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Original Article

The use of different otolith-shape analyses for stock discrimination of Yellowtail Snapper *Ocyurus chrysurus*, (Bloch, 1791) in the coastal waters of northeastern Brazil

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Abstract: In this study, the otolith morphology of yellowtail snapper (*Ocyurus chrysurus*) was used for stock identification based on different shape analyses viz. morphometric parameters with shape indices, Elliptical Fourier descriptors (EFD), wavelet transform (WT), and landmarks. The samples were collected from Fortaleza (Ceará) and Recife (Pernambuco) in the coastal waters of Brazil to identify stocks and determine the best method for stock discrimination of *O. chrysurus*. The result showed no significant difference between these two regions which was supported by the Linear Discriminant Analysis (LDA). To select the best discrimination methods a correct classification through jack-knifed and Wilks' λ test was performed. The morphometric parameters with shape indices showed a correct classification of 25% and the landmark method's correct classification was 33.1%. These two methods had a lower correct classification than the otolith contouring methods (EFD = 42.3% and WT = 43.5%). Also, the Wilks' λ test showed lower power discrimination for morphometric with shape indices and landmark method (λ= 0.904 and λ= 0.808, respectively), in comparison with the two contouring methods (EFD λ = 0.688 and WT λ = 0.601). These results indicate that the most suitable methods for observing small variations in *O. chrysurus* otoliths can be EFD and WT.

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Introduction

Stock identification and population structure are a critical issue for sustainable management and conservation of marine fish (Ider et al., 2017; Wang et al., 2018). The studies show that many marine fishes have a considerable spatial community structure. According to the level of communication, they could be divided into several relatively independent and related subpopulations (Li et al., 2020). Generally, to identify and discriminate fish stocks many methods are used, but most studies emphasize genetics and morphometric features (Mohaddasi et al., 2013; Nasri et al., 2013; Zamani Faradonbe et al., 2015; Jalili et al., 2015).

The morphological features of otoliths are one of the methods widely used in stock discrimination. Otoliths are calcium carbonate structures located in the inner ear of fishes (Campana and Casselman, 1993). They have valuable information about the fish's life history and identification purposes (Fashandi et al., 2019). The shape of otolith is affected by environmental conditions and a genetic characteristic, which indicates features stock-specific (Christina Treinen et al., 2012). It can be mentioned that the morphology of otolith in the same species in various regions can differ between populations (Zhang et al., 2020). Therefore, the otolith shape analyses are significant for stock discrimination (Berg et al., 2018; Szymon et al., 2019). Therefore, otolith shape analyses have an important role in stock-identification

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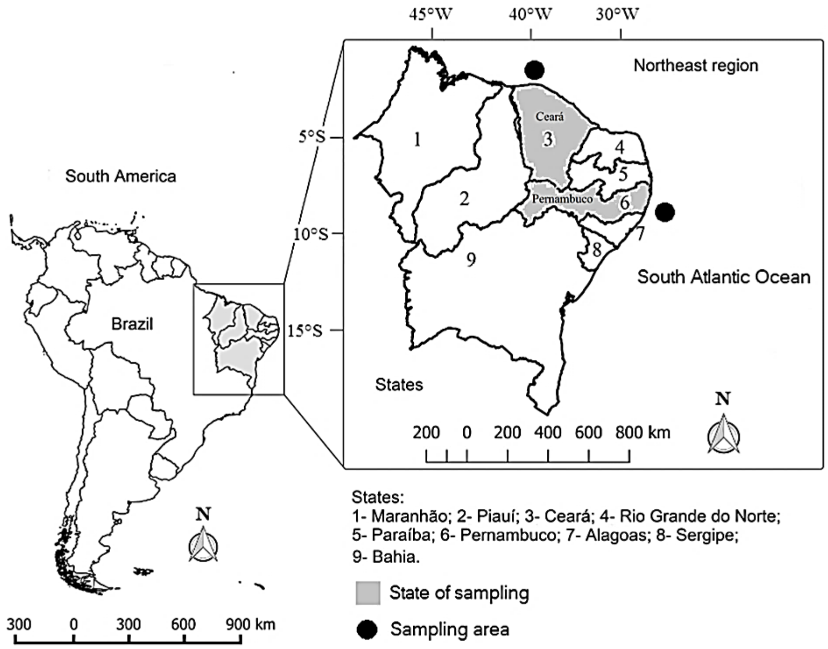

Figure 1. Localization map showing the sampling areas, where black points represent sampled sites. Abbreviations: CE= Ceará; PE= Pernambuco.

studies (Smith et al., 2002; Turan, 2006; Agüera and Brophy, 2011; Jemaa et al., 2015).

The yellowtail snapper, *Ocyurus chrysurus* (Bloch, 1791), a member of the Lutjanidae family, occurs from the USA (Massachusetts to Bermuda) to southeastern Brazil in the western Atlantic Ocean, and also in the Gulf of Mexico and the Caribbean Sea (Bester, 2015). The local name of this species in NE Brazil is Guaiúba (Freire and Carvalho-Filho, 2009). It is commercially an important species for the Brazilian coast (Begossi et al., 2011) playing a significant role in reef zones as a predator (Parrish, 1987). A study of this species based on a short-data series in northeastern Brazil from 1998 to 2000 showed the overexploitation by the hand-line fishery, which has reduced its mean abundance index (Mattos and Maynou, 2009). According to the IUCN Red List, *O. chrysurus* was listed as a Threatened Species and Data Deficient in 2016 (Lindeman et al., 2016).

There are many studies on the biological features of *O. chrysurus* (Araujo et al., 2002; Begossi et al., 2011), but evidence on the population structure of this species in the coastal water of Brazil is scarce. A previous study showed that there is only one stock of *O. chrysurus* found in the coastal waters of Brazil based on genetic data (Vasconcellos et al., 2008), albeit some other research showed the relatively limited movement in this species, which can lead to reduced gene flow between stocks (Da Silva et al., 2015). It should be noted that having a phenotypic adaptation does not necessarily imply genetic variations, and it probably takes years for phenotypic changes to appear in population genetics (Swain et al., 1991). Therefore, fish stock identification is necessary for accurate fisheries management, and ignoring the cognition of the fish stocks may lead to unpredictable risks such as overfishing (Zhang et al., 2020). Many species have been identified in northeastern Brazil with significant population structure separation, such as *Coryphaena hippurus* (Duarte-Neto et al., 2008), *Hepatus pudibundus* (Reis-Júnior et al., 2020) and P*aralonchurus brasiliensis* (Freire et al., 2020).

