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ONTOGENETIC DEVELOPMENT OF SAGITTA OTOLITHS IN EUGERRES BRASILIANUS (CUVIER, 1830) (ACTINOPTERYGII: GERREIDAE) WITH EMPHASIS ON SIZE AT FIRST SEXUAL MATURITY IN A COASTAL LAGOON IN SOUTHEASTERN BRAZIL

Desenvolvimento ontogenético dos otólitos sagitta de *Eugerres brasilianus* (Cuvier, 1830) (ACTINOPTERYGII: GERREIDAE) com ênfase no tamanho de primeira maturação sexual em uma lagoa costeira no sudeste do Brasil

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ABSTRACT

Otolith developmental assessments related to fish life history search to provide important information for understanding the intraspecific variability of these structures. In this context, the present work searched to identify ontogenetic and sexual patterns in the morphology of *Eugerres brasilianus* otoliths captured in Itaipu lagoon between July 2019 and April 2020. The size at first maturation (L_{50}) was determined for the grouped and separate sexes. Wavelet coefficients and shape descriptors were evaluated by total length (TL) classes. While sexes and maturation stages were evaluated only by shape descriptors. A total of 158 specimens were captured between 7.5 and 30.6 cm TL, with a length at first maturation for grouped sexes of 15.5 cm TL, 15.3 cm TL for females and 15.5 cm TL for males. The wavelet coefficients showed significant differences between the TL classes (PERMANOVA; p<0.001). Significant differences were also observed for the shape descriptors for the TL classes, sexes and maturation stages (PERMANOVA; p<0.001). Our results confirm the hypothesis of ontogenetic variations in the morphology of *E. brasilianus* otoliths, which can be attributed to variations in habitat use, metabolism, condition and growth rates. While the differences between the otoliths of mature females and males, as well as between maturation stages, are reflections of the energy expenditure arising from gonadal development.

Keywords: Carapeba, otolith shape, wavelet, multivariate analysis.

RESUMO

Avaliações do desenvolvimento de otólitos relacionado a história de vida de peixes buscam prover informações importantes para compreensão da variabilidade intraespecífica dessas estruturas. Neste contexto, o presente trabalho buscou identificar padrões ontogenéticos e sexuais na morfologia dos otólitos de Eugerres brasilianus capturados na lagoa de Itaipu entre julho de 2019 e abril de 2020. O comprimento médio de primeira maturação (C_{50}) foi determinado para os sexos grupados e separados. Coeficientes de wavelet e descritores de forma foram avaliados por classes de comprimento total (CT). Enquanto sexos e estágios de maturação foram avaliados apenas por descritores de forma. Foram capturados 158 exemplares variando entre 7,5 e 30,6 cm CT, apresentando C_{50} de 15,5 cm CT para sexos grupados, 15,3 cm CT para fêmeas e 15,5 cm CT para machos. Os coeficientes de wavelet apresentaram diferença significativa entre as classes de CT (PERMANOVA; $p<0,001$). Diferenças significativas também foram observadas para os descritores de forma para as classes de CT, sexos e estágios de maturação (PERMANOVA; $p<0,001$). Nossos resultados confirmam a hipótese de variações ontogenéticas na morfologia dos otólitos de E. brasilianus, o que pode ser atribuído a variações no uso de hábitat, metabolismo, condição e taxas de crescimento. Enquanto as diferenças entre os otólitos de fêmeas e machos maduros, assim como entre estágios de maturação, são reflexos do gasto energético proveniente do desenvolvimento gonadal.

Palavras-chaves: Carapeba, forma do otólito, wavelet, análise multivariada

INTRODUCTION

Otoliths are species-specific calcareous concretions with distinct shapes located in the inner ear of fish (Campana, 1999; Tuset *et al.*, 2003). They are responsible for balance and hearing (Popper *et al.*, 2005). The biomineralization and morphogenesis of otoliths result from multi-causal processes due to the interaction of numerous internal (physiological) and external (environmental) factors (Mille *et al.*, 2015). Otolith shape is a widely used tool for various studies with taxonomic purposes (Tombari *et al.*, 2005), stock identification (Avigliano *et al.*, 2015), trophic ecology of ichthyophagous species (Miotto *et al.*, 2017), bioecological aspects and life history (Elsdon *et al.*, 2008; Gholami *et al.*, 2015), growth and ontogeny (Vaz-dos-Santos & Rossi-Wongtschowski, 2007; Reichenbacher *et al.*, 2009; Vignon, 2012; Carvalho *et al.*, 2015; Callicó-Fortunato *et al.*, 2017). The sagitta otolith is the largest of the three pairs of otoliths (sagittae, asterisci, and lapilli) found in teleosts and is the primary one used in the afore mentioned studies. Thus, understanding and characterizing the variation in otolith shape during ontogeny is of significant interest, as many studies, based on various evidence, correlate otolith shape with age (Bird *et al.*, 1986), size (Campana & Casselman, 1993; Mérigot *et al.*, 2007), and habitat use (Vignon, 2012).

The use of bivariate morphometry to describe the relationships between fish and otolith measurements has been extensively employed, including shape indices (Tuset *et al.*, 2003; Perin & Vaz-dos-Santos, 2014; Almeida *et al.*, 2020). In this context, traditional morphometric analysis of otoliths has facilitated the identification of various ecological aspects of fish species, encompassing changes during fish development related to sexual maturation, diet shifts, or habitat use by swimming fish (Gagliano & McCormick, 2004; Vignon, 2012; Carvalho

et al., 2015). Combining traditional morphometry with shape analysis extends the use of otoliths as key elements for understanding various bioecological aspects of both species and populations (Biolé *et al*., 2019). Different morphometric techniques can be used to investigate variations in otolith structure, such as wavelet analysis (Tuset *et al*., 2019) and shape descriptors, including area, perimeter, circularity, roundness, form factor, rectangularity, and ellipticity (Tuset *et al*., 2003; Carvalho *et al*., 2020). Based on the one-dimensional decomposition of otolith contours, wavelet analysis (WA) relies on expanding the contour into a family of functions obtained through dilations and translations of a single function known as the mother wavelet (Mallat, 1991). Shape descriptors were calculated to analyze variations in structure throughout the ontogenetic development of the studied species (Callicó-Fortunato *et al*., 2017). Recent studies have applied the combination of both methods to assess changes in otoliths during fish development (Biolé *et al*., 2019; Almeida *et al*., 2020; Clark *et al*., 2021).

Coastal lagoons in southeastern Brazil serve various purposes and interests, such as fishing, and their characteristics attract tourism and real estate development, making them focal points for socio-environmental issues related to the degradation of these ecosystems (Barros & Mansur, 2018). Studies indicate that coastal lagoons in the eastern part of the state of Rio de Janeiro are crucial repositories of aquatic diversity (Mansur *et al*., 2012). In this context, the Itaipu coastal region is situated, where lagoon systems play a significant role in maintaining coastal biological processes, as they aggregate, produce, and regulate biological resources (Monteiro-Neto *et al*., 2008; Camacho-Valdez *et al*., 2014). The Itaipu Lagoon is described as mesohaline and hypertrophic, serving as habitat for many species of economic interest (Sergipense & Pinto, 1995; Silva & Molisani, 2019). However, the constant increase in population around the lagoon creates a highly stressed environment associated with anthropogenic actions and high levels of pollution from domestic sewage (Kjerfve, 1994).

