

The use of the shape and chemistry of fish otoliths as a subpopulational discrimination tool for *Eugerres brasiliensis* in lagoon systems in the Southwest Atlantic Ocean

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ARTICLE INFO

Handled by Ivone Figueiredo Rosa

Keywords:

Gerreidae

Sagittae

Natural tags

Contour and chemical analyses

ABSTRACT

The Brazilian mojarra, *Eugerres brasiliensis*, is an economically important species for the artisanal fisheries usually found in the estuarine waters along the southwest Atlantic Ocean. Despite this, knowledge about its population structure is scarce, and no management strategies have been applied to ensure the fisheries sustainability in Brazil. Thus, the present study intended to understand the population dynamics of *E. brasiliensis* in three coastal lagoons located in Rio de Janeiro, Brasil. A total of 90 individuals were collected in the lagoon systems of Itaipu, Saquarema and Araruama, between December 2019 and March 2020. A pre-selection of 30 individuals per location from the same age group (2 years old), following age estimation by counting the annual growth increments, were used. The contour of the sagittal otoliths was evaluated using elliptical Fourier descriptors (EFD), and the multi-elemental signatures (MES) of the whole otoliths were obtained using solution-based inductively coupled plasma mass spectrometry. Data were analyzed using univariate and multivariate statistics to assess the degree of separation between individuals from different lagoons. EFD revealed significant differences among individuals from the different sampling regions. MES exhibited distinct regional patterns, mainly driven by differences in Cu/Ca, Li/Ca, Mg/Ca, Mn/Ca, and Sr/Ca ratios. Reclassification accuracy rates obtained from linear discriminant function analyses using both EFD and MES of otoliths were 100% (Itaipu), 97% (Araruama) and 90% (Saquarema). Therefore, a clear distinction was observed among these groups, which was related to the inherent characteristics of each lagoon system, their semi-restricted connectivity with the adjacent coastal zone, as well as the estuarine-opportunistic behavior of the species. Thus, the results suggest that these fisheries should be managed as different subpopulation-units. However, more studies should be carried out about the fish movements and life history events of this species in southeastern Brazil.

1. Introduction

The Brazilian mojarra, *Eugerres brasiliensis* (Cuvier, 1830), is the largest species of the Gerreidae family (maximum fork length of 50 cm: [García-Arteaga et al., 1997](#)), being an estuarine-opportunistic demersal fish with a wide geographic distribution, ranging from South Carolina, USA to Santa Catarina, southern Brazil ([Figueiredo and Menezes, 1980](#);

[Eiras-Stofella and Charvet-Almeida, 2000](#); [Andrade-Tubino et al., 2008](#)).

It is one important ecological fish species in Brazil due to its occurrence, abundance and resilience in different marine and estuarine ecosystems, such as mangroves and tropical and subtropical lagoon systems ([Deckert and Greenfield, 1987](#); [Castro-Aguirre et al., 1999](#); [Paiva et al., 2013](#)). Despite its economic importance, information on its population dynamics is limited, and as such there is no rational management strategy

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<https://doi.org/10.1016/j.fishres.2023.106795>

Received 24 March 2023; Received in revised form 18 June 2023; Accepted 6 July 2023

Available online 24 July 2023

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for the resource in Brazil (Rodrigues et al., 2017).

Eugerres brasiliensis is frequently caught by sport fisheries, artisanal and industrial fleets in the estuarine and coastal waters of Northeast (Tischer and Santos, 2003; Barletta and Costa, 2009; Soares et al., 2009; Soares et al., 2011; Souza et al., 2018), Southeast (Clauzet et al., 2005; Pinheiro and Joyeux, 2007; Souza et al., 2018) and Southern (Chaves and Robert, 2003; IBAMA, 2009) Brazil. It constitutes an important demersal fishing resource in tropical and subtropical coastal lagoons and estuaries (Pérez-Hemández and Zavala-Hurtado, 1993; Rodrigues et al., 2017). In the northeast region of Brazil, the gerreids are highly prized species for human consumption (Menezes et al., 2009). In the Rio de Janeiro state, *E. brasiliensis* is captured mainly by the artisanal fleet, and occasionally collected as a by-catch by the industrial fleet. The total reported catches by the state government agency have shown a growing trend over the last years in Rio de Janeiro coastal areas, with estimated annual landings of 16 tonnes in 2018, 18 tonnes in 2019 and 24 tonnes in 2020 (FIPERJ, 2022). However, a recent study showed that these recorded landings are underestimated since in the lagoon systems of the eastern Rio de Janeiro state, it is one of the most representative fish species, with 95 tonnes captured annually by the local artisanal fleets through multispecific fisheries (Tubino et al., 2021). Moreover, it has potential interest in aquaculture, due to the ease of breeding in captivity, rapid growth, generalist feeding and resilience to environmental factors, although still in an experimental phase (Cavalli and Hamilton, 2007; Soares et al., 2016; Valenti et al., 2021).

Eugerres brasiliensis is a fish classified as marine estuarine-dependent and omnivore-bentivore (Andrade-Tubino et al., 2008; Ramos et al., 2016; Almeida et al., 2021). This species inhabits coastal waters and regularly enters in the estuaries, particularly as juveniles. However, they also use nearshore marine waters as alternative habitats. *Eugerres brasiliensis* can migrate between freshwater and marine coastal environments for reproductive purposes, relying on protected environments and resource availability in transition areas to complete its reproductive cycle (Ramos et al., 2016; Soares et al., 2016). However, a previous study found individuals exclusively in the middle estuary area, where its high densities suggest that the species is well adapted to the environmental constraints compared with other Gerreids species occurring exclusively in the lower estuary under strong marine influence (Franco et al., 2012). In the eastern Rio de Janeiro lagoon systems, *E. brasiliensis* individuals in early juvenile and mature stages were recorded showing that the species can occur in these environments throughout its life cycle (Almeida et al., 2021). This species exhibits split spawning, with reproductive activity occurring throughout the year, but with lower intensity during winter in the lagoon systems of eastern Rio de Janeiro. It reaches sexual maturity with 15.5 cm of total length (Andrade et al. Personal communication).

In the southeastern Brazil, the coastal lagoons located in the Rio de Janeiro have great ecological importance for the maintenance of coastal biological diversity (Franco et al., 2022). Several studies have reported that these coastal lagoons function as important hot-spots of aquatic biodiversity (Fortes et al., 2014; Guimarães et al., 2021; Camara et al., 2021). They are also considered aquatic ecosystems with high biological productivity (Knoppers et al., 1999), functioning as nursery areas and adult habitats for several fish species of economic interest (Prestrelo and Monteiro-Neto, 2016; Cruz et al., 2017; Costa et al., 2021). The artisanal fisheries taking place in these environments have a significant socio-economic importance, presenting typical small-scale characteristics and employing few technologies for navigation and capture of target species (Barroso et al., 2000; Tubino et al., 2021).

The connectivity between adjacent fish subpopulations through larval, juvenile and adult stages dispersal is a key-issue for marine metapopulations (Sale et al., 2005; Kritzer and Sale 2006; Camara et al., 2020). In this context, the identification of subpopulations and their degrees of connectivity can contribute to the establishment of distinct management units, and highlights the importance of specific habitats for the maintenance of the adult stocks (Lawton et al., 2010; Vignon, 2012;

Avigliano and Volpedo, 2016; Camara et al., 2021).