The specific objectives of this study were first quantifying the variation of sagitta shapes to identify stocks of *O. chrysurus* between Ceará and Pernambuco in the coastal waters of Brazil and, secondly, evaluation of different otolith shape analysis such as morphometric parameters with shape indices, Elliptical Fourier descriptors (EFD), Wavelet transform (WT) and landmarks, to determine the best method for stock discrimination of this species.

Materials and Methods

Samples collection: The northeastern Brazilian

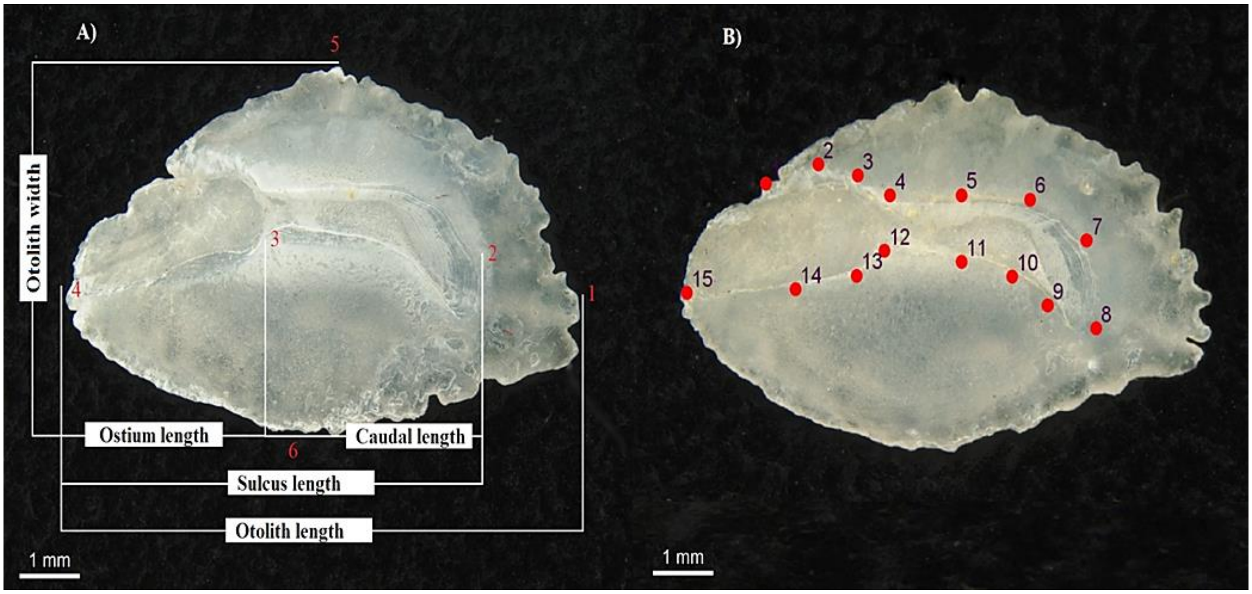

Figure 2. (A) Measurement of otolith dimensions and (B) Definition of otolith sulcus landmarks of *Ocyurus chrysurus.*

(NEB) coast spreads up to 3000 km along with the latitudinal range (01ºN to 18ºS) (Moura et al., 2013). Fish specimens were collected directly from artisanal and local small-scale fisheries at Fortaleza/ Ceará (CE) and Recife/ Pernambuco (PE) in 2019 -2020 (Fig. 1). The samples were caught with hand lines, gillnets, and traps from nearby localities (CE: 3°69'06.32"S, 38°.58'22.29"W, PE: 8°14 '86.23"S, 24°80'94.51"W). Data on weight (g), fork length (cm), and standard length (cm) of fishes were recorded in the field, and sex was determined by assessment of gonads.

Sagittal otoliths were removed from 200 samples (CE = 100; PE =100). After that 40 samples from each region (CE = 40; PE =40), with the same age and length (35 to 45cm) were selected in the ichthyology laboratory of the University Federal of Paraiba (UFPB), and separated into left and right sides, cleaned with running water, brushed, dried, and stored in labeled microtubules. Both left and right sagittae were weighed using a digital precision scale (0.0001 mg). Right otoliths were placed with the rostrum to the left side on a black background and photographed using a Leica stereomicroscope (M2005A) coupled to a Leica DFC450 digital camera (1213×937 pixels) with a magnification of 10X. The pixel-mm ratio for each image (jpg) was taken by ImageJ software a using 1 mm scale. The otolith outline shapes were automatically selected by two software SHAPE (Chalh et al., 2014) and Age & Shape (Sadighzadeh et al., 2012). The age determination of otoliths was done by two methods and compared. To increase the accuracy of the study and determine the best method to obtain the year lines, the burning (using flame) and the polishing method (with different sandpaper numbers such as 1500, 2000, 3000, and 6000) were used and transformed into thin slices. The otolith shape is age-dependent; therefore, we selected the samples that were 5+ years old. To enhance the image resolution of age lines, the images were transferred to the Adobe Photoshop CS5 software (Campana, 2004).

Morphometric analyses: According to the terminology of Avigliano et al. (2014a, 2015), measurements of otolith dimensions, such as Otolith length (OL, mm, points 1 to 4), Otolith width (OW, mm, points 5 to 6), otolith Perimeter (P, mm) and otolith Area (A, mm²). Also, the ostium length (OL, mm, points 3 to 4), caudal length (CL, mm, points 2 to3), and sulcus length (SL, mm, points 2 to 4) were taken manually by the ImageJ software (Fig. 2A). Shape indices of otoliths were calculated by the following formulas (Agüera and Brophy, 2011): Circularity = P/A^2, Form-factor = $4\pi A/p^2$, Ellipticity = $(OL\text{-}OW)/(OL+OW)$, Roundness = $4A/(OL)^2$, Rectangularity = $A/(OL{*}OW)$, and Aspect ratio = OL/OW.

