Fisheries Research 255 (2022) 106436

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Review

Identification of fish stock based on otolith as a natural marker: The case of *Katsuwonus pelamis* (Linnaeus, 1758) in the Southwest Atlantic Ocean

Gabriel Marcel de Souza Corrêa[a,*], Juliano Lauser Coletto[b], Jorge Pablo Castello[c], Nathaniel R. Miller[d], Rafael de Almeida Tubino[e], Cassiano Monteiro Neto[a], Marcus Rodrigues da Costa[a]

[a] *Laboratório de Biologia do Nécton e Ecologia Pesqueira, Departamento de Biologia Marinha, Universidade Federal Fluminense, Caixa Postal 100644, 24001-970 Niterói, RJ, Brazil*
[b] *Laboratório de Tecnologia Pesqueira e Hidroacústica, Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, 96201-900 Rio Grande, RS, Brazil*
[c] *Laboratório de Recursos Pesqueiros Pelágicos, Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG, 96201-900 Rio Grande, RS, Brazil*
[d] *Department of Geological Sciences, University of Texas at Austin, Austin, TX 78712, USA*
[e] *Departamento de Biologia Animal, Universidade Federal Rural do Rio de Janeiro, Rodovia BR 465, Km 07, 23890000 Seropédica, RJ, Brazil*

ARTICLE INFO

Handled by B. Morales-Nin

Keywords:
Skipjack tuna
Morphometric
Chemical analyses
Atlantic
Morphotypes

ABSTRACT

Morphological, morphometric, and chemical analysis of otoliths have proven to be useful tools for intraspecific comparison and identification of fish stocks. In the present study, we used otolith silhouette and morphometric analysis (n = 103) in addition to chemical signatures (n = 20) to test the hypothesis of a single stock unit of skipjack tuna (*Katsuwonus pelamis*) in the Southwest Atlantic Ocean (20–34°S). No significant differences were found between sexes (t-test $p > 0.05$), enabling analyses for all study specimens to be grouped. Cluster analysis using Fourier coefficients (30 first harmonics) differentiated three otolith morphotypes within South (28–34°S) and Southeast (20–28°S) regions of the study area. Harmonic principal component analysis using the first two significant axes showed no pattern of spatial separation by region. Among the seven shape descriptors examined, only circularity and form factor showed significant differences between the morphotypes (A and B). Microchemistry analysis (LA/ICP-MS) applied to the core portion of fish otoliths showed significant differences in Ba, Mn and Mg concentrations between the two regions and morphotypes ($p < 0.05$). These results support that the *Katsuwonus pelamis* population in the Southwest Atlantic Ocean belong to a single stock unit with a probable common area of origin. Specimens captured in the South and Southeast regions of Brazil had heterogeneous phenotypic attributes regarding otolith shape, probably determined by a temporal equilibrium selection process.

1. Introduction

An otolith is a small acellular concretion located in the fish's head, composed of calcium carbonate in the form of aragonite, associated with a protein matrix called otolin and other minerals in smaller proportions (trace elements) (Campana, 1999). Otoliths act as organs that perform the functions of balance (position perception) in the water column and sound detection (Campana and Thorrold, 2001; Schulz-Mirbach et al., 2014). Moreover, although otoliths are metabolically inert, they are influenced by the physical and chemical properties of the surrounding water; information and characteristics of environmental conditions are thus recorded throughout the ontogenetic development of the fish (Campana and Neilson, 1985; Campana, 1999; Elsdon and Gillanders, 2003).

Otolith shape is considered species-specific and is determined by extrinsic (e.g., water temperature, salinity) and intrinsic (rates of somatic and otolith growth) factors, which in turn can be altered by other attributes (i.e., depth, and diet) experienced by the fish (Campana and Casselman, 1993; Morales-Nin, 2000; Hüssy, 2008). Fish that prefer distinct water bodies have associated feeding habits (Duncan et al., 2018; Coletto et al., 2021) and often have otolith shapes that differ from the same species with preferences for other water bodies (Kikuchi et al., 2021). Otolith morphology has consequently been used in intraspecific comparisons to identify fish stocks (Campana and Casselman, 1993;

---

* Correspondence to: Universidade Federal Fluminense, Laboratório de Biologia do Nécton e Ecologia Pesqueira, Departamento de Biologia Marinha Caixa, Postal 100644, 24001-970, Niterói, RJ, Brazil.

E-mail addresses: gabrielmarcel12@hotmail.com, gabmarcel94@gmail.com (G.M. de Souza Corrêa).

https://doi.org/10.1016/j.fishres.2022.106436
Received 24 August 2021; Received in revised form 9 June 2022; Accepted 8 July 2022
Available online 25 July 2022

Cardinale et al., 2004; Elsdon et al., 2008; Almeida et al., 2020). Because of this specificity, several authors have tested the hypothesis of otolith morphological variation as a predictor of stock separation, because otolith shape is regulated by both genetic factors and environmental variables (Gauldie and Crampton, 2002; Volpedo and Echeverríááa, 2003; Cardinale et al., 2004; Vignon and Morat, 2010; Tuset et al., 2020). In addition, the application of morphometric analysis in otoliths allows a detailed description of their shape and contour, which can be compared intra- and/or interspecifically (Tuset et al., 2003; Almeida et al., 2020). Therefore, this is an efficient approach to determine and describe functional aspects of fish populations, as well as to differentiate stocks in order to support species and ecosystem management strategies (Campana and Casselman, 1993).

Chemical signatures of otoliths have been used as a tool to discriminate between fish stocks and migratory patterns and to determine the areas of origin and connectivity between habitats. This approach allows relating the occurrence of a species to the distinct habitats used during its development, thus acting as a natural marker of the species life cycle (Kitchens et al., 2018; Franco et al., 2019; Moura et al., 2020; Correia et al., 2021). Recent studies have integrated morphometric and chemical analysis of otoliths to characterize population structures, compare stocks, and identify habitat connectivity patterns for various fish species, including pelagic fish (*Thunnus thynnus, Xiphias gladius, Katsuwonus pelamis, Chaetodipterus faber, Scomber scombrus, Coryphaena hippurus*) (Brophy et al., 2016; Mahe et al., 2016; Wujdi et al., 2017; Soeth et al., 2019; Moura et al., 2020; Almeida et al., 2020), demersal fish (*Genypterus blacodes, Urophycis brasiliensis, Chelidonichthys lucerna*; Ladroit et al., 2017; Biolé et al., 2019; Ferreira et al., 2019), anadromous fish (*Salmo trutta*; Rashidabadi et al., 2020), and rocky substrate fish (*Sparus aurata;* Geladakis et al., 2021).

The skipjack tuna, *Katsuwonus pelamis* (Scombridae), is a cosmopolitan pelagic migratory species that belongs to the group of tuna and tuna-like fish, which is widely distributed in subtropical and tropical oceans (Murua et al., 2017). It has high commercial potential, ranking third among the most caught marine species worldwide (FAO, 2020). In Brazil, it is the most abundant tuna species. Much of its production comes from catches of the pole-and-line and live bait fleet (Castello, 2000; Madureira et al., 2016). The skipjack's reproductive strategy is opportunistic spawning, with spawning peaks in the summer period (January–February) in the Southwest Atlantic Ocean (Andrade, 2006; Soares et al., 2019). Its feeding habits are also opportunistic, relying mostly on crustaceans, fish, and cephalopods (Olson et al., 2016), and the composition of its diet varies according to the region (e.g., Varela et al., 2019). In the Southwest Atlantic, mesopelagic fish (e.g., *Maurolicus stehmanni*), krill (*Euphausia similis*) and small pelagic fish (e.g., *Engraulis anchoíta, Sardinella brasiliensis*) are the main prey (Ankenbrandt, 1985; Vilella, 1990; Coletto et al., 2021).

In the Atlantic Ocean, the International Commission for the Conservation of Atlantic Tunas (ICCAT) oversees management of tuna and tuna-like fish fisheries. For assessment and management purposes, ICCAT considers skipjack tuna to consist of eastern and western Atlantic stocks (ICCAT, 2019). Pole-and-line fishing is the main skipjack tuna method used in the western Atlantic Ocean (Almeida and Andrade, 2002). Fishing areas in the eastern Atlantic Ocean have recently expanded, in particular towards the western Central Atlantic (ICCAT, 2019). In addition, studies based on movement patterns inferred through the use of tagging programs suggested the adoption of more management units based on smaller and more homogeneous areas for future assessments in the Atlantic Ocean (Fonteneau, 2015). Thus, a tuna tagging program was launched in 2015 (Atlantic Ocean Tropical Tuna Tagging Programme) to generate new data on stock units. In the last technical report (ICCAT, 2019) a total of 112,757 individuals from different species of tropical tunas have now been tagged and released. Currently, 14,285 of these tagged fishes have been recovered. Nevertheless, the distribution of tagged fishes between the 3 main tropical species remains rather unbalanced: Skipjack (*Katsuwonus pelamis*) at ca 41%; Yellowfin (*Thunnus albacares*) at ca 33% and Bigeye (*Thunnus obesus*) at ca 19%. All the tagging release and recovery data are stored in a database at the ICCAT Secretariat, which will enable the implementation of assertive measures for fisheries management.

Despite the high commercial value of the skipjack tuna, little is known of its population (stock) identity in coastal Brazil fishery waters (Madureira and Monteiro-Neto, 2020). The lack of knowledge about exploited fish stocks can cause changes in the exploration activity and in the population structure of the species, thereby causing the depletion and/or overfishing of resources (Begg et al., 1999; Cadrin et al., 2014; Trindade-Santos and Freire, 2015). In the present study, we used otolith shape analysis and chemical signatures to test the hypothesis of a single stock in the Southwest Atlantic Ocean. We used catch data reported from the South (28–34°S) and Southeast (28–20°S) regions of the Brazilian coast, to determine if fish from both regions share the same origin. If the shape indices and chemical signatures are significantly different between the South and Southeast regions, the null hypothesis will be rejected. Otherwise, the shape and chemical indicators will support the hypothesis of a single stock unit for the Southwest Atlantic from a common area of origin in the South Atlantic.

