



Otolith shape analysis of *Lutjanus jocu* and *L. synagris* in the Abrolhos bank, Brazil

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Abstract: Fishes of the family Lutjanidae figure among the main fishing resources along continental shelves of tropical and subtropical regions. The information about of the morphology of the sagittal otolith of these species in the western Atlantic Ocean coast is still incipient. The objective of this study was to characterize the morphology, morphometry and ontogenetic variations of the sagittal otoliths of two Lutjanidae species (*Lutjanus jocu* and *L. synagris*) with commercial importance through the use of size descriptors, shape indexes and elliptical Fourier descriptors. We employed five shape-based indexes: roundness, circularity, ellipticity, rectangularity, and aspect ratio. Morphometric measurements of otoliths were used in predictive linear regression equations to estimate fish size. Regarding ontogeny, *L. jocu* exhibited higher morphological variation, while *L. synagris* maintained a generally invariant shape, growing in constant proportions. The T2 Hotelling multiparametric analysis revealed significant differences between the elliptical Fourier descriptors of both species, defining a phenotypic form for each species. The results of the study provide information on the morphometry and shape indexes of otoliths for two snapper species, representing the first description for *L. jocu* in this region. This information contributes to the discrimination and identification of these species for future research.

Key words: Morphology, Ontogeny, Shape indexes, Lutjanidae, Fourier analysis.

Análise da forma dos otólitos de *Lutjanus jocu* e *Lutjanus synagris* no Banco dos Abrolhos, Brasil. Resumo: Os peixes da família Lutjanidae estão entre os principais recursos pesqueiros ao longo das plataformas continentais das regiões tropicais e subtropicais. As informações sobre a morfologia do otólito *sagittae* dessas espécies na costa do Oceano

Atlântico Ocidental ainda são incipientes. O objetivo deste estudo foi caracterizar a morfologia, morfometria e variações ontogenéticas dos otólitos de duas espécies de Lutjanidae (*Lutjanus jocu* e *L. synagris*) com importância comercial, através do uso de descritores de tamanho, índices de forma e descritores elípticos de Fourier. Empregamos cinco índices baseados em forma: arredondamento, circularidade, elipticidade, retangularidade e proporção. Medidas morfométricas dos otólitos foram utilizadas em equações de regressão linear preditivas para estimar o tamanho do peixe. Em relação à ontogenia, *L. jocu* apresentou maior variação morfológica, enquanto *L. synagris* manteve uma forma geralmente invariável, crescendo em proporções constantes. A análise de T² Hotelling revelou diferenças significativas entre os descritores elípticos de Fourier de ambas as espécies, definindo uma forma fenotípica para cada espécie. Os resultados do estudo fornecem informações sobre a morfometria e índices de forma dos otólitos para duas espécies de lutjanídeos, representando a primeira descrição para *L. jocu* nesta região. Essas informações contribuem para a discriminação e identificação dessas espécies para futuras pesquisas.

Palavras-chave: Morfologia; Ontogenia; Índices do formas; Lutjanidae, análises de Fourier.

Introduction

Snappers (Lutjanidae) are prominent among the primary fishing resources in tropical and subtropical oceanic platforms worldwide (Allen 1985; Graham *et al.* 2008). In the coastal waters of the western Atlantic Ocean, nine species of the genus *Lutjanus* are intensely exploited by artisanal and commercial fishing (Rezende *et al.* 2003, Frédou *et al.* 2006). Examples of this group include the dog snapper (*L. jocu*) and the lane snapper (*L. synagris*), both considered vulnerable to fishing due to their large size, long life cycle, slow growth, and late maturation (Polovina & Ralston 1988, Sadovy 2001). Such characteristics place them in a delicate situation, especially under extensive fishing efforts (Bannerot *et al.* 1987, Aschenbrenner *et al.* 2016). Similarities in behavioral and environmental factors may lead them to share fundamental morphological characteristics in structures or organs strongly associated with the environment, as is the case with otoliths (Assis 2000).

Otoliths are polycrystalline structures mainly composed of calcium carbonate, playing essential roles in sound perception and balance (Platt & Popper 1981; García-Godos 2001). Otoliths possess complex morphology, extensively used in various fisheries biology studies, including age and growth (Previero *et al.* 2011), trophic ecology (L'Abée-Lund *et al.* 1992), population determination (Begg *et al.* 2001, Avigliano *et al.* 2015), species identification (Tuset *et al.* 2008, Volpedo & Vaz dos Santos 2015, Jawad *et al.* 2017), paleo-ichthyological descriptions (Nolf 1985, Murray & Wilson 1999), and ontogenetic studies (Carvalho *et al.* 2015, Callicó Fortunato *et al.* 2017). One of the most significant applications of otoliths is their use as a taxonomic tool for char-

acterizing and differentiating species (Garciaarena 1989, Martinez *et al.* 2007, Tuset *et al.* 2010). In particular, quantifiable otolith characteristics, encompassing traditional and shape morphometry, have established them as diagnostic traits, proving useful in identifying species, determinate age and growth and populations under fishing exploitation (Ihssen *et al.* 1981, Begg & Brown 2000, Galley *et al.* 2006, Previero *et al.* 2011).

The focus of otolith studies in recent decades has been to establish the relationship between shape, morphological characteristics, size of otoliths with the environment and the behavior of fish using techniques such as geometric morphometry (Lombarte & Cruz 2007, Volpedo & Fuchs 2010, Jaramilo *et al.* 2014, Morais *et al.* 2023). The available knowledge on the fishery biology of both snapper species in the western Atlantic remains limited. The objective of this work was to characterize the morphology and morphometry of the sagittal otoliths of *L. jocu* and *L. synagris*, and to describe their ontogenetic development. The study analyzed shape variations in otoliths throughout the fish's life stages, revealing key differences in size, shape, dorsal and ventral margins, and rostrum development between the two species.