The carapeba, *Eugerres brasilianus* (Cuvier, 1830), a species abundant in tropical and subtropical coastal lagoon regions of the Western Atlantic, ranges from the southern USA to southern Brazil (Menezes & Figueiredo, 1980). Within the Gerreidae family, this species is considered one of the largest representatives and is one of the most abundant and important demersal resources in the coastal lagoons of Saquarema and Araruama (Almeida *et al*., 2021) as well as in northeastern Brazil (Rodrigues *et al*., 2017). Considered a marine visitor or opportunist due to its tolerance of salinity variations, it can be found in various environments and exploited as a fishing resource (Andrade-Tubino *et al*., 2008). Besides its significant importance for artisanal fisheries, it also shows potential for aquaculture due to its socioeconomic value and biological characteristics (Menezes & Figueiredo, 1980; Rodrigues *et al*., 2018; Azevedo *et al*., 2021). Classified as an omnivore, it feeds on small organisms such as polychaetes and crustaceans (Eiras-Stofella & Charvet-Almeida, 2000). However, its diet may change throughout its developmental stages, with trophic level shifts reported among juveniles, subadults, and adults (Ramos *et al*., 2014). Habitat use by the species also varies ontogenetically, with juvenile individuals concentrating in the more internal zones of estuarine environments where they find shelter and resource availability, while subadults and adults may distribute throughout these environments and adjacent coastal areas (Ramos *et al*., 2016)

Given the ecological and economic importance of *Eugerres brasilianus* and the scarcity of studies in southeastern Brazil, we aimed to provide crucial information about otolith development and its relationship with life history, thereby facilitating a better understanding of the intraspecific variability of the species' otoliths. Assuming that otoliths are effective indicators of fish life habits, it is hypothesized that different life events experienced by

individuals may result in modifications to otolith shape, which can be identified using tools for morphological variation assessment and described in morphotypes. Population attributes such as somatic growth, sex, and maturation stages are key life history parameters directly related to development, metabolism, and energy utilization. Therefore, these factors are among the most important and prominent as potential drivers of morphological diversification in otoliths. Thus, this study aimed to identify ontogenetic and sexual patterns in the morphology of the sagitta otolith of *E. brasilianus* captured in the Itaipu Lagoon, a transitional environment subjected to numerous anthropogenic impacts, by examining variations in otolith shape among different size classes, sexes, and maturation stages.

MATERIAL E METHODS
Study area

Itaipu Lagoon (22º56'S and 43º02'O) (Figure 1) covers an area of 1 km² and is oriented in an east-west direction. It is one of the first lagoons in the complex of coastal lagoons in the eastern part of the state of Rio de Janeiro, formed by the Itaipu sub-basin, which has an area of 22.5 km². The lagoon has a permanent channel connecting it to the sea and is surrounded by an urban area that is part of the metropolitan region of Niterói (Silva & Molisani, 2019). Its surroundings are associated with ecosystems such as sand dunes, restinga, mangroves, and swamps, representing a site with high biotic and abiotic environmental diversity. These attributes make the region a prominent tourism and leisure hub, as it is situated within the Atlantic Forest biome (Brandon *et al*., 2005). Additionally, the study area is integrated into two conservation units (PESET - Serra da Tiririca State Park; RESEX-Itaipu - Itaipu Marine Extractive Reserve), where management actions involve controlling urban occupation and mitigating the impacts of irregular sewage connections, which affect the environmental quality of this system (Inea,2021).

Figure 1- Location of Rio de Janeiro in Brazil, the eastern part of the state of Rio de Janeiro, and the sampling lagoon (Itaipu Lagoon System), where *Eugerres brasilianus* were captured from July 2019 to April 2020
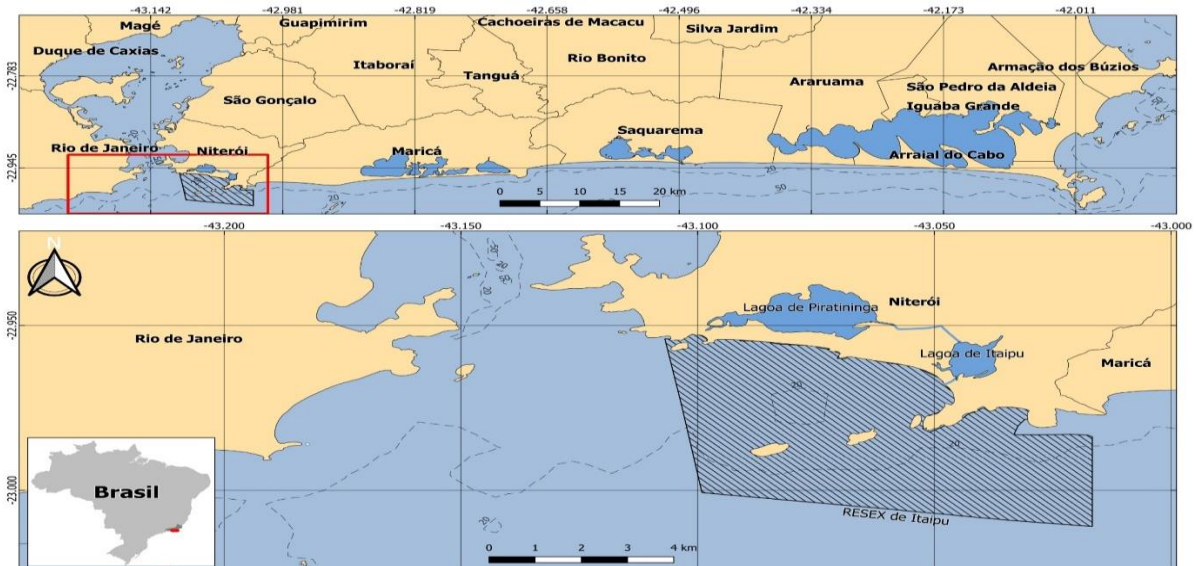
Sampling

The sampling program was conducted in two phases: 1) Seasonal collections (winter and summer) were carried out in Itaipu Lagoon using cast nets with various mesh sizes (5, 12, and 20 mm) to obtain the target specimens. After collection, all material was transported to the laboratory for biometric measurements and analysis. 2) Monthly acquisition (from July 2019 to April 2020) of fish from local artisanal fishing was conducted through the purchase of fish directly from fishermen, aiming to obtain samples across different size strata (juveniles and adults).

All fish were individually measured for the following dimensions: total length (TL), recorded in centimeters using an ichthyometer with a precision of 0.1 cm; total weight (TW), eviscerated weight (EW), and weights of the gonads (GW) and liver (LW) in grams (g), measured with a digital balance with a precision of 0.01 g. After dissection of each collected specimen, sex and maturity stages were identified based on the macroscopic descriptions proposed by Brown-Peterson *et al.* (2011): immature; developing (maturing); ripe (mature); spawned; and regenerating. Subsequently, the sagitta otoliths were extracted, cleaned, and stored in Eppendorf tubes until the time of analysis.