2. Materials and methods

2.1. Study area, sample collection and processing

Samples were obtained from catches of skipjack tuna conducted in offshore regions of South and Southeast Brazil, between 20°S and 34°S (Fig. 1). Two pole-and-line fleets based respectively in Rio Grande (RS) and Niterói (RJ) were used for the sampling program. The Southern fleet (RS) operates between latitudes 28°S and 34°S in summer months, then moves northward up to 21°S during winter (Coletto et al., 2019). The Southeastern fleet (RJ) operates mostly between latitudes 20°S and 26°S (Soares et al., 2019). These operational areas are characterized by two distinct oceanographic regimes, separated by the Santa Marta Grande Cape (~28°S), which marks a change in the orientation of the coastline, in coastal physiography (Muehe, 1998). The South region (28–34°S) is influenced by cold waters carried by the Malvinas Current during fall and winter (Garcia, 1997; Castro et al., 2006). The Southeast region, between 20° and 28°S, is heavily dominated by oligotrophic Tropical Water carried southward by the Brazil Current, being enriched by

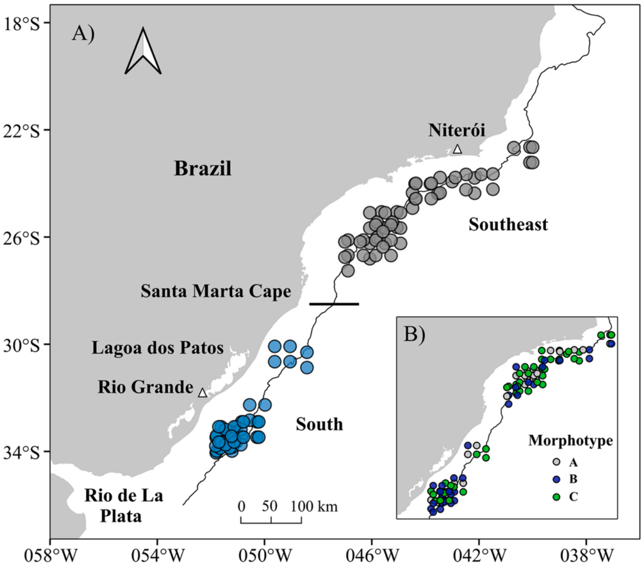

Fig. 1. A) Spatial distribution of skipjack (*Katsuwonus pelamis*) catches in the Southwest Atlantic off the Brazilian coast (A). The South (blue) and Southeast (dark grey) regions are separated by the Santa Marta Cape near 28°S (solid line). Inset shows otolith morphotypes spatial distribution (B).

coastal and shelf break upwelling events of cold and nutrient-rich South Atlantic Central Water – SACW (Lima et al., 1996; Garcia, 1997; Coelho-Souza et al., 2012).

Katsuwonus pelamis specimens were collected between March 2017 and May 2018 through a sampling program that included the systematic monitoring of the landings from vessels based in the South and Southeast regions. To avoid ontogenetic variations in otolith shape, only adult individuals were selected based on the size at first sexual maturation (Soares et al., 2019). The sex, furcal length (FL, cm), and total weight (TW, g) of collected specimens were recorded.

After collection, the specimens were stored on ice and transported to the Laboratory of Nekton Biology and Fisheries Ecology of the Universidade Federal Fluminense/RJ and to the Laboratory of Pelagic Fisheries Resources of the Universidade Federal do Rio Grande/RS, where they were processed. The sagittal otoliths of 103 adult individuals ($FL > 45$ cm) were removed through an opening in the dorsal region of the neurocranium to perform shape analysis. After removal, the otolith pairs were cleaned to remove organic tissues by washing with distilled water, then dried and stored.

2.2. *Shape analysis*

Only the left otoliths were separated for photographic records. They were arranged horizontally, with the rostrum and anti-rostrum positioned to the left and with the furrow upwards. The images were captured using a stereo microscope with an attached Zeiss Axiocam camera, with an incident light against a black background, and a micrometer ruler. The photographs were edited in the paint.NET software and analyzed (binarized) using the ImageJ software.

2.2.1. *Shape indices*

The shape indices were determined to assess the degree of similarity in otolith morphology between the different capture areas (South and Southeast regions). To evaluate the basic shape descriptors, otolith length (OL, mm), otolith width (OW, mm), otolith area (OA, mm^2) and otolith perimeter (OP, mm) were measured. All measurements of the main descriptors were generated using the ImageJ software. These measurements were used to calculate the shape indices: circularity, rectangularity, roundness, and form-factor (Table 1) according to the method described by Tuset et al. (2003). The fractal dimension of the otolith contour was calculated using the FracLac plugin (ImageJ software).

Rectangularity describes the variations in length and width relative to the area, where the value 1.0 indicates a perfect rectangle. Circularity and roundness provide information about the similarity of the analyzed object to a perfect circle or ellipse, respectively. The form factor estimates the irregularity of the surface area, with values of 1.0 for a perfect circle and less than 1.0 for an irregular shape (Tuset et al., 2003). In addition, the fractal dimension was determined. This is a measure of how much an object differs from its topological dimension, i.e., the greater the irregularity of a shape, the greater its fractal dimension, which reflects a more complex structure (Backes and Bruno, 2005; Duarte-Neto et al., 2008).

Table 1
Otolith shape indices calculated from morphometric measurements. Otolith area (OA, mm^2), otolith perimeter (OP, mm), otolith length (OL, mm), and otolith width (OW, mm).

Shape indices	Equation
Circularity	(OP^2/OA)
Rectangularity	$(OA/[OL*OW])$
Roundness	$(4OA/[\pi OL^2])$
Form factor	$(4\pi OA/OP^2)$

2.2.2. *Fourier coefficient derivation*

Fourier coefficients (FC) were calculated using elliptic Fourier analysis (EFA) generated by the SHAPE 1.3 software and the ChcViewer and CHC2NEF packages. This analysis allows extraction of the two-dimensional irregular otolith contour and forms a set of components called harmonics-Ha (Lestrel, 1997). FCs are defined as a sinusoidal series describing contours by means of a constant periodic function that is characterized by the sum of the sine and cosine functions of increasing frequencies that are retained by the addition of harmonics (Shen et al., 2009), where each Fourier descriptor is characterized by an amplitude and a phase angle. This method represents a fit of the harmonic functions with respect to the original otolith contour with an ellipse as the first approximation step, decomposing the contour by varying the x and y coordinates separately as parametric functions of the distance along the contour, where each harmonic *n* consists of four coefficients per individual *an*, bn, c*n*, and d*n* (Kuhl and Giardina, 1982).

2.2.3. *Statistical analysis of shape variables*

The shape index morphometric variables were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene test), and subsequently transformed to the logarithmic scale, except for Fourier coefficients. As the homogeneity and normality criteria were not met for all shape descriptors, only non-parametric tests were applied. Student's t-test was used to assess significant differences between otolith length and sexes.

The correlation between fish and otolith length and each shape descriptor was evaluated by analysis of covariance (ANCOVA). The descriptors that significantly correlated with fish length were corrected using the angular coefficient "***b***" of the equations. For the Fourier coefficients it was not necessary to perform any correction because FCs are interpreted as invariant in relation to length (Diaz et al., 1997).

Permutational multivariate analysis of variance (PERMANOVA and pairwise-test) were performed using the Euclidean distance as the similarity matrix, with 9999 random permutations to compare the shape descriptors area, perimeter, circularity, rectangularity, form-factor, roundness, and fractal dimension among the factors (years, regions, and morphotypes) (Clarke and Gorley, 2006; Anderson et al., 2008).

The shape patterns of the otoliths generated from the FCs were determined in an unbiased manner, based on two multivariate statistical techniques, using the data matrix composed of the first 30 harmonics, with 98% accuracy of shape reconstruction being obtained. The first multivariate analysis was a hierarchical cluster analysis using Ward's criterion (Reis, 1997), in which the Euclidean distance was used as a measure of similarity among otoliths to determine clusters. Finally, principal component analysis (PCA) was used, because this method describes variance in a descending order, thus reducing the dimensionality of the data, simplifying the analysis, and allowing the detection of differences between morphotypes and regions.

2.3. *Otolith microchemistry*

Otolith cores from 20 adult individuals (10 from the South and 10 from the Southeast) were analyzed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to characterize natal compositions and variability associated with juvenile habitats. In preparation for LA-ICP-MS, otoliths were embedded in crystal polyester resin. After drying, the resin blocks were cut with a low rotation metallographic saw (IsoMET Buehler), equipped with a 0.3 mm-thick diamond blade to obtain sections between 0.6 and 0.8 mm whose cross-sections revealed the otolith core. Following sanding with 200, 300, 500, and 600 grit sandpaper (D'água advance), otolith sections were polished using ultra-precision polishing films to provide a flat smooth surface exposure of the core. Otolith sections were glued onto glass slides (polished side up) using thermoplastic glue.

Elemental concentrations were determined using an Elemental Scientific NWR193UC (193 nm wavelength, < 4 ns pulse width) laser system, coupled to an Agilent 7500ce inductively coupled mass spectrometer at the University of Texas, Austin. The laser system was equipped with a large format two-volume laser cell with fast washout ($<$ 1 s), which accommodated all otolith samples and standards in two separate loadings. Laser ablation parameters were optimized for sensitivity and signal stability from test ablation transects on representative unknowns. Prior to analysis, samples and standards were pre-ablated to remove potential surface contamination. Laser analyses of otolith unknowns were bracketed hourly by standard measurements (MACS-3 and NIST 612, typically measured in triplicate for 60-s). Sample transects mainly consisted of otolith core traverses, with 16 spots from both sides of the primordia, to measure the chemical compositions of the core. *Katsuwonus pelamis* otoliths were analyzed using a 35 μm spot at a scan rate of 10 μm/sec. The quadrupole time-resolved method measured 7 masses using integration times of 10 ms (^{24}Mg, $^{43-44}$Ca, ^{88}Sr), 20 ms (^{55}Mn), and 50 ms (^{7}Li, ^{137}Ba). The sampling period of 0.4032 s corresponds to 94.25% quadrupole measurement time. The scan rates of 10 μm/sec respectively correspond to analyte measurements every 2.016 μm and 4.032 μm along transects. Time-resolved intensities were converted to concentration (ppm) equivalents using Iolite software (Univ. Melbourne, Hellstrom et al., 2008), with 43Ca set as the internal standard and a Ca index value of 38.3 wt%. Baseline intensity values bracketing otolith and standard analyses were determined from gas blank intervals (35 or 60 s), measured while the laser was off and all masses were scanned by the quadrupole. Average analyte recovery factors for all runs of NIST 612 were 0.98 ± 0.05 (N = 64) relative to GeoREM preferred values (http://georem.mpch-mainz.gwdg.de).

