

Discrimination of red porgy *Pagrus pagrus* (Sparidae) potential stocks in the south-western Atlantic by otolith shape analysis

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Funding information

E.K. received a CAPES scholarship (88882.347007/2019-01). M.H. (303561/2015-7) is a research fellow from the Brazilian National Scientific and Technological Research Council. Otoliths from Rio de Janeiro were collected during Project Multipesca, supported by Brazilian Fund for Biodiversity (FUNBIO) marine research grant 104/2016.

Abstract

Otolith shape analysis is a powerful method for fish stock identification. We compared the otolith shape of *Pagrus pagrus* (Linnaeus 1758) along with its distribution in four south-western Atlantic regions where it is commercially fished: Rio de Janeiro, Rio Grande do Sul in southern Brazil, the Argentine-Uruguayan Common Fishing Zone (UA) and the Argentinian Exclusive Fishing Zone (AR). Otolith shapes were compared by Elliptical Fourier and Wavelet coefficients among specimens in a size range with similar otoliths, morphometric parameters and ages. Four potential stocks were identified: one in the AR, a second along the UA which included specimens from southern Brazil with well-marked opaque bands in its otoliths (MRS), the third in southern Brazil with faint or absent opaque bands in its otoliths (FRS) and the fourth along Rio de Janeiro. The difference in the otolith shape among regions followed differences reported using other stock identification techniques. The similarity between otoliths from UA and MRS (ANOVA-like, $P > 0.01$) can be explained by seasonal short-range migrations. Otoliths shape differences between MRS and FRS (ANOVA-like, $P < 0.01$) suggest that *P. pagrus* does not form a homogeneous group in southern Brazil.

KEYWORDS

Fourier, marine fish, otolith morphology, *ShapeR*, stock identification, Wavelet

1 | INTRODUCTION

The red porgy *Pagrus pagrus* (Linnaeus, 1758) is a protogynous hermaphrodite coastal sparid fish (Aleksseev, 1983) commonly found on consolidated and sandy shelf bottoms in temperate and subtropical coastal regions of the Atlantic Ocean and the Mediterranean Sea. The red porgy is targeted by both commercial and recreational fisheries throughout its distribution (FAO, 2018). According to the FAO report, the south-western Atlantic contributed the highest percentage of global landings of the red porgy, comprising 58% of global production

reported between 1950–2011, 84% by Argentina and 16% by Brazil (Russell *et al.*, 2014).

There are four main fishing areas for the red porgy in the south-western Atlantic, from Espírito Santo state in Brazil to Buenos Aires province in Argentina (Cousseau & Perrotta, 2013). The two northernmost fishing areas are managed under Brazilian jurisdiction: from Espírito Santo to the north of Rio de Janeiro (RJ) in south-eastern Brazil, and south of Rio Grande do Sul (RS) in southern Brazil (Costa *et al.*, 1997; Haimovici, 1998). The Argentine-Uruguayan Common Fishing Zone (UA) is managed by both countries, and the

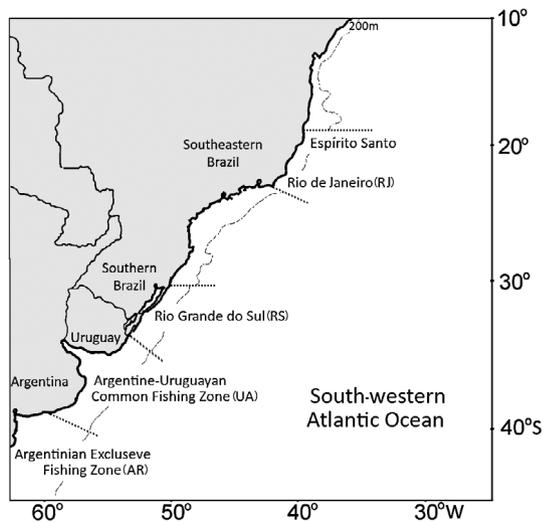


FIGURE 1 *Pagrus pagrus* main fishing areas in the south-western Atlantic Ocean. The dotted lines correspond to the different catch areas

southernmost fishing area (AR) is managed exclusively by Argentina (García & Molinari, 2015) (Figure 1).

The failure to recognize the population structure of exploited species can lead to dramatic changes in biological attributes, productivity rates and genetic diversity, as well as overfishing and depletion of the less productive population units (stocks *sensu*, Begg *et al.*, 1999; Cadrin *et al.*, 2014). Regarding red porgy populations, Ball *et al.* (2006) compared microsatellites and mitochondrial DNA markers among several stocks of the north-eastern, north-western and south-western Atlantic and observed differences in two loci across the three regions. Within the south-western Atlantic, several stocks were suggested based on phenotypical methods. Differences in red porgy growth parameters (García & Déspos, 2015) and body shape characterization (Porrini *et al.*, 2015) were observed between the Argentine-Uruguayan Common Fishing Zone and the exclusive fishing zone of Argentina. When analysing long-lived larval parasite assemblages, Soares *et al.* (2018) identified three distinct stocks: south-eastern Brazil, southern Brazil and Argentina. On the scales of red porgies fished in the Rio Grande do Sul, two *annuli* patterns were observed: well-marked and faint or absent. Although specimens with both patterns occur year-round, the proportion of specimens with faint or absent *annuli* is lower in the winter months (Haimovici, 1997; Haimovici *et al.*, 2020).

