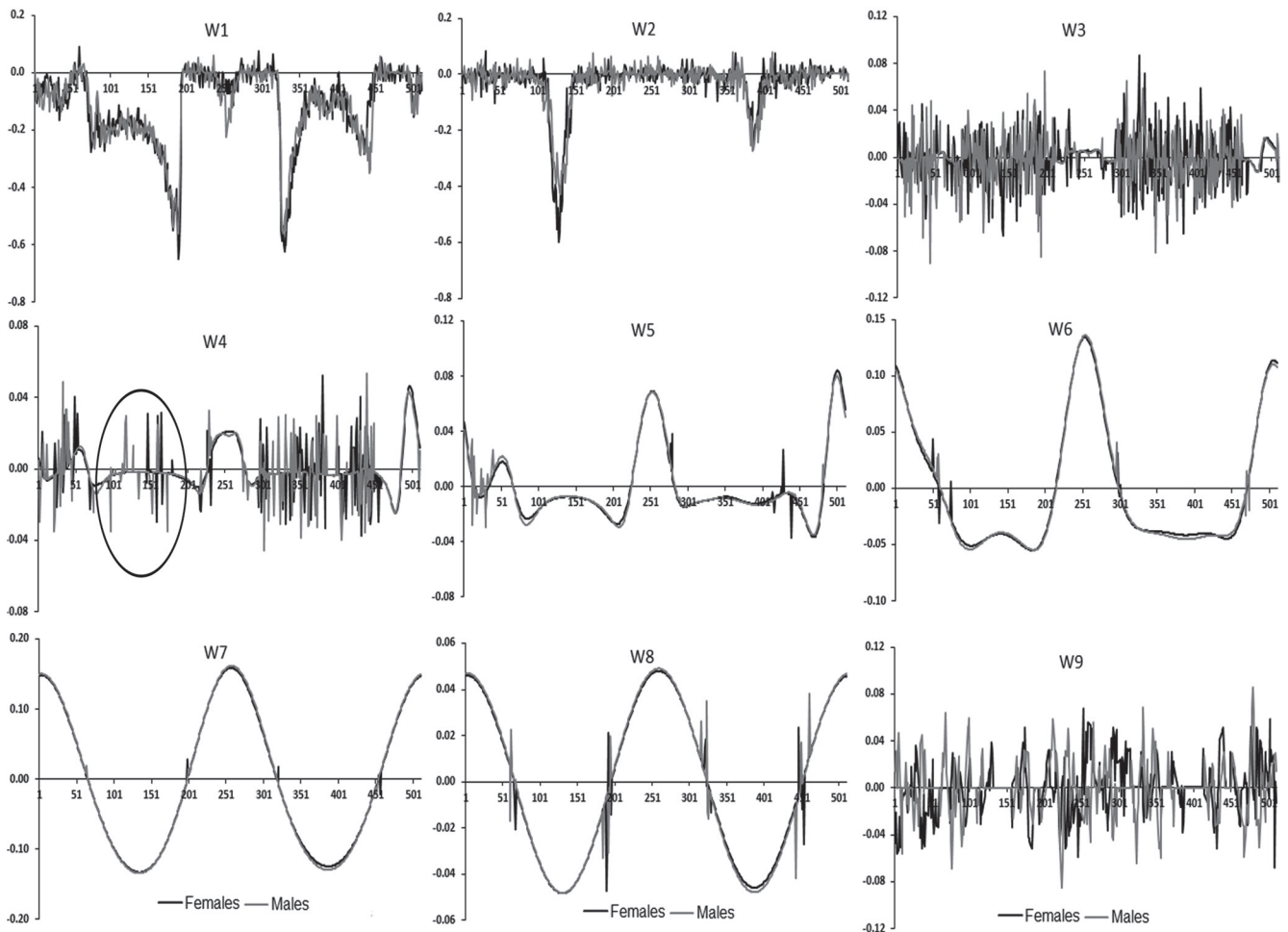


Figure 9. Decomposition of the contour of the *sagitta* otoliths of males and females of *Menticirrhus americanus*, on the x-axis there are 512 Cartesian coordinates of the contour, y-axis, the means of the points. Females are black lines and males are gray lines. Circle in wavelet 4 to highlight more discrepant region between males and females.

Table 5. Values of probability obtained through the PERMANOVA with the Bonferroni correction applied in the contour of the *Menticirrhus americanus* otoliths among the sex (M = males and F= females) and stage of life. Values $p < 0.05$ and $p < 0.001$ were considered significant.