The structural, shape and chemical properties of otoliths are a useful tool to unravel the population structure, movement patterns and habitat connectivity of fishes (Hoff et al., 2022; Muniz et al., 2020; Schroeder et al., 2022a). Genetic factors are largely responsible for inter-specific differences, but regional variations within species are almost controlled by the environment (Tuset et al., 2016; Vasconcelos et al., 2021; Soeth et al., 2022). This allows to unravel the life history of a species, since the otolith shape depends on fish growth rates, individual condition and environmental influences (Campana and Casselman, 1993; Cardinale et al., 2004; Salgado-Cruz et al., 2020). The morphology of otoliths can provide answers about the life history and behaviour of fish, because they suffer the influence of several abiotic and biotic variables, allowing to distinguish sets of individuals of the same species among different areas and periods (Lattuca et al., 2015). In this context, otolith shape analysis using geometric methods constitutes a practical and efficient way to define fish stocks, being fundamental in population and ecosystem management strategies (Corrêa et al., 2022; Hoff et al., 2020; Schroeder et al., 2022a). Different morphometric techniques have been applied to otoliths in order to investigate the structure of fish stocks, such as wavelet transformations (Tuset et al., 2019; Wiff et al., 2020; Soeth et al., 2022), elliptical Fourier descriptors (EFD) (Santos et al., 2017; Almeida et al., 2020; Muniz et al., 2020), and shape indices (Montanini et al., 2015; Callicó-Fortunato et al., 2017; Adelir-Alves et al., 2019).

The use of otolith geochemical signatures is another technique that has been frequently applied in ichthyology to study the movement patterns, habitat use and discrimination of fish stocks (Correia et al., 2021; Hoff et al., 2022; Moreira et al., 2022). In this context, four fundamental premises involve such structures as a natural marker of the environment that allows the reconstruction of the life history of a species: i) the otoliths are metabolic inert structures; ii) the otoliths grow throughout the life cycle of the fishes; iii) the composition of the otoliths reflect the chemistry of water; and iv) the environmental and physiological conditions affect the incorporation of the minor and trace elements in the otoliths (Kalish, 1989; Campana et al., 1999; Izzo et al., 2018). Several studies have been carried out in Brazil using otolith chemistry, highlighting the importance of this tool in approaches related to movement patterns (Avigliano et al., 2015; Franco et al., 2019; Soeth et al., 2020), nursery areas (Avigliano et al., 2016; Menezes et al., 2021; Avigliano et al., 2020), and population structure (Daros et al. 2016; Maciel et al. 2021; Schroeder et al. 2022b) of estuarine and coastal marine fish species of high economic and ecological importance. Some of the studies combined the analyses of the shape of otoliths and their elemental signatures as a practical and efficient way to assess issues related to population dynamics, movement patterns, habitat use and connectivity in fish (Soeth et al., 2019; Ferreira et al., 2019; Adelir-Alves et al., 2019).

Thus, the present study aimed to investigate the patterns of variation in the shape and multielemental signatures of the otoliths in individuals of *E. brasiliensis* captured in three coastal lagoon systems in Rio de Janeiro, Brazil, in order to obtain information about the population structure, habitat connectivity and fish movement. The final goal was to acquire fundamental information to support rational management measures for fisheries and conservation purposes.

2. Material and methods

2.1. Study area characterization

Eugerres brasiliensis adults were collected in three lagoon systems located in the eastern Rio de Janeiro state, Brazil, southwestern Atlantic Ocean: 1) Itaipu, 2) Saquarema and 3) Araruama (Fig. 1; Table 1). The climate gradient in the study area varies from humid to semi-arid, being governed by the presence of the continental equatorial air mass in the summer and the tropical Atlantic air mass during the rest of the year,

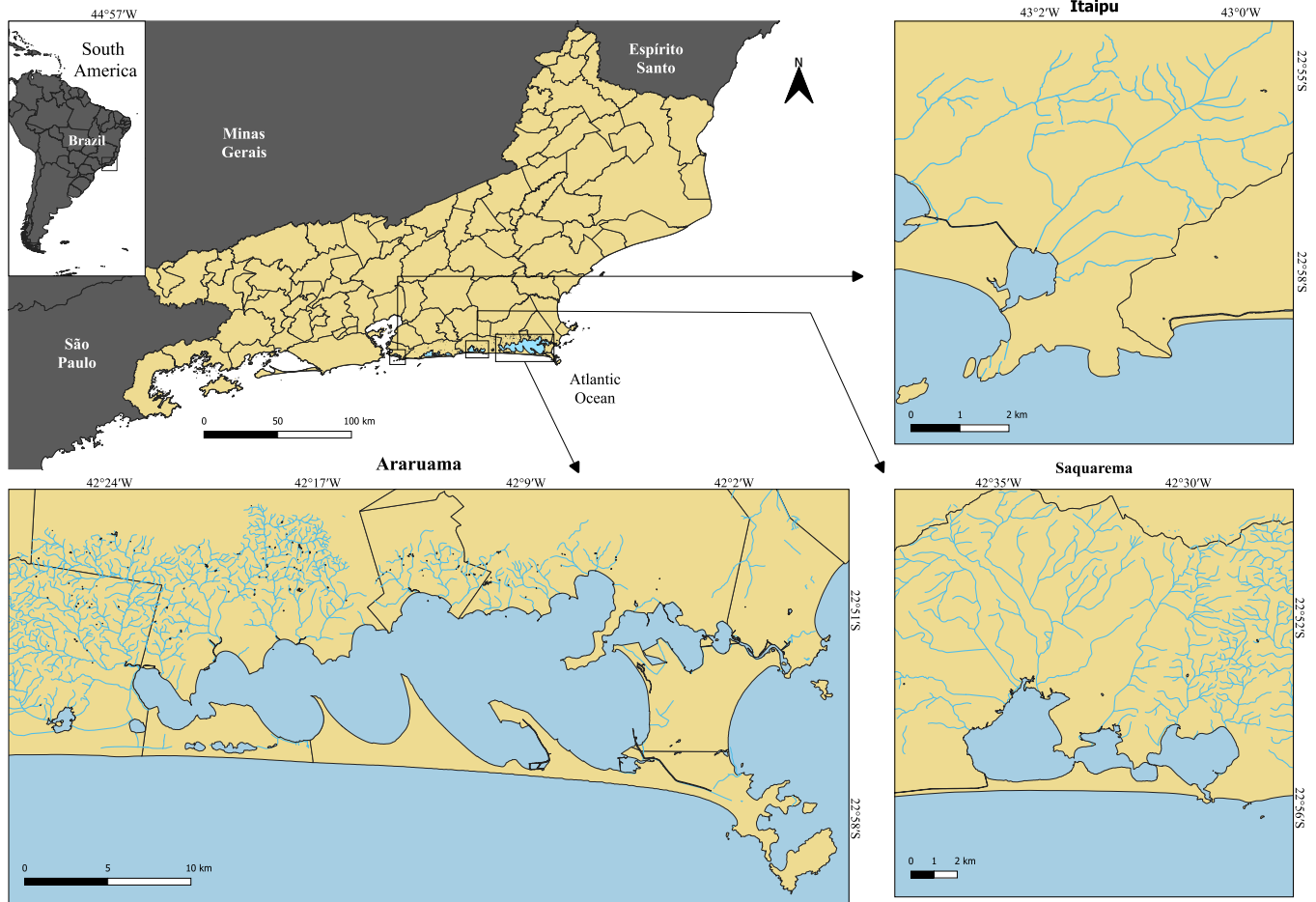


Fig. 1. Location of Brazil in the South America (inlet map), Rio de Janeiro and the three sampling locations (Itaipu Lagoon System, Saquarema Lagoon System and Araruama Lagoon System) of *Eugerres brasiliensis* caught on the Southwest Atlantic from December 2019 to March 2020.

Table 1

Geographic coordinates, descriptive parameters, trophic state and main anthropogenic contributions of the three lagoon systems of eastern Rio de Janeiro, Brazil.