Otoliths are acellular concretions of calcium carbonate and other inorganic salts, generally considered immune from modification once formed (Green *et al.*, 2009). Some studies have demonstrated that otoliths may exhibit species-, population- or stock-specific differences in their shapes (Campana & Casselman, 1993; Cardinale *et al.*, 2004; Libungan *et al.*, 2015). Currently, it is recognized that otolith shape is environmentally and genetically regulated (Vignon & Morat, 2010), but the variability in morphology is also related to environmental factors (Campana, 1999), differences in general lifestyle (Tuset *et al.*, 2016), growth rate variations (Campana & Casselman, 1993), food quality (Gagliano & McCormick, 2004) and

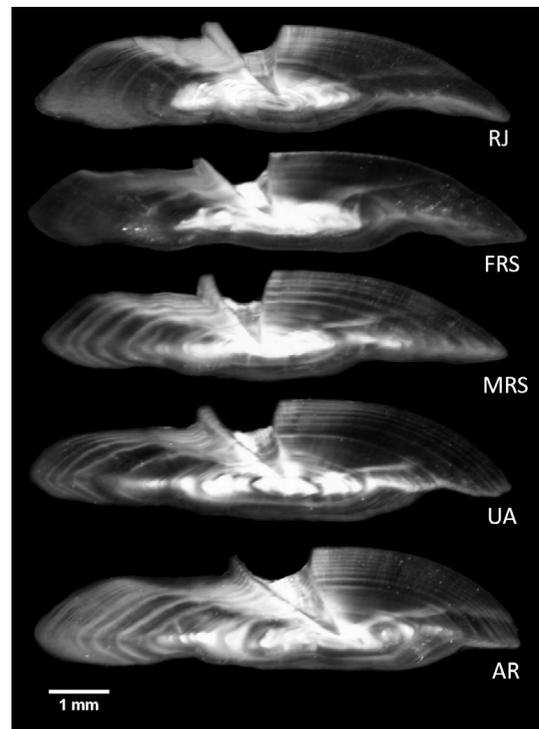


FIGURE 2 *Pagrus pagrus* sagittae otolith sections of 370 mm fish size from Rio de Janeiro (RJ), Rio Grande do Sul faint growth opaque bands (FRS) and well-marked growth opaque bands (MRS), the Argentine-Uruguayan Common Fishing Zone (UA), and the Argentinean Exclusive fishing Zone (AR)

stressful conditions (Vignon, Vignon, 2018). In this way, otolith morphology has been used to identify stocks of fish that are likely to have spent a significant part of their lives in different environments and therefore may provide an indirect basis for potential stock separation (Begg *et al.*, 1999).

The differences in otolith morphology among stocks can be assessed by different approaches, including statistical analysis of general shape parameters, landmark analysis (Cadrin *et al.*, 2014) and otolith shape analysis through Elliptical Fourier transforms (Campana & Casselman, 1993) and Wavelets (Libungan & Pálsson, 2015). Otolith shape analysis is a popular and well-established method for discrimination of fishes with distinct spatial distributions over part or all of their lifetime (Campana & Casselman, 1993; Libungan *et al.*, 2015). This method requires otolith images from which outlines can be extracted and quantified, so the shape variation patterns within and among groups can be evaluated based on a large number of independent variables (Libungan & Pálsson, 2015). The Wavelet method is useful for detecting shape differences at specific regions, which could be located at a given angle on the otolith outline; in contrast, the Fourier method only provides information about overall differences in otolith shape, but not in specific locations (Libungan & Pálsson, 2015). Environmental influences might induce an overall change in the otolith shape, while genetics might induce localized changes (Vignon & Morat, 2010). Therefore, in some cases, Wavelet might provide a better explanation of shape

differences, while for others, the Fourier method might be more powerful in distinguishing populations (Libungan & Pálsson, 2015).

This study aimed to evaluate the otolith shape as a stock marker for the red porgy in areas with potential mixing of neighbouring stocks in the south-western Atlantic Ocean. We used both Elliptical Fourier and Wavelet coefficients to compare differences among otolith shapes. Four distinct otolith shape patterns of *P. pagrus* were identified in which the discrimination among three groups reflected a geographical latitudinal gradient. In southern Brazil, two groups with differences in their life history evidenced by differences in the contrast of the opaque and translucent bands in their otoliths also had distinct otolith shape patterns.

2 | MATERIALS AND METHODS

Sagittae otoliths of 833 red porgies were collected between 2015 and 2019 in four regions, covering all the distribution range of the species in the subtropical and warm temperate south-western Atlantic Ocean: along Rio de Janeiro (22°S–23°S) and Rio Grande do Sul (32°S–34°S) in Brazil, in the Argentine-Uruguayan Common Fishing Zone (34°S–39°S), and in the exclusive fishing zone of Argentina (39°S–42°S) (Figure 1).

