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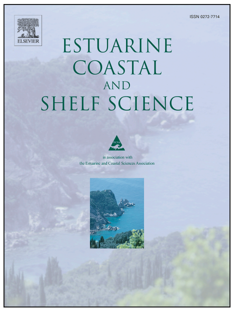

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Fish stocks of *Urophycis brasiliensis* revealed by otolith fingerprint and shape in the Southwestern Atlantic Ocean

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Abstract

Brazilian codling *Urophycis brasiliensis* is one of the main commercial coastal fish species from the Southwestern Atlantic Ocean. Regardless of its economic relevance, its stock structure remains largely unknown. In this study, we used the otolith shape and the core/outer edge multi-elemental fingerprints (Li:Ca, Mg:Ca, Mn:Ca, Fe:Ca, Zn:Ca, Rb:Ca, Sr:Ca, and Ba:Ca ratios) to evaluate the spatial segregation of young (nursery areas) and adult (stocks) stages of fish from the coast of northern Argentina, Uruguay, and southern Brazil. Otolith edge chemistry showed that several elemental ratios were significantly different between catching areas. Permutational multivariate analysis of variance (PERMANOVA) ($p<0.05$) and quadratic discriminant analysis (QDA), with jackknifed classification of 80.0% and 68.2% for otolith core and edge, respectively, were effective in discriminating between sampling sites considering young and adult life stages. PERMANOVA analysis of otolith shape revealed multivariate significant differences only between Argentina and Brazil ($p=0.0001$) individuals, whereas no differences were found between fish from Uruguay and Argentina ($p>0.05$). QDA classification rates were relatively low for Uruguay (48.0%) and values of 66.7 and 70.0% were determined for Brazil and Argentina, respectively. Our results not only show the presence of at least two fish stocks (Argentina and Brazil), with a third potential stock in Uruguay, but also suggest a strong spatial segregation during ontogeny.

Keywords: Brazilian codling; nursery; Southwestern Atlantic; population; *sagittae* otolith

1. Introduction

World fisheries have shown a consistent decline during the last three decades, where the biologically sustainable extraction of marine fisheries resources has been reduced from 90% in the 1970s to 70% in 2015 (FAO, 2018). Marine coasts have a high ecological relevance because they are suitable areas for spawning, feeding, and the development of numerous fish species (Blaber and Blaber, 1980; Shulman, 1985). On the other hand, coastal systems are vulnerable to environmental impacts and overexploitation due to increasing fishing pressure (Worm et al., 2006). This is especially critical in the coast of the Southwestern Atlantic Ocean (SAO), which are important commercial and artisanal fishery areas and where some relevant aspects for fisheries management such as spawning areas, presence of different stocks, connectivity, and stock structure are still unknown for several fish species. Brazilian codling *Urophycis brasiliensis* (Kaup, 1858) is one of the main commercial coastal species from SAO, registering industrial and artisanal landings that exceed 2,000 tons per year for Argentina, Uruguay, and Brazil (CEPERG, 2012; DINARA, 2016; MINAGRO, 2018; UNIVALI, 2014). This species inhabits relatively shallow waters from 23°S (Río de Janeiro State, Brazil) to 45°S (Patagonia, Argentina) (Bovcon et al., 2011; Goldstein, 1986) and can be considered as a cross-border resource. It follows then, that it is critical to determine the stock structure and distribution in order to develop comprehensive management plans (Cadrin et al., 2013; Ricker, 1981).

Several methods have been used to determine fish stocks, such as capture-recapture methods, population parameters (growth rate, reproductive characteristics), abundance and richness of parasites, genetics, otolith and scale shape analyses, fish morphometry, and microchemistry of otoliths and fin spines (Avigliano, et al., 2019; Cadrin et al., 2013; Niklitschek et al., 2010).

Otoliths are acellular and metabolically inert calcified structures present in the inner ear of teleostean fishes (Campana, 1999; Panfili et al., 2002). Trace elements dissolved in the water are incorporated and retained in the otolith structure as the fish grows (Avigliano et al., 2019; Campana, 1999; Kerr and Campana, 2014). Because the trace elements are acquired during ontogeny and are not resorbed after deposition (Campana, 1999; Elsdon et al., 2008) the chemistry of the otolith core reflects the environmental conditions during the early stage of life, whereas the outer edge represents the most recent period of life (fishing area). Different techniques have been used for measuring the concentration of trace elements in otolith zones, i.e., core and edge (Avigliano and Volpedo, 2016). Currently, Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) is considered the most powerful method because it provides a high precision, spatially resolved analysis of specific domains within the otolith, and which represent different ontogenetic stages. Hence, the chemistry of the core and outer edge are commonly used to discriminate nursery areas and fish stocks, respectively (Avigliano et al., 2017b; Avigliano et al., 2018b; Campana, 2014; Reis-Santos et al., 2015).