Lagoon System	Coordinates	Area (km ²) *	Drainage basin area (km ²)*	Depth range (m)*	Water residence time (days)*	Water temperature range (°C) ** ***	Salinity range*	Average rainfall (mm/year) ***	Trophic state* ** *	Predominant anthropogenic contribution* *
Itaipu	22° 96' S 43° 04' W	2	23	0.7–4.0	3	22–33	12–31	1.4	hypereutrophic	Domestic sewage
Saquarema	22° 93' S 42° 49' W	21.2	215	0.6–2.4	27	18–33	9–34	1.3	supereutrophic	Domestic sewage/ agricultural waste
Araruama	22° 88' S 42° 01' W	225	285	0.5–18	84	21–29	12–60	0.9	supereutrophic	Domestic sewage/ agricultural waste/ mineral extraction: salt and shell limestone

Source: The authors based this information on data from [Dias et al. \(2021\)*](#), [Barroso et al. \(2000\)* *](#), [Knoppers et al. \(2009\)* * *](#) and [Silva and Molisani \(2019\)* * * *](#).

including the passage of cold fronts mainly during the spring ([Barbiéri and Coe Neto 1999](#); [Alvares et al., 2013](#)). The geomorphological configuration, the tidal channels nature and the continental drainage runoff are the main factors that determine the hydrodynamics on these systems ([Dias et al., 2021](#)). These lagoon systems are characterized by one or several series of elliptical cells, connected to the adjacent ocean by a single narrow channel, which allows classifying them as the suffocated type, i.e., semi-restricted connectivity ([Miranda et al., 2002](#)). Variations in water level (mesotidal) and salinity occur as a function of small and large-scale weather events, river runoff and changes in the hydric gradient ([Nichols and Biggs, 1985](#); [Mansur et al., 2012](#)). These characteristics lead to salinity gradients that distinguish zones with

different levels of freshwater and marine influence in all lagoon systems.

Located in the Niterói city (516,981 inhabitants), the coastal lagoon of Itaipu is an estuarine ecosystem connected by artificial channels to the Piratininga lagoon (Camboatá channel) and to the sea (Itaipu channel) ([Fig. 1](#)). There is an intensely urbanized area in the surroundings, with accentuated loss of habitat ([Barroso et al., 2000](#); [Silva and Molisani, 2019](#)) ([Table 1](#)).

Located in the Saquarema city (91,938 inhabitants), the Saquarema lagoon system is an estuarine ecosystem composed of four coastal lagoons connected to each other and to the sea by an artificial channel ([Fig. 1](#)). Its surroundings have large rural occupation and some urban centers in certain areas ([Barroso et al., 2000](#); [Silva and Molisani, 2019](#))

(Table 1).

Inserted in the Araruama (136,109 inhabitants), Iguaba Grande (29,344 inhabitants), São Pedro da Aldeia (107,556 inhabitants), Cabo Frio (234,077 inhabitants) and Arraial do Cabo cities (30,827 inhabitants), the lagoon system of Araruama is an estuarine ecosystem composed of three compartments formed by seven elliptical cells connected to each other and to the sea by an artificial channel (Fig. 1). Its surroundings are mostly rural, but with increasing urbanization due to the constant development of tourism in the region (Barroso et al., 2000) (Table 1).

The distance between the Itaipu and Saquarema lagoons is approximately 65 km, while the distance between Saquarema and Araruama is less than 15 km, forming a mosaic of coastal lagoons known as the 'lagoon systems of the eastern Fluminense', which represents approximately 23.5% of the coastal zone of the state of Rio de Janeiro (Begot and Vianna, 2014). From a geomorphological point of view, these lagoon systems show a semi-restricted connectivity with the adjacent coastal zone, since they are characterised by a series of elliptical cells, connected to the adjacent ocean by a single narrow channel. These lagoons are typical of coastal regions with high gravity wave energy, significant coastal drift and moderate tide height. The channel acts as a dynamic filter, consequently, the effects of tidal oscillation and currents are very attenuated inside the lagoon. This type of lagoon is characterized by long fluvial discharge times, dominant wind effect, and intermittent variation of vertical stratification due to solar heating and freshwater discharge (Kjerfve, 1994; Miranda et al., 2002).

2.2. Fish sampling and age estimates

Individuals were acquired directly from artisanal fishermen in the three lagoon systems (Itaipu, Saquarema and Araruama) between December 2019 and March 2020. All fish were caught with a gill net during the rainy season. After the collection, specimens were stored in ice and transported to the laboratory. All individuals were measured in total length (TL, cm) and weighed (TW, g). Thereafter, the sagittal otoliths were extracted with plastic tweezers, washed with distilled water to remove organic tissues and stored dry in plastic tubes. The pairs of otoliths (left and right sagittae) were distinguished according to the position of the rostrum and sulcus acusticus (Secor et al., 1992). Only well preserved and entire otoliths were used for further shape and microchemical analyses.

For age readings, dry otoliths were positioned with the sulcus acusticus down, under transmitted light using a Leica S9i stereoscopic microscope with 10X magnification (Vaz-dos-Santos, 2015). The counts of the growth increments, assumed to be annual, were performed by two independent and experienced readers, and only otoliths with 100% agreement were used (Moura et al., 2020).

Thirty individuals from each lagoon (a total of 90 individuals: TL = 23.8 ± 0.3 cm), all adults [TL > size at first maturity recorded between 13 cm (Ramos et al., 2012) and 15 cm (García-Cagide et al., 1994) of fork length] from age group 2⁺, were pre-selected for further analyses to minimize ontogenetic influences, to neutralize sample size and to fulfill the bulk otolith approach (Table 2).

2.3. Otolith shape analysis

Unbroken left otoliths were placed with sulcus acusticus up and the rostrum pointing to the left. Orthogonal two-dimensional digital images of otoliths were captured using the Leica S9i stereoscopic microscope. Colored high resolution images were obtained using reflected light against a black background with 10X magnification. Thereafter, the images were processed in the Leica LAZ-EZ program, and scales in millimeters were applied. Then, edits were performed in ImageJ software (version 1.52o). Images were transformed into a grayscale, and thereafter binarized (threshold pixel value of 0.2) using shapeR (Libungan and Pálsson, 2015). The external contour of the otoliths was

Table 2

Sample size (N), total length (TL) and otolith mass (OM) of *Eugerres brasiliensis* individuals collected in the three lagoon systems of eastern Rio de Janeiro, Brazil. Values are presented as means, standard errors (SE) and ranges.

Lagoon System	N	TL (cm)			OM (mg)	
		Date of collection	Mean ± SE	Range	Mean ± SE	Range
Itaipu	18	Dec. 2019	22.0 ± 0.4	17.6 – 26.1	22.12 ± 0.86	12.70 – 29.00
	1	Jan. 2020				
	9	Fev. 2020				
	2	Mar. 2020				
Saquarema	17	Dec. 2019	22.9 ± 0.5	17.5 – 26.0	25.71 ± 1.04	15.90 – 39.20
	13	Fev. 2020				
Araruama	9	Dec. 2019	26.6 ± 0.3	23.9 – 30.5	38.69 ± 1.29	26.80 – 57.70
	5	Jan. 2020				
	4	Fev. 2020				
	12	Mar. 2020				

evaluated using elliptic Fourier descriptors (EFD) generated using the ShapeR package version 0.1–5 (Libungan and Pálsson, 2015) of the R programming environment (R Development Core Team, 2022). ShapeR consists of a tool specifically designed to study the morphometric variation of otoliths in fish populations and diagnose the structure of fish stocks. This package allows the user to automatically extract closed contours from a large number of images, being able to smooth them to eliminate pixel noise, extract EFD from the contours and visualize the average shape of the otoliths of the samples (Libungan and Pálsson, 2015). The EFDs were considered invariant in relation to the starting point of reading, scale, rotation and fish size, therefore, as a consequence of the normalization process, the first three coefficients (a1, b1 and c1) were omitted (Libungan and Pálsson, 2015). Five additional coefficients (a3, a4, b9, d2 and d7) showed significant interaction with TL and were removed (Libungan and Pálsson, 2015; Longmore et al., 2010). The first 12 harmonics reached 98.5% of the mean cumulative power. Therefore, 40 (48–3–5) normalized EFDs were used for the subsequent analyzes of *E. brasiliensis*.