Cross-sections of 0.20 mm passing through the otolith core were obtained with a low-speed precision saw to determinate the age of the red porgy sampled in all regions and classify the Rio Grande

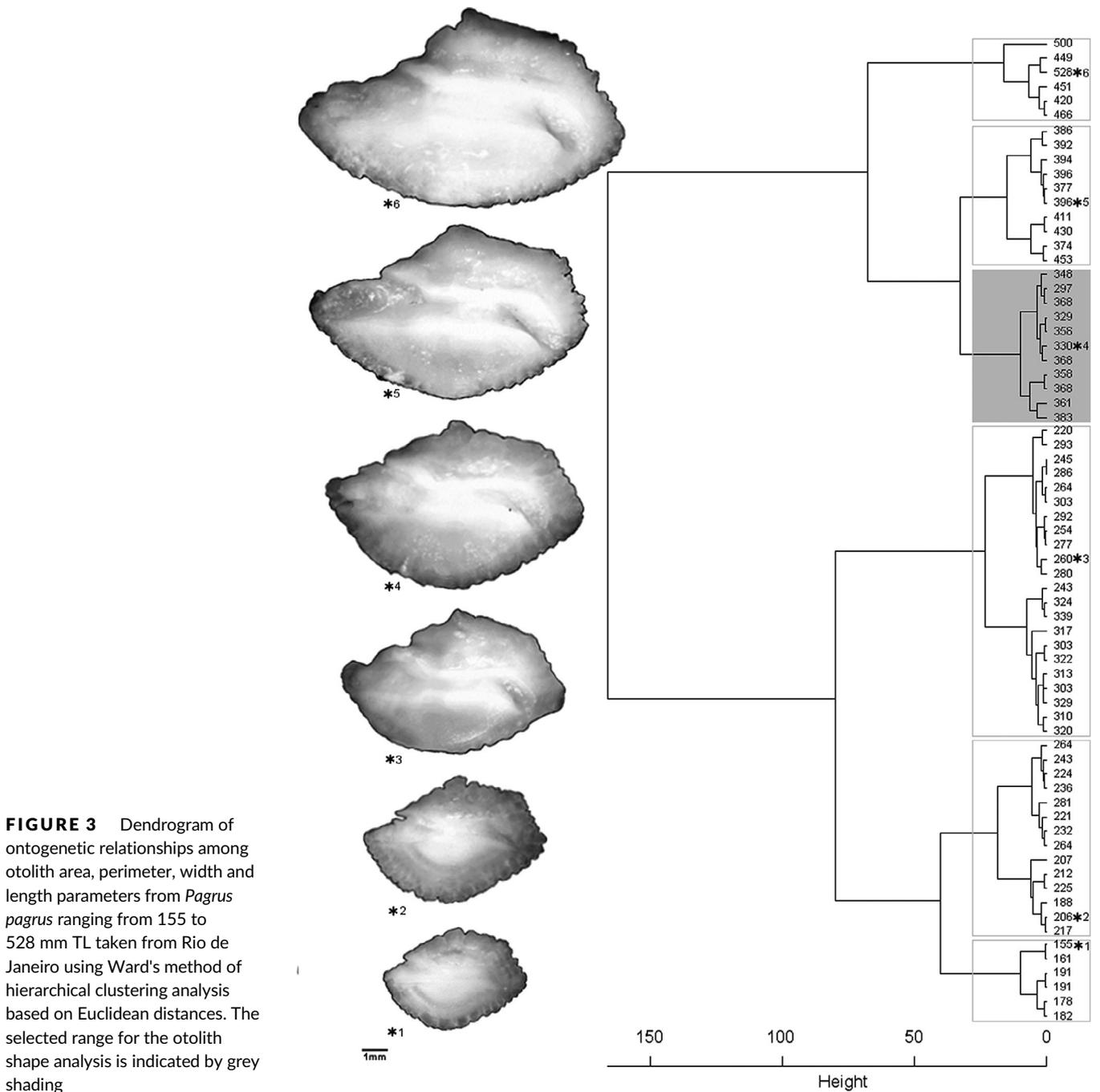


FIGURE 3 Dendrogram of ontogenetic relationships among otolith area, perimeter, width and length parameters from *Pagrus pagrus* ranging from 155 to 528 mm TL taken from Rio de Janeiro using Ward's method of hierarchical clustering analysis based on Euclidean distances. The selected range for the otolith shape analysis is indicated by grey shading