Material and Methods

Study area and sampling: The Abrolhos Bank (16° 40' - 19° 40'S; 39° 10' - 37° 20'W) is a 46,000 km² enlargement of the eastern Brazilian shelf (Fig. 1) with depths rarely exceeding 70 m (Moura *et al.* 2013). The region encompasses South Atlantic's largest reefs, rhodolith beds and unconsolidated substrate. Reef fisheries are predominantly artisanal

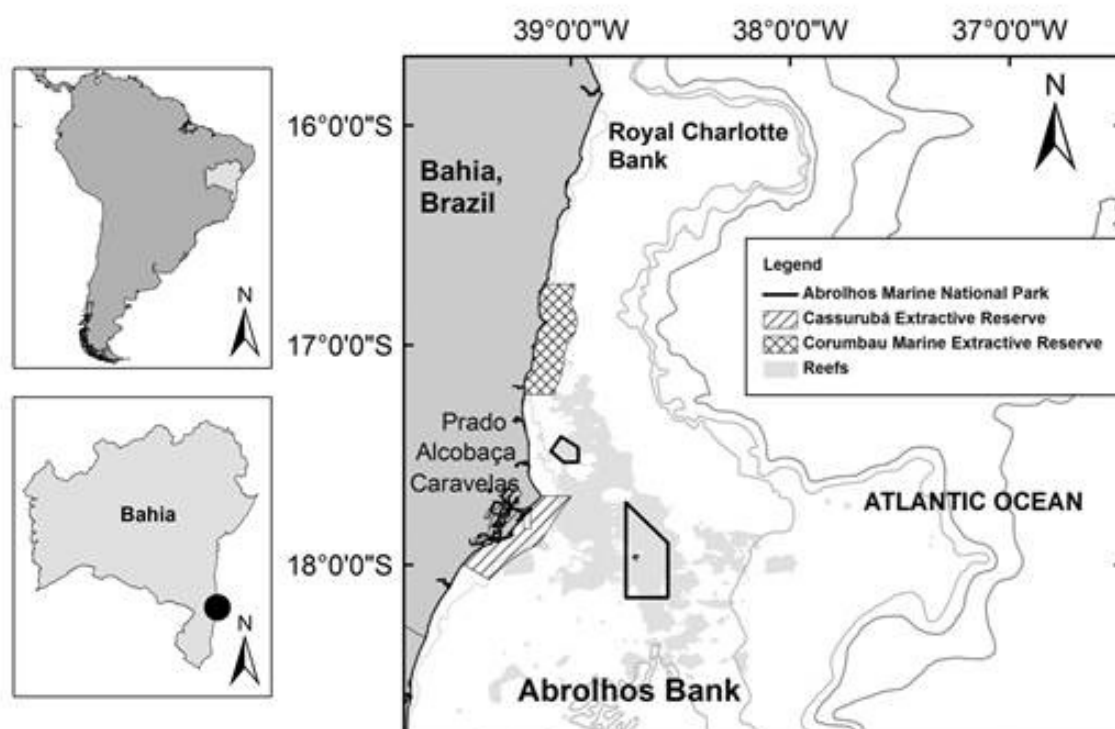


Figure 1. Map of the Abrolhos Bank, showing the municipalities where samples were collected.

(Freitas *et al.* 2011, 2014) and Abrolhos is Brazil's main reef fisheries ground (Freitas *et al.* 2014). The main landings occur in the coastal cities near to Abrolhos Bank (Fig. 1). In the Abrolhos shelf, many Marine Protected Areas have been created (National Marine Park - 910 km²; Community based Extractive Reserves Cassurubá - 1,010 km and Corumbau - 815 km). Specimens of *L. jocu* (n = 157) and *L. synagris* (n = 95) were obtained from weekly random sampling at the counties of Prado, Alcobaça and Caravelas (Fig. 1), between June 2005 and March 2007. For each sample, the sex was identified macroscopically (Freitas *et al.* 2011) and the total length (TL, mm) was measured. Both sagittal otoliths were removed, cleaned and stored in a dry container for further processing, labeled accordingly. **Morphology and morphometry of the otoliths:** For metrics analysis, only otoliths that did not have any damage in their structure were taken into account. Otoliths were photographed under stereomicroscope with incident light against dark background and positioned with the *sulcus acusticus* facing upward (Fig. 2a). A standardized scale was used in each of the photographs obtained to facilitate posterior measurement. Otolith morphological description followed Tuset *et al.* (2008). For the morphometric analysis, the weight of the otolith (OW, g.) was ob-

tained using an analytical scale (± 0.0001 g.) and its thickness (OT, mm) was determined using a digital caliper with 0.01 mm accuracy. Images were processed with the Image J software (Schneider *et al.*, 2012) to obtain the following morphometric variables: area (OA, mm²), perimeter (OP, mm), length (OL, mm) and height (OH, mm), as well as to determine the indices based on its shape: circular (OP^2/OA), ellipsoid ($((OL - OH) / (OL + OH))$), rectangular ($OA/[OL \times OH]$), Roundness ($[4 \times OA] / [\pi \times OL^2]$) and Aspect ratio (AR, OH/OL) (Fig. 2b), as suggested by (Tuset *et al.*, 2008).

Elliptic Fourier analysis: Otolith contour was determined by digitalization and simplification of the silhouette in the image, expressed as a sequence of coordinates that could be manipulated and mathematically adjusted in an equation derived from the Fourier functions (Claude, 2008, Kuhl & Giardina, 1982, Tracey *et al.* 2006). The Elliptic Fourier descriptors (EFD) are based on trigonometric and periodic functions called harmonics, whose combinations allow the reconstruction of the contour of the otolith (Farias *et al.* 2009). Each harmonic is composed of four elliptic Fourier coefficients (EFD), which are used to calculate the estimated contribution of each harmonic for the description of the otolith contour. The first three coefficients of the first harmonic,