Firstly, the Grubbs' test was performed for otolith morphometric and shape indices data to check for the existence of outliers. Then, Kolmogorov-Smirnov (K-S) and Levene´s tests were used for testing normality and homoscedasticity, respectively. Next, data were converted to log (x) to reduce the intragroup variability, and a Student T-test analysis was performed to distinguish the variation in *O. chrysurus* total length and fork length between the groups (CE and PE). Also, a T-test between left and right otolith measurements was calculated and when no significant differences were detected between them, the left otolith could be used in cases of missing (broken or damaged) values of the right otolith (only two were broken and flipped). Analysis of Co-Variance (ANCOVA) was performed using the total length as covariates to verify differences for each otolith dimension and shape index between CE and PE. The significant variables were corrected using the common within-group slope b (Lleonart et al., 2000) and the formula that was used to correct variables is: Vadjusted = Vi−b×TL, where Vadjusted is the adjusted value, Vi is the variable, and b is the slope value (Campana and Casselman, 1993; Ferguson et al., 2011). In addition, principal components analysis (PCA) was performed to summarize the data on otolith dimensions and shape indices. The Kaiser-Meyer-Olkin (KMO) was applied to determine the data set quality and the acceptability for PCA analysis ($P<0.05$). According to the KMO test, a KMO value of 0.90-1.00 is accepted as excellent, 0.80-0.89 as very good, 0.70-0.79 as good, 0.60-0.69 as a medium, and less than 0.60 as unsuitable. Linear discriminant analysis (LDA) was applied to demonstrate the variation and to evaluate the success in fish classification accuracy to the original region or group. Cross-validations were performed using jackknifed procedures (Correia et al., 2011). Wilks' λ was used to calculate the power of discrimination. If Wilks' λ approaches zero, the groups are well separated, and if there is no discrimination it approaches one (Canas et al., 2012).

Otolith outline methods

Elliptical Fourier descriptors (EFD): The otolith outline of *O. chrysurus* was extracted using the program SHAPE 1.3. For the software setup, all images were transferred to full-color (24-bit) bitmap format images with Microsoft Office Picture Manager (Iwata and Ukai, 2002). Otolith contour identification was provided with Fourier descriptors (SHAPE software). The software, using EFD generates 20 harmonics, which allowed us to draw the otolith contours (Tracey et al., 2006; Farias et al., 2009). Each harmonic represents four coefficients (A, B, C, and D) and thus 80 coefficients were obtained for each otolith. To choose the minimum number of harmonics, a level of 95% of the accumulated variance was used (Ferguson et al., 2011). According to the first harmonic, it normalizes the contours automatically (NEFDs in SHAPE package) normalizing the contour in relation to the first harmonic and consequently, they become invariant to size, rotation, and starting point, which causes the degeneration of the three first coefficients to fixed values: $a_1 = 1$, $b_1 = c_1 = 0$ (Iwata and Ukai, 2002). The small values of harmonics are generally important for explaining the observed morphological variations of the otolith by the last harmonic vs. the total harmonic number (*n*). The flowing process was done in the Print Print program in the SHAPE Software (Sadighzadeh et al., 2012; Pattuinan and Demayo, 2018).

Wavelet transforms (WLTs): WLTs is one of the important methods for identifying the outline shape of otoliths that can recognize stocks and species based on the structure. In the following formula, otoliths were contoured for each region, and different points of contours were compared and analyzed to identify differences between regions (Sadighzadeh et al., 2014).

$$\Psi_S(x) = \frac{1}{s}\Psi\left(\frac{\varphi}{s}\right)$$

Where, φ = otolith radius at scale s, and ΨS: wavelet function. A total of nine wavelet functions were calculated from one to three providing information about little differences of otolith contour, while wavelet functions from seven to nine indicate scant contour characteristics. We can choose wavelet number 5 as an intermediate function (tested) and

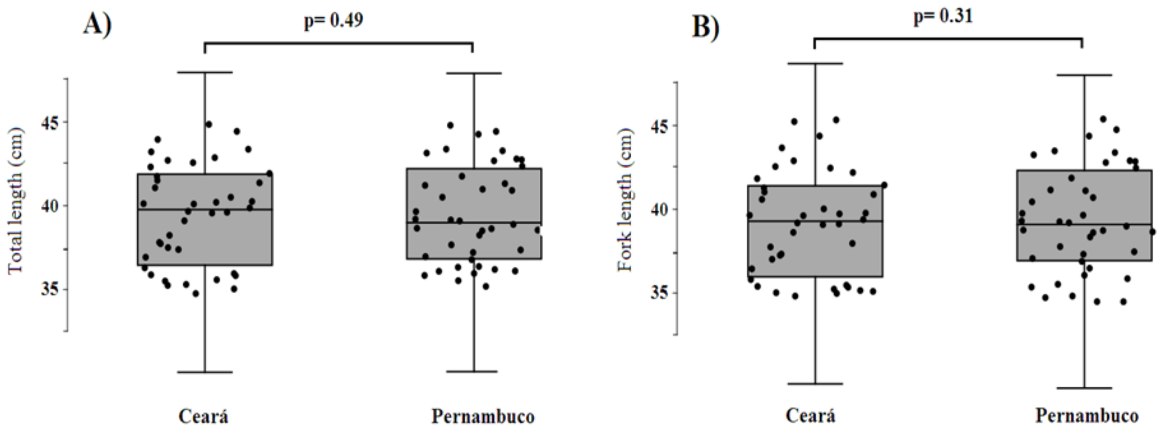

Figure 3. Boxplot with points graph from the T-test revealing homogeneous groups for Ceará and Pernambuco regarding to the *Ocyurus chrysurus* (A) total length and (B) fork length.