TABLE 1 Latitude range, year of data collection, total length (TL) ranges, number of samples, mean and standard deviation (s.d.) for age and otolith morphological parameters (in mm) of the sampled groups of *Pagrus pagrus*

| Region | Latitude range | Sampling year | Number of total samples (TL range = 455–528 mm) | OSA samples selected (TL range = 330–370 mm) | | | | |
|--|----------------|----------------------|---|--|--------------------------------|----------------------------------|---------------------------------|-------------------------------------|
| | | | | Age (mean ± s.d.) | Otolith area (mean ± s.d., mm) | Otolith length (mean ± s.d., mm) | Otolith width (mean ± s.d., mm) | Otolith perimeter (mean ± s.d., mm) |
| RJ (Rio de Janeiro) | 22° S–23° S | 2017 | 174 | 5.2 ± 0.9 | 58.3 ± 4.1 | 11.7 ± 0.5 | 7.3 ± 0.3 | 35.3 ± 2.1 |
| FRS (Rio Grande faint growth bands) | 32° S–34° S | 2018 | 160 | 4.5 ± 0.8 | 59.6 ± 4.2 | 11.6 ± 0.4 | 7.7 ± 0.4 | 37.6 ± 2.8 |
| MRS (Rio Grande well marked growth bands) | 32° S–34° S | 2016 2018 2019 | 16 196 37 | 5.8 ± 1.3 | 58.7 ± 4.2 | 11.6 ± 0.5 | 7.4 ± 0.3 | 35.6 ± 2.0 |
| UA (Argentine-Uruguayan Common Fishing Zone) | 34° S–39° S | 2018 2015 | 79 50 | 4.9 ± 1.3 | 59.2 ± 5.7 | 11.4 ± 0.7 | 7.5 ± 0.4 | 34.9 ± 2.4 |
| AR (Argentinian Exclusive Fishing Zone) | 39° S–42° S | 2018 | 121 | 5.2 ± 0.9 | 57.8 ± 5.1 | 11.7 ± 0.5 | 7.4 ± 0.4 | 34.9 ± 2.4 |

Note. OSA, otolith shape analysis.

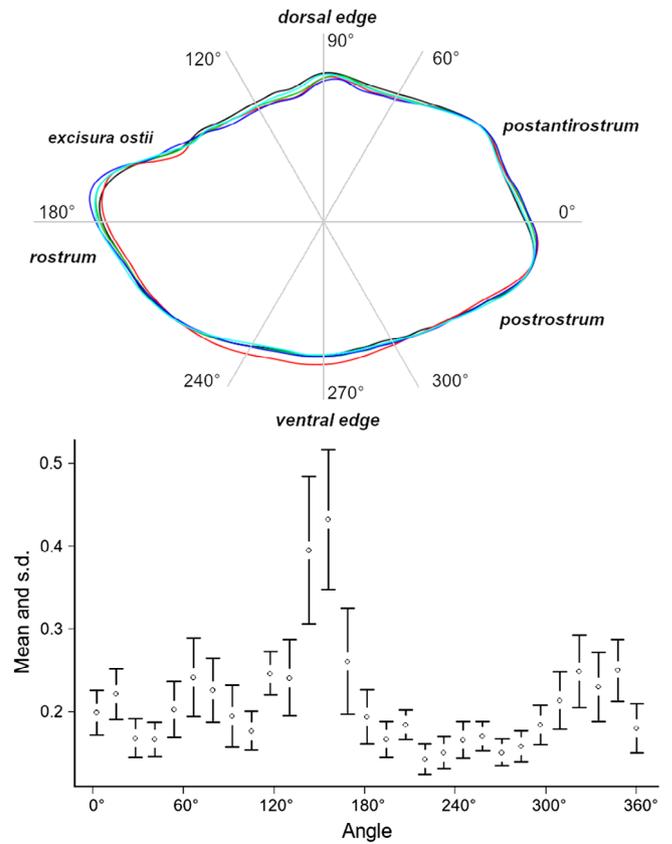


FIGURE 4 Mean otolith shape based on the Wavelet reconstruction for each group (above) and mean and standard deviation (s.d.) of the Wavelet coefficients among all groups (below) of *Pagrus pagrus*. Specimens were sampled from RJ (Rio de Janeiro), FRS (the Rio Grande do Sul faint) and MRS (well-marked otolith growth bands), UA (Argentine-Uruguayan Common Fishing Zone) and AR (Argentinian Exclusive fishing Zone). Degrees (°) represent angles on polar coordinates: (—) RJ, (—) FRS, (—) MRS, (—) UA, (—) AR

samples in two groups: those with well-marked growth bands and those with faint or absent growth bands (Figure 2). The age estimations based on sliced otoliths of red porgies were validated by García and Déspos (2015) and Haimovici *et al.* (2020).

For each specimen, the total length (TL, mm) was measured and one otolith had its inner surface recorded with a digital image captured with an AmScope MD500 camera magnified 10× times under a Nikon SMZ800 stereomicroscope.

Numerous studies have documented that otolith shape varies throughout the fish life cycle (e.g., Campana & Casselman, 1993; Mapp *et al.*, 2017; Simoneau *et al.*, 2000). However, early disruptive events can affect the otolith ontogenetic trajectory in the absence of any growth-related differences (Vignon, 2018). To reduce the effect of otolith ontogenetic changes, Ward's hierarchical clustering analysis was used to identify a size range of fishes in which the otoliths had similar morphometric characteristics. The area, perimeter width, and length of 70 otoliths of randomly selected fishes were used, considering the minimum and maximum total length observed (155–528 mm TL).