2.4. Otolith elemental analysis

Right otoliths were prepared for elemental analysis following standard protocols (Patterson et al., 1999; Rooker et al., 2001; Correia et al., 2021). The otoliths were cleaned in an ultrasonic bath with ultrapure water (H₂O, Milli-QR System, Milli-Q-Water) for 5 min, followed by immersion in 3% ultrapure hydrogen peroxide (H₂O₂, Fluka, Trace-Select) for 15 min to remove any organic residue remaining. The otoliths were immersed in a 1% (v/v) ultrapure nitric acid solution (HNO₃, Honeywell Fluka, TraceSELECT™, ≥69.0%) for 10 s to remove surface contamination, followed by a triple immersion in ultrapure water for 5 min to remove the acid (Rooker et al., 2001). The otoliths were then stored in previously acid washed Falcon tubes, where the otoliths were allowed to dry in a laminar flow hood. The decontaminated otoliths were weighed on an analytical balance (0.01 mg), dissolved for 15 min in 65% ultrapure HNO₃ (v/v), and diluted with ultrapure water (> 18.2 MΩ.cm at 25 °C) to a final volume of 15 mL (i.e., 2% HNO₃ v/v and 0.2% TDS m/v, Soeth et al., 2019). A pre-selected set of minor and trace elements (¹³⁷Ba, ⁵⁹Co, ⁶⁵Cu, ⁷Li, ²⁶Mg, ⁵⁵Mn, ⁶⁰Ni, ⁸⁸Sr and ⁶⁶Zn), including the calcium (⁴⁴Ca), usually found in informative levels (above the limits of detection, LOD) in fish otoliths (Correia et al., 2021), were assessed by solution-based inductively coupled plasma mass spectrometry (SB-ICP-MS). A iCAP™ Q (Thermo Fisher Scientific, Bremen, Germany) equipped with a concentric glass nebulizer, a Peltier-cooled deflector baffled cyclonic spray chamber, a standard quartz torch, and a

two-cone interface design (sample and skimmer cones) was used. High purity argon (99.9997%) (Gasim II, Leça da Palmeira, Portugal) was used as nebulizer and plasma gas. Equipment control and data acquisition were performed using Qtegra™ software (Thermo Fisher Scientific, Bremen, Germany). The instrument was operated under the following conditions: RF power, 1550 W; argon flow rate, 14 L/min; auxiliary argon flow rate, 0.8 L/min; nebulizer flow rate, 0.98 L/min. Indium (^{115}In), Scandium (^{45}Sc), Terbium (^{159}Tb) and Yttrium (^{89}Y) were monitored as an internal standards to minimize the effect of plasma fluctuations or different nebulizer aspiration rates among the samples (Moura et al., 2020). The otolith samples were analyzed in random order to avoid possible sequence influences. The LOD were calculated from the individual calibration of the curves using the three sigma criteria and were (in $\mu\text{g/L}$): ^{137}Ba (0.03), ^{44}Ca (8630), ^{59}Co (0.01), ^{65}Cu (0.07), ^7Li (0.02), ^{26}Mg (0.1), ^{55}Mn (0.03), ^{60}Ni (0.05), ^{88}Sr (0.025) and ^{66}Zn (0.18). Analytical accuracy (recovery rate, RR) was determined using a certified multi-element reference material for otoliths (NIES 22) and varied between 88% and 118%. The precision expressed as the relative standard deviation (RSD) obtained from an analytical triplicate, ranged between 1.21% and 4.10%. Both values (RSD and RR) are within the analytical accepted values (Dove et al., 1996: RSD: <20% and RR: 75%–125%). The concentration of the elements, originally in μg element/L solution, was transformed into μg element/g otolith and then into μg element/g Ca (Higgins et al., 2013).

2.5. Data analysis

Multielement signatures (MES) were evaluated for normality, homoscedasticity and the presence of outliers (Grubb's test) (Correia et al., 2021) before statistical analysis. To meet this assumptions Co/Ca, Cu/Ca, Li/Ca, Mg/Ca, Sr/Ca and Zn/Ca ratios were transformed by $\log(x + 1)$. In order to ensure that variations in the otolith mass (OM) (as proxy of the fish length) cannot influence the MES differences among the lagoon systems, the relationship between the OM and the element/Ca ratios were evaluated through analyzes of covariance (ANCOVA) with the OM as a covariate (Campana et al., 2000; Daros et al., 2016; Schroeder et al., 2022b). A positive relationship was observed for the Sr/Ca and Ni/Ca (ANCOVA, $p < 0.01$), and a negative relationship for Cu/Ca (ANCOVA, $p < 0.05$). The effect of OM on otolith chemistry was removed by subtracting the common within-group linear slope multiplied by the OM from the observed element/Ca ratios (Campana et al., 2000). Single elemental differences among lagoons were assessed by One-Way Analysis of variance (ANOVA), followed by a Tukey post-hoc test, if needed. For the MES and EFD, a Multivariate Analysis of Variance (MANOVA) were used. For MANOVA, the approximate F-ratio statistic (Pillai's trace) was reported, followed by pairwise comparisons using the Hotelling-Lawley test (Correia et al., 2021). A classic stepwise Linear Discriminant Function Analysis (LDFA) was applied to the EFD, MES and both combined in order to investigate spatial differences and to examine the accuracy of the reclassification matrix using the jackknife method, in order to allocate each individual to their original location (Adelir-Alves et al., 2019). All univariate and multivariate statistics were performed using the vegan (Oksanen et al., 2021) and exchange packages in the R programming environment (R Development Core Team, 2022) and SYSTAT 13 software. Results are presented as mean \pm standard errors. A significance level of $p < 0.05$ was adopted.

3. Results

3.1. Otolith shape analysis

The reconstruction of the otolith shape contour from the EFD means showed visual differences between the lagoon systems, especially in the excisura and in the postrostrum (Fig. 2A and B). EFD among the three lagoon systems showed significant differences in MANOVA (Pillai's Trace, $F_{1,374} = 3$; $df = 2$; $p < 0.05$). In the pairwise comparisons,

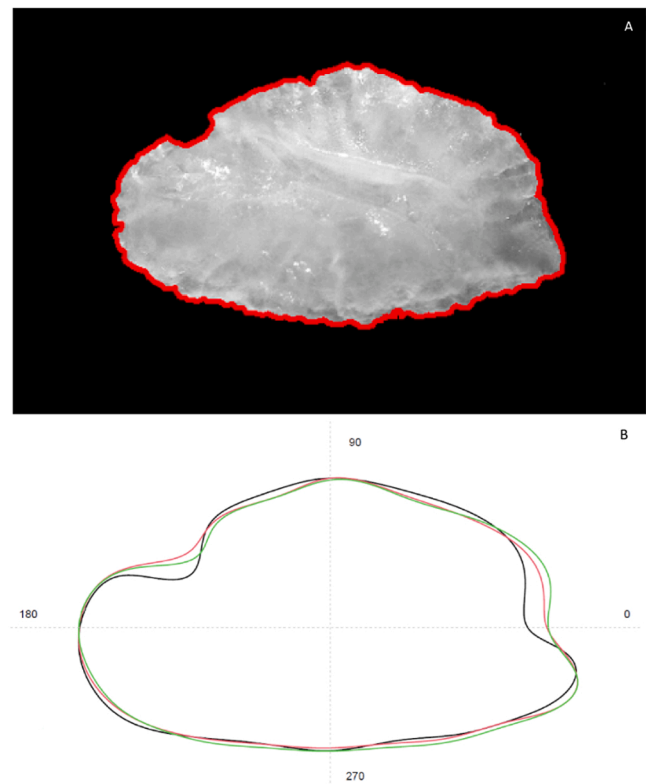


Fig. 2. Digital image of the inner face of a *Eugerres brasiliensis* otolith showing the outer contour (A). Mean otolith contours by lagoon system (B). Itaipu: red; Saquarema: green and Araruama: black.

Saquarema showed to be statistically different from the other two lagoons (Hotelling-Lawley test; $p < 0.05$) (Table 3). The LDFA using the EFD showed a clear discrimination of the Saquarema lagoon system, but a partial overlap between Itaipu and Araruama (Fig. 3A). The jackknife reclassification matrix obtained showed 76% of an overall reclassification of the individuals to their original locations (80% Saquarema, 77% Itaipu and 70% Araruama) (Table 4).

