

Article

Insights into Population Structure and Spatial Connectivity of the Bluefish *Pomatomus saltatrix* (Teleostei: Pomatomidae) Along the Southeastern–Southern Coast of Brazil Using Fourier- and Wavelet-Based Otolith Contour Descriptors

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Abstract

Otolith shape has been widely used as a tool to investigate the population structure of marine fish, as it reflects environmental, physiological, and genetic influences throughout the lives of individuals. The morphological variability of *Pomatomus saltatrix* sagittal otoliths along the southeastern–southern coast of Brazil was assessed using Elliptic Fourier Descriptors (EFDs) and Wavelet Transform Descriptors (WTDs). A total of 74 individuals from Rio de Janeiro (RJ, $N = 25$), São Paulo (SP, $N = 24$), and Santa Catarina (SC, $N = 25$) were analyzed. The coefficients obtained from both methodologies were subjected to multivariate analyses, including Permutational Multivariate Analysis of Variance (PERMANOVA) and Flexible Discriminant Analysis (FDA), combined with Jackknife Cross-Validation (JKC). The results showed high similarity in otolith shape among the sampled locations. Overall, PERMANOVA detected significant spatial differences for both EFD and WTD, although the proportion of variation explained by sampling location was low for both descriptors (<5%). After Bonferroni correction, no pairwise differences remained significant for EFD, whereas WTD indicated significant differences between SC and the other two sampling locations. Nevertheless, FDA indicated partial discrimination among sampling sites, with some overlap among groups for both descriptors. Despite this, JKC rates were high, reaching 92% for EFD and 87% for WTD. Overall, the results point to a pattern of weak spatial structuring in *P. saltatrix* along the southeastern–southern coast of Brazil. Although subtle morphological heterogeneity was detected among the analyzed locations, the evidence does not support the existence of completely isolated stocks. The observed patterns indicate weak spatial phenotypic differentiation and may be consistent with population connectivity; however, connectivity cannot be directly inferred from otolith shape alone and represents only one possible interpretation, as similar environmental conditions, shared growth histories, or limited discriminatory power of otolith morphology could also contribute to the observed scenario. Thus, the study reinforces the utility of otolith morphometry as a



Academic Editor: Jian Yang

Received: 31 July 2026

Revised: 7 September 2026

Accepted: 9 September 2026

Published: 10 September 2026

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complementary tool for population studies and highlights the importance of integrating it with other phenotypic and genotypic approaches for a more comprehensive understanding of the structure of the species' stocks.

Keywords: fish stocks; Atlantic Southwest; natural tags; Sagittae shape analysis; elliptic Fourier descriptors; wavelet transform descriptors

Key Contribution: This study provides the first assessment of the population structure of *Pomatomus saltatrix* along the southeastern–southern coast of Brazil by integrating two complementary otolith shape descriptors. The findings reveal a pattern of weak spatial structuring compatible with population connectivity, although this represents only one possible interpretation of the observed pattern, indicating that regional morphological heterogeneity occurs at a small scale and does not support the existence of completely isolated stocks, thereby reinforcing the value of otolith morphometry as a complementary tool for stock identification and underscoring the need for integration with genetic and phenotypic approaches to fully elucidate the species' population dynamics.

1. Introduction

Pomatomus saltatrix (Linnaeus, 1766), commonly known as the bluefish, is the sole extant representative of the family Pomatomidae and one of the most widely distributed coastal pelagic fish species worldwide [1–3]. The species inhabits subtropical and temperate waters of the Atlantic, Indian, and Western Pacific oceans, with a notable absence in the Eastern Pacific, colonizing an environmental range that extends from shallow estuaries and surf zones to the outer continental shelf [4–6]. In the Southwest Atlantic, *P. saltatrix* is distributed continuously along the Brazilian coast, from the Northeast region to the southernmost tip, with the highest abundance recorded between the states of Rio de Janeiro and Rio Grande do Sul [7]. This wide distribution, combined with gregarious behavior and remarkable migratory potential, makes the species an important resource for both commercial and recreational fisheries in southeastern–southern Brazil [8].

This species has supported artisanal and industrial fisheries along the Brazilian coast for decades. In southern Brazil, purse-seine and gillnet fleets exploit the resource concurrently and may exert differential fishing pressure on specific age classes throughout the year [8]. Historical landing records and local ecological knowledge from artisanal fishers have indicated marked regional declines in the abundance of the species [9]. In contrast, the most recent official fisheries statistics indicate that recorded catches of *P. saltatrix* increased from 5780 t in 2023 to 11,300 t in 2024, corresponding to an approximately 95.5% increase over the previous year [10]. However, this apparent increase should be interpreted cautiously, as Brazilian fisheries statistics are compiled from multiple monitoring programs and are still affected by spatial and temporal data gaps, which may limit direct robust assessments of stock exploitation status. These contrasting temporal patterns reinforce the need for a better understanding of population structure, as the identification of biologically meaningful stock units is fundamental for effective fisheries assessment and management and for reducing the risk of overlooking the depletion of spatially structured population components [11–13].