Size at First Maturity

The average size at first maturity (L_{50}) was determined for both pooled and separated sexes based on the adjustment of a logistic regression applied to the dataset. In addition to the total length (TL) of the individuals, the proportion of individuals that had already initiated the reproductive process (% of mature individuals) was considered, including individuals in development, ripe, spawned, and regenerating. The logistic regression used was based on a generalized linear model combined with a non-parametric bootstrap method implemented through the SizeMat package (Torrejon-Magallanes, 2020), which is integrated into the R software (R Core Team, 2022).

Otolith Processing

For the analyses, the right otoliths were positioned horizontally to be photographed with the rostrum and antirostrum facing left and the sulcus facing upwards against a black background, using a Leica Stereozoom S9iSZX12 stereomicroscope with an integrated camera. The photos were processed using the LAS EZ software, with scales in millimeters, and then edited using Paint.net (v4.2.15) (Figure 2A).

Figure 2 - (A) Internal face of a right sagitta otolith of *Eugerres brasilianus* (Cuvier, 1830) shown in the original photograph; (B) contour in red (PO), length (LO), and width (WO) of the otolith
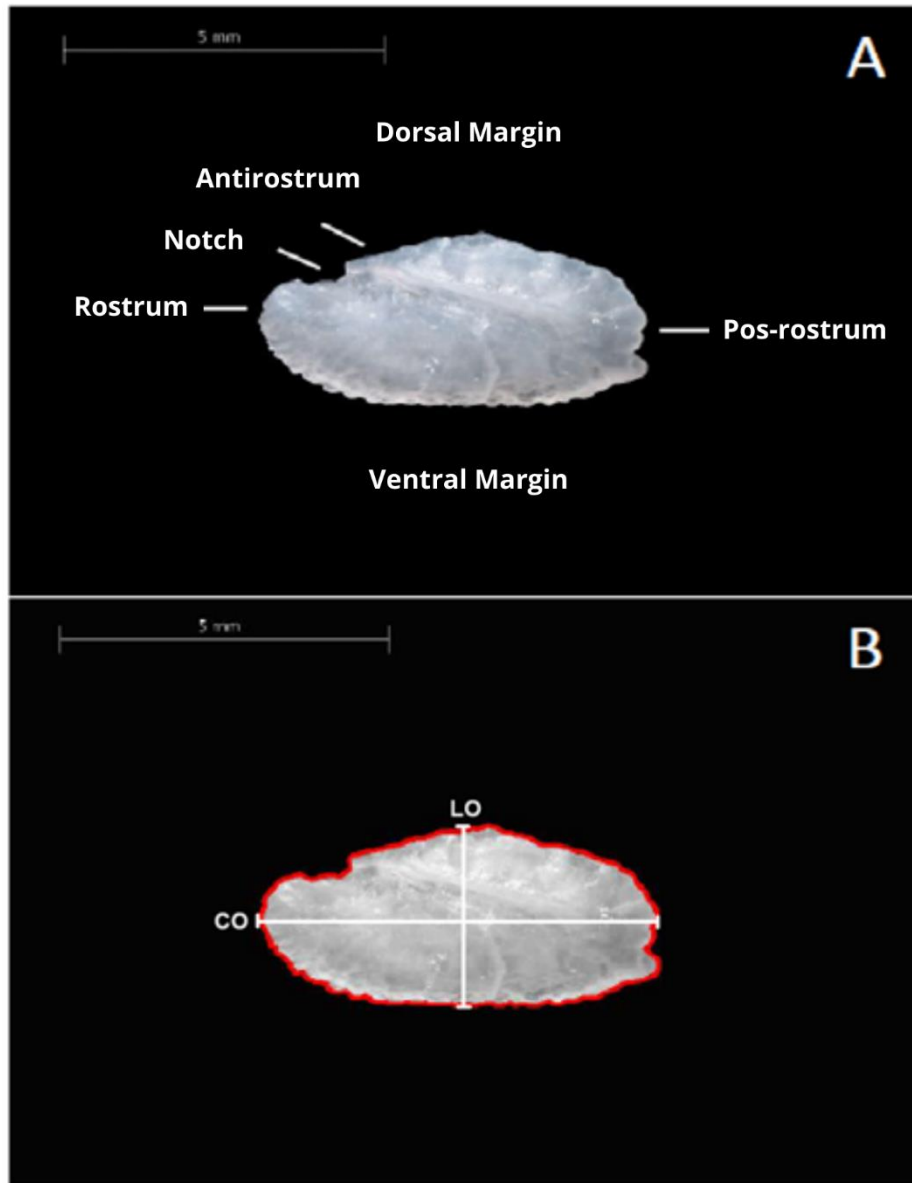
Contour Analysis

The ShapeR package, incorporated into the R software (R Core Team, 2022), is a commonly used tool for studying morphological variation of otoliths in fish populations through wavelet transformations. Wavelet transformations are a method of signal propagation into a set of functions that represent the expansions and translations of a single function known as the root wavelet (Libungan & Pálsson, 2015).

Shape Descriptors

Shape descriptors were determined to characterize the morphology of otoliths across different size classes, sexes, and maturity stages. For each otolith, the descriptors used were

area (AO, in mm^2), length (LO, in mm), width (WO, in mm), and perimeter (PO, in mm) (Figure 2B), which were generated using the ShapeR package version 0.1-5 (Libungan & Pálsson, 2015) in R (R Core Team, 2022). The indices of circularity (PO^2 / AO), roundness (4AO / [π LO^2]), form factor (4πAO / PO^2), rectangularity (AO / [LO × LO]), and ellipticity ([LO - LO] / [LO + LO]) were calculated according to the methodology proposed by Tuset *et al.* (2003). All morphometric variables were log-transformed (log(x+1)) for statistical analyses to meet the assumptions of normality and homoscedasticity, assessed using the Shapiro-Wilk and Levene tests.

Statistical Analyses

Allometric effects of fish length were corrected for wavelet coefficients and shape descriptors using the methodology described by Lleonart *et al.* (2000), which removes the size effect when comparing otolith contours and measurements. The allometric effects of fish length were adjusted for wavelet coefficients and shape descriptors using an algorithm integrated into the ShapeR package (Lleonart *et al.*, 2000; Libungan & Pálsson, 2015). Ten wavelet coefficients (Ws3c4, Ws3c5, Ws4c1, Ws4c8, Ws4c16, Ws5c8, Ws5c13, Ws5c14, Ws5c15, Ws5c25) that showed significant interaction with both groups and lengths were automatically removed (Libungan & Pálsson, 2015).

The intraclass correlation coefficient (ICC) was used to measure the reliability of measurements based on the variance ratio of wavelet transformations. Variation in otolith shape relative to size classes was assessed using Canonical Analysis of Principal Coordinates (CAP) based on wavelet coefficients with coordinates derived from Euclidean distances. The variation in distance between groups was tested with Permutational Univariate Analysis of Variance (PERMANOVA) using the R vegan package (Oksanen *et al.*, 2017), with 10,000 permutations. The classification rate of groups was estimated using Linear Discriminant Analysis (LDA) with the R MASS package (Venables & Ripley, 2002).