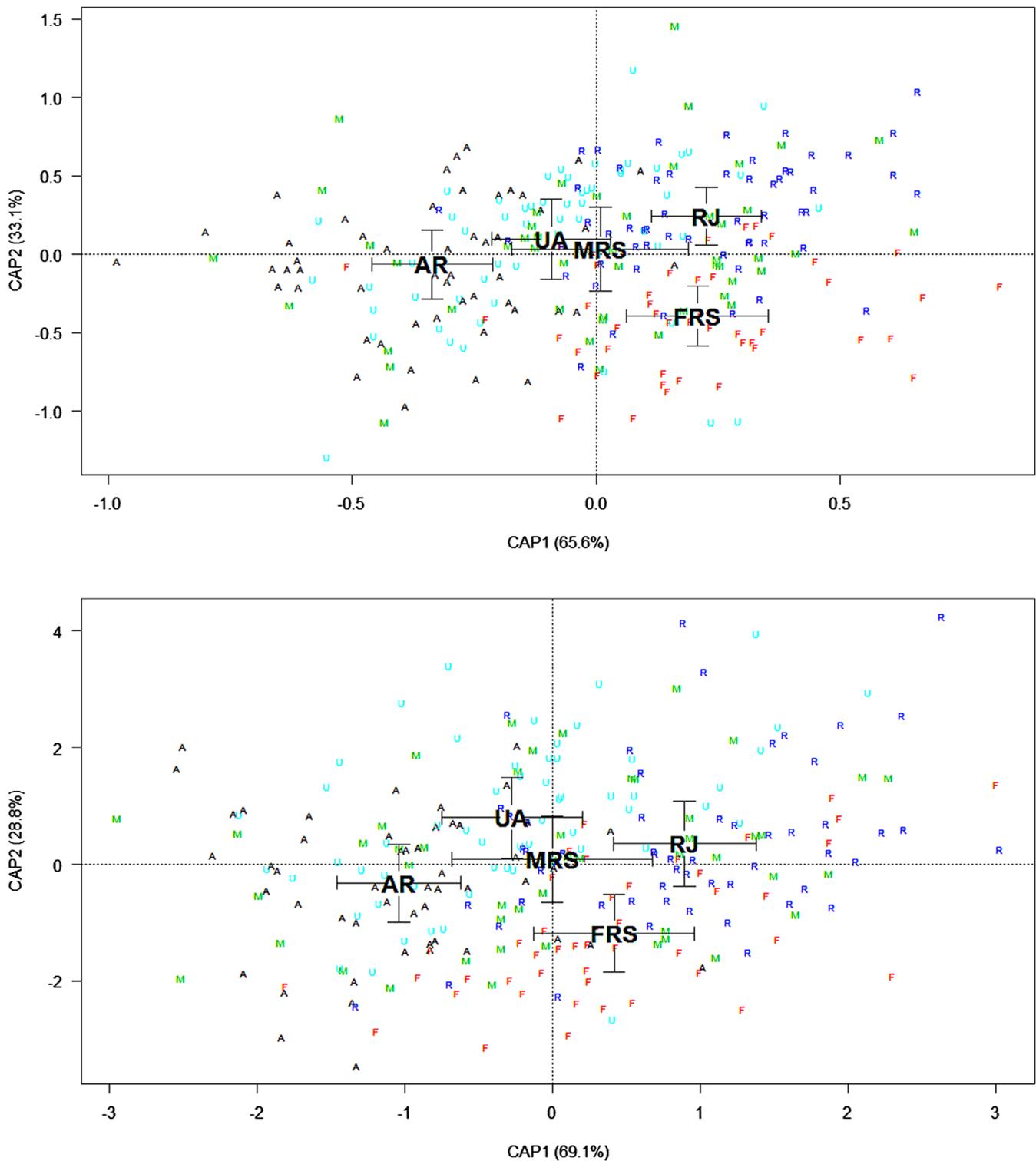


FIGURE 5 Otolith shape of *Pagrus pagrus* from the five sampled groups in the south-western Atlantic using canonical analysis for Fourier (above) and Wavelet (below) coefficients. Black letters represent the mean canonical coordinates surrounding by standard error for each group: RJ (Rio de Janeiro), FRS (the Rio Grande do Sul faint otolith bands), MRS (well-marked otolith bands), UA (Argentine-Uruguayan Common Fishing Zone) and AR (Argentinian Exclusive Fishing Zone)

The *ShapeR* package (Libungan & Pálsson, 2015) was used to analyse the images of each otolith and record the outlines as a matrix of x and y coordinates. The shape patterns of each of the five sample

groups were expressed as the Wavelet/Fourier coefficient: Rio de Janeiro (RJ), Rio Grande do Sul faint otolith growth bands (FRS) and well-marked otolith growth bands (MRS), Argentine-Uruguayan

Common Fishing Zone (UA) and Argentinian Exclusive Fishing Zone (AR). The Wavelet coefficients were obtained with the *wavethresh* package (Nason, 2012) and the normalized Elliptic Fourier technique was performed using the *iefourier* and *efourier* functions (Claude, 2008). The deviation of the reconstructed otoliths outline from the original outline was evaluated with the *ShapeR* package (Libungan & Pálsson, 2015) to determine the number of Wavelet and Fourier coefficients needed for the analysis. By using 64 Wavelet and 45 Fourier coefficients, an accuracy rate of 98.5% was obtained.