	F	M	Young
F			
M	0.01		
Young	1	0.07	
Small Females	0.83	0.2	1

The CVA presented 79.6% of correct reclassification of the otoliths between the sexes and life stages sampled. Young females and small young females had the highest correct percentage of classification (Table 6).

DISCUSSION

The morphological features of otoliths of *M. americanus* evaluated during their ontogenetic development, such as: absence of rostrum and excisura, heterosulcoid *sulcus acusticus* and round anterior region, can be considered diagnostic characteristics of this species, as also

Table 6. Correct reclassification of the contour of the otolith *Menticirrhus americanus* among the sex and stage of life obtained through the analysis of canonical variables (CVA). The number in parentheses corresponds to the frequency of reclassification.

	F	M	Young	Small Females	Total
F	21(72)	6(21)	1(3)	1(3)	29
M	10(33)	19(63)	1(3)	0	30
Young	0	0	27(93)	2	29
Small Females	1(3)	0	2(7)	27(90)	30
Total	32	25	31	30	118

described in other studies (Volpedo & Echeverría, 2000; Siliprandi *et al.*, 2014; Volpedo *et al.*, 2017).

The absence of rostrum and excisura indicates that *M. americanus* poorly uses water column to move, being a species associated with the substrate after the settlement. These characteristics of the otolith allow to differentiate *M. americanus* from species that use the water column, since these have a well-developed rostrum and excisura (Volpedo & Echeverría, 2003; Carvalho *et al.*, 2015).

The otolith of *M. americanus* showed ontogenetic variations in the same way as other authors observed