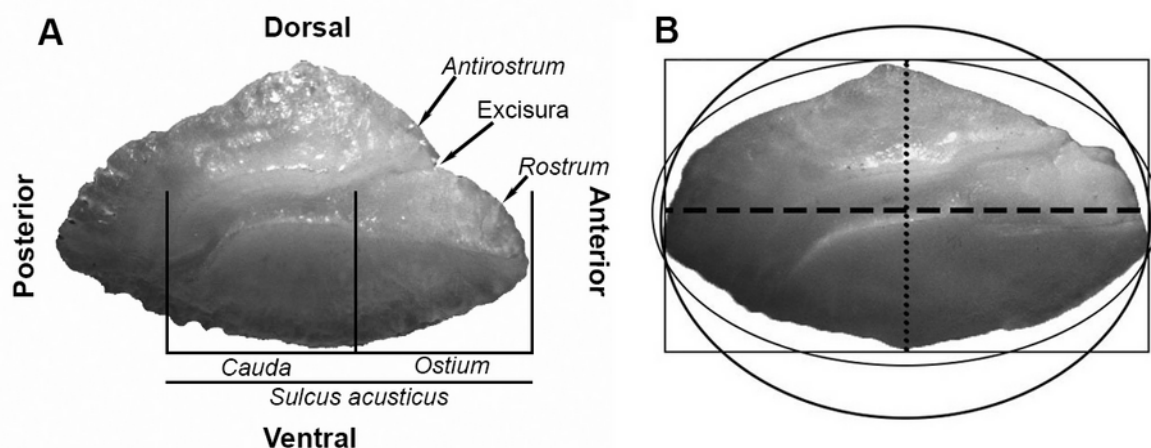


Figure 2. A left otolith of *L. jocu* showing morphological characteristics; B *L. synagris* left otolith showing morphometric measurements of full length and width (dotted lines), as well as shape indices; circularity, ellipticity and rectangularity.

which describe the ellipse that best fits the contour, were excluded in the normalization process to remove the effects of size, centroid, and rotation (Iwata & Ukai 2002, Kuhl & Giardina 1982).

Only the left otoliths were used to standardize the analysis (*L. jocu*, $n = 118$; *L. synagris* $n = 78$). With the Fourier coefficients obtained, an information matrix was built to perform a principal component analysis (PCA). The number of components was defined from the break-point (Muggeo 2003), where linear models were adjusted between the main components (PCA) and the angular coefficients were determined.

Differences between species were tested through multivariate analysis of variance (MANOVA) using T^2 Hotelling. All statistical procedures were performed with the software R, version 3.4.4 (R Development Core Team 2018) with the Momocs packages (Bonhomme & Claude 2014) and segmented (Muggeo 2008). The level of significance was set at $\alpha = 0.05$ for all statistical analyzes.

Analysis of morphometric data: Using the matrix of morphometric variables and shape indexes by species, the atypical or extreme values were eliminated (outliers). The effect of fish's allometric growth was also removed by the formula proposed by Lleonart (2000) ($Y_i^* = Y_i (X_0/X_i)^b$), where: Y_i is the original value of the variable, X_0 is the mean TL value, X_i the original fish TL value and b the parameter of the curve slope in the regression. The values of the morphometric measurements of the otoliths of each species (OL, OH, OT, OW, OP and OA) were related allometrically to the corresponding total length. The relations between weight of otolith (OW) and area of otolith (OA) to the fish length

(TL) was estimated using the linear equation $Y = aX^b$. Linear and non-linear regressions were used to correlate the variables with the development patterns of otolith in relation to fish growth compared between species. Normality and homogeneity of the variables were examined with the Kolmogorov-Smirnov and Levene tests, respectively. The student's t-test and univariate ANOVA were employed to differentiate shape indices corresponding to the left and right sides of the otolith while the Mann-Whitney U test was utilized to compare differences between sexes. A principal component analysis (PCA) was performed using the shape indices to determine the relationship of the species to the quantitative variables analyzed. To evaluate the discrimination of otoliths in each method, a linear discriminant analysis (LDA) was performed. All statistical analyses, including Mann-Whitney U test, PCA, ANOVA, MANOVA, and regressions, were performed using R software version 3.4.4 (R Development Core Team 2018) with the dplyr (Wickham *et al.* 2014), nlstools (Baty *et al.* 2015) and factoextra (Kassambara & Mundt 2017).

Results

A total of 252 specimens were analyzed. *Lutjanus jocu* specimens showed TL amplitudes between 140-855 mm (344.10 ± 145.87 mm; mean $\pm$ standard deviation), while *L. synagris* specimens varied between 211-560 mm TL (316.65 ± 81.90 mm) and did not present significant differences related to sex (*L. jocu*: Mann-Whitney, $U = 5923.5$, $P > 0.05$; *L. synagris*: Mann-Whitney, $U = 720$, $P > 0.05$).

Otoliths morphology: Otoliths of *L. jocu* present a triangular shape and a concave-convex profile, their posterior region rounded to oblique, and their anterior region rounded and slightly pointed. They also present dorsal and ventral margins from sinuous to crenulated borders. Their *sulcus acusticus* present *ostial* opening, medium position and inframedial curvature, with descending orientation beyond the heterosulcoid form, as well as the funnel-shaped ostium and the slightly curved tubular *cauda*. *Rostrum* and *antirostrum* are present and developed (Fig. 3a).

Ontogenetic differences were found in the shape of the otolith, border and *rostrum*, the form tends to change from elliptic in the smaller size classes (*L. jocu* :140-260-380-500-620-740 mm; *L. synagris* 220-310-400-490-580 mm) to the triangular shape in larger individuals. The border grew according to the ontogenetic development, being pronounced, and pointed in the larger classes as well as the *rostrum*, which also presented changes in size, being present and developed with descending orientation in the longest fish classes (Fig. 4a).