Bluefish exhibits a life-history strategy that may promote population connectivity across broad geographic scales. The species is highly migratory, undertaking seasonal movements associated with feeding and spawning activities throughout its distribution range [1,14,15]. In the southwestern Atlantic, reproductive activity occurs during different periods of the year, with spawning events recorded during autumn (April–June) and spring

(October–December) [3]. Furthermore, *P. saltatrix* is a multiple spawner with a pelagic larval phase, allowing early life stages to be dispersed over considerable distances by oceanographic currents before recruitment to coastal nursery habitats [4,16]. The species also displays schooling behavior and extensive seasonal movements along the continental shelf, characteristics that may facilitate demographic connectivity and gene flow among regions [6,17,18]. Nevertheless, evidence of reproductive fidelity and regional differences in environmental conditions suggests that some degree of population structuring may still occur despite the species' high dispersal potential [19–21].

Consistent with this apparent contrast between dispersal potential and regional differentiation, the population structure of *P. saltatrix* remains complex and has been the subject of considerable debate. Early mitochondrial DNA analyses suggested high connectivity within the northwestern Atlantic, supporting the existence of a largely panmictic regional stock, but also revealed pronounced genetic divergence among geographically isolated populations worldwide [22]. Subsequent studies identified significant phylogeographic breaks between eastern and western Atlantic populations and additional structuring within the Mediterranean Sea, highlighting the influence of historical climatic events and oceanographic barriers on population divergence [19]. More recently, evidence of sex-biased migration and asymmetric gene flow has suggested that connectivity patterns may vary among regions and demographic groups [20,23]. Likewise, DNA barcoding analyses revealed deeply divergent molecular lineages across the species' global distribution, with the southwestern Atlantic population representing a unique evolutionary unit of conservation concern [5]. In the southwestern Atlantic, where the present study was conducted, bluefish from southern Brazil and Argentina were historically considered to constitute a single stock. However, recent molecular evidence revealed deeply divergent lineages and two evolutionarily significant units distributed predominantly north and south of 23°40' S, although they are still treated by regulatory agencies as a single fishery stock [5,6,14]. While genetic markers provide evidence of evolutionary differentiation and historical or contemporary gene flow, otolith shape integrates phenotypic responses to genetic background, individual growth, and environmental conditions. Therefore, otolith morphology can provide complementary information on spatial phenotypic variation that may not necessarily coincide with patterns inferred from genetic markers. Importantly, such phenotypic differences should not, by themselves, be interpreted as evidence of reproductive isolation, distinct evolutionary units, or discrete fishery stocks.

In this context, otolith shape analysis represents a powerful complementary approach for investigating fish population structure and stock differentiation, as otoliths grow continuously throughout a fish's lifetime and retain phenotypic information associated with the environmental conditions experienced by individuals [24–26]. Although species-specific, otolith shape may exhibit intraspecific geographic variation driven by environmental conditions, feeding regime, growth patterns, and genetic factors, making it a valuable natural marker for identifying spatial phenotypic variation among groups [27–29]. Among the available quantitative approaches, Elliptic Fourier Descriptors (EFDs) characterize the overall contour geometry through harmonic decomposition [30–32]. In contrast, Wavelet Transform Descriptors (WTDs) preserve the spatial location of morphological variation and are particularly effective in detecting localized transitions and irregularities along the otolith contour [33–35]. Therefore, otolith contour descriptors provide a broad characterization of shape variation and represent valuable tools for assessing fish population differentiation [32,36,37].

Despite the growing body of literature on the reproductive biology, growth, and fisheries of *P. saltatrix* in the southwestern Atlantic [3,7,14], its population structure along the Brazilian coast remains poorly resolved. Otolith shape analysis provides a robust

framework for detecting spatial phenotypic variation in marine fish populations and has been increasingly applied to support stock identification and population structure assessments [25,37,38]. In this context, the combined application of EFD and WTD to sagittal otoliths collected across a broad latitudinal gradient encompassing three major fishing grounds (Rio de Janeiro, São Paulo, and Santa Catarina) along the southeastern–southern Brazilian coast provides a complementary framework for investigating subtle spatial structuring in *P. saltatrix*. Accordingly, this study aimed to assess geographic variation in sagittal otolith shape and to compare the performance of two contour-based approaches (EFD and WTD) for identifying spatial patterns of phenotypic variation. It was hypothesized that otolith shape would vary among sampling locations and that EFD and WTD would differ in their discriminatory performance. EFD and WTD were used as complementary approaches because EFDs primarily summarize broad-scale variation in overall contour geometry, whereas WTDs retain spatial information and are therefore more sensitive to localized changes along the otolith outline. Their combined application was expected to provide a more comprehensive assessment of spatial phenotypic variation than either descriptor alone.

2. Materials and Methods

2.1. Fish Sampling

Individuals of *P. saltatrix* were collected at three sites along the Brazilian Atlantic coast, namely Rio de Janeiro, São Paulo, and Santa Catarina, between August and October 2024 (Figure 1). A total of 74 individuals were analyzed, with 24–25 specimens collected at each sampling site (Table 1).