On the other hand, the otolith contour (shape), which is the result of both phenotypic and genetic factors, has also been used to delimit stocks (Reichenbacher and Reichard, 2014; Vignon and Morat, 2010). In recent years, the combination of chemistry and morphometry of otoliths has improved our understanding of stock structures for several fish groups from SAO (Avigliano et al., 2015, 2016, 2017; Avigliano et al., 2019; Soeth et al., 2019; Volpedo and Cirelli, 2006) and around the world (Ferguson et al., 2011; Soeth et al., 2019; Tanner et al., 2015).

The present study tests the hypothesis of the presence of different stocks and nursery areas of *Urophysis brasiliensis* in Southwestern Atlantic Ocean (Argentina, Uruguay and Brazil). In this regard, the stock structure of *U. brasiliensis* was studied by using multi-elemental fingerprints (Li:Ca, Mg:Ca, Mn:Ca, Fe:Ca, Zn:Ca, Rb:Ca, Sr:Ca, and Ba:Ca ratios) in otoliths (core and edge) from young and adult individuals. Moreover, Fourier elliptical analysis of otoliths was also used to assess the spatial segregation of adult stages.

2. Materials and Methods

2.1. Study area and sampling

The study area is located in the Southwestern Atlantic Ocean between 25°S and 38°S, covering both tropical and temperate regions. This coastal marine area presents a decreasing water temperature gradient from north (~18-30°C) to south (~7-24°C) (Avigliano et al., 2016; Guerrero et al., 2010; Lana et al., 2001).

A total of 175 fish were collected between July 2016 and July 2017 from catches by commercial trawlers operating at three specific locations: Villa Gesell (Argentina, AR), Piriápolis (Maldonado, Uruguay, UR) and (Itajaí, Paraná, Brazil, BR) (Figure 1). Fish were measured (total length = TL, cm), weighted (g) (Table 1), and dissected to extract both sagittal otoliths.

In order to evaluate fish stocks it is highly recommended to use individuals of similar age because both otolith chemistry and morphometry can change during ontogeny (Avigliano et al., 2017b; Kerr and Campana, 2014). Nonetheless, several authors have reported the presence of fake rings, not only in *U. brasilensis* (Acuña, 2000; Andrade et al., 2004; Cavole et al., 2018), but also in *U. tenuis* (Clay and Clay, 1991), *U. chuss* (Dery, 1988), and *U. cirrata* (Martins and Haimovici, 2000). Therefore, despite several attempts, a valid method to determine the age of *U. brasiliensis* (and of other species from the same genus) remains elusive (Cavole et al., 2018). Herein, only fish with a total length between 30 and 54 cm (TL, Table 1) were selected to reduce the potential effect of size/age on the studied variables (Avigliano et al., 2018b; Ferguson et al., 2011). This TL range is within the commercial size of *U. brasiliensis.*

2.2. Otolith chemistry

Eighty-five left sagittal otoliths were randomly sub-sampled, weighted, and cleaned by using 3% hydrogen peroxide and 2% HNO_3 (Merck KGaA, Garmstadt, Germany) (Avigliano et al., 2017a). Otoliths were later rinsed three times with ultrapure water (resistivity of 18.2 MOhm ·cm) and dried at room temperature. Otolith core-sections (thickness = 1000 µm) were obtained by embedding the sample in epoxy resin. Samples were later sectioned transversely using a Buehler Isomet low-speed saw (Hong Kong, China). Otolith sections were polished using a 9 µm-grit sandpaper and later cleaned using an ultrasonic cleaner with ultrapure water for 5 minutes.

Elemental concentrations were determined in otolith cores and edges by using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) following the procedure described in (Avigliano et al., 2019). The analytical isotopes were ^{7}Li, ^{34}Mg, ^{55}Mn, ^{57}Fe, ^{66}Zn, ^{85}Rb, ^{88}Sr, and ^{138}Ba. The first 300-500 μm from the core and the last 350-600 μm from the outer edge represent approximately the core radius and the last two complete *annuli*, respectively (Figure 3). The laser ablation system used is a Teledyne Analyte G2 ArF excimer (193 nm) coupled to an iCapQ ThermoFisher ICP-MS. The otolith was pre-ablated using a spot size of 85 μm at 10-20 μm/s in order to avoid possible surface contamination. Measurements were carried out using a circular aperture of 65 μm, an ablation rate of 5 μm/s, a repetition rate of 10 Hz, and an energy density of 5 J/cm^2.