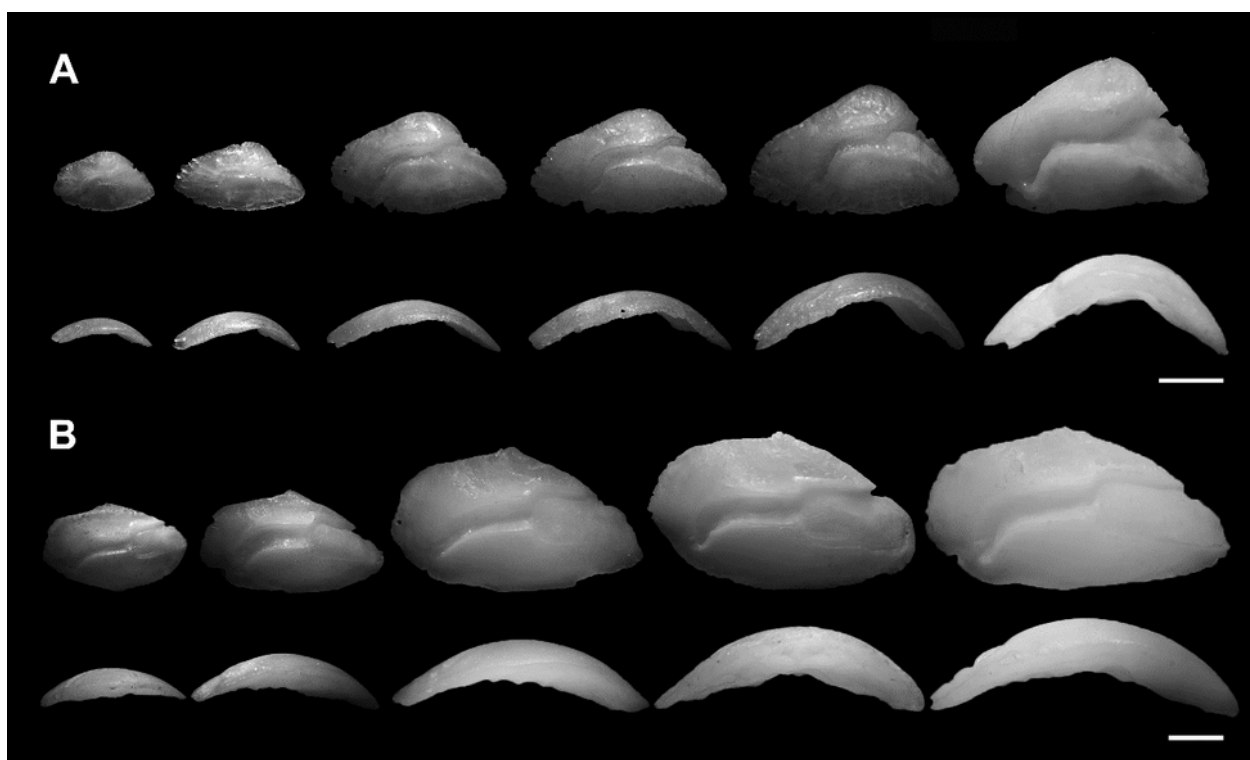


Figure 3. Internal face of sagitta otolith; A *L. jocu* (140-260-380-500-620-740 mm TL); B *L. synagris* (220-310-400-490-580 mm TL). Scale = 4 mm.

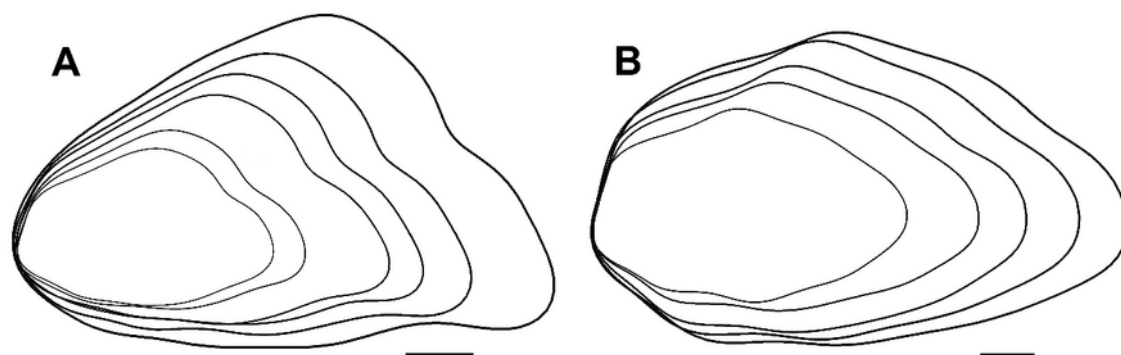


Figure 4. Changes in the shape of the otolith in relation to fish development; A *L. jocu* (min. size: 140 mm, max. size: 855 mm); B *L. synagris* (size min: 211 mm, max. size: 560 mm), scale 2 mm.

Table 1. Multiple comparison with one-way ANOVA for the shape indices of the snappers. Values reported include mean $\pm$ SD values. The bold values indicate statistical differences ($p < 0.05$).

Variable	<i>L. jocu</i>	<i>L. synagris</i>	<i>F</i>	<i>P</i>
Aspect ratio	1.73 $\pm$ 0.076	1.81 $\pm$ 0.06	71.9	0.001
Circularity	0.62 $\pm$ 0.05	0.72 $\pm$ 0.03	382.6	0.001
Ellipticity	0.26 $\pm$ 0.02	0.27 $\pm$ 0.01	7.896	0.005
Rectangularity	0.68 $\pm$ 0.01	0.71 $\pm$ 0.01	214.3	0.002
Roundness	0.57 $\pm$ 0.02	0.55 $\pm$ 0.01	71.49	0.002

Table 2. Correlation of the variables in the first two main components of the form indices, highlighting (in bold) the variables with greater weight in each component.

Shape indexes	PC1	PC2
Aspect ratio	0.56	-0.12
Roundness	-0.56	0.12
Circularity	0.18	0.73
Rectangularity	0.31	0.57
Ellipticity	0.5	0.35

On the other hand, *L. synagris* presented otoliths with elliptical shape and concave-convex profile, with posterior region rounded to oblique, and the pointed and split anterior region. Their dorsal and ventral margins presented asymmetrical curvature of sinuous to irregular form. Their *sulcus acusticus* presented ostial aperture and median position with inframedial curvature, descending orientation as well as heterosulcoid form. Its ostium is rectangular, and its tubular *cauda* slightly curved, while their *rostrum* and *antirostrum* are present and slightly developed.

Ontogenetic differences were found in the form of the anterior region related to their profile, *rostrum*, and size, and thus the shape of the otolith tends to change from elliptic in the smaller size classes to the oblique shape in larger individuals (Fig. 3b), being the part of the anterior region the most pronounced with the development. The profiles in the smaller size classes are plane-convex with the *rostrum* going from underdeveloped to absent, while

in classes larger than 300 mm they begin to present a convex concave profile with *rostrum* present and developed (Fig. 4b).