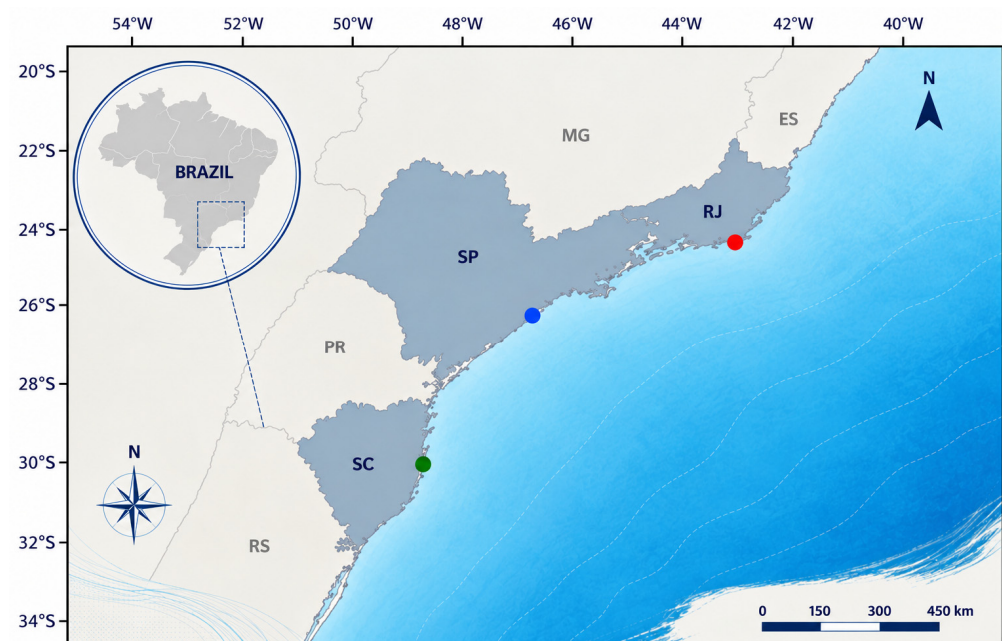


Figure 1. Map of southeastern and southern Brazil showing the three sampling sites [Rio de Janeiro (RJ, red dot), São Paulo (SP, blue dot), and Santa Catarina (SC, green dot)] where *Pomatomus saltatrix* specimens used in this study were collected between August and October 2024. The dashed square in the inset indicates the location of the study area within Brazil.

Specimens were captured by local small-scale fleets using longlines and gillnets and were obtained directly from artisanal fishers at the landing locations. Because these fishers operate locally and land their catches close to the respective fishing grounds, each landing

site could be reliably associated with the corresponding capture area, ensuring the accurate assignment of each specimen to its geographic origin.

Table 1. Sampling location, collection date, sample size (N), and total length (TL, cm) (mean values ± standard error) of *Pomatomus saltatrix* used for otolith shape analyses.

Site	Date	N	TL
Rio de Janeiro	October 2024	25	41.2 ± 0.8
São Paulo	August 2024	24	40.5 ± 0.6
Santa Catarina	September 2024	25	40.9 ± 0.5

In the laboratory, each specimen was measured for total length (TL, 0.1 cm). Individuals selected for otolith shape analysis ranged from 33.2 to 45.5 cm TL. No significant differences in TL were detected among sampling sites (ANOVA, $F_{2,71} = 0.364$, $P = 0.696$), indicating that body size was broadly comparable among the sampled groups. A restricted size range was used to reduce potential ontogenetic effects on otolith shape [25,26,39]. In addition, contour descriptors were normalized for size prior to statistical analysis, ensuring that subsequent analyses reflected shape variation rather than differences in otolith size. Moreover, most individuals (84.7%) had a TL equal to or greater than the overall estimated TL at which 50% of the population reaches sexual maturity ($TL_{50} = 37.4$ cm [3]).

Both sagittal otoliths were carefully removed from the cranial cavity using forceps, cleaned to remove adhering tissues, air-dried, and individually stored in labeled plastic tubes until shape analysis. Left and right sagittae were distinguished based on the orientation of the *sulcus acusticus* and rostrum [40].

Although the specimens were not sexed, previous studies on *P. saltatrix* reported no significant sexual dimorphism in otolith shape [41,42], indicating that sex is unlikely to have confounded the spatial patterns observed in the present study. Moreover, only one sagittal otolith per individual was used for the contour analysis. This approach was considered appropriate because previous studies on *P. saltatrix* reported no significant differences between left and right sagittal otoliths in terms of morphometric measurements, indicating negligible side-related variation [43].

2.2. Otolith Shape Contour Analyses

2.2.1. Otolith Preparation and Image Acquisition

Prior to contour analysis, paired sagittal otoliths were visually inspected for structural integrity, and only otoliths with intact margins were retained for analysis. Otoliths exhibiting fractures, erosion, or incomplete margins were excluded because contour discontinuities may introduce artificial variation into shape descriptors and compromise quantitative outline characterization [44–46]. This procedure ensured that the contours used in the subsequent analyses represented well-preserved otolith morphology and minimized potential variation associated with structural damage. The left sagitta was preferentially selected for image acquisition whenever it presented a complete and well-preserved contour.

Selected sagittae were positioned in a standardized anatomical orientation, with the *sulcus acusticus* facing upward and the rostrum directed to the right (Figure 2A). Standardized otolith positioning was maintained throughout image acquisition to obtain comparable two-dimensional contour projections among specimens, following procedures commonly applied in otolith shape studies [24,26,45]. Two-dimensional orthogonal digital images were acquired using a binocular stereomicroscope under reflected light against a dark background using a Sony IMX363 high-resolution digital camera at 5× magnification (Sony Corporation, Tokyo, Japan), equipped with a 12 MP CMOS sensor and an image resolution of 4032 × 3024 pixels (Sony Corporation, Tokyo, Japan). Digital images were converted into

black-and-white binary silhouettes using Paint 3D (v.2024.2410.13017.0), providing clearly defined outlines for subsequent contour extraction. The resulting standardized binary images (Figure 2B) were used as the common input for both EFD and WTD analyses.