To evaluate variations in the elemental concentrations recorded in the otolith cores and to make comparisons between catches from each region and morphotype, 32 readings around the primordium were established as standard measurements. Univariate analysis (Kruskal-Wallis test) was used for the comparisons of the element:Ca ratios in the otolith cores. Statistical analyses were performed using the RStudio, PRIMER 7, PAST version 4.03. and the SHAPE version 1.2 softwares.

3. Results

A total of 103 left otoliths were used for shape analysis, 44 from the South and 59 from the Southeast. Furcal length ranged from 46 to 71.5 cm in the Southeast and from 46 to 67 cm in the South (Table 2). Student's t-test showed no differences between sexes ($p > 0.05$), which allowed grouping of the sexes for further analysis.

3.1. Fourier coefficient analysis

Cluster analysis using the amplitude of the first 30 harmonics led to the identification of three morphotypes for the skipjack tuna, which were classified into morphotypes A, B and C (Fig. 1B and Fig. 2). Morphotype A exhibits a deep excisure on the posterior part and a greater elevation on the dorsal posterior part compared to morphotypes B and C. Morphotype B has a deep excisure near the anterior dorsal region compared to morphotypes A and C. Finally, morphotype C exhibits a shallow excisure on the anterior dorsal part (Fig. 3).

The first seven principal components generated from Fourier coefficient analysis were significant. The first two axes explained 75.8% of the total variation. The first component (PC1) explained 49.3% and the second component (PC2) explained 26.6%. From the third component onward, the eigenvalues did not vary significantly. The main variations demonstrated by the mean shape (± standard deviation) of the contours of the first five components occurred in the size of the rostrum and antirostrum, and in the height and shape of the posterior part of the otoliths (Fig. 4).

Table 2
Number of individuals, amplitude, average, standard deviation (SD) and median fork length for *K. pelamis*, by region and for all specimens analyzed in the Southeast Atlantic.

Region	Southeast	South	All
Number of otoliths	59	44	103
Amplitude (cm)	46.0–71.5	46.0 – 67.0	46.0–71.5
Average	54.0	52.6	53.0
Standard deviation	6.1	5.3	5.8
Median	52.0	51.0	52.0

The first two axes of the principal component analysis did not show a pattern of data separation between the South and Southeast regions (Fig. 5A). However, the first two principal component axes showed a separation between morphotypes A, B, and C (Fig. 5B).

3.2. Shape indices

The shape descriptors perimeter, form-factor, and fractal dimension showed no correlation with otolith length according to ANCOVA ($p > 0.05$). The variables that had some degree of correlation with otolith length were corrected using the angular coefficient (***b***), thus minimizing distortions between otolith length and the variable. The first two axes of PCA analysis applied to the shape descriptors explained 92.4% of the total variation. The first component (PC1) explained 73.6% and the second component (PC2) explained 18.8% between the two regions. Roundness ($r = -0.13$) and form factor ($r = -0.65$) were negatively correlated, whereas circularity ($r = 0.65$) was positively correlated with the first axis (Fig. 6). Area ($r = 0.88$) and perimeter ($r = 0.39$) were positively correlated with the second axis. Rectangularity ($r = 0.03$) and fractal dimension ($r = 0.01$) showed negligible correlations. Comparisons performed using three-factor PERMANOVA for all variables among years, morphotypes, and regions showed no significant differences ($p > 0.05$), with the exception of circularity and form-factor, which showed differences among morphotypes (pairwise test = $p < 0.001$; types A and B only) (Supplemental data, Table S1).

3.3. Chemical signature of the core

Only Mn:Ca and Ba:Ca from cores of the *K. pelamis* otoliths differed significantly between specimens collected in South and Southeast regions. The Sr:Ca ratio was slightly higher in the otoliths from the Southeast region than in those from the South region, (Kruskal-Wallis – $p = 0.28$) (Fig. 7A). The Li:Ca ratio was similar in both regions (KW – $p = 0.67$) (Fig. 7B). The Mg:Ca ratio also did not differ between the regions, although the otoliths from the Southeast region showed a greater interquartile variation range (KW – $p = 0.87$) (Fig. 7C). The Mn:Ca and Ba:Ca ratios were significantly different between the two regions (KW – $p = 0.04$; $p = 0.001$) (Fig. 7D, E). Higher Ba:Ca ratios were observed in the otoliths from the South region, while the Mn:Ca ratio was higher in the Southeast region.

Elemental concentrations in the core of skipjack otoliths differed among morphotypes for Mg:Ca and Ba:Ca. The Sr:Ca concentration was slightly higher in morphotype C in comparison to morphotypes A and B, although the difference was not significant (KW – $p = 0.35$) (Fig. 8A). Li:Ca and Mn:Ca concentrations were not different between morphotypes (KW – $p = 0.53$; $p = 0.60$, respectively) (Fig. 8B, C). The elemental ratios of Mg:Ca and Ba:Ca were different among morphotypes (KW – $p = 0.01$; $p = 0.03$) (Fig. 8D, E). Mg:Ca ratio in morphotypes A and B were different from morphotype C ($p = 0.01$). Ba:Ca ratios differed between morphotypes A and B ($p = 0.01$).

4. Discussion

Our results are consistent with the hypothesis that a single stock unit of *Katsuwonus pelamis* occurs in the Southwest Atlantic Ocean, corroborating the idea of a common area of origin at low latitudes. Pinto et al. (2002) documented the predominance of *K. pelamis* larvae in the exclusive economic zone of Northeast Brazil (2ºS - 14ºS). Andrade and

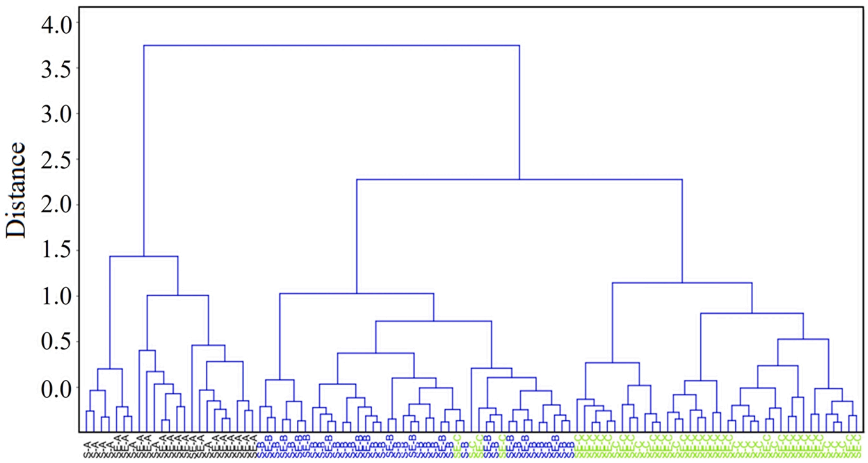

Fig. 2. Dendrogram based on Euclidean distances of the 30 first harmonics and respective morphotypes A (grey), B (blue), and C (green).

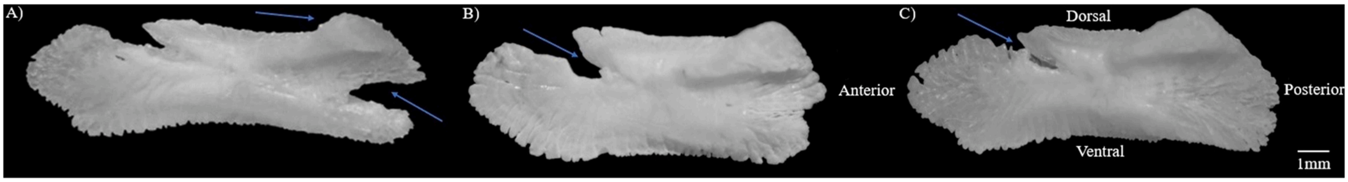

Fig. 3. Sagitta otoliths of *K. pelamis* referring to morphotypes A, B and C recorded in the Southwest Atlantic Ocean. Arrows indicate main differences among morphotypes (excisures). Fork length (FL) for each specimen in the images were: Morphotype A – FL = 59 cm; morphotype B - FL= 48 cm, and morphotype C - FL= 52 cm. White bar indicates image scale.

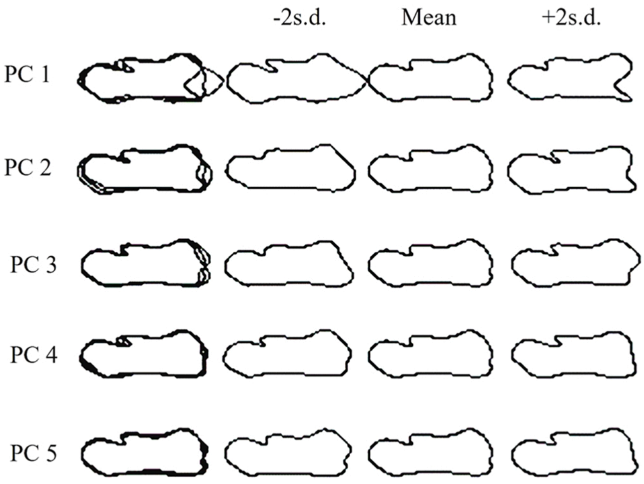

Fig. 4. Variations in the shape of the *K. pelamis* sagitta otoliths explained by each principal component (PC) and mean variation (± 2 SD) of the shape contours.

Santos (2004), based on studies of skipjack tuna recruitment in the Southwest Atlantic, advocated a low latitude breeding area. According to these authors, the species exhibits erratic recruitment throughout the year in the region (5–22ºS). Our morphometric, shape, and chemical signature analyses reiterate that *K. pelamis* is a single stock distributed in the Southwest Atlantic. Although otolith shape indicated intraspecific variations, consistent with a phenotypically heterogeneous population, otolith core compositions (element:Ca ratios) showed no significant variations with the exception of the Mg:Ca, Mn:Ca and Ba:Ca ratios. These enrichments may indicate an association between larval rearing areas and areas with high nutrient concentrations (estuarine zones, shelf break/topographic upwellings) (Pinto et al., 2002). These findings are supported by genetic marker studies indicating a single stock of skipjack tuna off the Brazilian coast (Carvalho et al., 2020a, 2020b).