The ICP-MS was operated at a power of 1500 W using helium as carrier gas with a flow of 6,000 mL/min. Prior to each analytical session, the LA-ICP-MS was tuned and monitored by analyzing the NIST SRM 610 reference standard ($^{238}U^{+}/^{232}Th^{+}$ ratio between 0.95 – 1.05), oxide production (ThO^{+}/Th^{+} < 0.5%), and double-charged production ($^{22}M^{+}/^{44}Ca^{++}$ < 0.01%). The USGS MACS-3 and the NIST SRM 612 reference materials were used as a primary and secondary standard, respectively (Jochum et al., 2011; NIST, 2012; Pearce et al., 1997). The USGS MACS-3 is a synthetic calcium carbonate pellet and was used as a primary standard because it has a similar composition as fish otoliths, thereby reducing matrix effects (Avigliano, et al., 2019; Avigliano et al., 2018a). Data reduction was performed using Iolite (Paton et al., 2011) and the X_Trace_Elements_IS DRS (Longerich et al., 1996). The concentration (mg/kg) of the different elements was determined by using ^{43}Ca (38.8 wt.%) as the internal standard (Yoshinaga et al., 2000).

Replicate analyses of the NIST SRM 612 reference material show the following recoveries: 92% for Li, 85% for Mg, 96% for Mn, 87% for Zn, 100% for Sr and 97% for Ba. The Fe and Rb concentration of NIST SRM 612 determined in this study was within reported values (Jochum et al., 2011). Copper was not considered because at least 30% of the values were below detection limit (0.07-0.18 mg/kg). Estimates of precision were determined by the relative standard deviation percentage (RSD, %) of quadruplicate samples. RSD values below 7% were obtained (Table S1), with the data indicating good precision (Currie, 1999). The detection limits (DL) were estimated from the standard deviation of the background intensity (Campana et al., 1997). The DL (in mg/kg) for the analyzed elements in otoliths were: Ba: 0.006, Fe: 7, Li: 0.05, Mg: 0.04, Mn: 0.2, Rb: 0.02, Sr: 0.04, and Zn: 0.4. Elemental concentrations were reported as molar ratios relative to Ca (mmol/mol).

2.3. Otolith shape analysis

The internal side (Tuset et al., 2008) of each right sagittal otolith (N=175, Table 1) was photographed using a Nikon Coolpix L110 (15x optical zoom wide) digital camera at the same focal length and all images were taken with a black background. Then, the fields of the images were digitally cleaned and a scale was added (1 x 1 cm). Finally, the images were saved as BMP files.

Elliptic Fourier analysis (EFA) was used for assessing differences in the otolith contour between sampling sites (Avigliano et al., 2018c; Crampton, 1995). This analysis allows the shape of an otolith to be represented as a closed curve in a two dimensional outline. This outline is a combination of sine and cosine functions harmonically related (descriptors), where each one is composed of 4 Fourier coefficients (FC) (Crampton, 1995).

Otolith images were digitized using the Shape 1.3 software to perform the EFA (Iwata and Hukai, 2002). The numerical contour of each otolith was extracted by using a chain coding algorithm (Crampton, 1995). According to Fourier power spectrum (Crampton, 1995), the first 28 harmonics achieved 99.99% of the cumulated power (Figure 2) and hence, the otolith outline is represented by 112 FCs. The first harmonic was used to normalized the FCs, transforming these into invariant with respect to size and rotation (Ferson et al., 1985). This method transforms the first three FCs into constants, resulting in a total of 109 variables instead of 112.

2.4. Statistical analysis

Elemental ratios and FCs were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests.

Only the Li:Ca ratio of the otolith edge fulfilled the assumptions of normality and homogeneity (Shapiro-Wilk and Levene's tests, $p<0.05$). After log(x+1) transformation, only the otolith edge Mg:Ca and core Sr:Ca ratio met the assumptions (Shapiro-Wilk and Levene's tests, $p>0.05$). For this reason, univariate differences between sampling sites were assessed by using parametric tests for Li:Ca and Log(Mg:Ca+1) for edge and Log(Sr:Ca+1) for core, whereas non-parametric statistics were used for all other ratios.

The total length (TL) and otolith weight effect on the element:Ca ratios were assessed by using Spearman or Pearson correlations, according to the fulfillment of the normality and homogeneity assumptions. No significant correlation were found between TL or otolith weight and elemental ratios for both core and edge ($p>0.05$). ANOVA, followed by the Bonferroni test, was used to evaluate univariate differences between sites for Li:Ca and Log(Mg:Ca+1) (edge) and Log(Sr:Ca+1) (core). Kruskal-Wallis was used to test univariate comparisons between sampling sites for all other elemental ratios. Permutational multivariate analysis of variance (PERMANOVA), based on Mahalanobis distance (Anderson, 2006) with 9999 permutations, was employed to test multi-elemental differences in otolith core and edge fingerprints between catch areas. Because the assumption of homogeneity of variance-covariance matrices was not met (Box test, $p<0.001$), quadratic discriminant analysis (QDA) was used instead of linear model to test the ability of the ratios to classify fish into specific sampling sites using core and edge fingerprints, separately.