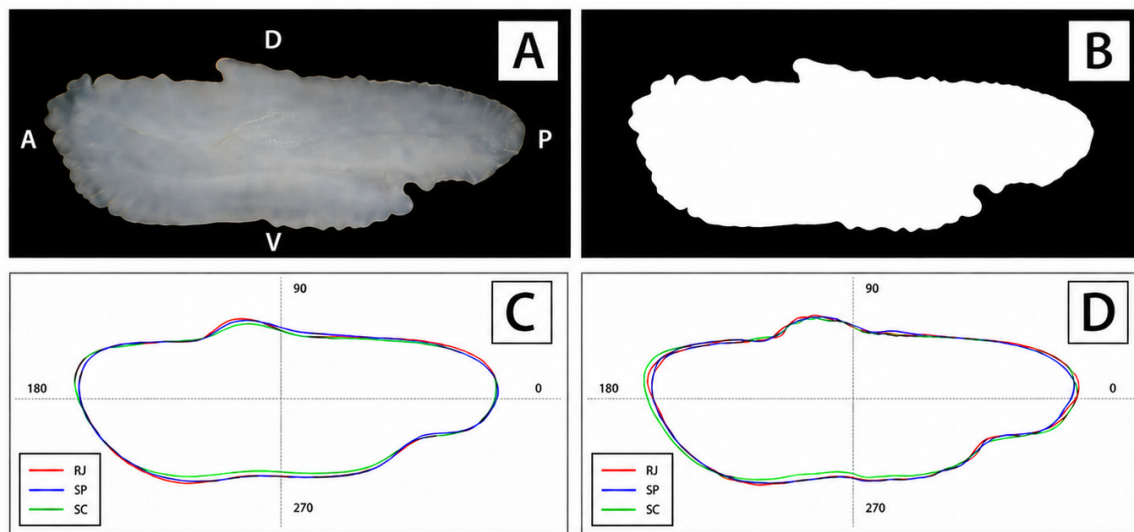


Figure 2. Left sagittal otolith of *Pomatomus saltatrix*: (A) original photograph; (B) corresponding binary digital image; (C) Elliptical Fourier Descriptors (EFDs); (D) Wavelet Transform Descriptors (WTDs). Abbreviations: anterior (A); posterior (P); dorsal (D); ventral (V); Rio de Janeiro (RJ: red line); São Paulo (SP: blue line); Santa Catarina (SC: green line).

2.2.2. Elliptic Fourier Descriptors (EFDs)

Otolith outlines were quantified using EFD, a contour-based approach that represents a closed outline as a series of harmonically related ellipses [30,47,48]. This approach enables complex biological outlines to be described quantitatively while retaining information on overall contour geometry and has been widely applied to otolith shape analysis and fish stock discrimination [45,49,50].

The minimum number of harmonics required to adequately reconstruct the otolith contour was determined from cumulative Fourier power, an approach commonly used to retain sufficient shape information while reducing descriptor dimensionality [24,28,32]. The first six harmonics, excluding coefficient d_6 , accounted for 95% of cumulative Fourier power. Accordingly, otolith contours were summarized by 20 EFD coefficients [6 harmonics $\times$ 4 coefficients – 3 invariant coefficients (a_1 , b_1 , and c_1) – d_6]. Mean otolith contours for each sampling site were reconstructed from the standardized EFD coefficients [28] (Figure 2C).

2.2.3. Wavelet Transform Descriptors (WTDs)

Otolith contours were additionally characterized using WTDs, which provide a multi-scale representation of outline variation while retaining the spatial position of morphological features along the contour [34,35,51]. Unlike EFD, in which localized contour variation may be distributed across several harmonics, wavelet-based analysis allows changes in contour geometry to be associated with specific regions of the otolith outline [34,46,52].

The wavelet transform decomposes the otolith contour across successive spatial scales, enabling broad-scale shape patterns and localized marginal features to be characterized within the same analytical framework [34,51,53]. This spatially explicit representation is particularly useful for identifying regions of the otolith contour that contribute to morphological differentiation among groups and may provide complementary information to Fourier-based descriptors [35,46,51].

In the present study, the same standardized binary otolith contours used for EFD were analyzed using WTD implemented in the ShapeR package in R (version 1.0.2) (Figure 2D). Effects of size on shape were adjusted with the otolith mean radius. A total of 63 wavelet coefficients were used to describe the otolith outline. Wavelet coefficients that showed significant interactions between subpopulation units and TL ($P < 0.05$) were omitted automatically. The resulting wavelet coefficients were used to quantify localized contour variation among sampling sites and input variables in subsequent multivariate analyses. The combined application of EFD and WTD allowed overall and spatially localized components of otolith shape variation to be assessed using complementary contour descriptors [34,35,46].