3.2. Otolith elemental analysis

3.2.1. Single elemental analysis

The Cu/Ca, Li/Ca, Mg/Ca, Mn/Ca and Sr/Ca ratios showed differences among locations (One-Way ANOVAs; $p < 0.05$) (Table 5). However, the Ba/Ca (overall range: 5.89–52 $\mu\text{g/g}$) (Fig. 4A), Co/Ca (overall range: 1.08–1.56 $\mu\text{g/g}$) (Fig. 4B), Ni/Ca (overall range: 9.92–15 $\mu\text{g/g}$)

Table 3

Pairwise MANOVA comparisons among the three lagoon systems regarding the elliptic Fourier descriptors (A), multielemental signatures (B) and combining both techniques (C). Significant statistical differences ($p < 0.05$) were marked in bold.

	Itaipu	Saquarema	Araruama
A			
Itaipu		< 0.001	0.360
Saquarema	< 0.001		< 0.001
Araruama	0.360	< 0.001	
B			
Itaipu		< 0.001	< 0.001
Saquarema	< 0.001		< 0.001
Araruama	< 0.001	< 0.001	
C			
Itaipu		< 0.001	< 0.001
Saquarema	< 0.001		< 0.001
Araruama	< 0.001	< 0.001	

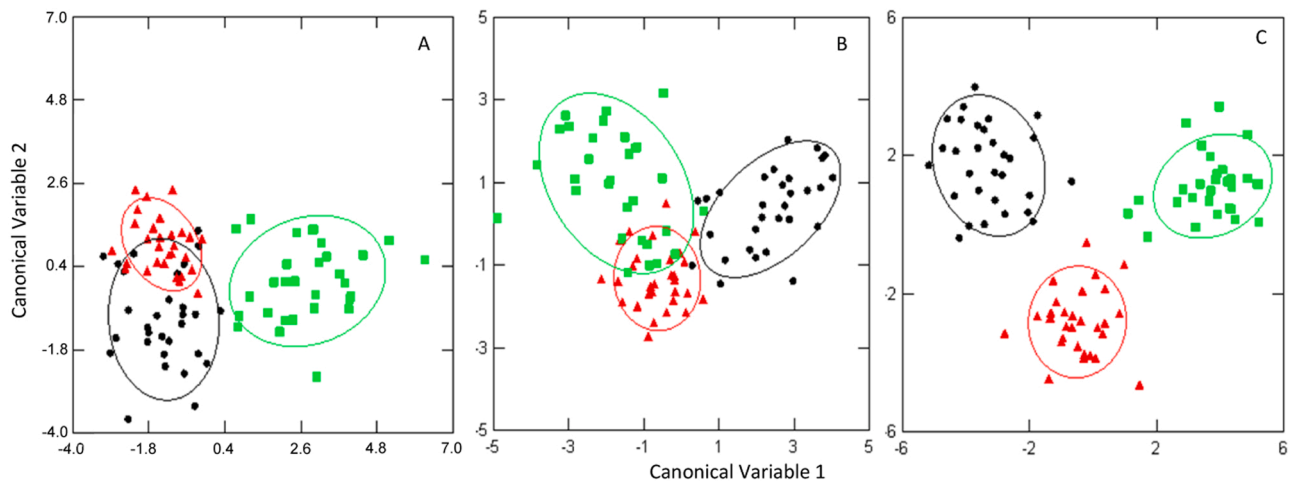


Fig. 3. Linear discrimination function analyses showing spatial differences in the analysis of *Eugerres brasiliensis* otoliths using elliptic Fourier descriptors (A), multielement signatures (B) and combining both techniques (C). Itaipu: red; Saquarema: green and Araruama: black.

Table 4

Jackknife reclassification matrix for *Eugerres brasiliensis* based on elliptic Fourier descriptors (A), multielemental signatures (B) and combining both techniques (C).

Original locations	Predicted locations			% Correct
	Itaipu	Saquarema	Araruama	
A				
Itaipu	23	0	7	77
Saquarema	2	24	4	80
Araruama	8	1	21	70
Total	33	25	32	76
B				
Itaipu	27	3	0	90
Saquarema	8	21	1	70
Araruama	5	0	25	83
Total	40	24	26	81
C				
Itaipu	30	0	0	100
Saquarema	3	27	0	90
Araruama	1	0	29	97
Total	34	27	29	96

(Fig. 4 G) and Zn/Ca (overall range: 0.18–4.22 $\mu\text{g/g}$) (Fig. 4I) ratios did not show any significant differences among locations (One-Way ANOVAs; $p > 0.05$) (Table 5). Spatial variability was observed for Cu/Ca, Li/Ca, Mg/Ca, Mn/Ca and Sr/Ca (Fig. 4 C, D, E, F and H, respectively). The Cu/Ca ratio (overall range: 0.64–5.11 $\mu\text{g/g}$) differed significantly between Itaipu and Saquarema lagoon systems (Tukey test; $p < 0.05$; Fig. 4 C), but no differences were found between Araruama and the other lagoon systems (Tukey tests; $p > 0.05$; Fig. 4 C). The Li/Ca ratio (overall range: 0.32–0.82 $\mu\text{g/g}$) was higher in the Araruama lagoon system, intermediary in Itaipu and lower in Saquarema (Tukey test; $p < 0.05$; Fig. 4D). The Mg/Ca ratio (overall range: 21–56 $\mu\text{g/g}$) was lower in Araruama compared to the other two lagoon systems (Tukey test; $p < 0.05$; Fig. 4E), and no differences were found between Itaipu and Saquarema (Tukey test; $p > 0.05$; Fig. 4E). The Mn/Ca ratio (overall range: 1.23–15.00 $\mu\text{g/g}$) was higher in Saquarema lagoon system, intermediary in Araruama and lower in Itaipu (Tukey test; $p < 0.05$; Fig. 4 F). The Sr/Ca ratio (overall range: 4273–9958 $\mu\text{g/g}$) was higher in the Araruama compared to the other lagoons (Tukey test; $p < 0.05$; Fig. 4H), and no differences were found between Itaipu and Saquarema (Tukey test; $p > 0.05$; Fig. 4H).

3.2.2. Multi-elemental analysis

Significant differences among the lagoon systems for the MES were

Table 5

Comparison of *Eugerres brasiliensis* element/Ca ratios among locations by One-Way ANOVA. Significant statistical differences ($p < 0.05$) were marked in bold.

Element	Source	df	SS	MS	F	p
Ba/Ca	Location	2	272.000	136.220	1.46	0.238
	Residual	87	8113.000	93.250		
	Total	89	8385.000			
Co/Ca	Location	2	0.004	0.002	2.78	0.131
	Residual	87	0.088	0.001		
	Total	89	0.092			
Cu/Ca	Location	2	0.343	0.171	4.25	< 0.05
	Residual	87	3.510	0.040		
	Total	89	3.853			
Li/Ca	Location	2	0.403	0.202	53.43	< 0.001
	Residual	87	0.328	0.004		
	Total	89	0.731			
Mg/Ca	Location	2	0.131	0.066	13.66	< 0.001
	Residual	87	0.417	0.005		
	Total	89	0.549			
Mn/Ca	Location	2	273.200	136.600	17.22	< 0.001
	Residual	87	690.000	7.930		
	Total	89	963.200			
Ni/Ca	Location	2	2.230	1.117	0.91	0.405
	Residual	87	106.380	1.223		
	Total	89	108.610			
Sr/Ca	Location	2	0.368	0.184	56.14	< 0.001
	Residual	87	0.285	0.003		
	Total	89	0.653			
Zn/Ca	Location	2	0.069	0.035	0.22	0.806
	Residual	87	13.955	0.160		
	Total	89	14.024			

found using a MANOVA (Pillai's Trace; $F_{1,23294} = 14$; $df = 2$; $p < 0.05$). In the pairwise comparisons, all lagoon systems showed statistically differences among them (Hotelling-Lawley test; $p < 0.05$) (Table 3). The LFDA using the MES showed almost a complete distinction of the Araruama lagoon system and a partial overlap between Itaipu and Saquarema (Fig. 3B). The values of the jackknife reclassification matrix showed an overall reclassification of 82% for the individuals to their original lagoon systems (90% Itaipu, 83% Araruama and 70% Saquarema) (Table 4).