indicate special differences depending on the important characteristics of the otolith contour (Sadighzadeh et al., 2012). For determining the otolith contours, a total of 512 cartesian coordinates were extracted for each wavelet to draw the main outline shape of each region (Schwarzhans, 1980; Nolf, 2013). The following process was carried out using Age and Shape software. The wavelet transforms analysis was performed to specify the differences in average outline points between regions. To determine which points, discriminate among regions and rank wavelet by points, a single-factor ANOVA was applied to the frequency graph (WT-waves). Also, the F-score of points was compared and the highest F-score was selected as variation points. For EFD coefficients and wavelet transform data, Kolmogorov-Smirnov and Levene's tests were performed for normality and homogeneity, respectively. The effect of allometric growth was also reduced from all data by fish length standardization as a reference (Lleonart et al., 2000; Tuset et al., 2006). Principle component analysis (PCA) was performed for EFD and WT to explain the features of the outlines. Then to determine the quality of the data, the KMO test was performed. A linear Discriminant Analysis (LDA) was carried out for EFD and WT data to verify the differences. For evaluating the success classification accuracy cross-validation was also performed using jackknifed procedures. A Wilks' λ was used to calculate the power of discrimination (Tuset et al., 2006; Correia et al., 2011).

Landmark method: The surface images of otoliths (internal side) were taken by a digital camera. With the TpsUtil software, all images were transformed into tps format (Grandos- Amores et al., 2020). Then the otolith sulcus part (ostium = 8 and caudal = 7) was selected, to digitize 15 landmark points (Tuset et al., 2016; Chollet-Villalpando et al., 2019) (Fig. 2B). After landmarking by the TpsDig2 software manually, Procrustes analysis was generalized and the new coordinates (X, Y) was captured from landmark whose digitization were transformed (Gower, 1975). Because the processed x and y coordinates are different from image to image.

Procrustes superimposition method was used to omit the shape, rotation, and size (Zelditch, 2004). The illustration and analysis of sulcus differences were calculated by the PAST 3.22 software (Tabatabaei Yazdi et al., 2012). In addition, PCA was performed to summarize the data multivariable for otolith sulcus landmarks, and to determine the quality of data the Kaiser-Meyer-Olkin (KMO) test was performed. Linear Discriminant Analysis (LDA) was performed to discriminate fishes between CE and PE. Also, LD1 (density) draws the overlap degree between regions. Wilks' λ was used to calculate the power of discrimination.

Results

Morphometric analyses: There were no significant

Table 1. ANCOVA analysis for otolith morphometric parameter and shape indices between Ceará and Pernambuco. Values are given in Mean ± Standard-Deviation. Abbreviations: ns = not significant; *= significant (P<0.05).

Otolith morphometric parameters	Ceará (CE)	Pernambuco(PE)	ANCOVA
Otolith weight (gr)	0.099 ±	0.120 ± 0.0522	ns
Area (m^2)	45.681 ±	42.171± 6.655	*
Perimeter (m)	32.828 ±	31.154 ± 2.703	*
otolith length (mm)	11.922 ±	10.592 ± 1.832	ns
otolith width (mm)	6.858 ±	5.775 ± 0.892	ns
Sulcus length(mm)	8.510 ±	8.524 ± 0.424	ns
Ostium length (mm)	4.239 ±	4.194±0.376	ns
Codal length (mm)	4.454 ±	4.485±0.761	ns
Shape indices			
Aspect ratio	0.768 ±	0.573 ± 0.014	*
Circularity	19.092 ±	19.903 ± 0.672	ns
Ellipticity	0.581 ±	0.389 ± 0.018	*
Form factor	0.780 ±	0.611 ± 0.042	*
Rectangularity	0.851 ±	0.877 ± 0.014	ns
Roundness	3.688 ±	3.755 ± 0.191	ns
Age structure			
Age (year+month)	5.537 ±	5.278±2.325	ns

Values are given in Mean ± SEM; Abbreviations: ns = not significant; * = significant (P<0.05)

differences for the *O. chrysurus* total length and fork length between CE and PE (P = 0.31 and P = 0.49, respectively), with only one homogenous group recorded (Fig. 3A, B). The average total length in Ceará was 32.81±12.70 cm and in Pernambuco 31.15±12.54 cm (mean±standard deviation). Also, the average fork length in Ceará was 27.33±9.96 cm, and in Pernambuco 26.11±9.98 cm. The age structure between Ceará and Pernambuco also showed no significant difference (P>0.05). Measurements and analyses of right and left otoliths indicated no significant differences between right and left otolith in each region, thus the right otolith was considered for the next analysis (t-test, P>0.05).

Also, there were no significant differences between male and female otolith (about weight and length) (t-test, P>0.05) (Fig. 3).

The ANCOVA showed significant differences in some otolith morphometric parameters correlated to the total length such as area (m^2) and perimeter (m), and for the shape indices such as aspect ratio, ellipticity, and form factor between Ceará and Pernambuco (area: P<0.05 F = 2.65; perimeter: P<0.05 F =3.54; aspect ratio: P<0.05 F = 2.45; ellipticity: P<0.05 F = 2.87; form factor: P<0.05 F = 2.76) (Table 1). The common within-group slope b was used to correct the variables that were significantly correlated with length.

The first two components of the PCA explained the total variation of otolith morphometric parameters and shape indices (56.7%). The consequence of the factor analysis demonstrated that the KMO coefficient was 0.71 at a good level, showing that the data were appropriate for factor analysis. The first principal component (PC1) explained 33.5% of the variation in the data set.This variation is strongly influenced by the following variables; otolith weight, length, and width. The second principal component (PC2) explained 23.2% of the total variance and it is influenced by the variables related to form factor and area (Fig. 4A). LDA for morphometric parameters and shape indices showed 25% correct classification through the Jackknife of individuals into their original region and the Wilks' λ was 0.904, confirming that the

Table 2. Percentage of cumulative variance of otolith geometric morphometric coefficients of *Ocyurus chrysurus* by using Principal Component Analysis (PCA) (*>77: the PCA coefficients selected from 77 Fourier descriptors).