Otoliths morphometry: The left and right otoliths showed no significant difference in relation to the variables of size and shape (*L. jocu*: $t = 1.7114$, $df = 227$, $P > 0.05$; *L. synagris*: $t = 1.1242$, $df = 227$, $P > 0.05$). The five shape indices presented a normal distribution ($P > 0.05$) and ANOVA showed significant differences ($P < 0.05$) on the means of all shape indices (Table 1), between the two species studied. Otoliths of *L. jocu* presented a greater dispersion in all indices and obtained higher roundness (Fig. 5), compared to *L. synagris*, that present higher average circularity and aspect ratio (AR). Ellipsoidal index was similar between species.

The principal component analysis explained 88.94% of the total variance in the first two components, which were relevant according to the break point model. The first component (PC1) explained the 62.12% of the variance, where the aspect ratio and ellipticity presented the highest relevance together with the roundness index, which presented a negative correlation (Table 2). The component two (PC2) explained 26.8% of the variance and indicates greater relevance to circularity and rectangularity (Fig. 6). Linear discriminant analysis (LDA) showed a reliable identification of 98.41% for the shape indices of the two species. For *L. jocu* the LDA presented 97.45% of the individuals classified correctly in comparison to *L. synagris* that presented 100% in the classification.

It was possible to find significant relationships between the TL and the variables of otolith size (OL, OH, OA, OP, OW and OT) for both species ($P < 0.001$, $R^2 > 0.85$) (Fig. 7). The relationship between total length of the fish and otolith thickness showed a low coefficient of determination for *L. synagris* ($R^2 = 0.746$) in relation to the values obtained in the other variables, while the ratio TL/OA presented values of determination coefficient higher among all the morphometric variables for the two species. The equation of the lines obtained from the variables was linear: $y = a + bx$, with exception of TL/OW and TL/OA, where the regression type was non-linear with the equation: $y = ax^b$. The results of these calculations showed that, observing through the slope parameter b , *L. synagris* presented greater slope in all regressions, with the exception of thickness.

Otolith shape: The analysis identified that the first 12 harmonics assure 99% of the contribution of the contour, thus, the principal components analysis (PCA) determined 45 components. Using the graphi-

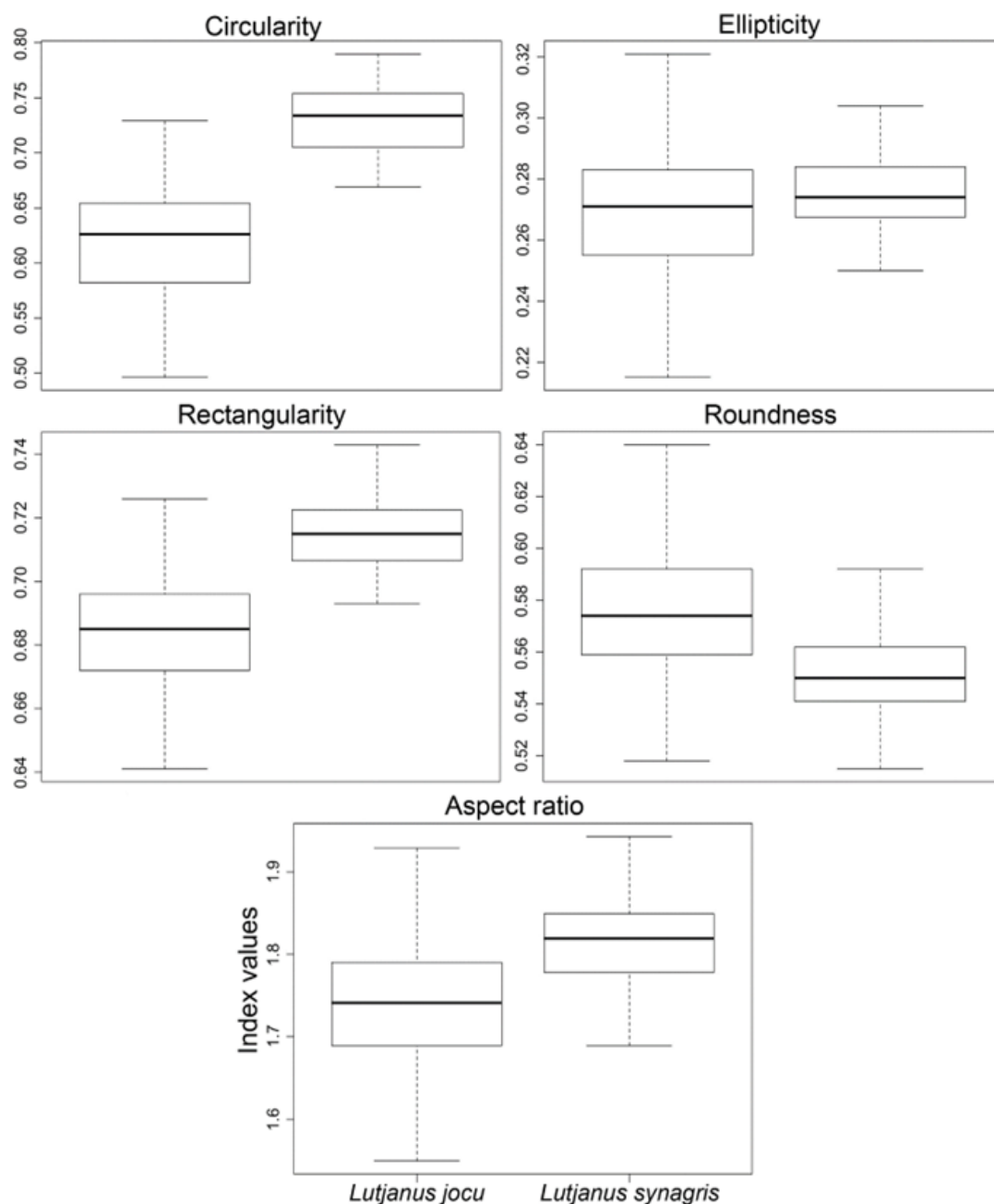


Figure 5. Boxplot comparing the distribution of the indices of form between the two species.

cal model of the break point, it was identified that the values stabilized from the fourth component, which together explain 60.11% of the total accumulated variance.