3.2.3. Otolith shape and chemical analyses combined

Significant differences were also found among locations when combining the EFD and MES data running a MANOVA (Pillai's Trace; $F_{1,75242} = 6$; $df = 2$; $p < 0.05$). In the pairwise comparisons, all lagoon systems showed significant differences among them (Hotelling-Lawley test; $p < 0.05$) (Table 3). The LFDA plot draw using the combined data

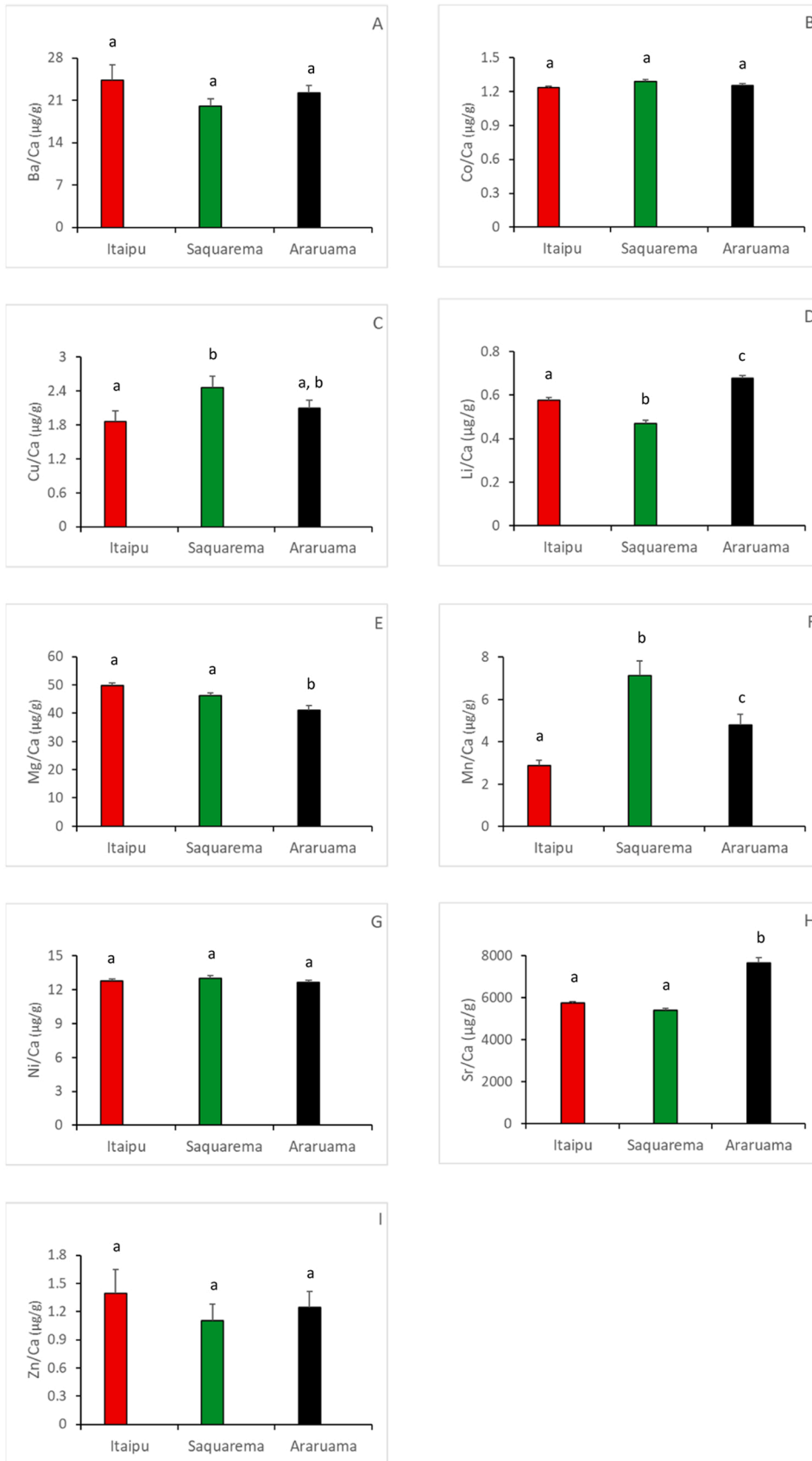


Fig. 4. Element/Ca ratios in otoliths of *Eugerres brasiliensis* collected in the lagoons systems of eastern Rio de Janeiro, Brazil. (A) Ba/Ca ratios. (B) Co/Ca ratios. (C) Cu/Ca ratios. (D) Li/Ca ratios. (E) Mg/Ca ratios. (F) Mn/Ca ratios. (G) Ni/Ca ratios. (H) Sr/Ca ratios. (I) Zn/Ca ratios. Different letters above the columns indicate significant statistical differences observed between locations (One-Way ANOVA: $p < 0.05$, followed by a Tukey test: $p < 0.05$). Elemental concentrations (detrended concentrations for Sr/Ca, Ni/Ca and Cu/Ca ratios) are showed as mean values \pm standard errors.

of the EFD and MES showed a clear separation among the individuals of the three lagoon systems (Fig. 3C). The jackknife reclassification matrix showed an overall reclassification of 96% of the individuals to their original locations (100% Itaipu, 97% Araruama and 90% Saquarema) (Table 4).

4. Discussion

The EFD and MES analyses applied to the sagittal otoliths of *E. brasiliensis* proved to be efficient tools to discriminate different sub-populations (defined here as an arbitrary spatially-delimited subset of individuals within a population: Wells and Richmond, 1995) of the species in the eastern Rio de Janeiro coastal lagoon systems. Previous studies allowed the discrimination of populations using otoliths applying the EFD (Pérez and Fabr e, 2013; Hoff et al., 2020; Muniz et al., 2020), MES (Geffen et al., 2003; Lawton et al., 2010; Murphy et al., 2012) or both, including in Brazilian fish species (Adelir Alves et al., 2019; Soeth et al., 2019; Maciel et al., 2020). Appropriate analyses of the contour and composition of fish otoliths supported the discrimination of phenotypically different groups of individuals which is probably related with distinct regional environmental variables (Izzo et al., 2018; Vasconcelos et al., 2021; Corr ea et al., 2022). Recent studies carried out in the lagoon systems of eastern Rio de Janeiro support these findings, since they recorded distinct physiographic characteristics and environmental conditions for these lagoons (Camara et al., 2021; Franco et al., 2022; Dias et al., 2021).