Subject to the influence of endogenous and environmental factors, otolith shape analysis has been advocated as a stock discrimination tool (Tuset et al., 2003; Cardinale et al., 2004; Capoccioni et al., 2011; Vignon, 2015; Moreira et al., 2019). Morphometric indices may vary according to ontogeny, feeding regime, water properties, and geographic location (Cardinale et al., 2004; Carvalho et al., 2020a, 2020b; Almeida et al., 2020). Intraspecific differences may further be related to sex, presence of distinct cohorts, and age class (Campana and Casselman, 1993; Ladroit et al., 2017). As the skipjack tuna is considered an opportunistic spawner, the morphology of its otolith is defined at the hatching of the larvae, which may occur under different environmental conditions influencing otolith shape, along with genetic factors. Our analysis of otolith morphology (e.g., circularity and form-factor) supports the existence of three morphotypes within the study area.

Our findings demonstrate that *Katsuwonus pelamis* otoliths are characterized by complex and variable shapes, with a well-marked excisure between the rostrum and anti-rostrum. Using morphometric indices and silhouette analysis, Wujdi et al., (2017, 2018) similarly documented the morphologic variability in skipjack tuna otoliths for four Indian Ocean localities off Indonesia. According to them, otoliths were non-rounded, nearly oval, elongated, and showed irregular surface. In addition, the authors did not observe statistically significant differences among the shape descriptors, which indicates that this is a single stock unit for the region. Our results agree with the general description of the shape indices observed for the Indian Ocean population, except for the fact that one of the morphotypes recorded in the Southwest Atlantic (morphotype A) has a deep excisure in the posterior region.

Furthermore, the absence of significant differences between areas (South and Southeast), observed both in the analysis of the shape descriptors (area, circularity, form-factor, rectangularity, roundness, and fractal dimension) and in the Fourier coefficient analysis, consolidates

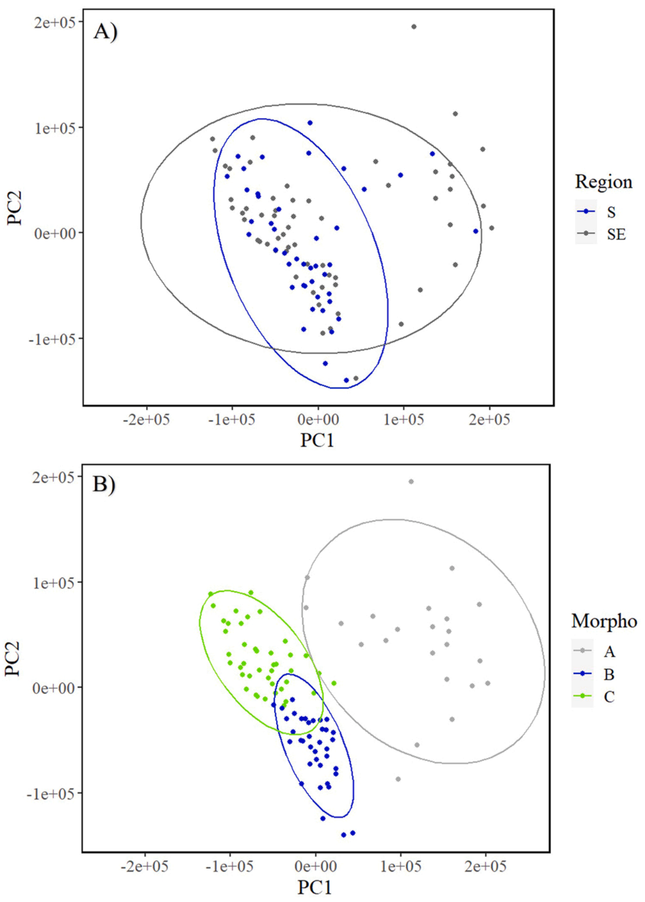

Fig. 5. Ordering from the principal components analysis of Fourier variables according to regions (A) (SE = Southeast and S= South) and morphotypes (B) (types A = grey, B = blue and C = green).

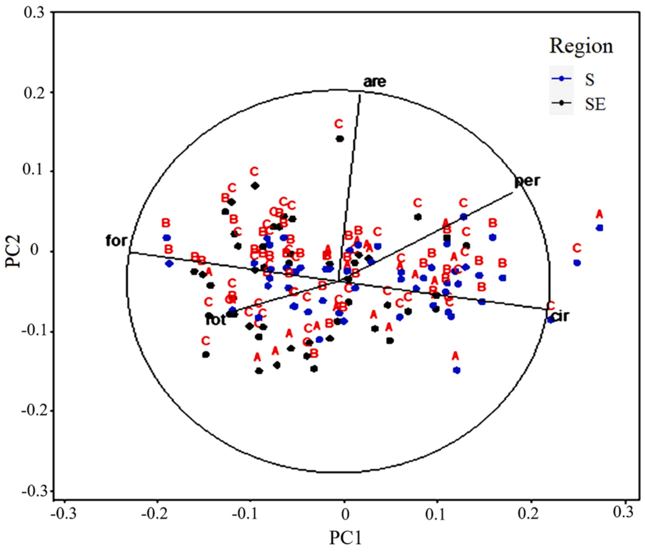

Fig. 6. Ordering diagram of the first two axes of the principal components analysis of the morphometric variables of the sagitta otoliths of *K. pelamis*. Area coded samples (SE = Southeast, S = South, cir = circularity, per = perimeter, are = area, rot = roundness, for = form factor).

the hypothesis of a single stock for the Southwest Atlantic. In addition, the presence of the three morphotypes is explained by a temporal equilibrium selection process based on a genetic polymorphism that is persistent in the population. In this case, distinct morphotypes occur alternately in the same time interval, influenced by individual genotypic and/or phenotypic variations, associated with natural or stochastic selection effects (Gauldie and Crampton, 2002; Almeida et al., 2020), as the reproductive process of this species.

The chemical composition of the otolith is determined by the composition of the surrounding water, and when associated with a unit of time (standardized for all sampled individuals, i.e., the initial development period measured in the core of the specimens around the primordium), it can be used to indicate the environmental or residence conditions during the specific life stage of a species (Campana, 1999). The incorporation of trace elements into the otolith structure is influenced by several factors, including ontogenetic and physiological events, diet, and environmental constraints such as salinity and temperature (Walther et al., 2010; Chang et al., 2012; Thomas et al., 2017). Among the existing methods to assess the population dynamics of a fish stock (e.g., life history parameters, tagging, and recapture), the identification of source areas based on otolith microchemical analysis allows an understanding of dispersal patterns and their relationship with production and capture (Cadrin and Secor, 2009; Secor, 2010). In addition, multi-element otolith profiles can be used for environmental and life history reconstructions of the fish species (Elsdon et al., 2008).

In our study, otolith cores differed in their Ba:Ca and Mn:Ca ratios between regions, and Ba:Ca and Mg:Ca among morphotypes. Artetxe-Arrate et al. (2021) also reported overlapping chemical signatures of skipjack tuna otoliths in three areas of origin in the Indian Ocean, suggesting low geographic separation among nursery areas. The incorporation of Ba and Sr into otoliths usually reflects environmental constraints (Loewen et al., 2016; Izzo et al., 2018), and these elements are considered reliable "geographic markers" (Thomas et al., 2017). Sr concentration is positively correlated with water salinity (Elsdon and Gillanders, 2004; Walther and Thorrold, 2006) and, to a lesser extent, with temperature (Walsh and Gillanders, 2018). Ba concentration, however, is often negatively correlated with salinity because Ba in nearshore environments originates from terrigenous materials transported by rivers to estuaries and coastal regions (Walther and Limburg, 2012; Sarimin and Mohamed, 2014). In the case of the Ba:Ca ratio in oceanic fish, the increase in Ba concentration in the otolith core may reflect periods of juveniles foraging near upwelling zones (Wang et al., 2009; Artetxe-Arrate et al., 2019) or in areas with a high concentration of chlorophyll-a, with a consequent increase in primary production at the base of pelagic food webs used for foraging in the Southwest Atlantic (Coletto et al., 2021).

Our results demonstrate that otolith Ba:Ca is higher for fish captured in the South region than for fish from the Southeast region, and among morphotypes. This difference may reflect different spawning and/or larval growth sites combined with higher nutrient availability due to estuarine influence and/or shelf break or topographic upwellings (Pinto et al., 2002) that promote an increase in the concentration of inorganic nutrients. Matsuura (1982) found high larvae concentrations near the shelf break between Abrolhos Bank (18°S) and the Vitória-Trindade seamounts (21°S). This region is proximal to Rio Doce continental outflow and is also influenced by cyclonic vortices originating from eddies associated with areas of upwelling (Campos, 2006; Arruda et al., 2013; Aguiar et al., 2014). Nonaka et al. (2000) reported Scombridae larvae in samples collected from the Abrolhos Bank and adjacent waters in eastern Brazil (17ºS - 23ºS). Leis et al. (1991) documented *K. pelamis* breeding areas near oceanic islands of French Polynesia. These regional patterns may indicate that the species uses the confluence area of the South Equatorial Current as a spawning area. Artetxe-Arrate et al. (2021) documented interannual Ba:Ca patterns (in otoliths) among three breeding areas (West, Central, and East) in the Indian Ocean, with overlapping chemical signatures overall (Sr:Ca, and Mg:Ca). These authors also reported variations in chemical signatures between annual cohorts associated with seasonal variations in the physicochemical properties of water. These patterns may be associated with the “income