2.3. Statistical Analysis

EFD and WTD were analyzed separately to assess spatial variation in otolith shape among the three sampling sites. Multivariate differences in otolith contour among sites were assessed using Permutational Multivariate Analysis of Variance (PERMANOVA), based on Euclidean distance matrices. This non-parametric, permutation-based approach was chosen for its robustness in multivariate data analysis and because it does not require strict adherence to the assumption of multivariate normality. Statistical significance was estimated using 9999 random permutations, with the sampling site included as a fixed factor with three levels [25,54]. Homogeneity of multivariate dispersion among sampling sites was assessed using PERMDISP, based on distances to group centroids and 9999 permutations; dispersion was homogeneous for EFD ($P = 0.102$), whereas significant heterogeneity was detected for WTD ($P = 0.032$), which was considered when interpreting the corresponding PERMANOVA results. When the overall PERMANOVA was significant ($P < 0.05$), pairwise PERMANOVA comparisons were performed to identify which pairs of sampling sites differed in otolith shape. To account for multiple pairwise testing, P -values were adjusted using the Bonferroni correction. These pairwise comparisons allowed the spatial patterns detected by the overall PERMANOVA to be further examined by testing differences between each pair of sampling sites [32,55,56].

The ability of shape descriptors to discriminate individuals according to their geographic origin was also evaluated using a Flexible Discriminant Analysis (FDA), a nonlinear extension of traditional discriminant analysis based on smoothing functions and adaptive regression methods [57]. FDA was used to visualize the multivariate distribution of the groups in canonical space and to investigate the degree of morphological separation between the sampled localities.

Classification accuracy performance was assessed using a jackknifed cross-validation (JKC) based on a leave-one-out procedure. In this procedure, individuals were sequentially excluded from model fitting and subsequently classified according to the discriminant model derived from the remaining observations. The percentage of individuals correctly reassigned to their original sampling site was calculated for each site and for the overall dataset and was used to compare the discriminatory performance of EFD and WTD [32,35,38]. All multivariate analyses were performed separately for the EFD and WTD datasets using R version 4.3.0 (R Core Team, Vienna, Austria, 2023), following analytical frameworks previously applied to morphometric and otolith shape datasets [35,38,58]. A statistical significance level of $\alpha = 0.05$ was adopted for all analyses.

3. Results

3.1. Mean Otolith Outlines

The mean otolith outlines of *Pomatomus saltatrix* from Rio de Janeiro (RJ), São Paulo (SP), and Santa Catarina (SC) exhibited a high degree of morphological similarity, with sub-

stantial overlap among the mean curves reconstructed using EFD and WTD (Figure 2C,D). Overall, otolith shape was highly conserved across sampling sites, maintaining the characteristic sub-oval profile and the main anatomical features of the contour. Nevertheless, subtle localized variations were observed, mainly in the dorsal prominence and along the ventral and posterior margins of the otolith, indicating only minor spatial differences in contour shape (Figure 2C,D).

3.2. Elliptic Fourier Descriptors

PERMANOVA based on EFD coefficients detected significant differences in otolith shape among sites ($R^2 = 0.045$, $F = 1.664$, $P = 0.026$; Table 2). Despite statistical significance, the sampling site accounted for only a small proportion (<5%) of the overall variation in otolith shape. The unadjusted pairwise comparison indicated a significant difference between RJ and SC ($P = 0.039$); however, this difference was no longer significant after Bonferroni correction (adjusted $P = 0.117$).

Table 2. Results of the overall PERMANOVA testing differences in otolith shape among sampling sites using Elliptic Fourier Descriptors (EFDs) and Wavelet Transform Descriptors (WTDs). Pairwise PERMANOVA results are shown below each overall analysis. P -values were adjusted for multiple comparisons using the Bonferroni correction. Abbreviations: DF , degrees of freedom; SS , sums of squares; R^2 , proportion of explained variation; $Pseudo-F$, pseudo-F statistics; P , permutation-based P -value; P -Adjusted, Bonferroni-adjusted P -values; RJ, Rio de Janeiro; SP, São Paulo; SC, Santa Catarina.

EFD						
Overall PERMANOVA	DF	SS	R^2	$Pseudo-F$	P	
Sites	2	0.086	0.045	1.664	0.026	
Residual	71	1.844	0.955			
Total	73	1.930	1			
Pairwise PERMANOVA	DF	SS	$Pseudo-F$	R^2	P	P -Adjusted
RJ vs. SC	1	0.061	2.040	0.041	0.039	0.117
RJ vs. SP	1	0.046	1.521	0.031	0.327	0.981
SC vs. SP	1	0.045	1.439	0.030	0.378	1.000
WTD						
Overall PERMANOVA	DF	SS	R^2	$Pseudo-F$	P	
Sites	2	2.027	0.046	1.717	0.039	
Residual	71	41.914	0.954			
Total	73	43.941	1			
Pairwise PERMANOVA	DF	SS	$Pseudo-F$	R^2	P	P -Adjusted
RJ vs. SC	1	1.237	2.134	0.043	0.031	0.046
RJ vs. SP	1	0.522	0.851	0.018	0.558	0.558
SC vs. SP	1	1.276	2.208	0.045	0.030	0.046

FDA showed partial separation among sites, with substantial overlap in the multi-variate distribution of individuals from RJ, SP, and SC (Figure 3A). The first two canonical variables explained 53.5% and 46.5% of the discriminant variation, respectively. Individuals from SC were predominantly distributed towards negative values of the first canonical variable, whereas those from RJ were mostly associated with positive values. SP individuals were primarily distributed along positive values of the second canonical variable.

JKC yielded an overall correct reclassification rate of 91% (Table 3). Site-specific correct reclassification rates were 90% for SP, 90% for SC, and 95% for RJ.