Delineation and discrimination of estuarine and marine coastal fish stocks using otolith morphometry were performed by several authors on the Brazilian coast for several species (*Micropogonias furnieri*: Santos et al., 2017; *Abudefduf saxatilis*: Adelir-Alves et al., 2019; *Chaetodipterus faber*: Soeth et al., 2019; *Isopisthus parvipinnis*: Hoff et al., 2020; *Genidens genidens*: Maciel et al., 2020; *Scomberomorus brasiliensis*: Soeth et al., 2020; *Sardinella brasiliensis*: Schroeder et al., 2022a). These intraspecific phenotypic variations were attributed to the physical-chemical parameters of the different water masses, which appear to influence the fish metabolism and growth rates, affecting the pattern of incorporation of calcium carbonate and the otolith's morphology (Schulz-Mirbach et al., 2019). Regional variations in the shape of the otoliths of *Gadus mohua* collected off the coast of Iceland, for instance, appear to be influenced by several environmental conditions, such as water temperature and dynamics of the oceanographic currents (J nsson et al., 2006). Moreover, life history parameters such as recruitment, reproduction and feeding regime altered the morphology of the sagitta of *Trachurus picturatus* in the northeast Atlantic Ocean (Tuset et al., 2019). The shape and symmetry of the otoliths of juveniles of *Amphiprion akindynis* and *Pomacentrus amboinensis* reflected the individual's body condition (Gagliano and McCormick, 2004). In this context, aquatic ecosystems with environmental differences are able to influence the life history of the individuals within a population, which could result in morphological variations of the otoliths among groups of individuals (Cardinale et al., 2004; Almeida et al., 2020; Corr ea et al., 2022). The Itaipu lagoon is an environment with a small area, carrying capacity and water residence time. It is considered a eutrophic environment that is subjected to strong events of sedimentation, leading to the closure of the channel connecting the lagoon to the sea, interrupting the natural flow of water and causing fish mortality events (Knoopers and Kjerfve 1999; Barroso et al., 2000; Cerda et al., 2013). The Saquarema lagoon is the most environmentally preserved area and the least impacted by human activities. This lagoon is connected to the sea through a channel that is 1.4 km long and has a variable depth ranging from 1 to 6 m (Knoopers and Kjerfve 1999; Mendes and Soares-Gomes, 2011). The Araruama lagoon is a hypersaline environment and has the largest area among the systems studied, with a high capacity to support the species that inhabit it, even though it is subject to numerous anthropogenic impacts. Its communication with the sea occurs through an extensive channel approximately 5 km in length, with variable width (60–350 m) and

depth (3–7 m) (Barroso et al., 2000; Souza et al., 2003; Silva and Roman, 2016). It means that the lagoon systems studied hereby have different ecosystem attributes being capable of affecting in different ways the individuals of *E. brasiliensis* and consequently the morphology of their otoliths. Studies on bioecological aspects of Gerreidae species in Brazil have shown that *E. brasiliensis* have preferences for estuarine areas, spending several stages of their life cycle in these environments (Ramos et al., 2014; Rodrigues et al., 2017; Franco et al., 2012). It is well known that estuaries tend to restrict gene flow, which can result in unique selection regimes and lead to the formation of physiologically adapted populations that diverge from their marine counterparts. These populations can become partially or completely isolated, creating opportunities for in situ speciation (Bilton et al., 2002).

The MES for *E. brasiliensis* otoliths presented an average composition similar to other studies carried out in the Rio de Janeiro state (Franco et al., 2019, 2022; Soeth et al., 2019; Maciel et al., 2020; Maciel et al., 2021; Schroeder et al., 2022b). The main factors capable of influencing the chemical composition of otoliths are the physicochemical characteristics of the water, endogenous physiological variables and life history attributes of populations, such as feeding, reproduction and metabolism (Campana et al., 2000; Albuquerque, 2015; Schroeder et al., 2022b). Nonetheless, the relative importance of each factor is species-dependent. In this case, we are dealing with a marine estuarine-dependent species that can make significant excursions on short timescales in different dynamic environments (Ramos et al., 2016). However, to interpret the otolith chemical signatures of *E. brasiliensis* without having a prior water characterization of the different lagoons is challenging, since the physicochemical properties of the water masses have a significant influence on the chemistry of the otoliths (Elsdon and Gillanders, 2003). Furthermore, differential fishing pressure among lagoons can alter fish mortality and growth rates, which may also affect otolith microchemical patterns (Catalan et al., 2018). The obtained results showed significant differences for the Sr/Ca, Li/Ca, Mg/Ca and Mn/Ca ratios. Except for Mn/Ca, the other ratios showed conservative patterns associated with the salinity gradient, i.e. Sr/Ca and Li/Ca showed higher values for the Araruama lagoon, followed by Itaipu and Saquarema. On the other hand, Mg/Ca concentrations were higher in Itaipu, Saquarema and Araruama. Water concentrations of Sr, Li and Mg are generally positively correlated with salinity (Campana, 1999; Avigliano et al., 2014). Such patterns observed in the otoliths correspond to the characteristics of the evaluated lagoons, Araruama being a hypersaline system with the largest area (km²) while Itaipu is the smallest system in terms of drainage basin, area and time of residence of the water suffering strong influence from the adjacent oceanic zone (See Table 1). In contrast, the Mn/Ca ratio was higher in Saquarema, Araruama and Itaipu respectively. Such variations could be magnified during summer due to increased microbial activity, anoxic conditions and increased amounts of Mn in interstitial water (Dellwig et al., 2007). Such observed pattern reflects the trophic state conditions of these systems (see Table 1) and their incorporation into otoliths facing hypoxic environments, especially in the hottest months of the year (Limburg et al., 2015). The absence of significant differences in Ba/Ca, Co/Ca, Cu/Ca, Ni/Ca and Zn/Ca concentrations in otoliths among the lagoons could be explained by the water balance, tidal variations and hydrodynamics of each lagoon system associated with the different forms of connection with the sea. Furthermore, the similar values observed between the lagoons for these elements can be result of various anthropic activities, such as mining and proximity to urban and/or industrial areas (see Table 1) (S ndergaard et al., 2015; Andronis et al., 2017). The element/Ca differences observed intraspecifically among the lagoon systems may also be explained by other processes related to the habitat use patterns of this species. Indeed, this species uses estuarine habitats during distinct ontogenetic phases that are related to salinity gradients and seasonal changes in rainfall (Ramos et al., 2014, 2016). Therefore, the incorporation of the minor and trace elements into otoliths can be regulated by fish physiology, environmental factors (such as salinity,

dissolved oxygen, and temperature), and water composition. Li showed a similar pattern to Sr, consistent for an element whose Li/Ca ratios increases with salinity (Hicks et al., 2010), which justifies the higher concentrations found in the Araruama Lagoon. Samples collected in the Araruama lagoon showed a higher concentration of Sr/Ca compared to the other environments, as previously recorded for *Micropogonias furnieri* (Franco et al., 2019). Sr is an element directly related to water salinity, usually associate to marine waters origin because the oceans are large reservoirs of this element (Elsdon and Gillanders, 2004; Webb et al., 2012). Indeed, the Araruama lagoon consists of a hypersaline environment, whose evaporation rates exceed those of precipitation in the region due to coastal upwelling of Southern Atlantic Central Water (SACW) (Kjerfve et al., 1996; Nascimento et al., 2019). On the other hand, Itaipu lagoon and Squarena showed higher concentrations of Mg/Ca compared to the Araruama lagoon probably because of their larger capacity to renew its waters with the sea (Dias et al., 2021). Mg concentrations in otoliths are highly dependent of physiological factors, due to the function of this element in the fish organism, whose variation is little affected by external sources such as food or water (Woodcock et al., 2012). It means that the differences found for the Mg/Ca ratios could reflect the physiological variation among the individuals of each lagoon system. However, distinct environmental influences are also capable of affecting the life history of individuals and the incorporation of this element in the aragonitic matrix (Knoppers and Kjerfve, 1999; Hüsey et al., 2021). Mn is an element whose concentration in the otoliths can be related to continental contribution (Elsdon et al., 2008; Laugier et al., 2015), physiological factors (Elsdon and Gillanders, 2003) or hypoxic environments (Limburg et al., 2015). The differences found between the lagoon systems were expected, with the Saquarema lagoon showing higher concentrations of Mn/Ca due to the intense fluvial input rich in sediments that it receives from its drainage basin (Dias et al., 2017); while the higher concentrations in the Araruama lagoon compared to the Itaipu lagoon are probably due to the longer residence time of its waters and proximity an urban centers (Silva and Molisani, 2019). The Ba and the Sr water concentrations are usually inversely related, with Ba decreasing and Sr increasing across the salinity gradient (Walsh and Gillanders, 2018). High levels of Ba in coastal lagoons are expected to occur mainly due to the input of terrigenous material carried by rivers (Albuquerque et al., 2010; Webb et al., 2012; Moreira et al., 2022), although its enrichment too can arise through contamination of the waters by pollutants (Gomes et al., 2017; Daros et al., 2022), contribution of water from aquifers (Shaw et al., 1998) or even through coastal upwelling (Artetxe-Arrate et al., 2019). However, the absence of significant differences in Ba/Ca concentrations among the lagoons can be explained by the low contribution of continental drainage due to the order of the tributaries (1, 2 and 3 orders, creeks and streams mostly), except during extreme hydrological events. Co, Cu and Zn are trace elements susceptible to the influence of physiological factors for its deposition in fish otoliths, due their roles as biomolecule co-factors (Daverat et al., 2012; Thomas et al., 2017; Hüsey et al., 2021). The highest Cu/Ca ratio in the Saquarema lagoon in relation to the Itaipu lagoon could be attributed to environmental conditions. Cu is a trace element whose concentrations in the otoliths vary according to physiological factors, playing an important role in the biomineralization of the organic matrix of otoliths (Thomas et al., 2020). Thus, the observed differences could be result of biomineralization regional differences and/or exposure to water bodies or sediments contaminated with metals from anthropic activities such as mining and proximity to urban and/or industrial areas (Søndergaard et al., 2015; Andronis et al., 2017). Ni is a trace element whose concentration in the otoliths is mainly related to environmental concentrations (Campana, 1999). The Ni/Ca ratios showed no differences among locations. Similar results were observed by Friedrich and Halden (2010), who found metals at low concentrations in otoliths from lakes near to mining areas, suggesting that chemical signature was attenuated. Zn is an element whose incorporation into the otoliths is associated with physiological variations and is