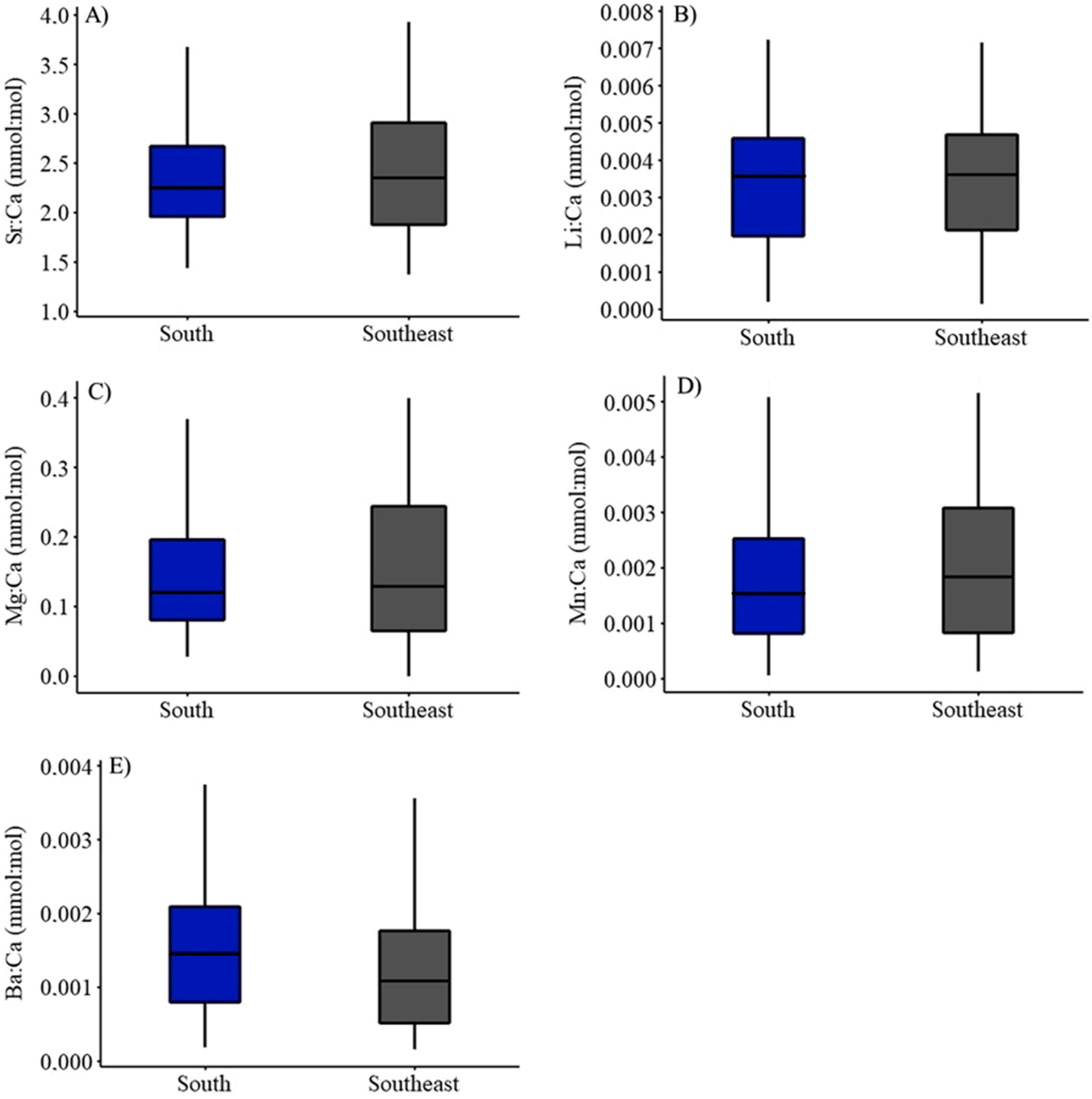

Fig. 7. Skipjack tuna otolith nucleus elementary ratios of the elements:Ca between the South and Southeast regions off Brazil in the Southwest Atlantic Ocean. Median, 1st and 3rd quartiles, minimum and maximum.

breeding" reproductive strategy of this species, whereby reproduction efforts are closely related to simultaneous food intake during the breeding season (Soares et al., 2019) at high production sites (e.g., seasonal Somali upwelling in the Indian Ocean and the Cabo Frio upwelling in the Southwest Atlantic). In our study, individuals captured in the Southeast region also had higher Mn:Ca than those in the South region. Mn incorporation in otoliths is not fully understood but may be related to reproductive mode and/or use of habitats with distinct chemical characteristics (Brophy et al., 2004; Ruttenberg et al., 2005; Artetxe-Arrate et al., 2019). Brophy et al. (2004) and Ruttenberg et al. (2005) suggested that high levels of Mn produced in otolith cores may reflect a strong maternal effect, associated with embryological development and physiological factors. Manganese is very abundant in the marine environment and required in many metabolic pathways, regulating cell function, bone development, growth, and reproductive processes (Ashford et al., 2007; DiMaria et al., 2010; Martinho et al., 2020). Additionaly, this element also reflects multiple environmental and physiological responses of individual fish to the surrounding ambient water conditions and can aid in environmental reconstructions (Martin and Thorrold, 2005; Tanner et al., 2013). Thus, the differences of Mg:Ca ratio among morphotypes may be linked to spawning, which occurs between the spring and early summer, with spawning peaks in January (Soares et al., 2019). Distribution of Mg:Ca may therefore reflect the relative amount of time lapsed for dispersal of spent spawners associated with each peak. We assume that the contrasts in otolith Ba/Ca and Mn/Ca between the Southeast and South study regions and between morphotypes for Ba/Ca and Mg/Ca are related to individuals belonging to different cohorts originated from different reproductive pulses, under the influence of different oceanographic conditions. These findings support our hypothesis of temporal selection, which is made evident by the presence of the three morphotypes (range of FC = 46.0–62.5 cm morphotype A; 46.0–67.0 cm morphotype B; 46.0–71.5 cm morphotype C) in the study area.

Our results are in line with previous findings regarding the presence of one stock in the Southwest Atlantic, and highlight the importance of maintaining this fishery resource of circumtropical distribution. Carvalho et al. (2020a), (2020b) used genetic markers to identify stocks of skipjack tuna in three areas on the Brazilian coast (South, Southeast, and Northeast). Their results also indicated single stock, with high genetic connectivity. However, the study suggests caution due to the low

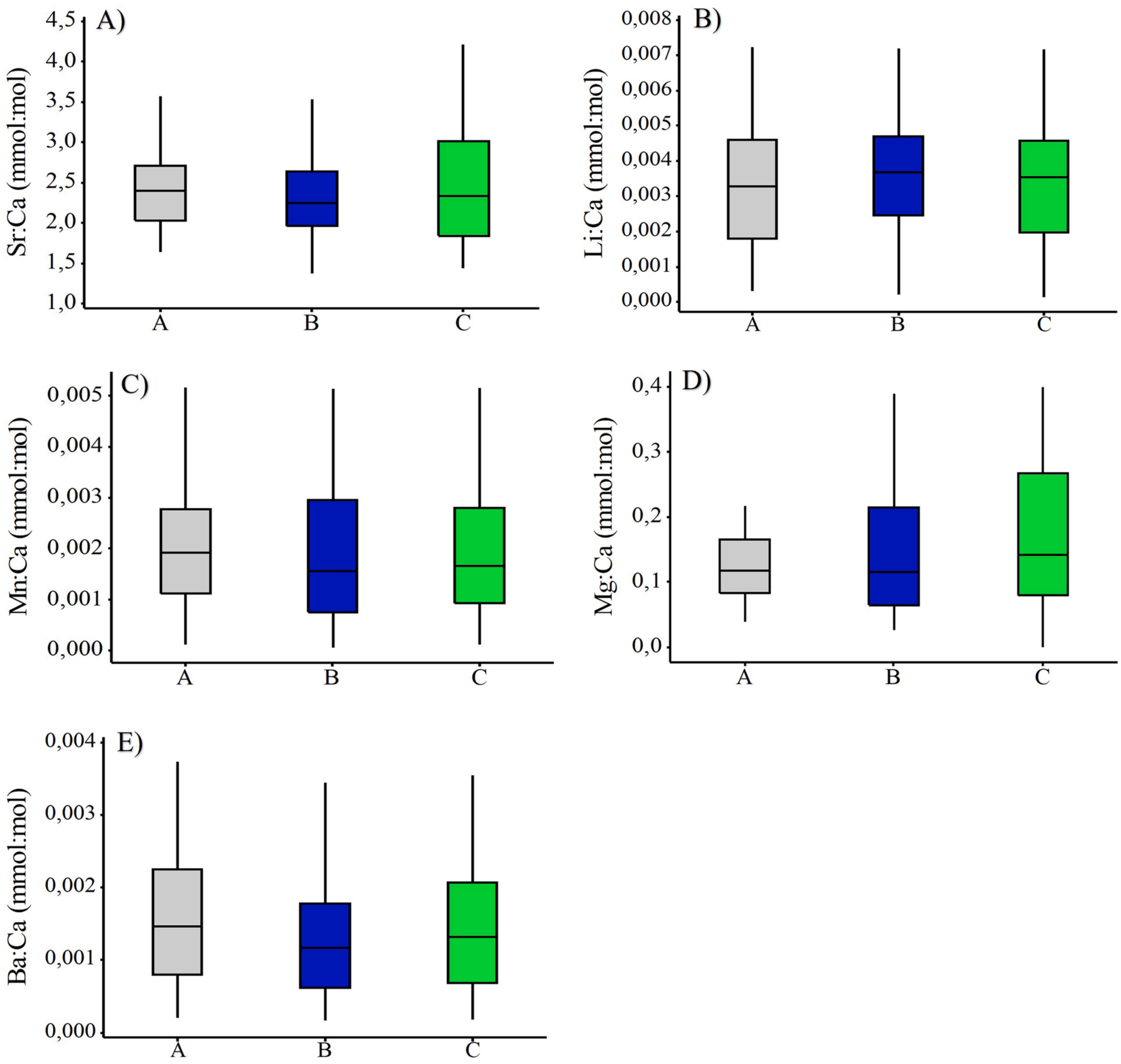

Fig. 8. Skipjack tuna otolith nucleus elementary ratios of the elements:Ca between morphotypes A (grey), B (blue), and C (green). Median, 1st and 3rd quartiles, minimum and maximum.

number of samples analyzed, and indicates that the data evaluated are not sensitive to changes that may have occurred recently in the structure of the stock or in the pattern of genetic connectivity (Carvalho et al., 2020a, 2020b).