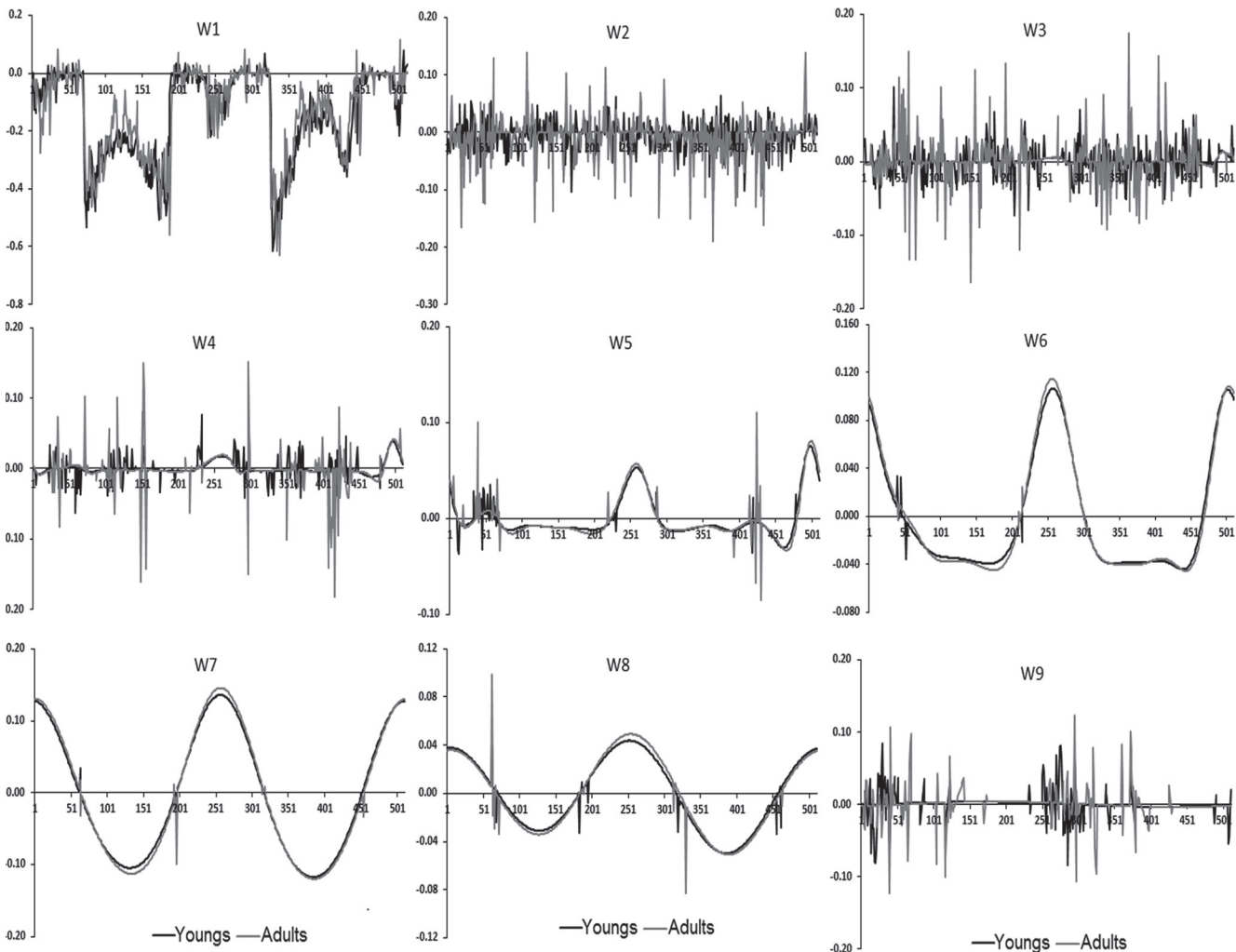


Figure 10. Decomposition of the contour of the *sagitta* otoliths of adult and young females of *Menticirrhus americanus*, on the x-axis there are 512 Cartesian coordinates of the contour, y-axis, the means of the points. Young females are the black lines and adult females, the gray lines.

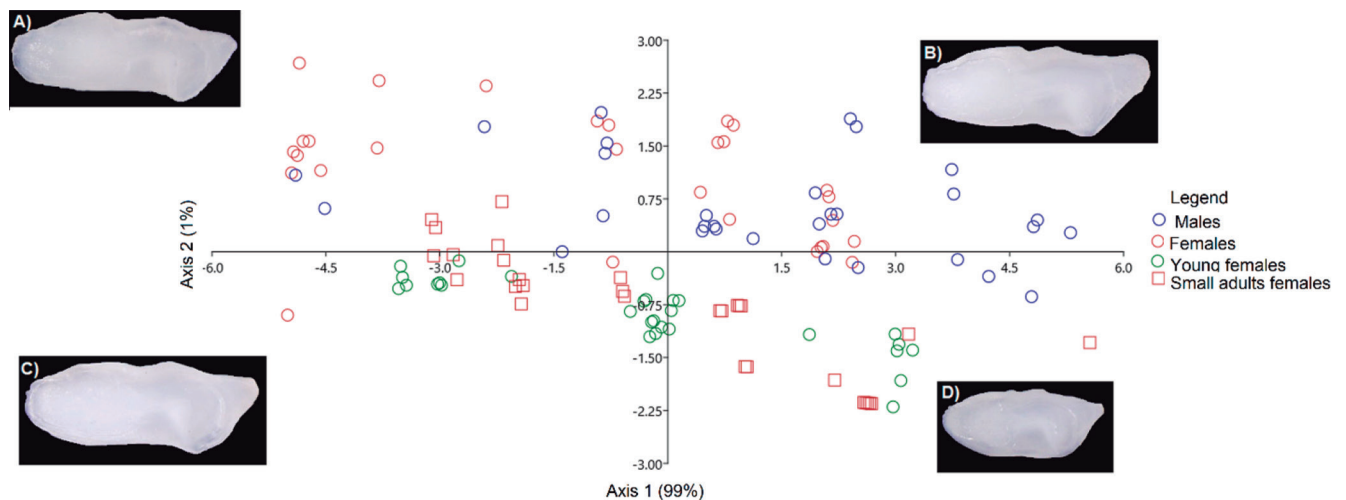


Figure 11. Scatterplot of the principal component analysis of the contour of the *sagitta* otolith of males (blue circles), females (red circles), young females (green circles) and small adult females (red square) *Menticirrhus americanus* in an estuarine beach of a subtropical environments in the Subtropical Southwestern Atlantic.

for other marine species (Volpedo & Echeverría, 1999; Vignon, 2012; Carvalho *et al.*, 2015). *Menticirrhus americanus* is a species of isometric positive growth ($b > 3$) (Dias *et al.*, 2014; Froese & Pauly, 2017), this characteristic is corroborated by the otolith growth change, which stops growing in the dorsoventral axis and begins to grow in the anteroposterior axis as also observed for *Paralonchurus brasiliensis*, another species belonging to the family Sciaenidae (Oliveira *et al.*, 2009). According to Avigliano *et al.* (2012), elliptical otoliths are correlated with species that inhabit more saline environments, as is the case of the *M. americanus* otolith that has elliptical otoliths and has a preferential environment estuarine mouths and shallow internal shelf (Barletta *et al.*, 2008).