Univariate Permutational Analysis of Variance (PERMANOVA) and Multivariate Permutational Analysis of Variance (PERMANOVA) were employed to test for differences between size classes, sexes, and maturity stages for shape descriptors, using the permuco (Frossard *et al.*, 2019) and vegan (Oksanen *et al.*, 2017) packages, respectively, in the R software. Principal Component Analysis (PCA) was used to visualize patterns among size classes and sex/maturity stages, utilizing the MASS package (Venables & Ripley, 2002) in R. A test for multivariate dispersion homogeneity was also applied to the shape descriptors to determine which type of discriminant analysis would be used (Misra, 1985). As the null hypothesis was accepted (p > 0.05), Linear Discriminant Analysis (LDA) combined with the jackknife method (Manly, 1986) was employed using the MASS package.

Interrelationships between morphological parameters and otolith length were determined using the lm package in R. Regression analysis was used to differentiate growth trends between males and females based on shape descriptors.

RESULTS

Between July 2019 and April 2020, 158 *Eugerres brasilianus* specimens were captured from the Itaipu Lagoon, with total lengths ranging from 7.5 to 30.6 cm and total weights from 3.0 to 439.0 g. The logistic curve for *E. brasilianus* indicated a length at first sexual maturity (L$_{50}$) of 15.5 cm total length for both sexes combined (n=158; females, males and indeterminate sex), 15.3 cm total length for females (n=69), and 15.5 cm total length for males (n=54) (Figure

3).

Figure 3 - Length at first sexual maturity (L_{50}) for both sexes combined (A), females (B), and males (C) of *Eugerres brasilianus* (Cuvier, 1830) captured in the Itaipu Lagoon from July 2019 to December 2020
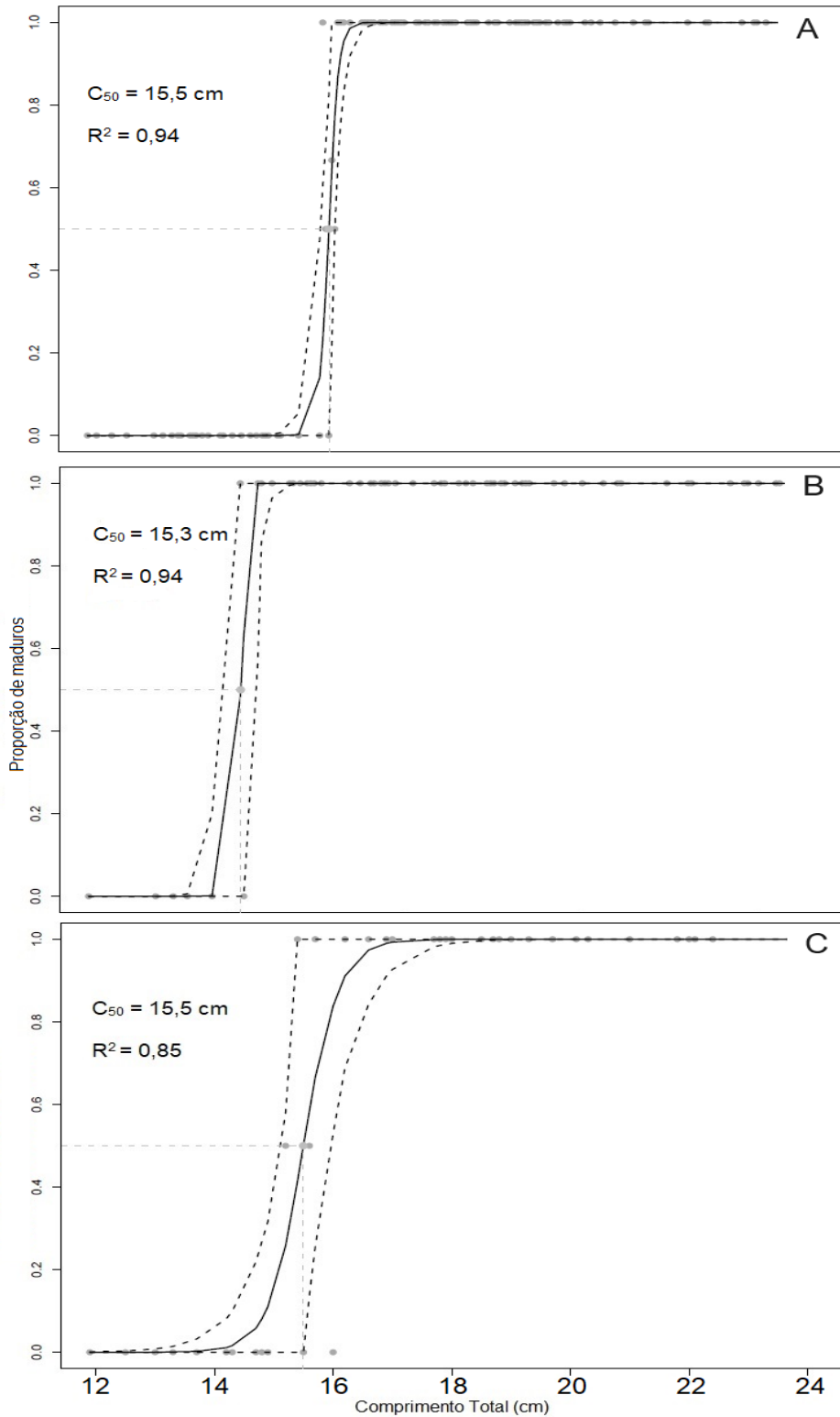
**Otolith Ontogeny** For ontogenetic analyses, 104 *Eugerres brasilianus* individuals

collected between September 2019 and March 2020 were selected. Five size classes were established with 5 cm intervals (C1, C2, C3, C4, and C5), with a size range varying from 7.5 to 30.6 cm total length (Table 1).

Table 1 - Pre-defined size classes, number of individuals (N), total length ranges (AMP), and means and standard deviations (MD±SD) for *Eugerres brasilianus* (Cuvier, 1830) collected in the Itaipu Lagoon

CLASSES	N	AMP	MD ± DP
C1 (6,0-11,0)	12	7,5 - 10,9	9,6 ± 1,2
C2 (11,0-16,0)	33	11,0 - 15,9	13,8 ± 1,5
C3 (16,0-21,0)	26	16,0 - 20,8	17,6 ± 1,4
C4 (21,0-26,0)	24	21,0 - 25,6	22,8 ± 1,4
C5 (26,0-31,0)	9	26,1 - 30,6	28,8 ± 1,4

The average otolith contours obtained from wavelet coefficients showed differences among the size classes. Morphologically, there is a dorsoventral flattening, accompanied by an anteroposterior elongation (extension of the rostrum and antirostrum) as the fish grows. In the post-rostrum region, a protrusion with the development of a pronounced indentation (0º angle) is observed during growth (Figure 4).