Other data reinforce our hypothesis, such as the findings of Andrade (2003) based on capture data that showed a clear pattern of latitudinal shifts of skipjack tuna between 1982 and 1986. According to this author, the highest concentrations (catch-per-unit effort - CPUE) varied between the South and Southeast regions according to the seasons and sea surface temperature (SST), which indicated a regular habitat use pattern. Coletto et al. (2019) evaluated the latitudinal shift of the tuna fleet based in Rio Grande do Sul and found that during the summer the fisheries were more intensive in the South region below latitude 30ºS. After the autumn, the fisheries intensified off the Southeast region, which suggests a pattern of seasonal movement of this population in the Southwest Atlantic. Another indication of habitat use for this region was based on tagging and recapture surveys conducted by the ICCAT. Juvenile individuals were tagged in the South region and recaptured in the Southeast region (Luckhurst, 2014; Fonteneau, 2015), providing evidence that the skipjack tuna migration process occurs in the north-south direction in the Southwest Atlantic (Madureira et al., 2016). In this context, evidence based on isotopic analysis of fish captured in both regions demonstrate the movement of fish from South to Southeast between March and June (Coletto et al., 2021). In this period, there is a decrease in temperature in the South region, as a result of the retraction of the Brazil Current and the northward displacement of the Subtropical Convergence Area to lower latitudes. Thus, we refute the alternative hypothesis and accept the null hypothesis, which assumes that a single stock unit occurs off the southwest coast of Brazil. Further studies are needed on the behavioral patterns of this stock, regardless of the modus operandi of the fleets operating in the region, which must obey the single set of rules governing fishing.

Funding

This research was supported by the Project Bonito: Ecologia e socioeconômica da pesca de katsuwonus pelamis na costa do Rio de Janeiro visando a avaliação de estoque, o manejo sustentável e sua utilização na alimentação escolar is an environmental offset measure established through a Consent Decree/Conduct Adjustment Agreement between PetroRio and the Brazilian Ministry for the Environment.” Technical and financial support contract No. 082/2016 in the scope of the “PROJETO DE APOIO À PESQUISA MARINHA E PESQUEIRA NO RIO DE JANEIRO – SARDINHA-VERDADEIRA”, FUNBIO - Brazilian Biodiversity Fund.

CRediT authorship contribution statement

Gabriel Marcel de Souza Corrêa: Conceptualization, Methodology, Writing – original, Software, Formal analysis. **Juliano Lauser Coletto:** Methodology, Software, Writing, Supervision. **Jorge Pablo Castello:** Methodology, Supervision, Writing – review & editing. **Nathaniel R. Miller:** Analysis, Methodology. **Rafael de Almeida Tubino:** Supervision, Writing – review & editing. **Cassiano Monteiro-Neto:** Supervision, Project Administration, Funding acquisition, Writing – review & editing. **Marcus Rodrigues da Costa:** Supervision, Writing – review & editing.

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.

Acknowledgements

We thank all the team at the Projetc “Bonito-Listrado”. This research is part of the master’s degree written by GMSC under the guidance of MRC. CMN held a Research Productivity Fellowship from CNPq (Grant# 305934/2019-8). We would like to thank the crews of the vessels from the Rio de Janeiro and Rio Grande pole and line fleets for their collaboration during the samplings.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fishres.2022.106436.

References

Aguiar, A.L., Cirano, M., Pereira, J., Marta-Almeida, M., 2014. Upwelling processes along a western boundary current in the Abrolhos – Campos region of Brazil. Cont. Shelf Res. 85, 42–59. https://doi.org/10.1016/j.csr.2014.04.013.

Almeida, L.R., Andrade, H.A., 2002. Comparação entre a eficiência de captura das frotas de vara e isca-viva e de cerco na pescaria do bonito-listrado (*Katsuwonus pelamis*): análise preliminar. Notas Téc. Facimar 6, 59–64.

Almeida, P.R., Monteiro-Neto, C., Tubino, R.A., Costa, M.R., 2020. Variações na forma do otólito sagitta de *Coryphaena hippurus* (Actinopterygii: Coryphaenidae) em uma área de ressurgência na costa Sudoeste do Oceano Atlântico. Iheringia. Série Zool. 110. https://doi.org/10.1590/1678-4766e2020019.

Anderson, M., Gorley, R., Clarke, K.P., 2008. PRIMER: guide to software and statistical methods. Plymouth, Prim.E 218.

Andrade, H.A., 2003. The relationship between the skipjack tuna (*Katsuwonus pelamis*) fishery and seasonal temperature variability in the south-western Atlantic. Fish. Oceanogr. 12 (1), 10–18. https://doi.org/10.1046/j.1365-2419.2003.00220.x.

Andrade, H.A., 2006. Diagnostico do estoque e orientações párea o ordenamento da pesca de Katsuwonus pelamis (Linnaeus, 1758). Análise das principais pescarias comerciais da região Sudeste-Sul do Brasil: Dinâmica populacional das espécies em explotação–II. Série documentos REVIZEE: Score Sul. São Paulo. Instituto Oceanográfico–USP, 28–37.

Andrade, H.A., Santos, J.A.T., 2004. Seasonal trends in the recruitment of skipjack tuna (*Katsuwonus pelamis*) to the fishing ground in the southwest Atlantic. Fish. Res. 66, 185–194. https://doi.org/10.1016/S0165-7836(03)00199-1.

Ankenbrandt, L.I.S.A., 1985. Food habits of bait-caught skipjack tuna, *Katsuwonus pelamis*, from the southwestern Atlantic Ocean. Fish. Bull. 83 (3), 379–393.

Arruda, W.Z., Campos, E.J., Zharkov, V., Soutelino, R.G., da Silveira, I.C., 2013. Events of equatorward translation of the Vitoria Eddy. Cont. Shelf Res. 70, 61–73. https://doi.org/10.1016/j.csr.2013.05.004.

Artetxe-Arrate, I., Fraile, I., Crook, D.A., Zudaire, I., Arrizabalaga, H., Greig, A., Murua, H., 2019. Otolith microchemistry: a useful tool for investigating stock structure of yellowfin tuna (*Thunnus albacares*) in the Indian Ocean. Mar. Freshw. Res. 70 (12), 1708–1721. https://doi.org/10.1071/MF19067.

Artetxe-Arrate, I., Fraile, I., Farley, J., Darnaude, A.M., Clear, N., Rodríguez-Ezpeleta, N., Murua, H., 2021. Otolith chemical fingerprints of skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean: First insights into stock structure delineation. Plosone 16 (3), e0249327. https://doi.org/10.1371/journal.pone.0249327.

Ashford, J.R., Arkhipkin, A.I., Jones, C.M., 2007. Otolith chemistry reflects frontal systems in the Antarctic Circumpolar Current. Mar. Ecol. Prog. Series 351, 249–260. https://doi.org/10.3354/meps07153.

Backes, A.R., Bruno, O.M., 2005. Técnicas de estimativa da dimensão fractal: um estudo comparativo. INFOCOMP J. Comput. Sci. 4 (3), 50–58.

Begg, G.A., Friedland, K.D., Pearce, J.B., 1999. Stock identification and its role in stock assessment and fisheries management: an overview. Fish. Res. 43 (1–3), 1–8. https://doi.org/10.1016/S0165-7836(99)00062-4.

Biolé, F.G., Thompson, G.A., Vargas, C.V., Leisen, M., Barra, F., Volpedo, A.V., Avigliano, E., 2019. Fish stocks of *Urophycis brasiliensis* revealed by otolith fingerprint and shape in the Southwestern Atlantic Ocean. Estuar. Coast. Shelf Sci. 229, 106406 https://doi.org/10.1016/j.ecss.2019.106406.

Brophy, D., Jeffries, T.E., Danilowicz, B.S., 2004. Elevated manganese concentrations at the cores of clupeid otoliths: possible environmental, physiological, or structural origins. Mar. Biol. 144 (4), 779–786. https://doi.org/10.1007/s00227-003-1240-3.

Brophy, D., Haynes, P., Arrizabalaga, H., Fraile, I., Fromentin, J.M., Garibaldi, F., Santos, M.N., 2016. Otolith shape variation provides a marker of stock origin for north Atlantic bluefin tuna (*Thunnus thynnus*). Mar. Freshw. Res. 67 (7), 1023–1036. https://doi.org/10.1071/MF15086.

Cadrin, S.X., Secor, D.H., 2009. Accounting for spatial population structure in stock assessment: past, present, and future. In: Beamish, R.J., Rothschild, B.J. (Eds.), The future of fisheries science in North America. Springer, Verlag, Berlin, pp. 405–426. https://doi.org/10.1007/978-1-4020-9210-7_22.

Cadrin, S.X., Karr, L.A., Mariani, S., 2014. Stock identification methods: an overview. Stock Identif. Methods 1–5. https://doi.org/10.1016/B978-0-12-397003-9.00001-1.

Campana, S.E., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Series 188, 263–297. https://doi.org/10.3354/meps188263.

Campana, S.E., Casselman, J.M., 1993. Stock discrimination using otolith shape analysis. Can. J. Fish. Aquat. Sci. 50 (5), 1062–1083. https://doi.org/10.1139/f93-123.

Campana, S.E., Neilson, J.D., 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42 (5), 1014–1032. https://doi.org/10.1139/f85-127.

Campana, S.E., Thorrold, S.R., 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations. Can. J. Fish. Aquat. Sci. 58 (1), 30–38. https://doi.org/10.1139/f00-177.

Campos, E.J., 2006. Equatorward translation of the Vitoria Eddy in a numerical simulation. Geophys. Res. Lett. 33 (22) https://doi.org/10.1029/2006GL026997.

Capoccioni, F., Costa, C., Aguzzi, J., Menesatti, P., Lombarte, A., Ciccotti, E., 2011. Ontogenetic and environmental effects on otolith shape variability in three Mediterranean European eel (*Anguilla anguilla*, L.) local stocks. J. Exp. Mar. Biol. Ecol. 397 (1), 1–7. https://doi.org/10.1016/j.jembe.2010.11.011.

Cardinale, M., Doering-Arjes, P., Kastowsky, M., Mosegaard, H., 2004. Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. Can. J. Fish. Aquat. Sci. 61 (2), 158–167. https://doi.org/10.1139/f03-151.

Carvalho, B.M. de, Volpedo, A.V., Fávaro, L.F., 2020b. Ontogenetic and sexual variation in the sagitta otolith of Menticirrhus americanus (Teleostei; Sciaenidae) (Linnaeus, 1758) in a subtropical environment. Papéis Avulsos De. Zool. 60, e20206009 https://doi.org/10.11606/1807-0205/2020.60.09.

Carvalho, P.H., Pozzobon, A.P.B., Vasconcellos, A.V., & Silva, G.B., 2020a. Genética do bonito-listrado na costa brasileira: conectividade e aspectos demográficos Sustentabilidade da pesca do bonito-listrado no Brasil, 89.