The mean otoliths reconstructed by normalized Wavelet coefficients for each group of samples were plotted and the differences among the groups evaluated visually. To estimate which part of the otolith outline contributed most to the difference between the potential stocks, mean shape coefficients and their standard deviation (s.d.) were plotted against the angle of the outline from where the coefficients were extracted, using the function *plotCI* from the *gplots* package (Warnes et al., 2014).

The normality and homogeneity of variances for each Wavelet/Fourier coefficient were examined using Shapiro–Wilk's and Levene's tests, respectively. A correlation matrix were used to verify the pairwise correlation between coefficients to look at possible multicollinearity. The number of coefficients was so high that those which did not meet the assumptions of normality and homoscedasticity, and showed multicollinearity ($r > 0.7$) were eliminated. In addition, the interaction between the total length of the red porgies and the remaining Wavelet/Fourier coefficients was investigated by analysis of covariance (ANCOVA). When there was a significant interaction ($P < 0.05$), those coefficients were excluded from the analysis to reduce the allometric relationship (Libungan & Pálsson, 2015). Among the 64 Wavelet and 45 Fourier coefficients integrated within the *ShapeR* package, only 27 Wavelet and 17 Fourier coefficients met these assumptions and therefore were used in the following statistical analysis.

The shape variation among the groups was compared by a canonical analysis of principal coordinate (CAP) applied on the Wavelet/Fourier coefficient using the *capscale* function in the *vegan* package (Oksanen et al., 2013). Ordination of the stock averages along the first two canonical axes was evaluated graphically with shape descriptors. An ANOVA-like permutation test for CAP was used to assess the significance of constraints using 1000 permutations. A minimum of 1% was adopted as a level of significance.

Linear discriminant analysis (LDA) was applied to the Wavelet/Fourier coefficients to analyse the accuracy of the otolith shape classification in their sampling origin (Libungan & Pálsson, 2015). The LDA classification method discriminates predefined groups based on sample observations from each group. It was calculated here with leave-one-out cross-validation using the *errorest* function in the *ipred* package (Peters & Hothorn, 2013), which returns the posterior probabilities for the groups. In each pairwise comparison, a Box's *m* test was applied to verify the equality of covariance matrices ($P > 0.01$). In all comparisons, this assumption was confirmed.

All statistical analysis was performed in the software R (R Core Team, 2019), version 3.6.0.

2.1 | Ethical statement

The research did not involve animal experimentation or harm, and required no permits under Brazil animal welfare laws.

3 | RESULTS

The choice of the dataset for the comparisons took into account the potential shape changes along the total fish length gradient. Six total

TABLE 2 Analysis of variance (ANOVA-like) permutation test of otolith shape among *Pagrus pagrus* sample groups in the *a priori* comparisons, and the percentage of classification success (C.s.) of linear discriminant analysis of Rio de Janeiro (RJ), Rio Grande faint otolith bands (FRS), Rio Grande well marked otolith bands (MRS), Argentine-Uruguayan Common Fishing Zone (UA) and Argentinian Exclusive Fishing Zone (AR)

| Comparisons | d.f. | Fourier | | | | Wavelet | | | |
|-------------|------|---------|--------|------------------|------|---------|--------|------------------|------|
| | | SS | F | P | C.s. | MS | F | P | C.s. |
| All groups | 4 | 0.112 | 19.375 | <0.001 | | 8.653 | 13.552 | <0.001 | |
| MRS vs. UA | 1 | 0.003 | 1.804 | 0.138 | 54% | 0.489 | 2.798 | 0.04 | 65% |
| MRS vs. RJ | 1 | 0.012 | 8.201 | <0.001 | 70% | 1.196 | 7.055 | <0.001 | 71% |
| MRS vs. FRS | 1 | 0.020 | 13.247 | <0.001 | 79% | 1.115 | 6.684 | <0.001 | 74% |
| MRS vs. AR | 1 | 0.020 | 12.948 | <0.001 | 79% | 1.557 | 9.440 | <0.001 | 73% |
| UA vs. AR | 1 | 0.013 | 9.116 | <0.001 | 71% | 1.649 | 10.649 | <0.001 | 70% |
| UA vs. RJ | 1 | 0.021 | 15.230 | <0.001 | 78% | 2.253 | 13.993 | <0.001 | 84% |
| UA vs. FRS | 1 | 0.035 | 24.567 | <0.001 | 88% | 2.837 | 18.099 | <0.001 | 87% |
| AR vs. FRS | 1 | 0.056 | 44.347 | <0.001 | 91% | 3.150 | 21.640 | <0.001 | 88% |
| AR vs. RJ | 1 | 0.064 | 52.161 | <0.001 | 91% | 5.975 | 37.193 | <0.001 | 90% |
| RJ vs. FRS | 1 | 0.034 | 28.722 | <0.001 | 83% | 1.661 | 10.980 | <0.001 | 73% |
| Residual | 252 | 0.36591 | | | | 40.228 | | | |