Figure 4 - Average contours of *Eugerres brasilianus* sagitta otoliths based on wavelet transformation, with each color representing the mean otoliths according to size classes (C1, C2, C3, C4, and C5). The centroid of the otolith (center of the cross) is the central point of the coordinates
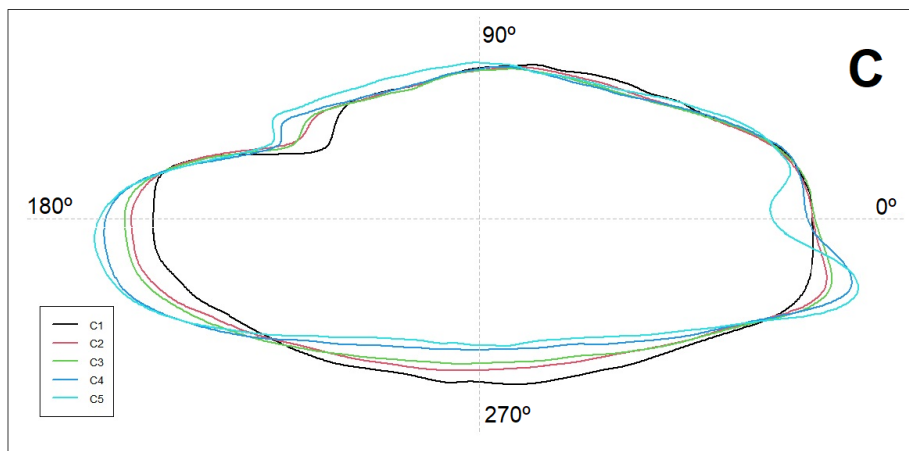
The average contours reveal significant variation in the regions near the 180º and 360º angles among the size classes, corroborated by the intraclass correlation coefficient (ICC) with high means/standard deviations and low ICC values. As the contour lines become more spaced, the intraclass correlation decreases, and the mean/standard deviation increases. This indicates that the measurements generated by the ICC are inversely proportional to the mean/standard deviation, suggesting that the size classes are distinct. The intraclass correlation coefficient showed higher means and deviations at 0º, 180º, and 360º angles, with low ICC values, indicating high variation in contour lines (Figure 4). After the 0º and 180º angles, there were

the highest peaks in ICC, indicating greater overlap of contour lines and highlighting a higher correlation between size classes.

Figure 5 - Intraclass correlation coefficient for the otoliths of *Eugerres brasilianus* (Cuvier, 1830) captured in Lagoa de Itaipu. Means and standard deviations of the wavelet coefficients (bars/circles) representing the average shape of all collected otoliths, and the intraclass correlation coefficient, indicated by the solid black line
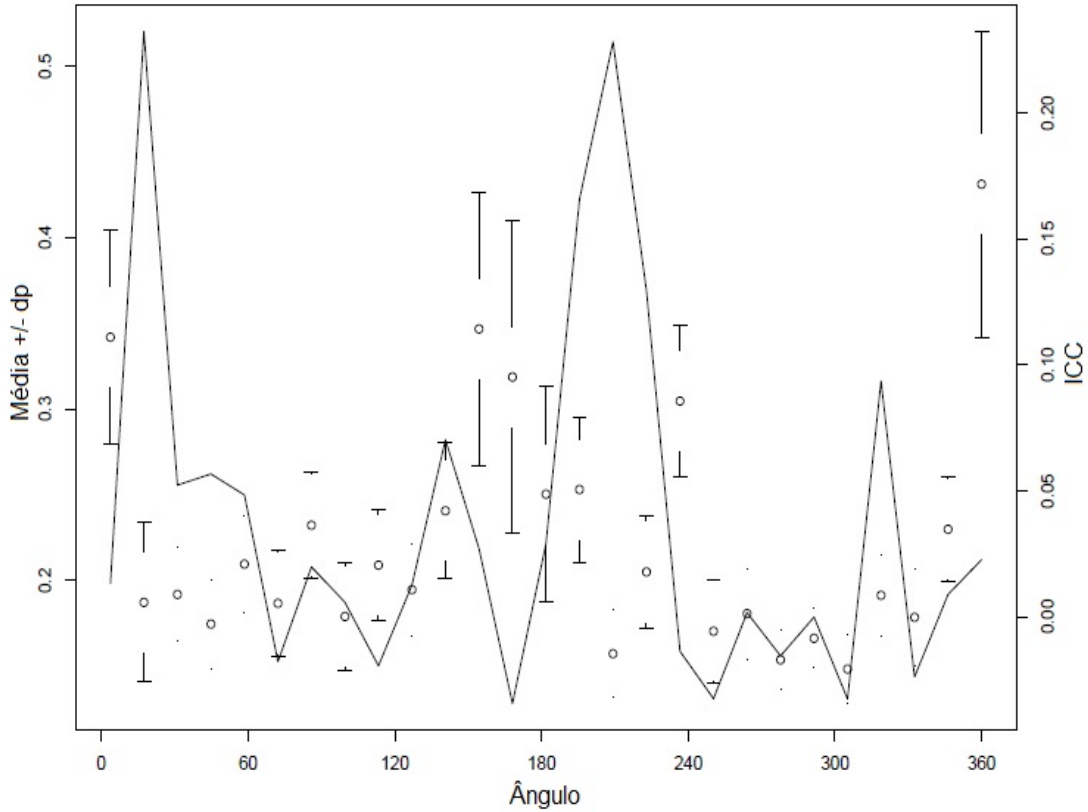
The permutational ANOVA conducted for the wavelet coefficients among all size classes revealed a significant difference (F: 22.693; p < 0.001). Differences were also observed when the classes were evaluated pairwise (p < 0.01) (Table 2).

Table 2 - Results of the permutational analysis of variance for the wavelet coefficients obtained from the contours of *Eugerres brasilianus* (Cuvier, 1830) otoliths by size class

Classes	pseudo-F	p
C1xC2	29,723	0.001 ***
C1xC3	25,628	0.001 ***
C1xC4	53,181	0.001 ***
C1xC5	48,239	0.001 ***
C2xC3	3,708	0.002 **
C2xC4	12,214	0.001 ***
C2xC5	27,790	0.001 ***
C3xC4	11,132	0.001 ***
C3xC5	27,394	0.001 ***
C4xC5	12,981	0.001 ***

Figure 5 shows a Canonical Analysis of Principal Coordinates (CAP) using wavelet

coefficients, where axis 1 explained 80% of the variation and axis 2 explained 13%. Classes C1 and C5 were distinct from the other classes along the first canonical axis (CAP1), while C2, C3, and C4 differed minimally from each other in relation to both axes. The classification rate established by Linear Discriminant Analysis (LDA) for the canonical axes was 100% for class C1, 71% for C2, 29% for C3, 68% for C4, and 100% for C5, resulting in an overall classification accuracy of 66% (Figure 6).