	Eigenvalue	Variance (%)	Cumulative (%)	*>77
PC1	2.389467E-003	48.305	48.305	*
PC2	7.963281E-004	16.098	64.403	*
PC3	5.303817E-004	10.722	75.125	*
PC4	3.077048E-004	6.220	81.346	*
PC5	1.861272E-004	3.762	85.109	*
PC6	1.510541E-004	3.053	88.162	*

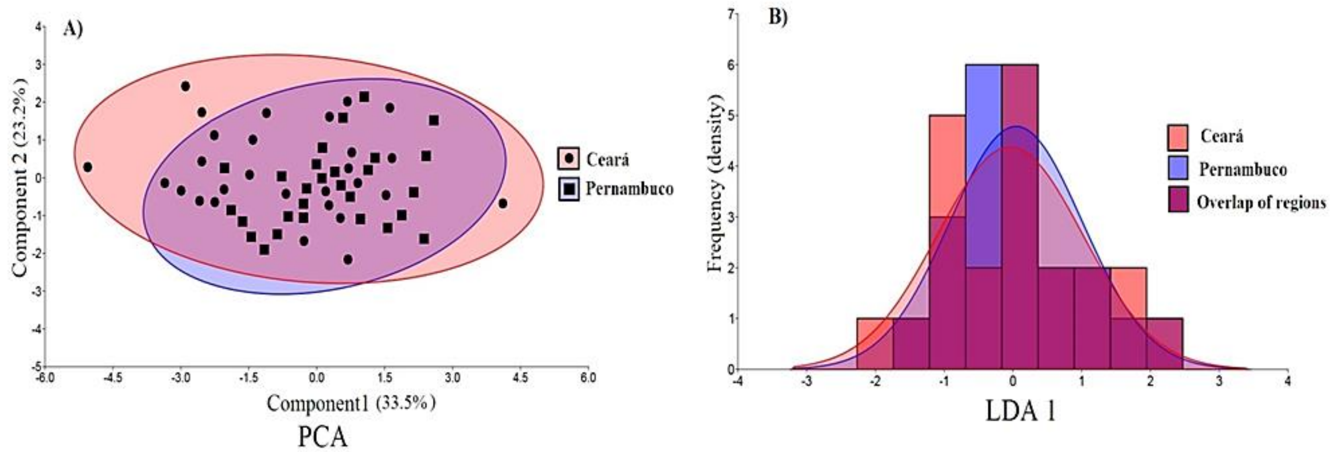

Figure 4. (A) Principal Component Analysis (PCA) and (B) Linear Discriminant Analysis (LDA) of otolith morphometric parameter and shape indices for *Ocyurus chrysurus* between Ceará and Pernambuco.

discriminatory power of the model is too low. The LD1 (density) recorded a high overlap degree (95.6%) for the CE and PE groups (Fig. 4B).

Otolith outline methods

Elliptical Fourier Descriptors (EFD): A program called Prin Comp (in SHAPE software) performs the main component analysis of EFD coefficients. The normalized EFD coefficients still cannot be directly used as shape features, because usually the number of coefficients is too large and it is difficult to describe the morphological meaning of each coefficient. So, the eigenvalue was calculated through formulas by the software to sum up the coefficient. From 77 EFD coefficients only six of them showed 88% variation with PC1 (va = 48.30%, eg= 2.38. cu= 48.30) and PC2 (va= 16.09%, va= 16.09, cu= 64.40) (Table 2).

According to the chain codes program (in SHAPE software), six Fourier descriptors coefficients and 20 harmonics could draw the mean shape of otoliths. In this case, PC1 to PC6 showed 98% variation in the outline shape. Also, we observed that as the eigenvalue increases, the shape changes decrease and the average shape of the otolith outline is closer to reality. In the mean shape of the otolith outline, the first two components showed that 73% of variation with PC1 (va= 55%, eg= 0.0023) and PC2 (va= 18%, eg= 0.00077) between rostrum, anti-rostrum and, post-rostrum (Fig. 5). The KMO coefficient was 0.89 at an excellent level demonstrates that the data were appropriate for factor analysis.

Wavelet Transform Analysis (WLTs): In wavelet transform analysis, wavelet 5 (WLT-5) was selected to describe the variation of the otolith contour means between regions. The normality and homogeneity of the wavelet point's variances were tested using the Kolmogrov-Smirnov and Leven's tests, respectively. All the variables that did not match these assumptions were removed. Analysis of ANCOVA (P<0.05) was performed to indicate the effect of fish length on variables (Total fish length was considered as a