Two significant components were identified by MANOVA with T^2 from Hotelling-Lawley (Hotelling-Lawley = 5.0106, DF = 193, $P < 0.05$) (Table 3). To analyze the reconstructed form (mean $\pm$ standard deviation) from the first two principal components (Fig. 8), it was identified that the main char-

acteristics displayed by components are; PC1, which delimits mainly the shape of dorsal and ventral portion, as well as the format of otolith, while the PC2 characterizes the anterior margin, including the expression of *antirostrum*. The dispersion diagrams of the first two components (PC1 and PC2) were able to differentiate the two species, according to their importance for the contribution of the specific information of the species (Fig. 9). The extreme variation along the first principal component indicates that the

contour of the otolith varies in developing the *rostrum* and *antirostrum*. The discriminant analysis (LDA) differentiated the two species correctly with an error level of 3.06% from the contour descriptors. The average values of the Fourier coefficients determined a phenotypic form for each species, allowing its differentiation and classification (Fig. 10).

Discussion

We have carried out the first morphological metric and morphological study of the sagitta otoliths in specimens of *L. jocu* in the western Atlantic Ocean. The otoliths of both species show similarity in the morphology described for otoliths of snapper species in southern Brazil (Brenha-Nunes *et al.* 2016).

The morphological characteristics generally present in otoliths are strongly related to genetics and phylogeny, but they can also be influenced by environmental factors and may be related to the way of life of the species (Campana 2005, Monteiro *et al.* 2005, Vignon & Morat 2010). The intraspecific vari-

ability at the level of the otoliths, can be related to the morphological characteristics and the shape indices, determining differences that allow the identification of species (Assis 2000).

The results of the morphological analysis showed that the otoliths of the two species are similar in relation to the characteristics of the *sulcus acusticus*, both having ostial opening in a median position with descending orientation and slightly curved tubular *cauda* with heterosulcoid format. These characteristics are similar to those described for snapper species of the family Lutjanidae of Southeastern-Southern Brazil (Brenha-Nunes *et al.* 2016). This similarity in form may correspond to interspecific kinship relationships, noting that environmental characteristics do not change the shape of the *sulcus* in species of this genus. As *sulcus acusticus* is in direct contact with the macula, responsible for hearing, its structure is related to predation and social behavior, and fish with large *sulcus acusticus* are associated with the formation of shoals (Bani *et al.* 2013; Jaramilo *et al.* 2014, Popper & Lu 2000).

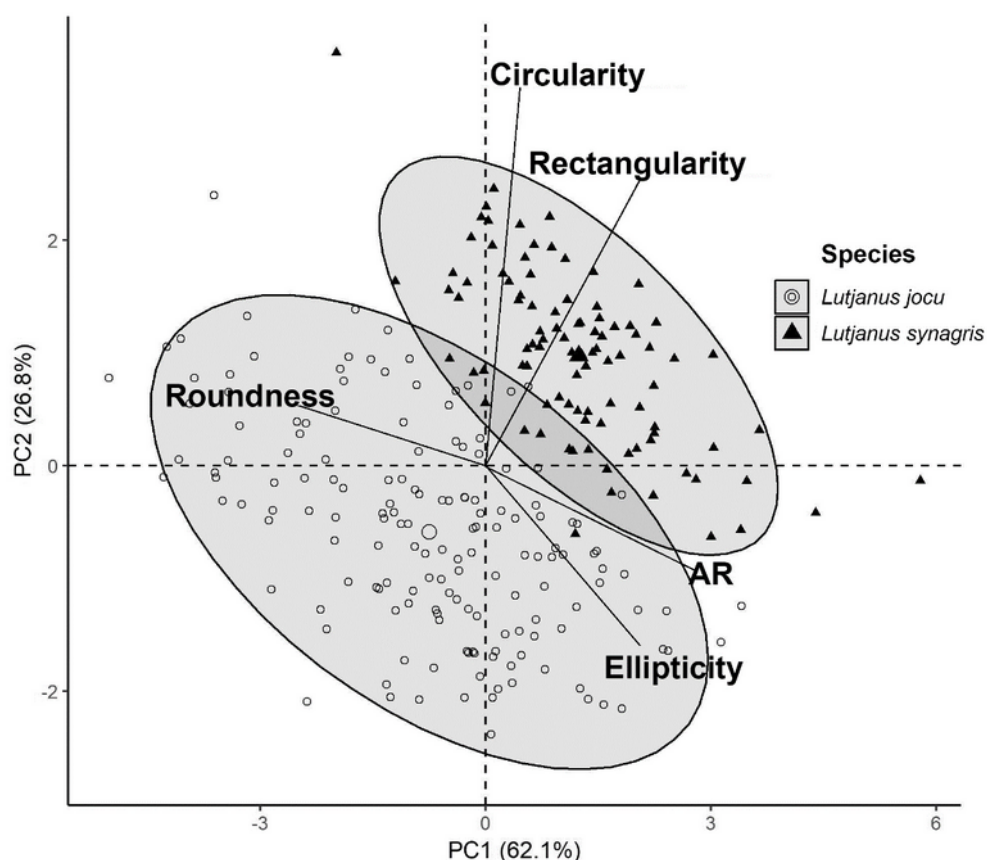
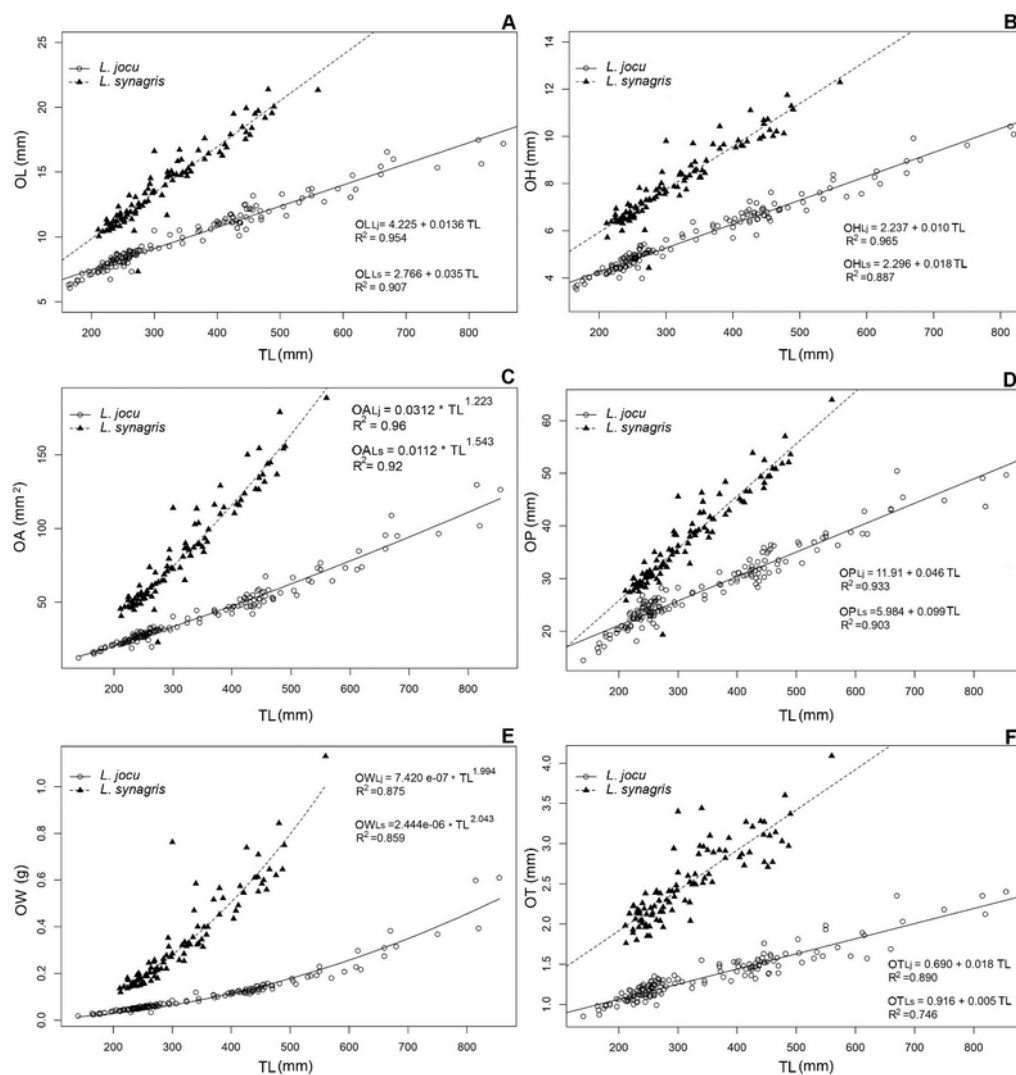