Figure 6 - Canonical Analysis of Principal Coordinates (CAP) among the wavelet coefficients obtained from the otoliths of *Eugerres brasilianus* (Cuvier, 1830) captured in the Itaipu Lagoon
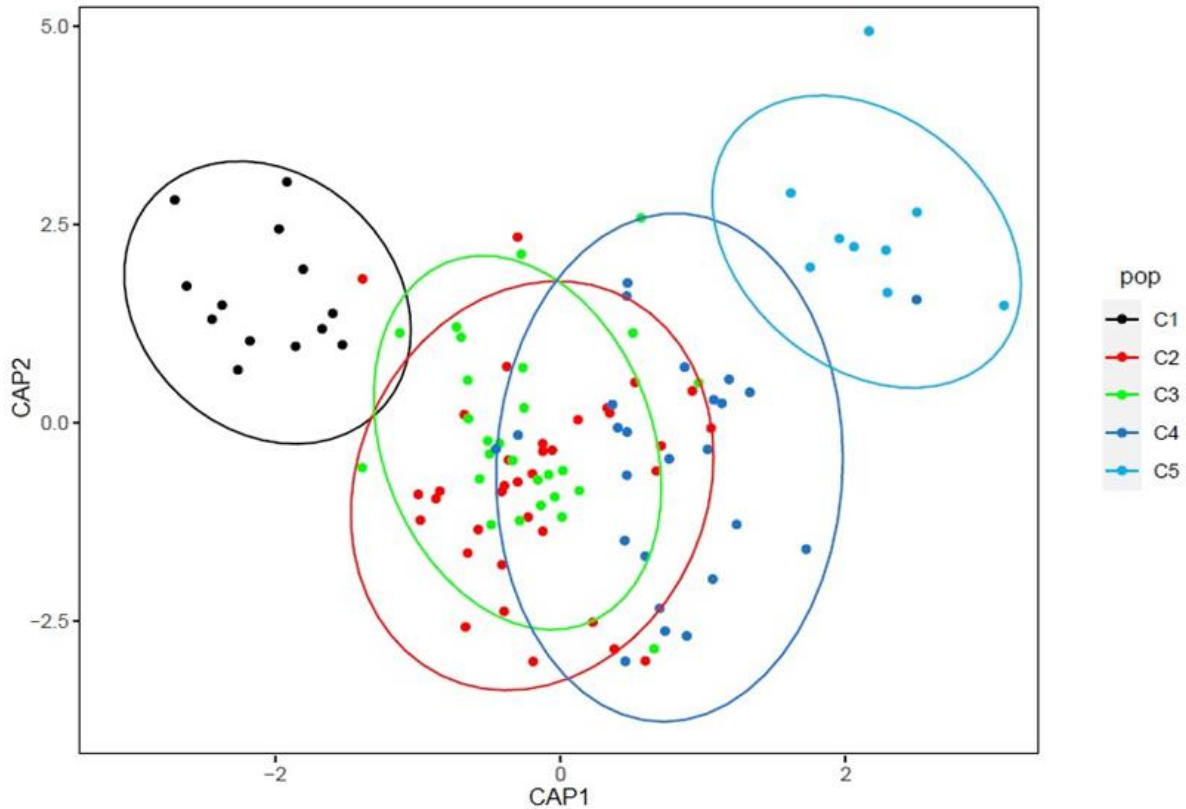
Significant differences were also observed for the shape descriptors among the five size classes when compared to each other using PERMANOVA (F: 98.961; p < 0.001), as well as when analyzed pairwise (p < 0.001) (Table 3).

Table 3 - Multivariate Permutational Analysis of Variance performed on the shape descriptors obtained from the otoliths of *Eugerres brasilianus* (Cuvier, 1830) by size classes

CLASSES	pseudo-F	p
C1xC2	55,128	0.001 ***
C1xC3	126,300	0.001 ***
C1xC4	268,460	0.001 ***
C1xC5	298,600	0.001 ***
C2xC3	23,729	0.001 ***
C2xC4	116,280	0.001 ***
C2xC5	138,190	0.001 ***
C3xC4	37,296	0.001 ***
C3xC5	77,791	0.001 ***
C4xC5	21,594	0.001 ***

The permutational ANOVA also revealed significant differences between the size classes

for each shape descriptor ($p < 0.05$). Pairwise comparisons between the classes showed that C2xC3 and C4xC5 did not exhibit significant differences ($p > 0.05$) for the descriptors of roundness and rectangularity for both pairs, while circularity and form factor differed only between C2xC3, and ellipticity differed between C4xC5. All other pairings showed significant differences for all descriptors ($p < 0.05$). Rectangularity was the descriptor with the fewest significant differences between pairings, whereas area and perimeter showed significant differences across all pairings.

In the Principal Component Analysis (PCA) conducted for shape descriptors, the first axis explained 92.1% of the total variance, while the second axis accounted for 4.8%. Along the first axis, a clear separation was observed between size classes C1 and C2 on the left side of the plot, as well as between size classes C4 and C5 on the right side. The centrally located class C3 exhibited overlap with classes C2 and C4. The Jackknife classification matrix yielded an overall classification rate of 76%, with the following classification values for size classes: C1 = 100%, C2 = 81%, C3 = 57%, C4 = 79%, and C5 = 77% (Figure 7). The shape descriptors that best explained the observed variation were: area (PC1: $r = 0.79$; PC2: $r = -0.41$), circularity (PC1: $r = 0.13$; PC2: $r = 0.53$), and perimeter (PC1: $r = 0.46$; PC2: $r = 0.05$). Other descriptors showed lower correlations with the first two axes: ellipticity (PC1: $r = 0.29$; PC2: $r = 0.36$), roundness (PC1: $r = -0.16$; PC2: $r = -0.33$), form factor (PC1: $r = -0.13$; PC2: $r = -0.10$), and rectangularity (PC1: $r = -0.01$; PC2: $r = -0.10$).

Figure 7 - Principal Component Analysis (PCA) using shape descriptors for the size classes of *Eugerres brasilianus* (Cuvier, 1830) captured in Lagoa de Itaipu
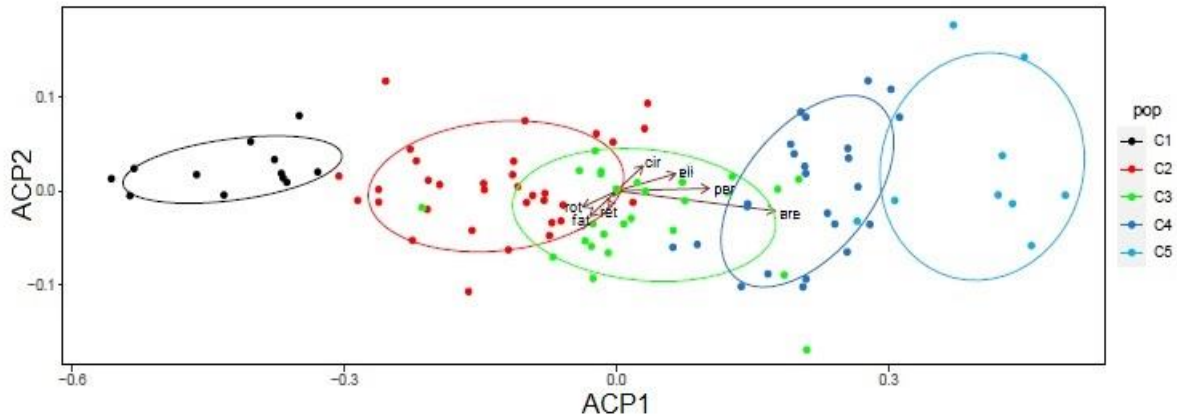
Sex and Maturity Stages

For the assessment of sexual variation, a total of 100 specimens were used, including 41 females, 25 males, and 34 immature individuals whose sex was not determined. Maturity stages were evaluated by categorizing individuals into "maturing" (equivalent to young individuals) and "mature" (corresponding to adults capable of reproduction). *Eugerres brasilianus* individuals were classified into five groups based on sex and maturity stage (I1, F2, M3, F4, and M5), according to the predefined size classes: I1 ≥ C1, C2, and C3; F2 and M3 ≥ C2, C3, and C4; F4 ≥ C3, C4, and C5; M5 ≥ C2, C3, and C4 (Table 4).