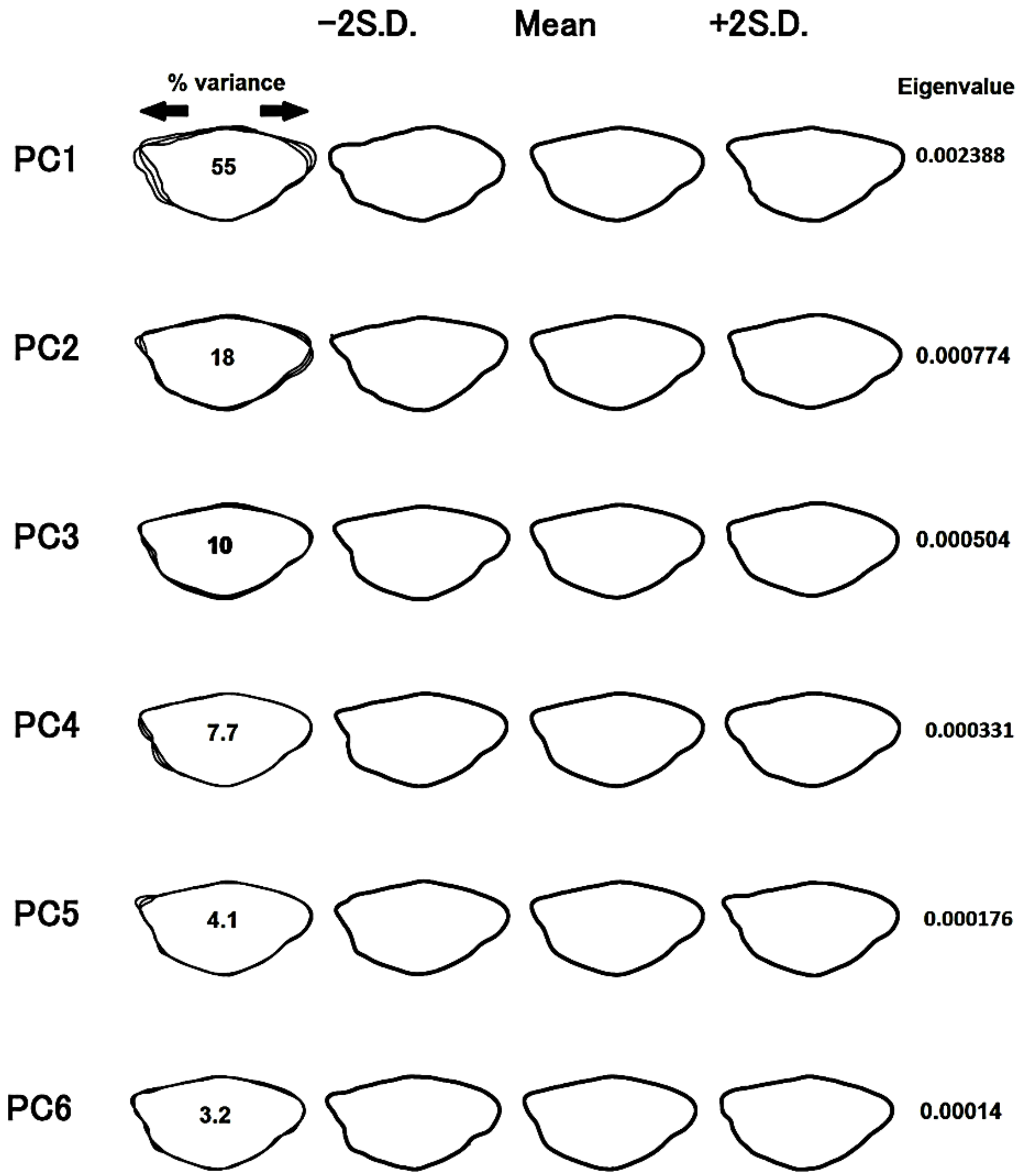

Figure 5. Otolith contour reconstructions by EFD method. Contours under the Mean column represent the average otolith shape in *Ocyurus chrysurus*. Contours on either side of the mean column illustrate the effect of each PC on otolith shape.

covariate). Also, wherever the effect of fish length was observed, the variables (points) were omitted. After that, a one-factor ANOVA was performed to determine which points could be used as a discriminator between regions to obtain potential predictors. These points included the numbers 176, 215, 258, 422, and 450 for WLT-5. The result indicated significant differences between all considered points ($P<0.05$). The contour information for wavelet transform was able to show the differences in otoliths from Ceará in comparison to Pernambuco (Fig. 6A). The highest F scores were 19.787 and 10.345 which were for points 258 and 450, respectively (Fig. 6B). These points were selected to describe variation in the Posterior and Antirostrum, respectively. Otolith from Pernambuco had a smaller size, with low variation. On the other hand, otoliths from Ceará were bigger with a larger variety in otolith contour.

The first two principal component axes (PC1 and PC2) described over 86.78% of the variation in otolith wavelet data between the two groups. The KMO

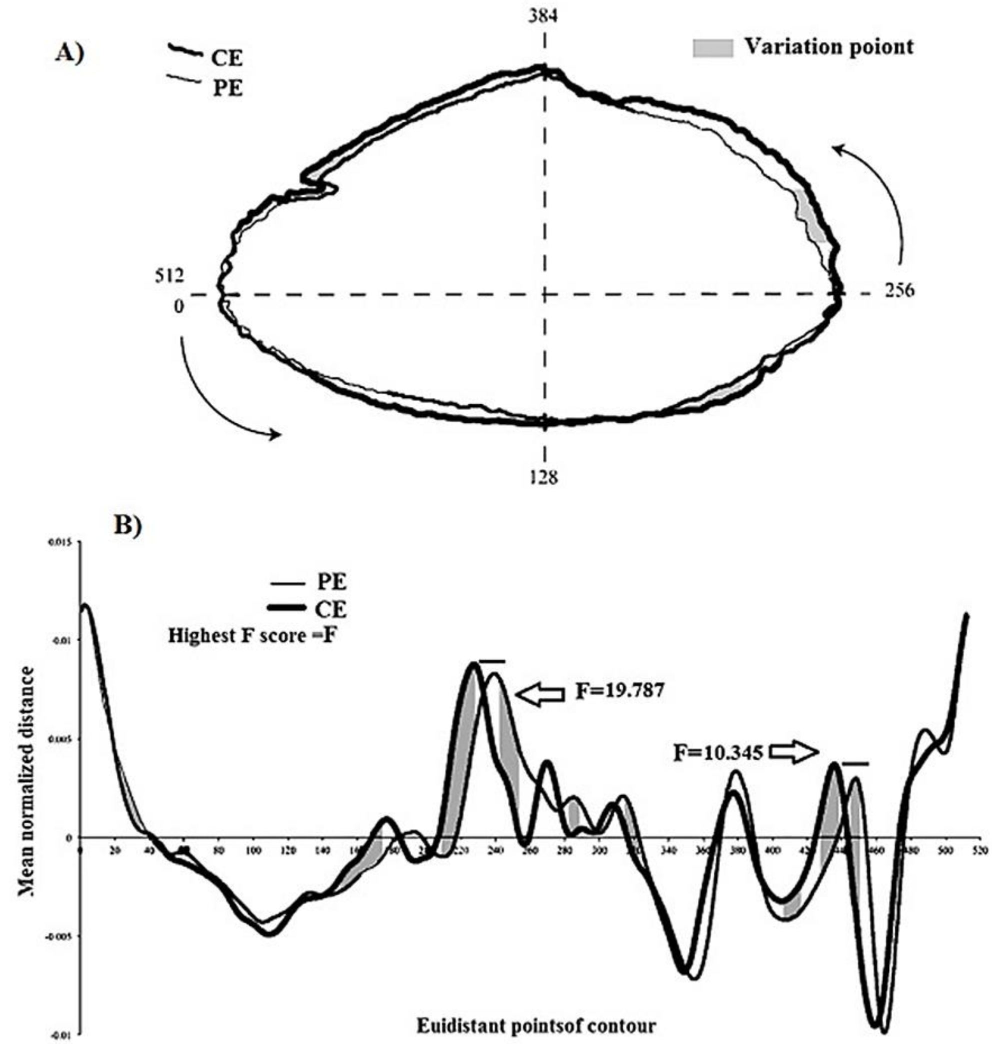

Figure 6. (A) The contour means information from Wavelet transform (WLT- 5) and (B) The highest F score points by frequency waves of *Ocyurus chrysurus* otoliths from Ceará in comparison to Pernambuco.