Figure 6. Scatter plot representing the first component (PC1) and the second (PC2) using the five form indices of the sagitta otolith, accounting for 88.94% of the total variance.

Table 3. Multivariate analysis of variance (MANOVA) with T2 from Hotelling-Lawley delimiting three significant components. DF = degree of freedom. The bold values indicate statistical differences ($p < 0.05$).

Responses	DF	Sum squared	Mean squared	F Value	P Value
PC1: Species	1	1221.59	1221.59	349.56	0.001
Residuals	194	677.96	3.49		
PC2: Species	1	155.57	155.571	45.664	0.001
Residuals	194	660.92	3.407		
PC3: Species	1	6.51	65.061	17.508	0.1873
Residuals	194	720.91	37.161		
PC4: Species	1	3.02	30.175	13.883	0.2401
Residuals	194	421.68	21.736		

**Figure 7.** Relationship between total fish length (TL) and otolith variables, R^2 = coefficient of determination.

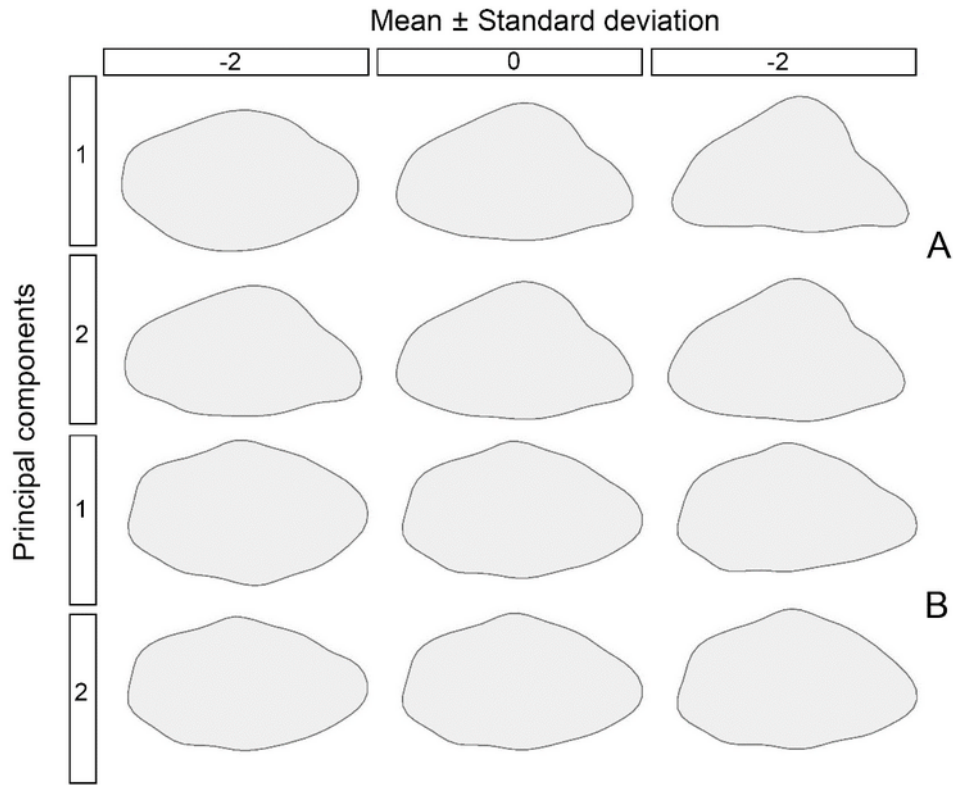


Figure 8. Representation of PC contributions (mean $\pm$ standard deviation); A *L. jocu*; B *L. synagris*.