Table 4 - The 5 codes used and their corresponding sex (M = Male; F = Female; Ind. = Indeterminate) and gonadal maturity stages (Im. = Immature; Em. Mat. = Maturing; Mad. = Mature), along with the total number (N) of individuals per code, the range (AMP) of total lengths, and the mean and standard deviation (MD±SD) for *Eugerres brasilianus* (Cuvier, 1830) collected from the Itaipu Lagoon

CODE	SEX	STAGE	N	AMP	MD ± DP
I1	Ind.	Im.	34	7,5 - 17,6	13,8 ± 2,7
F2	Fêmea	Em Mat.	16	13,8 - 21,1	16,9 ± 2,1
M3	Macho	Em Mat.	16	12,5 - 21,0	16,3 ± 2,3
F4	Fêmea	Mad.	25	19,4 - 30,6	24,1 ± 3,3
M5	Macho	Mad.	9	13,3 - 24,0	17,7 ± 3,1

Shape descriptors showed significant differences among the five groups classified by sex and maturation stage as determined by PERMANOVA (F: 97.612; p<0.001). When analyzed in pairwise comparisons, only the mature females and males (F2xM3) did not show significant differences (Table 5).

Table 5 – Permutational Multivariate Analysis of Variance (PERMANOVA) results for shape descriptors obtained from the otoliths of *Eugerres brasilianus* (Cuvier, 1830) across maturation groups

CODE	pseudo-F	p	CODE	pseudo-F	p
I1xF2	22,009	0.001 ***	I1xF2	22,009	0.001 ***
I1xM3	15,667	0.001 ***	I1xM3	15,667	0.001 ***
I1xF4	127,310	0.001 ***	I1xF4	127,310	0.001 ***
I1xM5	36,664	0.001 ***	I1xM5	36,664	0.001 ***
F2xM3	0,174	0,809	F2xM3	0,174	0,809

Permutational ANOVA also revealed significant differences between the groups classified by sex and maturation stage for all shape descriptors (p<0.05). Pairwise comparisons indicated that F2xM5 did not show differences in circularity, rectangularity, and form factor, while F4xM5 did not show differences in roundness, rectangularity, and ellipticity. The F2xM3 pairing was the only one that exhibited significant differences across all descriptors (p<0.05). Rectangularity showed fewer significant differences between pairings, whereas area and perimeter showed significant differences in all pairings (p<0.001) (see Table Appendix I).

In the principal component analysis (PCA) conducted for shape descriptors separated by maturation stage and sex, the first axis explained 92.3% of the total variation, while the second axis explained 4.6% (Figure 8). Along the first axis, a clear separation is observed between I1 and F4, whereas the other groups overlap across both axes. The linear discriminant analysis (LDA) yielded a jackknife classification matrix with classification rates of 79% for I1, 25% for F2, 31% for M3, 80% for F4, and 11% for M5, resulting in an overall classification rate of 57%. The shape descriptors that best explained the observed variation were area (PC1: r=0.79; PC2: r=-0.42), perimeter (PC1: r=0.46; PC2: r=0.05), circularity (PC1: r=0.13; PC2: r=0.53), and form factor (PC1: r=-0.13; PC2: r=-0.53). Other descriptors showed weak correlations with the second axis: ellipticity (PC1: r=0.29; PC2: r=0.36), roundness (PC1: r=-0.16; PC2: r=-0.33), and rectangularity (PC1: r=-0.01; PC2: r=-0.10).

Figure 8 - Principal component analysis (PCA) using shape descriptors for the sexes and maturation stages of *Eugerres brasilianus* (Cuvier, 1830) collected from the Itaipu Lagoon. Shape descriptors include otolith area (are), perimeter (per), circularity (cir), roundness (rot), form factor (fat), rectangularity (ret), and ellipticity (eli).
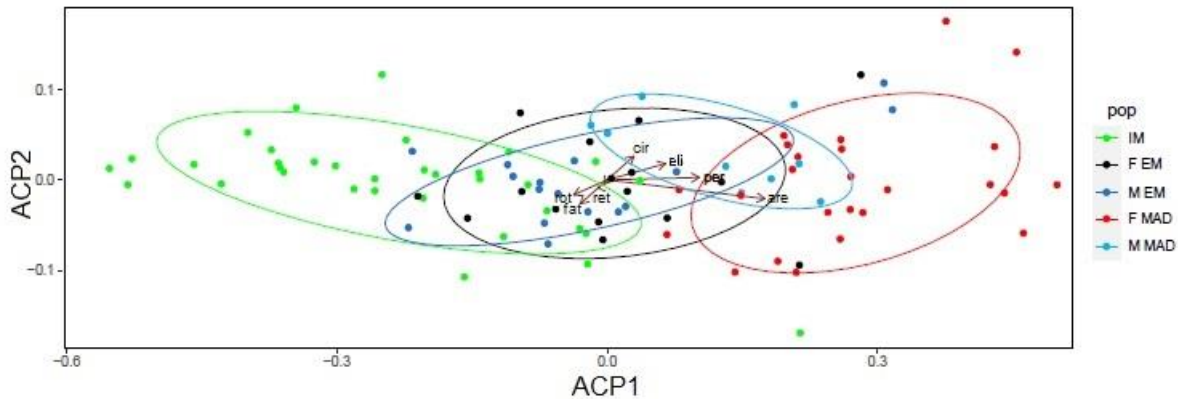
DISCUSSION