coefficient was 0.94 at an excellent level demonstrating that the data were appropriate for factor analysis. The PC1 and PC2 accounted for 53.50 and 33.287% of the total variance, respectively. LDA analysis was performed for wavelets transform (WT) and EFD countors through the Jackknife that showed a correct classification of 43.5 and 42.3%, respectively (Fig. 7A, B). Also, the Wilks' λ test scoured 0.601 and 0.688, confirming that the linear discriminatory power of the model is low. The LD1 (density) recorded a high overlap degree for CE (82.3%) and PE (83.5) between regions. Although different point degrees were obtained by wavelet analysis and Fourier descriptors countors, these differences were not strong enough to mention separation (Fig. 7).

Landmark points: The first two axes of PCA explained 73.2% of the total variation of otolith sulcus landmark data, with PC1 (44.4%) related to the rostrum and anti-rostrum (landmark points 1 and 15), and PC2 (28.8%) associated with the back of the sulcus (landmark points 7 and 8) that showed changes in the size of the sulcus between CE and PE (Fig. 8A). A high overlap degree was recorded for the CE and PE groups. As the factor showed that the KMO coefficient was 0.81 at a very good level, it was decided that the data were suitable for factor analysis. Linear Discriminant Analysis for 15 landmarks was performed and showed 33.1% correct classification through the Jackknife (Fig. 8B). Although a variation between four landmark points (1, 7, 8, and 15) was

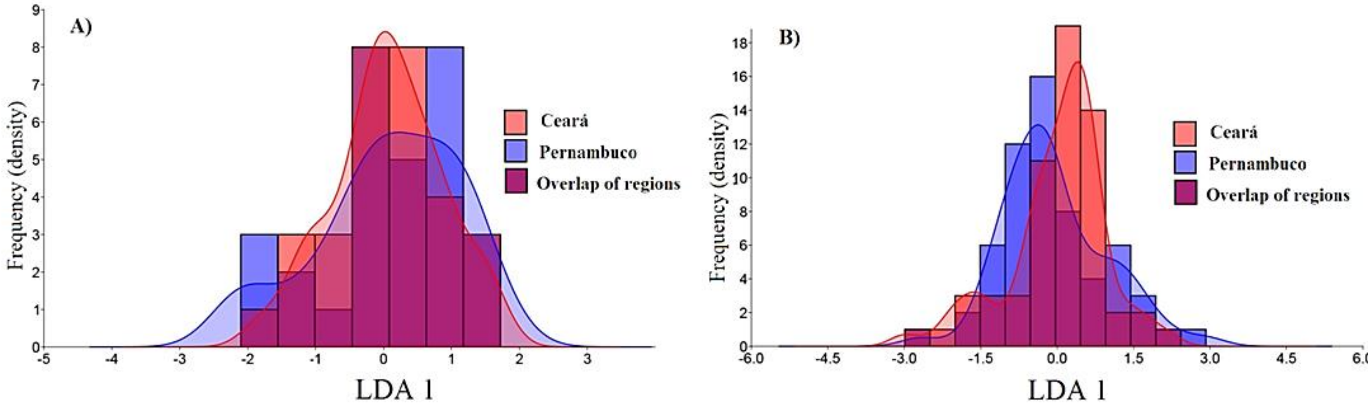

Figure 7. Linear Discriminant Analysis of otolith by (A) elliptical Fourier descriptors and (B) Wavelet Transforms (WLTs) countors for *Ocyurus chrysurus* between Ceará and Pernambuco.

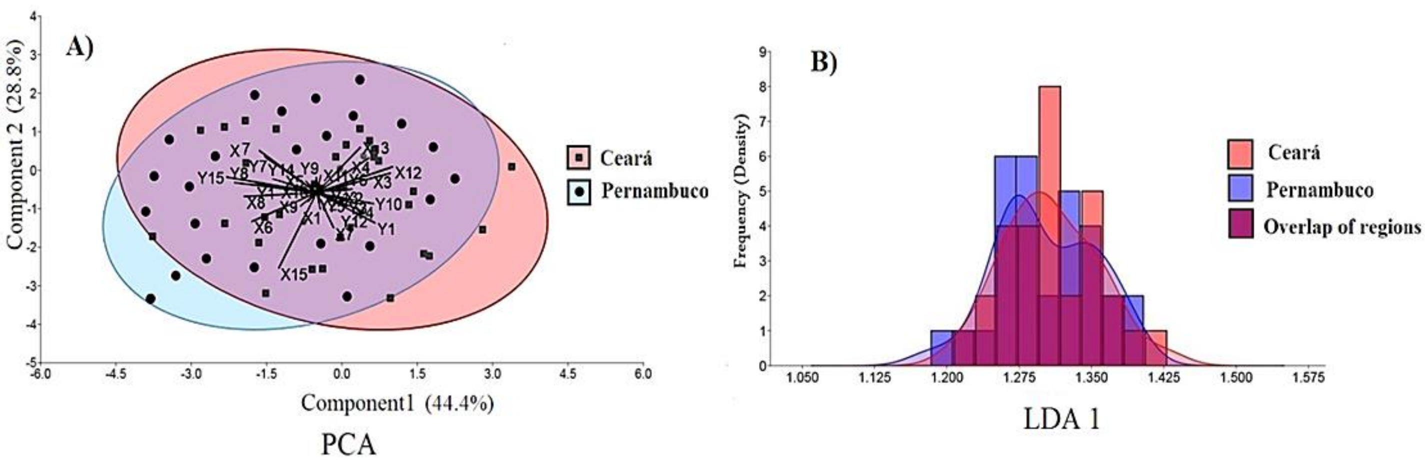

Figure 8. (A) Principal Component Analysis (PCA) plot of otolith sulcus according to PC1 and PC2. (B) Linear Discriminant Analysis of otolith sulcus landmark for *Ocyurus chrysurus* between Ceará and Pernambuco.

observed, the Wilks' λ test scoured 0.808, confirming that the linear discriminatory power of the model is too low and these differences could not support separation between Ceará and Pernambuco (high overlapping (LDA1=density) for landmark method = 92.7%).