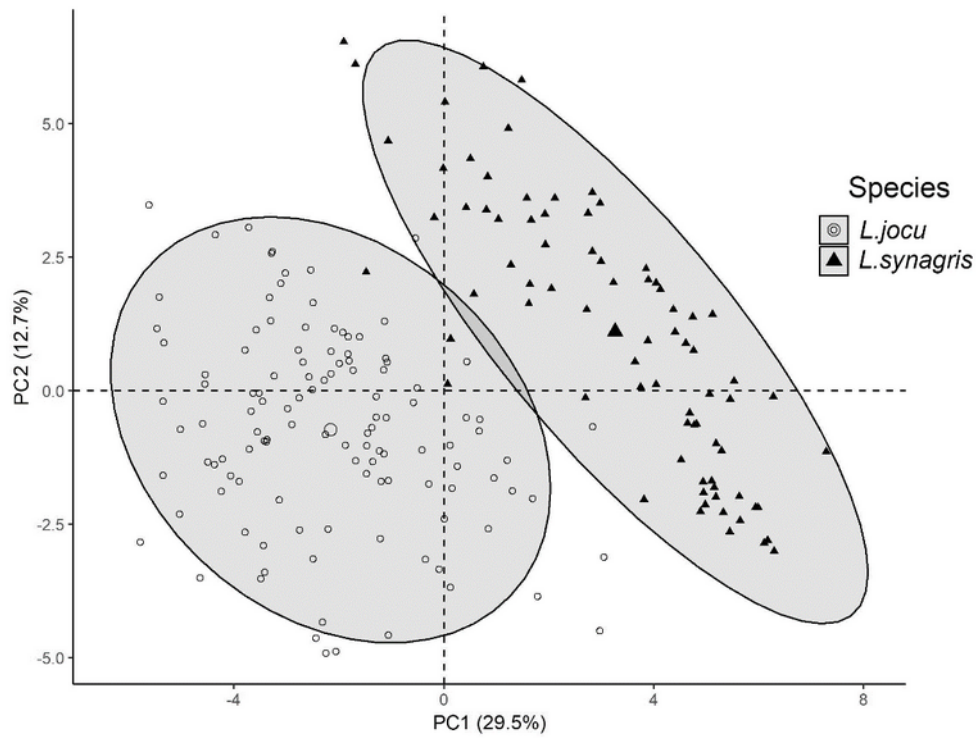


Figure 9. Dispersion graph of the main components PC1 and PC2 in the contour analysis.

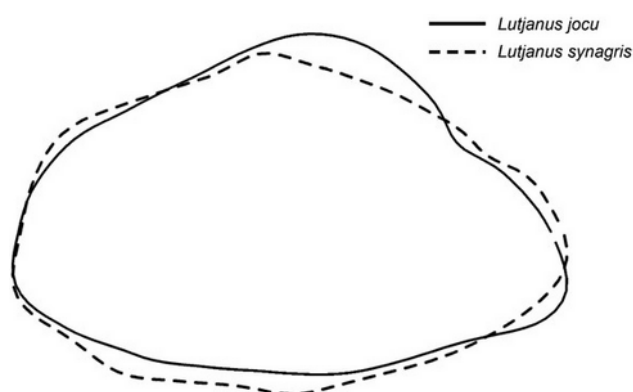


Figure 10. Phenotypic form of the sagitta otolith of *L. jocu* and *L. synagris* for the extreme south of Bahia, Brazil.

This is similar to species in the Lutjanidae, as these fish have seasonal migrations for food purposes and the formation of reproductive aggregations (Luckhurst 1998).

The shape variations found between the different size classes of individuals of *L. jocu* can be connected with the first sexual development (L_{50} = 324 and 344 mm of TL for females and males respectively, (Freitas *et al.* 2011). Since it was observed that in small organisms (140 – 310 mm) they have a more elliptical form and in juvenile organisms (after sexual maturity > 330 mm) the otolith shape changes to a more triangular shape. In this way, environmental factors influence somatic growth and generate changes in metabolism rate to assimilate and utilize reproductive energy along with hormone levels during fish development directly affecting otolith growth (Fablet *et al.* 2011).

There were no significant shape differences between the left and right otoliths for both species, therefore, any one may be used in future studies. Methodologies such as the morphometric characterization of the sagittal otoliths proved to be an efficient method used to discriminate fish species (Bani *et al.* 2013, Tuset *et al.* 2003, Zhuang *et al.* 2014), and the use of indices such as circularity, rectangularity and aspect ratio have been efficient in the characterization and discrimination of populations (Ponton 2006, Tuset *et al.* 2003).

In this study, the shape indices, size parameters and elliptic Fourier descriptors showed significant differences in the sagitta of the two species corroborating this efficiency. The shape variation of the sagittal otoliths of *L. jocu* and *L. synagris* was tested using five shape indices, of which circularity, roundness and rectangularity were the most relevant in the principal components analysis. Although the otoliths

of *L. jocu* are closer to a circle by the roundness index, while *L. synagris* otoliths approach a rectangular shape, explaining 88.94% of the total variance, being efficient in discriminating the species and can also be used in the interspecific discrimination (Stransky *et al.* 2008).

We used the principal component analysis of the coefficients of Fourier, as an effective tool for studying and describing forms of contour in otoliths (Cadrin & Friedland 1999, Campana & Casselman 1993). The results of the analyses identified the different ontogenetic variations in each species and defined a phenotypic average form for each size class. These findings are highly relevant for practical applications in differentiating snapper species, characterizing ontogenetic development, and highlighting the specific characteristics that sagittal otoliths present for each taxonomic group, making them fundamental for taxonomic identification and research on the diets of ichthyophagous marine animals.

The results of the analyses identified the ontogenetic variations among the species and established a phenotypic average form for each size class. The results are useful for practical applications such as differentiating snapper species, characterizing ontogenetic development, and identifying the features of sagittal otoliths specific to each taxonomic group, making these insights important for taxonomic identification and research on the diets of ichthyophagous marine animals.

Ethical statement

All specimens used in this study were provided by local fishermen. No experimental collections or procedures were conducted on live animals; therefore, ethical approval was not required.

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