Castello, J.P., 2000. Síntese sobre distribuição, abundância, potencial pesqueiro e biologia do bonito-listrado (*Katsuwonus pelamis*). Análise/Refinamento dos Dados Pretéritos Sobre Prospecção Pesqueira. Avaliação do Potencial Sustentável de Recursos Vivos na Zona Econômica Exclusiva MMA – REVIZEE, Rio Grande, p.13.

Castro, B.D., Lorenzzetti, J.A., Silveira, I.D., Miranda, L.D., 2006. Estrutura termohalina e circulação na região entre o Cabo de São Tomé (RJ) e o Chuí (RS). O ambiente oceanográfico da plataforma continental e do talude na região sudeste-sul do Brasil, 1, 11–120.

Chang, M.Y., Geffen, A.J., Kosler, J., Dundas, S.H., Maes, G.E., 2012. The effect of ablation pattern on LA-ICPMS analysis of otolith element composition in hake, *Merluccius merluccius*. Environ. Biol. Fishes 95 (4), 509–520. https://doi.org/10.1007/s10641-012-0065-7.

Clarke, K.R., Gorley, R.N., 2006. Primer. PRIMER-e, Plymouth.

Coelho-Souza, S.A., López, M.S., Guimarães, J.R.D., Coutinho, R., Candella, R.N., 2012. Biophysical interactions in the Cabo Frio upwelling system, Southeastern Brazil. Braz. J. Oceanogr. 60 (3), 353–365.

Coletto, J.L., Pinho, M.P., Madureira, L.S.P., 2019. Operational oceanography applied to skipjack tuna (*Katsuwonus pelamis*) habitat monitoring and fishing in south-western Atlantic. Fish. Oceanogr. 28 (1), 82–93. https://doi.org/10.1111/fog.12388.

Coletto, J.L., Botta, S., Fischer, L.G., Newsome, S.D., Madureira, L.S., 2021. Isotope-based inferences of skipjack tuna feeding ecology and movement in the southwestern Atlantic Ocean. Mar. Environ. Res. 165, 105246 https://doi.org/10.1016/j.marenvres.2020.105246.

Correia, A.T., Moura, A., Triay-Portella, R., Santos, P.T., Pinto, E., Almeida, A.A., Muniz, A.A., 2021. Population structure of the chub mackerel (*Scomber colias*) in the NE Atlantic inferred from otolith elemental and isotopic signatures. Fish. Res. 234, 105785 https://doi.org/10.1016/j.fishres.2020.105785.

Diaz, G., Cappai, C., Setzu, M.D., Sirigu, S., Diana, A., 1997. Elliptic Fourier descriptors of cell and nuclear shapes. Fourier Descriptors and Their Applications in Biology. Cambridge University Press, pp. 307–321.

DiMaria, R.A., Miller, J.A., Hurst, T.P., 2010. Temperature and growth effects on otolith elemental chemistry of larval Pacific cod, *Gadus macrocephalus*. Environ. Biol. Fish. 89, 453–462. https://doi.org/10.1007/s10641-010-9665-2.

Duarte-Neto, P., Lessa, R., Stosic, B., Morize, E., 2008. The use of sagittal otoliths in discriminating stocks of common dolphinfish (*Coryphaena hippurus*) off northeastern Brazil using multishape descriptors. ICES J. Mar. Sci. 65 (7), 1144–1152. https://doi.org/10.1093/icesjms/fsn090.

Duncan, R., Brophy, D., Arrizabalaga, H., 2018. Otolith shape analysis as a tool for stock separation of albacore tuna feeding in the Northeast Atlantic. Fish. Res. 200, 68–74. https://doi.org/10.1016/j.fishres.2017.12.011.

Elsdon, T.S., Gillanders, B.M., 2003. Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. Rev. Fish. Biol. Fish. 13 (3), 217–235. https://doi.org/10.1023/B:RFBF.0000033071.73952.40.

Elsdon, T.S., Gillanders, B.M., 2004. Fish otolith chemistry influenced by exposure to multiple environmental variables. J. Exp. Mar. Biol. Ecol. 313 (2), 269–284. https://doi.org/10.1016/j.jembe.2004.08.010.

Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E., Walther, B.D., 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol. Annu. Rev. 46 (1), 297–330.

FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.

Ferreira, I., Santos, D., Moreira, C., Feijó, D., Rocha, A., Correia, A.T., 2019. Population structure of *Chelidonichthys lucerna* in Portugal mainland using otolith shape and elemental signatures. Mar. Biol. Res. 15 (8–9), 500–512. https://doi.org/10.1080/17451000.2019.1673897.

Fonteneau, A., 2015. On the movement patterns and stock structure of skipjack (*Katsuwonus pelamis*) in the Atlantic: how many Skipjack stocks in the Atlantic Ocean. Collect. Vol. Sci. Pap. ICCAT, 71(1), 205–220.

Franco, T.P., Albuquerque, C.Q., Santos, R.S., Saint'Pierre, T.D., Araújo, F.G., 2019. Leave forever or return home? The case of the white mouth croaker *Micropogonias furnieri* in coastal systems of southeastern Brazil indicated by otolith microchemistry. Mar. Environ. Res. 144, 28–35. https://doi.org/10.1016/j.marenvres.2018.11.015.

Garcia, C.A.E., 1997. Physical oceanography. In: Seeliger, U., Odebrecht, C., Castello, J. P. (Eds.), Subtropical Convergence Environments: The Coastal and Sea in the Southwestern Atlantic. Springer, Berlin, pp. 94–96.

Gauldie, R.W., Crampton, J.S., 2002. An eco-morphological explanation of individual variability in the shape of the fish otolith: comparison of the otolith of *Hoplostethus atlanticus* with other species by depth. J. Fish. Biol. 60 (5), 1204–1221. https://doi.org/10.1111/j.1095-8649.2002.tb01715.x.

Geladakis, G., Somarakis, S., Koumoundouros, G., 2021. Differences in otolith shape and fluctuating-asymmetry between reared and wild gilthead seabream (*Sparus aurata* Linnaeus, 1758). J. Fish. Biol. 98 (1), 277–286. https://doi.org/10.1111/jfb.14578.

Hellstrom, J., Paton, C., Woodhead, J., Hergt, J., 2008. Iolite: software for spatially resolved LA - (quad and MC) ICPMS analysis. Mineral. Assoc. Can. Short. Course series 40, 343–348.

Hüssy, K., 2008. Otolith shape in juvenile cod (Gadus morhua): Ontogenetic and environmental effects. J. Exp. Mar. Biol. Ecol. 364, 35–41. https://doi.org/10.1016/j.jembe.2008.06.026.

ICCAT, 2019. International Commission for the Conservation of Atlantic Tunas. ICCAT Atlantic Ocean Tropical Tuna Tagging Programme (AOTTP). Evidence based approach for sustainable management of tuna resources in the Atlantic. Technical report, July 2019. 59p.

ICCAT, 2019. International Commission for the Conservation of Atlantic Tunas. Report of the Standing Committee on Research and Statistics (SCRS). Accessed April 2021.

Izzo, C., Reis-Santos, P., Gillanders, B.M., 2018. Otolith chemistry does not just reflect environmental conditions: a meta-analytic evaluation. Fish Fish 19, 441–454. https://doi.org/10.1111/faf.12264.

Kikuchi, E., Cardoso, L.G., Canel, D., Timi, J.T., Haimovici, M., 2021. Using growth rates and otolith shape to identify the population structure of Umbrina canosai (Sciaenidae) from the Southwestern Atlantic. Mar. Biol. Res. 1–14. https://doi.org/10.1080/17451000.2021.1938131.

Kitchens, L.L., Rooker, J.R., Reynal, L., Falterman, B.J., Saillant, E., Murua, H., 2018. Discriminating among yellowfin tuna *Thunnus albacares* nursery areas in the Atlantic Ocean using otolith chemistry. Mar. Ecol. Prog. Series 603, 201–213. https://doi.org/10.3354/meps12676.

Kuhl, F.P., Giardina, C.R., 1982. Elliptic Fourier features of a closed contour. Comput. Graph. Image Process. 18 (3), 236–258. https://doi.org/10.1016/0146-664X(82)90034-X.

Ladroit, Y., Maolagáin, C.Ó., Horn, P.L., 2017. An investigation of otolith shape analysis as a tool to determine stock structure of ling (*Genypterus blacodes*). N.Z. Fish. Assess. Rep. 24, 16.

Leis, J.M., Trnski, T., Harmelin-Vivien, M., Renon, J.P., Dufour, V., El Moudni, M.K., Galzin, R., 1991. High concentrations of tuna larvae (Pisces: Scombridae) in near-reef waters of French Polynesia (Society and Tuamotu Islands). Bull. Mar. Sci. 48 (1), 150–158.

Lestrel, P.E., 1997. Fourier Descriptors and Their Applications in Biology. Cambridge University Press.

Lima, I.D., Garcia, C.A., Möller, O.O., 1996. Ocean surface processes on the southern Brazilian shelf: characterization and seasonal variability. Cont. Shelf Res. 16 (10), 1307–1317. https://doi.org/10.1016/0278-4343(95)00066-6.

Loewen, T., Carriere, B., Reist, J., Halden, N., Anderson, W., 2016. Linking physiology and biomineralization processes to ecological inferences on the life history of fishes. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 202, 123–140. https://doi.org/10.1016/j.cbpa.2016.06.017.

Luckhurst, B.E., 2014. Elements of the ecology and movement patterns of highly migratory fish species of interest to ICCAT in the Sargasso Sea. Collect. Vol. Sci. Pap. ICCAT, 70(5), 2183–2206.

Madureira, L., Monteiro-Neto, C., 2020. Sustentabilidade da Pesca do Bonito-Listrado no Brasil. Walprint Gráfica e Editora. Rio De. Jan. 256.

Madureira, L., Coletto, J., Pinho, M., Weigert, S., Llopart, A., 2016. Pole and Line Fishing and Live Baiting in Brazil. INFOFISH International, pp. 14–17.

Mahe, K., Evano, H., Mille, T., Muths, D., Bourjea, J., 2016. Otolith shape as a valuable tool to evaluate the stock structure of swordfish *Xiphias gladius* in the Indian Ocean. Afr. J. Mar. Sci. 38 (4), 457–464. https://doi.org/10.2989/1814232X.2016.1224205.