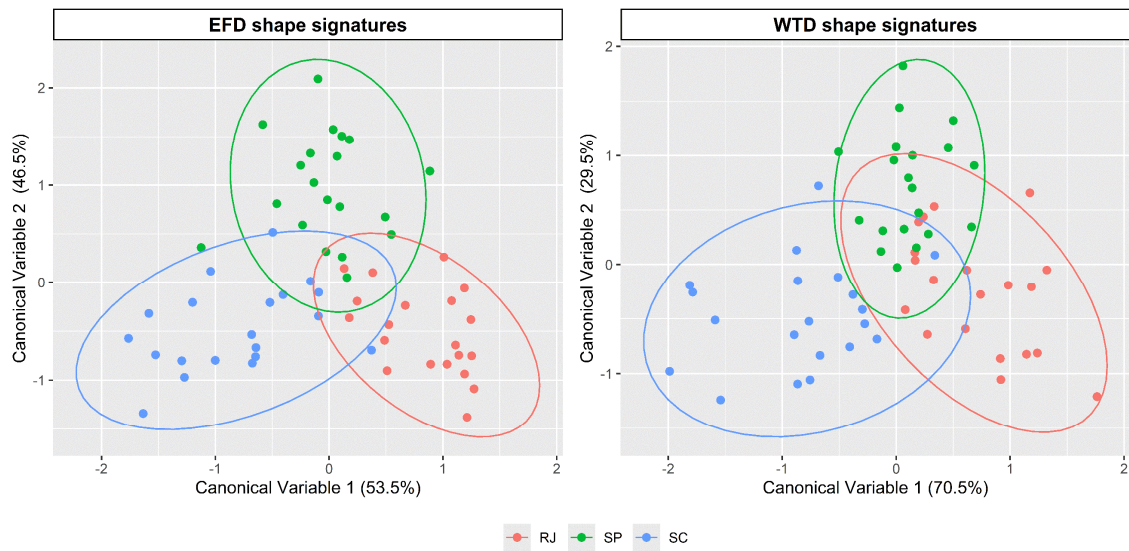


Figure 3. Flexible Discriminant Analysis (FDA) plots displaying spatial differences for otolith shape using (A) Elliptic Fourier Descriptors (EFDs) and (B) Wavelet Transform Descriptors (WTDs). Each data point represents an individual fish. Abbreviations: Rio de Janeiro (RJ: red line and circles); São Paulo (SP: blue line and circles); Santa Catarina (SC: green line and circles).

Table 3. Jackknifed cross-validation confusion matrices obtained from Flexible Discriminant Analysis (FDA) based on otolith shape descriptors derived from Elliptic Fourier Descriptors (EFDs) and Wavelet Transform Descriptors (WTDs). Samples originated from Rio de Janeiro (RJ), Santa Catarina (SC), and São Paulo (SP).

Predicted Site										Re-Classification (%)		
Original Site	Training Set			Test Set			Total			Specific	Overall	
	RJ	SP	SC	RJ	SP	SC	RJ	SP	SC			
EFD	RJ	19	1	0	5	0	0	25	0	0	95	91
	SP	1	18	1	0	5	0	0	25	0	90	
	SC	1	1	18	0	0	5	0	0	25	90	
WTD	RJ	16	4	0	5	0	0	25	0	0	80	87
	SP	2	18	0	0	5	0	0	25	0	90	
	SC	1	1	18	0	0	5	0	0	25	90	

3.3. Wavelet Transform Descriptors

PERMANOVA based on WTD coefficients detected significant differences in otolith shape among sites ($R^2 = 0.046$, $F = 1.717$, $P = 0.039$; Table 2). As for EFD, the sampling site explained only a small proportion (<5%) of the overall variation in otolith shape, indicating weak spatial differentiation. The unadjusted pairwise comparisons indicated significant differences between RJ and SC ($R^2 = 0.043$, $F = 2.134$, $P = 0.031$) and between SC and SP ($R^2 = 0.045$, $F = 2.208$, $P = 0.030$), with both differences remaining significant after Bonferroni correction (*adjusted P* = 0.046 for both comparisons). No significant difference was detected between RJ and SP ($R^2 = 0.018$, $F = 0.851$, $P = 0.558$; *adjusted P* = 0.558; Table 2).

FDA revealed partial discrimination among sampling sites, although overlap among individuals was observed (Figure 3B). The first two canonical variables explained 70.5% and 29.5% of the discriminant variation, respectively. Individuals from SC were predominantly distributed towards negative values of the first canonical variable, whereas those from RJ were mostly associated with positive values. SP individuals were primarily distributed towards positive values of the second canonical variable.

JKC yielded an overall correct reclassification rate of 87% (Table 3). Site-specific correct reclassification rates were 80% for RJ, 90% for SP, and 90% for SC.

4. Discussion

The present study provides the first assessment of spatial phenotypic variation in *P. saltatrix* along the southeastern–southern Brazilian coast based on the complementary analysis of EFD and WTD of the sagittal otoliths. The broadly consistent outcomes obtained with the two contour descriptors indicate that spatial variation in otolith shape is subtle and dependent on the scale at which contour geometry is quantified, a pattern increasingly recognized in otolith-based population studies using complementary shape descriptors [34,35,46]. The significant differences detected among sites for both EFD and WTD, together with the low proportion of variation explained by sampling site ($R^2 < 5\%$), suggest that spatial variation in otolith shape is detectable but weak. Pairwise differences did not remain significant after Bonferroni correction for EFD, whereas WTD detected significant differences between SC and both RJ and SP. Although both descriptors achieved high overall jackknifed reclassification success (92% for EFD and 87% for WTD), these results should be interpreted with caution, as they are insufficient to support the existence of discrete population units. Moreover, the significant heterogeneity in multivariate dispersion detected for WTD indicates that the corresponding PERMANOVA result may partly reflect differences in within-group dispersion rather than exclusively differences among group centroids, further supporting a cautious interpretation of the spatial variation detected with this descriptor.