Note: Statistically significant *P* values are in bold.

length intervals were clustered (Figure 3). The 330–370 mm total length interval, which included 257 individuals, was chosen for the shape analysis because it had the highest number of sampled specimens among all sampled groups. Additionally, the age ranges of the samples selected were similar among regions (Table 1). Furthermore, eight Wavelet and two Fourier coefficients showed an interaction with fish total length (ANCOVA, $P < 0.05$) and were thus omitted from the analysis to reduce the allometric effects.

The Wavelet reconstruction showed differences among the mean otolith outlines for all sampled groups, except between MRS and UA, which showed a similar otolith shape (Figure 4). The highest variation was observed for the *excisura ostii* (angle 140–150°) followed by the *dorsal edge* (angle 90–135°). In the *ventral edge* (angle 225–290°) the distance from the otolith centroid was higher in the FRS when compared to all the other sampled groups. There was high overlap in the *postantirostrum* (angle 40–70°), the *rostrum* (angle 200–225°) and the *postrostrum* (angle 300–320°) of all the sampled groups (Figure 4).

The canonical analysis of the principal coordinates showed that otolith shape was not homogeneous among the five sampled groups for both coefficients (ANOVA-like, $P < 0.01$), where 98.7% of the variation was explained by the first two axes for the Fourier and 97.9% for the Wavelet (Figure 5). For both canonical analyses AR, RJ and FRS appeared in different panels in which there was no overlap of confidence intervals represented by standard errors of the group mean. The ANOVA-like permutation test for the Fourier and Wavelet coefficients showed significant differences among all paired sampled groups ($P < 0.01$) except between UA and MRS (Table 2).

Among the sampled groups that showed a significant difference in the ANOVA-like test, the LDA pairwise classification success was between 70% and 91% for the Fourier coefficient and between 71% and 90% for the Wavelet coefficient. Classification success rates between the UA and MRS, for which the ANOVA-like test was not significant, were only 54% and 65%, respectively.

4 | DISCUSSION

Otolith shape analysis was effective in discriminating both geographically and ecologically distinct stocks of the red porgy and, together with other tools, may contribute to support management. In the south-western Atlantic, otolith shape has been helpful for stock identification of coastal fishes, such as *Urophycis brasiliensis* (Biolé *et al.*, 2019) and *Chaetodipterus faber* (Soeth *et al.*, 2019). Currently, it is recognized that overall otolith shape is genetically regulated, but there is a strong variability related to environmental factors (Vignon & Morat, 2010). As with other phenotypic-based methods, otolith shape analysis is a more powerful method to study short-term environmentally induced differences between fish groups than presently used genetic markers (Begg *et al.*, 1999). The latter are more suitable for long-term changes of fully isolated groups, as in the case of red porgy populations among the north-eastern, north-western and south-western Atlantic and Mediterranean Sea (Ball *et al.*, 2006), than for

nearby stocks such as those of southern (AR) and northern (UA) Argentina, which could not be discriminated by microsatellite DNA markers (Porrini *et al.*, 2015).

Differences in otolith shape between AR and UA showed 70% classification success. Environmental characteristics define two different oceanographic regimes between these regions, with distinct water recirculation and multispecific nursery and feeding areas, favouring larval retention (Acha *et al.*, 2004). Porrini *et al.* (2015) also found phenotypic differences, such as body shape, between red porgies from AR and UA. These authors suggested the presence of two different spawning areas for the red porgy along the Argentinian coast, which could affect the early stages of development and be responsible for morphological differentiation in adult individuals. Likewise, spatial variations in spawning among fish stocks may contribute to variation in otolith shape (Libungan *et al.*, 2015). Information on the early growth stages for red porgy are not available, however. Differences in somatic growth were observed in later stages between the northern and southern groups in Argentina (Cotrina & Raimondo, 1997) and between AR and UA (García & Déspos, 2015). Several studies have demonstrated that fish growth influences the otolith structure and shape (*e.g.*, Mapp *et al.*, 2017; Simoneau *et al.*, 2000).

No difference in otolith shape was observed between red porgies from the UA and those with well-marked *annuli* from southern Brazil. Most of the red porgy catches in the late 1970s and early 1980s in southern Brazil were performed by bottom trawlers near the northern Uruguayan border. Catches were 10-fold higher during the winters in comparison with the summer catches (Barcellos *et al.*, 1991). Haimovici (1997) suggested that part of the winter catches was composed of migrants from UA waters with well-marked *annuli* on their scales. Long-distance migrations of red porgy are not referred to in the literature, but Afonso *et al.* (2009) showed that tagged specimens could move several tens of kilometres in short periods. Limited displacements were shown to occur by García and Molinari (2015), who reported a red porgy seasonal extension of the southward distribution limit from 41°S to >42°S in the warm season and associated it with species reproduction.