Martin, G.B., Thorrold, S.R., 2005. Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile spot *Leiostomus xanthurus*. Mar. Ecol. Prog. Ser. 293, 223–232. https://doi.org/10.3354/meps293223.

Martinho, F., Pina, B., Nunes, M., Vasconcelos, R.P., Fonseca, V.F., Crespo, D., Primo, A. L., Vaz, A., Pardal, M.A., Gillanders, B.M., Tanner, S.E., Reis-Santos, P., 2020. Water and otolith chemistry: implications for discerning estuarine nursery habitat use of a juvenile Flatfish. Front. Mar. Sci. 7, 347. https://doi.org/10.3389/fmars.2020.00347.

Matsuura, Y., 1982. Distribution and abundance of skipjack (Katsuwonus pelamis) larvae in eastern Brazilian waters. Bol. do Inst. Oceano 31, 05–07. https://doi.org/10.1590/S0373-55241982000200002.

Morales-Nin, B., 2000. Review of the growth regulation processes of otolith daily increment formation. Fish. Res. 46, 53–67. https://doi.org/10.1016/S0165-7836(00)00133-8.

Moreira, C., Froufe, E., Vaz-Pires, P., Correia, A.T., 2019. Otolith shape analysis as a tool to infer the population structure of the blue jack mackerel, *Trachurus picturatus*, in the NE Atlantic. Fish. Res. 209, 40–48. https://doi.org/10.1016/j.fishres.2018.09.010.

Moura, A., Muniz, A.A., Mullis, E., Wilson, J.M., Vieira, R.P., Almeida, A.A., Correia, A. T., 2020. Population structure and dynamics of the Atlantic mackerel (*Scomber scombrus*) in the North Atlantic inferred from otolith chemical and shape signatures. Fish. Res. 230, 105621 https://doi.org/10.1016/j.fishres.2020.105621.

Muehe, D., 1998. O litoral brasileiro e sua compartimentação. In: Cunha S.B., Guerra A.J. T. (eds) Geomorfologia do Brasil. Editora Bertrand Brasil S.A, Rio de Janeiro, pp 273–349.

Murua, H., Rodriguez-Marin, E., Neilson, J.D., Farley, J.H., Juan-Jordá, M.J., 2017. Fast versus slow growing tuna species: age, growth, and implications for population dynamics and fisheries management. Rev. Fish. Biol. Fish. 27 (4), 733–773. https://doi.org/10.1007/s11160-017-9474-1.

Nonaka, R.H., Matsuura, Y., Suzuki, K., 2000. Seasonal variation in larval fish assemblages in relation to oceanographic conditions in the Abrolhos Bank region off eastern Brazil. Fish. Bull. 98, 767–784.

Olson, R.J., Young, J.W., Ménard, F., Potier, M., Allain, V., Goñi, N., Galván-Magaña, F., 2016. Bioenergetics, trophic ecology, and niche separation of tunas. Adv. Mar. Biol. 74, 199–344. https://doi.org/10.1016/bs.amb.2016.06.002.

Pinto, N.C.T., Mafalda Jr., P., Medeiros, C., Moura, G., Souza, C.S., 2002. Distribuição de larvas de *Katsuwonus pelamis* (Pisces, Scombridae), em larga escala, na Zona Econômica Exclusiva do Nordeste do Brasil. Trop. Oceanogr. Recife 30 (2), 171–184.

Rashidabadi, F., Abdoli, A., Tajbakhsh, F., Nejat, F., Avigliano, E., 2020. Unravelling the stock structure of the Persian brown trout by otolith and scale shape. J. Fish. Biol. 96 (2), 307–315. https://doi.org/10.1111/jfb.14170.

Reis, E., 1997. Estatística multivariada aplicada. Sílabo, 343 p.

Ruttenberg, B., Hamilton, S., Hickford, M., 2005. Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. Mar. Ecol. Prog. Series 297, 273–281. https://doi.org/10.3354/meps297273.

Sarimin, A.S., Mohamed, C.A.R., 2014. Sr/Ca, Mg/Ca and Ba/Ca ratios in the otolith of sea bass in Peninsular Malaysia as salinity influence markers. Sains Malays. 43 (5), 757–766.

Schulz-Mirbach, T., Ladich, F., Plath, M., Metscher, B.D., Heß, M., 2014. Are accessory hearing structures linked to inner ear morphology? Insights from 3D orientation patterns of ciliary bundles in three cichlid species. Front. Zool. 11 (1), 1–20. https://doi.org/10.1186/1742-9994-11-25.

Secor, D.H., 2010. Is otolith science transformative? new views on fish migration. Environ. Biol. Fishes 89, 209–220. https://doi.org/10.1007/s10641-010-9683-0.

Shen, L., Farid, H., McPeek, M.A., 2009. Modeling three-dimensional morphological structures using spherical harmonics. Evol. Int. J. Org. Evol. 63 (4), 1003–1016. https://doi.org/10.1111/j.1558-5646.2008.00557.x.

Soares, J.B., Monteiro-Neto, C., da Costa, M.R., Martins, R.R.M., dos Santos Vieira, F.C., de Andrade-Tubino, M.F., de Almeida Tubino, R., 2019. Size structure, reproduction, and growth of skipjack tuna (*Katsuwonus pelamis*) caught by the pole-and-line fleet in the southwest Atlantic. Fish. Res. 212, 136–145. https://doi.org/10.1016/j.fishres.2018.12.011.

Soeth, M., Spach, H.L., Daros, F.A., Adelir-Alves, J., de Almeida, A.C.O., Correia, A.T., 2019. Stock structure of Atlantic spadefish *Chaetodipterus faber* from Southwest Atlantic Ocean inferred from otolith elemental and shape signatures. Fish. Res. 211, 81–90. https://doi.org/10.1016/j.fishres.2018.11.003.

Tanner, S.E., Reis-Santos, P., Vasconcelos, R.P., Thorrold, S.R., Cabral, H.N., 2013. Population connectivity of *Solea solea* and *Solea senegalensis* over time. J. sea Res. 76, 82–88. https://doi.org/10.1016/j.seares.2012.11.005.

Thomas, O., Ganio, K., Roberts, B., Swearer, S., 2017. Trace element–protein interactions in endolymph from the inner ear of fish: implications for environmental reconstructions using fish otolith chemistry. Metallomics 9, 239–249. https://doi.org/10.1039/c6mt00189k.

Trindade-Santos, I., Freire, K.D.M.F., 2015. Analysis of reproductive patterns of fishes from three large marine ecosystems. Front. Mar. Sci. 2, 38. https://doi.org/10.3389/fmars.2015.00038.

Tuset, V.M., Lozano, I.J., González, J.A., Pertusa, J.F., García-Díaz, M.M., 2003. Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). J. Appl. Ichthyol. 19 (2), 88–93. https://doi.org/10.1046/j.1439-0426.2003.00344.x.

Tuset, V.M., Lombarte, A., Bariche, M., Maynou, F., Azzurro, E., 2020. Otolith morphological divergences of successful Lessepsian fishes on the Mediterranean coastal waters. Estuar. Coast. Shelf Sci. 236, 106631 https://doi.org/10.1016/j.ecss.2020.106631.

Varela, J.L., Cañavate, J.P., Medina, A., Mourente, G., 2019. Inter-regional variation in feeding patterns of skipjack tuna (*Katsuwonus pelamis*) inferred from stomach content, stable isotope and fatty acid analyses. Mar. Environ. Res. 152, 104821 https://doi.org/10.1016/j.marenvres.2019.104821.

Vignon, M., 2015. Disentangling and quantifying sources of otolith shape variation across multiple scales using a new hierarchical partitioning approach. Mar. Ecol. Prog. Series 534, 163–177. https://doi.org/10.3354/meps11376.

Vignon, M., Morat, F., 2010. Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. Mar. Ecol. Prog. Series 411, 231–241. https://doi.org/10.3354/meps08651.

Vilella, M.J.A., 1990. Idade, crescimento, alimentação e avaliação do estoque de bonito listado, Katsuwonus pelamis (SCOMBRIDAE: THUNNINI), explorado na região sudeste – sul do Brasil. In: Dissertation, Universidade Federal do Rio Grande.

Volpedo, A., Echeverría, D.D., 2003. Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentine. Fish. Res. 60 (2–3), 551–560. https://doi.org/10.1016/S0165-7836(02)00170-4.

Walsh, C.T., Gillanders, B.M., 2018. Extrinsic factors affecting otolith chemistry–implications for interpreting migration patterns in a diadromous fish. Environ. Biol. Fishes 101 (6), 905–916. https://doi.org/10.1007/s10641-018-0746-y.

Walther, B.D., Limburg, K.E., 2012. The use of otolith chemistry to characterize diadromous migrations. J. Fish. Biol. 81 (2), 796–825. https://doi.org/10.1111/j.1095-8649.2012.03371.x.

Walther, B.D., Thorrold, S.R., 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. Mar. Ecol. Prog. Series 311, 125–130. https://doi.org/10.3354/meps311125.

Walther, B.D., Kingsford, M.J., O`Callaghan, M.D., McCulloch, M.T., 2010. Interactive effects of ontogeny, food ration and temperature on elemental incorporation in otoliths of a coral reef fish. Environ. Biol. Fishes 89 (3), 441–451. https://doi.org/10.1007/s10641-010-9661-6.

Wang, C.H., Lin, Y.T., Shiao, J.C., You, C.F., Tzeng, W.N., 2009. Spatio-temporal variation in the elemental compositions of otoliths of southern bluefin tuna *Thunnus maccoyii* in the Indian Ocean and its ecological implication. J. Fish. Biol. 75, 1173–1193. https://doi.org/10.1111/j.1095-8649.2009.02336.x.

Wujdi, A., Setyadji, B., Nugroho, S.C., 2017. Identifikasi struktur stok ikan cakalang (*Katsuwonus pelamis* Linnaeus, 1758) di Samudra Hindia (WPP NRI 573) menggunakan analisis bentuk otolith. J. Penelit. Perikan. Indones. 23 (2), 77–88. https://doi.org/10.15578/jppi.23.2.2017.77-88.

Wujdi, A., Agustina, M., Jatmiko, I., 2018. Otolith shape indices of skipjack tuna, *Katsuwonus pelamis* (Linnaeus, 1758) from the Indian Ocean. J. Iktiologi Indones. 18 (2), 151–163. https://doi.org/10.32491/jii.v18i2.312.