EFD detected differences in overall contour geometry despite the substantial overlap observed among mean otolith outlines and individual distributions in the FDA plot. EFD characterizes closed biological contours through harmonic decomposition and is particularly effective in summarizing broad-scale variation in overall outline geometry, although localized contour features may be distributed across several harmonics and therefore be less spatially explicit [30,34,35]. Similar limitations in resolving subtle spatial differentiation have been reported when phenotypic differences among fish groups are restricted to specific portions of the otolith margin rather than affecting the entire outline [35,46,51]. Accordingly, the significant EFD result should be interpreted cautiously because the sampling site explained only a small proportion of the total variation, and the unadjusted pairwise difference between RJ and SC did not remain significant after Bonferroni correction, indicating that broad-scale contour geometry remained largely shared among the three sampling sites [35,44,45].

WTD also detected significant otolith shape variation among sites. Pairwise comparisons indicated significant differences between SC and both RJ and SP, which remained significant after Bonferroni correction, whereas RJ and SP did not differ significantly. Unlike EFDs, WTDs retain the spatial position of contour variation across scales and can identify localized morphological transitions that may be less readily resolved by harmonic representations of the entire outline [34,46,51]. The spatial pattern observed here therefore suggests that specific regions of the otolith contour contributed disproportionately to the weak spatial pattern involving SC individuals, rather than a uniform modification of overall otolith morphology [35,46,59]. Comparable differences in the discriminatory information recovered by alternative contour descriptors have been reported in otolith studies, reinforcing that descriptor choice can influence the spatial patterns resolved from the same biological structure [35,38,46].

The weak spatial pattern, particularly involving SC and, to a lesser extent, RJ, may reflect the environmental sensitivity of otolith growth and contour development. Otolith shape is influenced by interactions among genetic background, somatic growth, ontogeny,

and environmental conditions, particularly temperature and habitat-related variation in growth dynamics [60–62]. The southeastern and southern Brazilian shelf encompasses pronounced oceanographic heterogeneity associated with latitudinal thermal gradients, seasonal circulation, and regional hydrographic processes, which can generate contrasting growth environments for marine fishes [4,63,64]. Because otolith morphology integrates conditions experienced throughout individual growth, regional differences in environmental exposure may generate phenotypic signals even in populations that remain demographically connected [35,61,65]. Thus, the patterns detected by WTD and EFD, particularly involving SC and RJ, respectively, should be viewed as evidence of spatial phenotypic heterogeneity rather than as a demonstration of reproductive isolation or independent stock structure [11–13]. However, because environmental variables were not explicitly incorporated into the present analyses, these mechanisms should be regarded as plausible hypotheses rather than demonstrated drivers of the observed spatial variation in otolith shape. Moreover, although the analysis was restricted to individuals mostly at or above the estimated size at sexual maturity within a relatively narrow body-size range and contour descriptors were normalized for size, individual age was not available. Therefore, potential age- or growth-related effects on otolith shape cannot be completely excluded and should be considered when interpreting the subtle spatial differences detected.

This interpretation is particularly relevant considering the life-history characteristics of *P. saltatrix*. Individuals undertake extensive seasonal movements, occupy coastal and shelf habitats, and exhibit dispersive early life stages, all of which may promote connectivity across broad geographic scales [1,4,14]. The pelagic larval phase can facilitate transport across coastal regions, whereas the schooling and migratory behavior of juveniles and adults may further increase demographic exchange among sites [15,18,66]. Such dispersal processes may contribute to the extensive phenotypic overlap observed among RJ, SP, and SC, despite the high reclassification success obtained with both descriptor sets [13,35,44].

However, high dispersal potential does not necessarily imply complete population homogeneity in *P. saltatrix*. Genetic studies have revealed complex patterns of population differentiation across the species' global distribution, including major phylogeographic breaks and regionally structured evolutionary lineages [5,6,19]. In the southwestern Atlantic, recent molecular evidence indicates deep genetic divergence within the species, demonstrating that substantial evolutionary differentiation may persist despite the species' migratory capacity [5,6,67]. These findings emphasize that dispersal ability, demographic connectivity, phenotypic differentiation, and evolutionary divergence represent related but non-equivalent components of population structure and may produce apparently contrasting signals depending on the marker and spatial scale examined [11–13].

An important feature of the present results is the apparent contrast between weak group-level differentiation and high individual classification performance. Statistical tests of group differences and classification procedures address distinct questions: PERMANOVA evaluates whether the multivariate distributions associated with predefined groups differ, whereas cross-validated classification assesses the ability of the measured variables to predict the group membership of individual observations [68–70]. Consequently, statistically detectable differentiation may coexist with extensive overlap among individual phenotypes, as group-level differences do not necessarily imply complete morphological separation among all individuals [35,71,72]. Accordingly, the high cross-validated reclassification rates observed here should be interpreted as reflecting the discriminatory performance of the multivariate shape descriptors rather than, by themselves, as evidence of strong biological differentiation or discrete population units. Although the leave-one-out jackknife procedure reduces optimistic bias by classifying each individual using a model fitted without that observation, the relatively limited sample size (24–25 individ-

uals per site) may still constrain the generalizability of the classification performance to independent samples.