The northward winter displacement of the red porgy from Uruguay to southern Brazil suggested by Haimovici (1997) could be associated with northward running of cold shelf waters under the influence of the inner branch of the Malvinas current. This marine current reaches its maximum extension towards southern Brazil in the cold season, mixing the subtropical and sub-Antarctic waters that favour the concentration of nutrients (Ciotti *et al.*, 1995). Based on survey data from 1981 to 1987, Martins and Haimovici (2016) showed that 74% of the biomass of the red porgy on the continental shelf in southern Brazil belong to a “cold shelf” assemblage only present in the cold season associated with northward displacement of the western boundary of the subtropical convergence, and 26% to assemblages that were present year-round. Capitoli and Haimovici (1993) found differences in the feeding intensity and diet among the red porgy sampled in winter in southern Brazil near the Uruguayan border, which showed heavier stomach contents and more nutritious preys than those fished year-round in shallow waters and further north. The shifts of entire assemblages in southern Brazil

may occur as a consequence of feeding dispersions, as the optimal temperature conditions expand to a larger area (Martins & Haimovici, 2016). Thus, the similarity in otolith shape between UA and MRS supports the hypothesis of seasonal short-range migration associated with the cold season shift of more productive waters towards southern Brazil.

Otolith shape differences between FRS and MRS are more difficult to explain. Among the red porgies fished in the Rio Grande do Sul in the 1970–1980s, almost a quarter had faint or absent annuli in their scales, appearing more frequently in the warm season and in shallow waters (Haimovici, 1997). It could be hypothesised that the difference in otolith shape between FRS and MRS could be attributed to temperature and feeding conditions, which are recognized as important factors that regulate otolith growth and shape (Cardinale *et al.*, 2004). However, both FRS and MRS were observed among red porgies spawning in southern Brazil (Haimovici *et al.*, 2020). The degree of reproductive and geographic overlap between these two groups must be further studied. In addition, FRS was characterized by lower average age (Table 1), therefore observed differences between FRS and other groups may be caused by the age effect. The applied clustering method and selection of the fish from a similar ontogenetic group only partly prevent this problem.

To date, there have been no published genetic studies comparing red porgies from the fishing grounds in Rio de Janeiro with those from southern Brazil to Argentina; it is possible that the large distance between these regions results in genetic isolation, as the red porgy has limited migratory behaviour (Afonso *et al.*, 2009). Genetic isolation between south-eastern and southern Brazil has been observed for other coastal fishes, including *Macrodon atricauda* (Rodrigues *et al.*, 2013) and *Micropogonias furneri* (Vasconcellos *et al.*, 2015). However, some genetic flow through larval displacement associated with the southward running Brazil Current cannot be eliminated.

No differences in growth were observed between sexes in southern Brazil (Haimovici *et al.*, 2020) and difference were small in the other regions. On the other hand, as red porgies are hermaphrodites and we ignore the trajectory of the sex changes of each individual, it is difficult to associate them with otolith shape. Even if real differences should exist, they should increase variability and reduce the chance of distinguishing groups, but not question the robustness of the conclusions.

In conclusion, four potential stocks were identified by otolith shape analysis. The isolation of the Rio de Janeiro group can be attributed to its large geographic distance from the other groups. The difference in otolith shape between the Argentine-Uruguayan Common Fishing Zone and the Argentinian Exclusive Fishing Zone is in accordance with differences in growth and body shape of the red porgy between these regions. The similarity between the otolith shape of Argentine-Uruguayan Common Fishing Zone samples and those from southern Brazil with well-marked *annuli* from southern Brazil can be explained by the seasonal short-range migrations associated with the cold season shift of more productive waters towards southern Brazil. Differences between the otoliths with well-marked and faint or absent *annuli* from

southern Brazil suggest that red porgies in this region do not form a homogeneous group.

This study was possible through the collaboration of scientists of Brazil and Argentina. It provides information on the biological units that may be taken into account in the future if shared management is desired. International management of shared stocks is a complex issue that takes into account many political aspects that are beyond the scope of our study.

ACKNOWLEDGEMENTS

E.K. received a CAPES scholarship (88882.347007/2019-01). M.H. (303561/2015-7) is a research fellow from the Brazilian National Scientific and Technological Research Council. Otoliths from Rio de Janeiro were collected during Project Multipesca, supported by Brazilian Fund for Biodiversity (FUNBIO) marine research grant 104/2016.

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How to cite this article: Kikuchi E, García S, da Costa PAS, Cardoso LG, Haimovici M. Discrimination of red porgy *Pagrus pagrus* (Sparidae) potential stocks in the south-western Atlantic by otolith shape analysis. *J Fish Biol.* 2020;1–9. <https://doi.org/10.1111/jfb.14598>