Our results confirm the hypothesis of ontogenetic variation in the otolith shape of *Eugerres brasilianus* (Cuvier, 1830), similar to what has been observed in other marine fish species (Vignon, 2012; Carvalho *et al.*, 2015). As the fish grows, the shape of the otolith undergoes morphological changes (Lombarte *et al.*, 2006), associated with size variations, particularly emphasized during sexual maturation. Analysis of the wavelet means of *E. brasilianus* sagitta otoliths demonstrated such changes in both the length and contour of the otoliths. Shape descriptors also highlighted significant differences between size classes, with area, circularity, and perimeter best explaining the variations in the species, confirming changes in otolith shape related to fish size. In Itaipu Lagoon, *E. brasilianus* exhibits a resident habit (Almeida *et al.*, 2023). However, adult individuals of the species (concentrated in the more internal areas of estuarine environments) use the habitat and feed differently from juveniles (concentrated in the more external areas of estuarine environments) (Ramos *et al.*, 2014). As the fish grows, its swimming ability increases, allowing migrations that could reflect in otolith morphology, such as the development of the rostrum (Carvalho *et al.*, 2015). Galley *et al.* (2006) suggests that variations in otolith shape occur primarily along the antero-posterior axis of the structure rather than along the dorsal-ventral axis. This structural development, which accompanies the growth of the animal, results in an increase in its size in area and consequently in its perimeter. The circular shape found in the larval stage is progressively reduced as the individual grows (Joh *et al.*, 2015). This assumption indicates a negative allometric relationship between length and width, characterizing a growth pattern of the structure similar to that observed in other species (Simoneau *et al.*, 2000). The presence of a rostrum without a deep excisure suggests that the species is not an exceptional swimmer and has demersal habits (Carvalho *et al.*, 2015). Species in the family Sciaenidae typically lack a rostrum with an excisure, such as *Micropogonias furnieri* (Santos *et al.*, 2017) and *Menticirrhus americanus* (Carvalho *et al.*, 2020), indicating that they are demersal explorers (Ramos *et al.*, 2014). These otolith characteristics differentiate *E. brasilianus* from species with pelagic habits. For example, the dolphinfish (*Coryphaena hippurus*) and the true sardine (*Sardinella brasiliensis*) (Almeida *et al.*, 2020; Schroeder *et al.*, 2022) are species with a pronounced rostrum with excisure, indicating they are proficient pelagic swimmers.

The shape of otoliths exhibits ontogenetic development that is genetically predetermined, which is associated not only with the early stages of the species but can also be remodeled on a small scale due to environmental conditions experienced by individuals at different life stages, thereby increasing intraspecific variability (Vignon, 2012). Consequently,

it can be asserted that the reproductive process influences the shape of the sagitta otolith in *Eugerres brasilianus*, as changes occur in both the species' habits and habitat following the onset of the reproductive phase (Teixeira & Helmer, 1998; Rodrigues *et al.*, 2017). Another indication is the potential reallocation of energy from growth towards gamete formation, leading to modifications in the calcium deposition rate in the otoliths, as calcium is crucial for oocyte formation (Carvalho *et al.*, 2020).

The higher number of females captured compared to males may reflect favorable habitat conditions (Raposo & Gurgel, 2001) or be related to the reproductive potential of older, larger females (Chaves *et al.*, 2018). Similar to other species, females of *Eugerres brasilianus* attain larger sizes, making them more susceptible to capture by fishing gear (Rodrigues *et al.*, 2017). The mean size at first sexual maturity (C50) is one of the reproductive strategies influenced by the environment-genotype interaction (Vazzoler, 1996), representing the size at which at least 50% of individuals in the population are considered capable of reproduction (Fonteles Filho, 2011). Our C50 values were similar to those reported in the literature for the species; Soares *et al.* (2016) found C50 values of 17.0 cm for females and 15.0 cm for males of *E. brasilianus* in the northeastern region of Brazil. This length also affects the onset of gonadal maturation, helping to separate juvenile and adult guilds, thereby defining the reproductive stock. Thus, C50 provides insights into how the species is utilizing its environment, supporting the hypothesis that *E. brasilianus* uses the lagoon as a spawning ground, as evidenced by the high number of females with mature gonads recorded.

Both analyses (wavelet coefficients and shape descriptors) of the sagittal otoliths of *Eugerres brasilianus* revealed significant differences among individuals categorized as immature, juvenile, and adult. Clear patterns of distinction were also corroborated by ordination analyses (CAP and PCA), integrating pre-established size classes with gonadal development stages. The size class C1, represented solely by immature individuals, differed from the other classes. Conversely, individuals in maturation stages, represented by classes C2 and C3, did not exhibit differences between sexes. However, reproductively mature fish (classes C3, C4, and C5) showed differences among themselves. Some authors suggest that reproduction is a physiological factor that influences otolith morphology (Tombari *et al.*, 2005; Carvalho & Correa, 2014; Carvalho *et al.*, 2015). Our findings support this hypothesis, as the observed development of both body and otoliths, along with the established C50, allowed us to differentiate between young and adult fish. These stages of development are associated with distinct habitats and feeding habits (Ramos *et al.*, 2014), which, in turn, affect otolith morphology (Biolé *et al.*, 2019; Carvalho *et al.*, 2020).

Other researchers have recognized that sex can induce changes in otolith shape, as differences in size between genders are particularly notable after sexual maturation (Begg & Brown, 2000; Simoneau *et al.*, 2000; Cardinale *et al.*, 2004; Carvalho *et al.*, 2020). Our results showed significant differences in otoliths between sexes only in mature individuals, where sexual dimorphism was also observed. Thus, the patterns recorded here align with studies of marine species that exhibit sexual dimorphism in otolith shape (Mille *et al.*, 2015; Carvalho *et al.*, 2020). These morphometric variations are attributed to physiological changes or endogenous factors that affect the precipitation of calcium carbonate differently in juveniles and adults (Vignon, 2012; Carvalho *et al.*, 2015). Another valid assumption for this argument relates to species that use vocalization as a behavioral reproductive strategy (Carvalho *et al.*, 2020). Thus, hearing/absorbing sound during the reproductive process may determine variations in otolith shape, especially when males intensify vocalizations to attract females (Tellechea & Norbis, 2012; Parmentier *et al.*, 2014). Generally, these differences between male and female otoliths are related to each sex's lifestyle, including habitat use, preferred food items, energy expenditure on gamete production, reproductive aggregations, growth rates, and hormonal levels, which can contribute to morphological differentiation of the structure (Cardinale *et al.*, 2004; Tuset *et al.*, 2016; Parmentier *et al.*, 2018; Vaux *et al.*, 2019).

It is important to emphasize that the physiological characteristics of a fish's life history will manifest impressions on the otolith from its early stages through to advanced stages

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(senility), alongside ecological effects that vary in impact at different life stages of the individual. In summary, the factors influencing otolith shape include genetic factors, fish size (Vignon & Morat, 2010; Reichenbacher & Reichard, 2014), environmental conditions (Longmore *et al.*, 2010), and metabolism (Grønkjær, 2016). Reproduction is a physiological factor that influences otolith morphometry (Tombari *et al.*, 2005) because, with the onset of sexual maturation, there is a reallocation of energy investment, which may result in reduced somatic growth and alter otolith shape (Carvalho & Corrêa, 2014). Thus, differences in otolith shape between mature females and males, as well as among maturation stages, reflect the sexual maturation process and the development of this structure. Consequently, otolith growth is also regulated by the fish's physiological activity, which is influenced by environmental conditions. Analyses of sagittal otoliths provide insights into various aspects of fish biology (Morales-Nin, 1987) due to the relationship between otolith growth and fish body growth, making the structure a valuable source of information about the growth processes of these animals. Thus, not only environmental but also physiological data are incorporated into otoliths, generating a structure rich with information about the fish's life history. This knowledge enhances our understanding of the species' biology and underscores the importance of sustainable fishing practices to ensure the future viability of the species.

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