mainly attributed to fish food sources (Halden et al., 2000; Ranaldi and Gagnon, 2008; Thomas et al., 2017). Therefore, the absence of differences between the lagoon systems is probably due to the generalist diet of *E. brasiliensis*, whose ability to consume a wide range of food items may be a factor capable of making it difficult to distinguish Zn/Ca concentrations among individuals (Ramos et al., 2014; Almeida et al., 2021). Thus, our results suggest a high segregation (low connectivity) among estuaries, in which populations remain relatively isolated throughout the life cycle, having as a major barrier to adjacent oceanographic conditions.

Estuarine fish populations are generally delimited by water masses, hydrography and geomorphological characteristics of the coastal zone, which may have a dependency relationship with this type of ecosystem (Bilton et al., 2002; Kritzer and Sale, 2004; Silva-Junior et al., 2013). The fact that *E. brasiliensis* adults are rarely captured outside the estuarine environments reflect the species dependence on this ecosystem characteristics (Franco et al., 2012; Ramos et al., 2014; Ramos et al., 2016). Furthermore, different water bodies and associated physical-chemical barriers are capable of limiting the dispersion of larvae and juvenile individuals of fish species (Cowen and Sponaugle, 2009; Weersing and Toonen, 2009). The coastal upwelling of SACW may be an environmental component capable of restrain the movement of adult individuals of *E. brasiliensis*, as well as the dispersion of their larvae, among the different lagoon systems in the study region. This is due to the fact that *E. brasiliensis* prefers warmer waters, between 23.9 °C and 28.1 °C (Kaschner et al., 2016), while the area under the greatest influence of SACW in eastern Rio de Janeiro, between Guanabara Bay and Cabo Frio, has average sea surface temperatures below 21 °C in summer (Castro et al., 2006; Madureira et al., 2020). In a previous study, the upwelling of SACW was considered as a limiting factor for the dispersal of a highly important coastal species (*Chaetodiptyeris faber*) captured off the southwestern Atlantic coast (Soeth et al., 2019). Moreover, an experimental study showed a greater fluctuating asymmetry in body morphometrics of *E. brasiliensis* larvae exposed to the lowest temperature (20 °C) (Evangelista et al., 2019). In this way, the dependence of this species on environments with estuarine characteristics is highlighted, whereas the occurrence of different water masses can be a limiting factor to the dispersion of larvae and movement of adults of *E. brasiliensis*. Furthermore, the fact that the studied environments consist of suffocated coastal lagoons, with limited exchange of water, nutrients and biota with the sea, it could affect the dispersion of all aquatic organism (Kjerfve, 1986). Franco et al., 2022 found that fish assemblages of the lagoon systems of the southeastern state of Rio de Janeiro are highly influenced by local factors, such as abiotic conditions and resource availability between and within each lagoon system. It means that the hereby coastal lagoons could favor the differentiation of groups of organisms, due to barriers to dispersion, issues of physiological tolerance, or other environmental filters, where the flow of individuals is restricted and different selective regimes are imposed, thus generating physiologically adapted and phenotypically divergent populations (Bilton et al., 2002).

The hereby findings confirmed significant differences among individuals collected in the distinct coastal lagoons as depicted from LDA. A clear and consistent spatial pattern emerged among the coastal lagoons, compatible with the environmental drivers (e.g. salinity, temperature, transparency, pH, and conductivity) that characterize each aquatic compartment (Franco et al., 2022). Additionally, the combined use of these two natural tags allowed for a high reclassification rate of the individuals to the original locations, suggesting the existence of distinct subpopulations of Brazilian mojarra along the coastal lagoons of the Southwest Atlantic. The recognition of different subpopulations of *E. brasiliensis*, necessarily implies that management strategies can be applied independently to each coastal lagoon. The lagoon systems of eastern Rio de Janeiro suffer different environmental and fisheries pressures that can compromise the sustainability of the local halieutic resources if management is carried out in a generalized way (Bertucci

et al., 2016). All these systems have generic legislation to protect their resources. However, specific laws based on the life history attributes of the main fishing resources in these localities are still scarce. Recently, in Araruama, the closed period for fish and crustaceans was separated (Brasil, 2002), while in Saquarema, laws are prohibitive only for gear and fishing sites (Brasil, 1996). In contrast, Itaipu presents legislation aimed more at aspects of the local culture than its fishing resources (Niterói-RJ, 2011). Fisheries without proper management can lead to overexploitation of stocks as results of overfishing, capture of juveniles, or because environmental events that can lead to a decrease in the primary productivity (Waldman, 1999). In this context, the otolith morphology and microchemistry revealed as an efficient tool for the identification of *E. brasiliensis* subpopulations in the studied lagoon systems. However, further studies are needed to understand how the species uses the lagoon systems during its life cycle, namely regarding its habitat use and movement patterns, namely in geographic areas with different coastal-lagoon hydrological regimes and physiographic characteristics. Moreover, although several environmental and anthropogenic differences among the hereby lagoons have been pointed out, a full controlled field work is advisable to determine which abiotic variables, in particular temperature and salinity (and their direct effect on the fish growth rate), could explain the site-specific shape and chemical variations found in *E. brasiliensis* otoliths.

CRedit authorship contribution statement

Paulo Almeida: Conceptualization, Writing – original draft, Methodology, Investigation, Formal analysis. **Marcus Costa:** Conceptualization, Writing – review & editing, Supervision, Project administration. **Raiane Oliveira:** Investigation, Formal analysis. **Edgar Pinto:** Formal analysis, Resources. **Agostinho Almeida:** Formal analysis, Resources. **Rui Azevedo:** Formal analysis. **Cassiano Monteiro-Neto:** Writing – review & editing, Supervision, Project administration. **Alberto Teodorico Correia:** Conceptualization, Writing – review & editing, Methodology, Formal analysis, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

The Coordination for the Improvement of Higher Education Personnel (CAPES) provided financial support to the Universidade Federal Fluminense (UFF) and the Brazilian Fund for Biodiversity (FUNBIO) for the Sub-Project "Sistemas Lagunares do Leste Fluminense-SLLF (short title)" within the scope of the Project of Support to Marine and Fisheries Research in Rio de Janeiro. The project, entitled "Mecanismos Reguladores da Produção Pesqueira nos Sistemas Lagunares do Leste Fluminense: estado atual e cenários futuros", is an environmental offset measure established through a Consent Decree/Conduct Adjustment Agreement between PRIO and the Brazilian Ministry for the Environment. This study was supported by national funds through FCT—Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

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