Ontogenetic variations of otoliths indicate important characteristics of the ecology of *M. americanus*. According to Volpedo & Echeverría (2003), values in the aspect ratio (OH/OL%) between 30 and 50 indicate species associated with unconsolidated substrates (composed of silt, clay and sand), the values of *M. americanus* for this index varied between 35 and 45, reflecting that this species throughout its ontogeny is associated with unconsolidated substrates. This preference of *M. americanus* during all stages of life for this type of substrate is corroborated by its presence in the accompanying fauna in the Atlantic seabob (*Xiphopenaeus kroyeri*) shrimp fishery (Bernardo *et al.*, 2011; Cattani *et al.*, 2011).

According to Volpedo *et al.* (2008), the SRS index describes how much of the otolith surface comes into contact with the macular nerve through the *sulcus acusticus*. From this relation, it is possible to suggest the auditory capacity of fish; *M. americanus* presented higher values of this index than species of the genus *Cynoscion* (Aguirre, 2003), suggesting a greater auditory capacity of *M. americanus* in relation to the species of this genus. These observed differences are the result of several ecological factors. First, species of the genus *Cynoscion* forage in the water column (Rondineli *et al.*, 2007) requiring greater visual acuity and may have a lower auditory capacity, while *M. americanus* is a benthivorous species (Rondineli *et al.*, 2007) associated with environments with higher turbidity,

thus requiring a greater auditory than visual capacity. Another factor is the detection of predators by hearing (Popper *et al.*, 2005), as *M. americanus* is prey to several species of top predators, including marine mammals (Di Benedetto & Siciliano, 2007; Bornatowski *et al.*, 2014; Miotto *et al.*, 2017) its keen hearing favors its escape. Finally, a greater auditory capacity facilitates intraspecific recognition for mating, essential for *M. americanus* that forms reproductive aggregates through vocalization (Ramcharitar *et al.*, 2006).

Otoliths of males and females of *M. americanus* showed a sexual dimorphism in both the contour and the SRS index. The species of the family Sciaenidae are known to have the ability to vocalize, and this vocalization may be for defense of the territory or for reproduction (Montie *et al.*, 2015). In some species, male and female vocalize, but in the reproductive age, males increase the intensity of vocalization (Tellechea *et al.*, 2011; Parmentier *et al.*, 2014). In addition to the behavioral differences, there is a morphological distinction in the size of the sound muscle between the sexes (Ramcharitar *et al.*, 2006; Tellechea & Norbis, 2012). The sexual dimorphism in otoliths of *M. americanus* may be a result of the need to capture sound since females are attracted to males for mating by their vocalization. Species that form reproductive aggregates become more susceptible to fishing in this period, causing impacts for future cuts to withdrawals from individuals able to spawn.

As well as *M. americanus*, other species of demersal fish have demonstrated sexual dimorphism in the *sagitta* otolith contour (Mille *et al.*, 2015; Bose *et al.*, 2016). However, the small number of studies that verify the existence or not of sexual dimorphism in the otoliths of demersal species makes it impossible to conclude that this is a characteristic of demersal fish.

Both shape indices and contour analysis demonstrated significant differences in otoliths between young females and small adult females of *M. americanus*. With these analyses, the influence of the reproductive process on the otolith shape of the females of *M. americanus* is confirmed by the reduction of energy invested in somati-

ic growth for the formation of oocytes and a change in the rates of calcium deposition on the otolith, once this element is of great importance for oocyte formation.

From the results of this study it is possible to conclude that *M. americanus* presents an ontogenetic variation of the otolith shape. There is a sexual dimorphism between female and male otoliths, suggesting that these sexual variations are related to sound uptake into the formation of reproductive aggregates. Stages of life also showed differences in the shape of otoliths suggesting an influence of reproduction and somatic growth in this structure.

ACKNOWLEDGMENTS

The authors are grateful for funding from the project CAFB-BA/SPU and 292/14 UFPR/Fundação Araucária. They also thank CONICET (PIP112-20120100543CO), Universidad de Buenos Aires (UBACYT 20020150100052BA), Agencia Nacional de Promoción Científica y Técnica (ANPCyT PICT 2015-1823); CNPQ for the PhD degree scholarship to the first author (CNPQ 141267/2015-1), LAFMA (CEM-UFPR) for the stereomicroscope and the Olympus DP71 camera and the Centro de Microscopia Eletrônica (CME-UFPR) for the micrographs. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES) – Finance Code 001.

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