From a fisheries perspective, these results do not provide sufficient phenotypic evidence to delineate independent management units among RJ, SP, and SC. Stock boundaries should ideally be supported by concordant evidence from complementary markers because individual approaches may reflect different temporal and biological components of population structure [5,42,73]. Otolith shape primarily reflects integrated phenotypic responses to growth history, environmental exposure, and genetic influences, whereas genetic and otolith chemistry approaches can provide information on evolutionary differentiation, natal origin, and habitat use at different temporal scales [44,62,74]. Integrating contour analysis with genomic data, otolith elemental signatures, and movement information would therefore provide a stronger framework for evaluating whether the regional shape differentiation represents environmentally induced phenotypic variation, persistent demographic structuring, or a component of the evolutionary differentiation recently reported for southwestern Atlantic bluefish [5,6,75].

Overall, the complementary application of EFD and WTD revealed subtle spatial phenotypic heterogeneity in the otolith morphology of *P. saltatrix* along the southeastern–southern Brazilian coast. Rather than unequivocally supporting discrete stocks, the present findings are compatible with different scenarios of population organization, including spatially heterogeneous phenotypes within a single population or weakly differentiated population components that cannot be resolved from otolith shape alone. The weak spatial differentiation observed here should therefore not be interpreted as direct evidence of connectivity, as it may also reflect similar environmental conditions, shared growth histories, or the limited discriminatory power of otolith morphology. The spatial and temporal scope of the present sampling design should also be considered when interpreting these results. Sampling was restricted to three locations and a single, relatively short period in 2024 (SP in August, SC in September, and RJ in October) and therefore may not fully disentangle spatial from short-term temporal variation nor capture seasonal or interannual variability in otolith shape. Given the highly migratory behavior and broad distribution of *P. saltatrix*, broader spatial coverage and temporally replicated sampling would be valuable to assess the temporal stability and geographic extent of the phenotypic patterns identified here. Distinguishing between these alternative scenarios will require an integrative stock-identification framework combining phenotypic, genomic, chemical, and movement-based evidence across broader temporal and spatial sampling scales [6,12,13].

5. Conclusions

The combined analysis of EFD and WTD revealed weak spatial structuring in *P. saltatrix* along the southeastern–southern Brazilian coast. However, despite the high reclassification success obtained with both descriptors, the low proportion of variation explained by sampling site and the limited pairwise differentiation—restricted after Bonferroni correction to WTD comparisons involving SC—indicate that the observed spatial structuring is weak and insufficient to conclusively define distinct population units. The observed pattern is consistent with weak spatial phenotypic heterogeneity but does not provide sufficient evidence to determine whether the sampled groups represent distinct demographic units. Integrative studies combining otolith shape with genetic, chemical, and movement-based natural tags are therefore needed to clarify the stock structure of *P. saltatrix* in the southwestern Atlantic.

Author Contributions: Conceptualization, B.M.N., F.A.D. and A.T.C.; Methodology, B.M.N., F.A.D., R.S., R.G.K., M.R.C. and A.T.C.; Validation, B.M.N., R.S., R.G.K. and A.T.C.; Formal Analysis, B.M.N., R.S., R.G.K. and A.T.C.; Investigation, B.M.N. and A.T.C.; Resources, F.A.D., R.S., M.R.C. and A.T.C.;

Writing—Original Draft, B.M.N.; Writing—Review & Editing, A.T.C.; Visualization, B.M.N., R.S., R.G.K. and A.T.C.; Supervision, F.A.D. and A.T.C.; Project Administration, A.T.C.; Funding Acquisition, A.T.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by national funds through FCT—Fundação para a Ciência e a Tecnologia, I.P., and by the European Commission’s Recovery and Resilience Facility, within the scope of UID/04423/2025 (<https://doi.org/10.54499/UID/04423/2025>), UID/PRR/04423/2025 (<https://doi.org/10.54499/UID/PRR/04423/2025>), and LA/P/0101/2020 (<https://doi.org/10.54499/LA/P/0101/2020>).

Institutional Review Board Statement: Ethics Committee approval was not required for this study because no live animals were captured or handled specifically for research purposes. All fish specimens were obtained by purchasing individuals from local commercial fishermen after their capture during routine commercial fishing activities. Therefore, no experimental procedures or interventions involving live animals were performed as part of this study.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request. Public access to the dataset is currently restricted because the data form part of an ongoing PhD thesis that has not yet been publicly defended.

Acknowledgments: During the preparation of this manuscript, the authors used ChatGPT (OpenAI, GPT-5.5) to assist with improving the clarity, grammar, and readability of the text. The authors critically reviewed and edited all outputs and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

DF	Degrees of Freedom
EFDs	Elliptic Fourier Descriptors
FDA	Flexible Discriminant Analysis
JKC	Jackknifed Cross-Validation
PERMANOVA	Permutational Multivariate Analysis of Variance
R ²	Coefficient of Determination
RJ	Rio de Janeiro
SC	Santa Catarina
SP	São Paulo
TL	Total Length
WTDs	Wavelet Transform Descriptors

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