Discussions

Otoliths have hard structures that grow along the life cycle of fish, and the characteristics of these ingredients are different in fish stocks. Therefore, otolith morphology plays an important role in stock discrimination (Tuset et al., 2012; Avigliano et al., 2014). Since the use of each method has specific challenges and characteristics such as discriminatory power, spatiotemporal variations, ecological interpretation, and related expenses, choosing the best method for stock discrimination is significant (Tanner et al., 2016). This study is the first research to compare real data with mathematical techniques related to four different methods (morphometric, Elliptical Fourier Descriptors, wavelet transform, and landmark) and several different analyses (LDA cross-validation, Wilks' λ test, PCA, and KMO) that were applied to investigate the separation between samples of CE and PE in coastal waters of Brazil. The results of the morphological study showed that otoliths of yellowtail snappers from the CE and PE are very similar in morphological parameters (otolith contour, type of sulcus acusticus, ostium, and caudal), but there was variation in the rostrum and post- rostrum rims of the otolith. According to the otolith data set, that has been provided by different methods, the KMO coefficient test for the morphometric method showed a good level (0.66) and for the landmark method with a very good level (0.81). These two methods showed less efficiency in comparison to the Elliptical Fourier Descriptors (EFD) and wavelets transform (WT) with an excellent level (0.88 and 0.94 respectively). These levels generally indicated which method is more useful for factor analysis (Morawicki et al., 2022).

LDA for morphometric parameters and shape indices also showed a correct classification through jack-knifed of 25%, and the landmark method's correct classification was 33.1%. These two methods had a lower correct classification in comparison to the otolith countering methods (EFD = 42.3% and WT = 43.5%). Wilks' λ test was used to evaluate the discrimination power of LDA analysis in different methods. When Wilks' λ approaches near 0 the groups are well separated and if there is no discrimination it comes to 1, although in our study there was no significant discrimination observed. The morphometric and landmark methods had lower power discrimination (λ = 0.904 and λ = 0.808, respectively), in comparison with the two countering methods (EFD λ = 0.688 and WT λ = 0.601). These results indicate that the best method could be EFD and WT for observation of small variations in *O. chrysurus* otoliths according to classification and Wilks' λ test. The analysis of the otolith contour has revealed a low variation in the samples. Similar results about discriminating by using countering methods among yellow croaker along the Chinese coast were reported recently by Song et al. (2018) which indicated a higher classification success rate than using the morphometric method. Sadighzadeh et al. (2012) in comparison of different otolith shape descriptors and morphometrics for the identification of *Lutjanus* spp. from the Persian Gulf also observed that contour analysis (EFD and WT) can indicate the variation between groups and stocks according to the correct classification. On the contrary, Forsberg and Neal (1993) and Tuset et al. (2006) proposed that otolith weight is a more powerful discriminator than Fourier descriptors and can show a better stock structure. Zischke et al. (2016) reported an overall classification success of 96% in their application of otolith morphology to differentiate four *Scomberomorus* species (Scombridae) from northern Australia.

EFD only gives a general explanation of the otolith counter and does not pay attention to details (Reig-Bolaño et al., 2010). On the other hand, EFD does not require equal intervals along the otolith contour and can draw more complex forms of the Fourier functions, which better describe the contours (Tracey et al., 2006; Stransky et al., 2008). The advantage of using multiscale analysis (WT) is that it enables us to identify specific morphological points along the contour (x-axis) where the rostrum is the origin of the contour (Parisi-Baradad et al., 2005; Lombarte et al., 2006). The form of otolith is influenced by environmental, ontogenetic, ecological, and genetic factors (Cadrin and Friedland, 2005).

Ocyurus chrysurus is found in tropical and subtropical coastal waters, which have sandy bottoms and coral reef structures (Da Silva et al., 2015). Coral reefs may function as ecological corridors to allow gene migration in this species (Feitoza et al., 2005; Ortiz-Lozano et al., 2013) and *O. chrysurus* may use the coastal patch reefs along the CE and PE to move between these regions. This may be the reason for the high overlapobservation in this study for all methods. Also, it has to be mentioned that Fourier + wavelet methods in our study have demonstrated primary variation in otolith contours between the two regions. We know that this variation was not strong enough to support the discrimination (LDA analysis) between the two regions. However, this primary variation in otoliths may indicate primary changes in the ecosystem. Some studies in the northeast of Brazil reported on the destruction of the marine ecosystem and corals, which are the main habitats of this species. Feitosa et al. (2003) asserted that reefs located near the population of coastal waters in northeastern Brazil are showing different levels of stress. Overfishing of reef fish populations generally decreased. Also, fishermen, by catching small fish with small mesh-sized traps, kill almost all fish and damage corals in the surrounding area. Barradas et al.(2012) proposed that increasing human activity, especially during the tourism seasons causes changes in the ecosystem and consequently increases stress and destroys corals. According to this study, we suggest that more studies in phenotypic (morphology and geometric morphometric) have to be performed in the coastal waters of Brazil to observe the probability variations in this species.

As a result, the outcome of our study indicates that there is a single stock of *O. chrysurus* between Ceará and Pernambuco, and according to our study most suitable methods for identification of stocks of these fishes could be Elliptical Fourier Descriptors (EFD) and wavelets transform (WT). In this way, it can be concerned that achieving the best results for more accurate stock identification based on otolith shape, requires the use of different methods on each species.

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