FCs were normalized to TL (mean 47.1 cm) for discarding allometric effects taking into account the allometric relationship (*b*) (Lleonart et al., 2000). PERMANOVA was employed to test differences in the otolith shape between catch areas, whereas QDA (Box test, $p<0.001$) was used to test the ability of data to classify fish into sampling sites.

Prior to QDA analysis, multicollinearity was assessed by obtaining the tolerance (Hair et al., 2014). The classification prior probabilities were calculated based on group numbers and sample sizes

(White and Ruttenberg, 2007). Discriminant results were verified using the leave-one-out cross-validation (Jackknifed classification matrix).

Statistical analyses were performed by using Systat 13 and SPSS 19 softwares.

3. Results

3.1. Otolith chemistry

The otolith core Fe:Ca ratio was significantly lower in Brazilian waters than in Argentinian and Uruguayan waters.(p <0.05). Otolith core Zn:Ca and Ba:Ca ratios were significantly higher in Argentina than in Brazil and Uruguay ($p < 0.05$). Rb:Ca ratio was high in Argentina, intermediate in Uruguay, and low in Brazil ($p < 0.05$), while Sr:Ca was higher in Argentina that in Brazil ($p < 0.05$). No significant differences were found between sites for otolith core Log (Mg:Ca+1), Mn:Ca and Li:Ca ratios ($p > 0.05$) (Table 2).

Regarding the otolith edge chemistry, the Li:Ca ratio was significantly higher in Argentina than in Uruguay and Brazil ($p < 0.05$), whereas the Mn:Ca ratio was high in Brazil, intermediate in Uruguay, and low for Argentina ($p < 0.05$). The otolith edge Fe:Ca ratio was significantly lower in Brazil than in Argentina and Uruguay ($p < 0.05$). The Rb:Ca, Sr:Ca and Ba:Ca ratios were significantly higher in Argentina than in Uruguay and Brazil ($p < 0.05$). No significant differences were found between sites for the otolith edge Mg:Ca and Zn:Ca ratios ($p > 0.05$, Table 2). Multivariate analyses were effective in discriminating between the three sampling sites for both edge and core. Specifically, PERMANOVA analysis shows significant multivariate differences between the three sampling sites for both edge and core ($4.4<,F_{2:82}<6.1$, $p < 0.05$).

Mean cross-classification rates of QDAs were high/moderate for both edge (mean = 80.0%) and core (mean = 68.2%) (Table 3 and Figure 5). For core, the percentage of well classified individuals was lower for Uruguay (48.0%) than for Argentina and Brazil (60.0 and 93.3%, respectively). Based on the QDA coefficients, the order of the discriminatory power of the variables was: Rb:Ca (-0.59), Fe:Ca (-0.55), Sr:Ca (0.49), Ba:Ca (-0.47), Mn:Ca (0.29), Zn:Ca (-0.048), Mg:Ca (-0.037) and Li:Ca (-0.003). For edge, the percentage of correctly classified individuals ranged from 68.0 to 86.7% (Table 3 and Figure 5). For otolith edge, the QDA coefficient order was: Li:Ca (0.64), Mn:Ca (-0.62), Ba:Ca (0.60), Rb:Ca (0.20), Fe:Ca (0.17), Mg:Ca (-0.16), Sr:Ca (-0.084) and Zn:Ca (-0.082).

The prior probabilities were 0.29 for Uruguay, 0.35 for Argentina and Brazil.

3.2. Otolith shape analysis

Multivariate analyses were effective to discriminate sites using FCs (Table 3). PERMANOVA revealed multivariate significant differences between Argentina-Brazil and Uruguay-Brazil ($p < 0.05$), but no differences were found between Uruguay and Argentina ($p > 0.05$). QDA classification rates were relatively low to moderate (mean = 61.5%), ranging from 48.0 to 70.0% (Table 3 and Figure 5).

4. Discussion

The results obtained from otolith edge microchemistry and shape analysis suggest the presence of at least two fish stocks of *U. brasiliensis* in the SAO. Otolith microchemistry and shape analysis are effective and widely used tools for the discrimination of fish stocks and nursery areas (Avigliano et al., 2018b; Avigliano et al., 2017b; Callicó Fortunato et al., 2017; Soeth et al., 2019). In this study, the otolith edge chemical signature was an effective approach to discriminate Brazilian codling stocks in three study sites.

In addition, core analysis revealed a marked segregation during the early stage of life for Argentina and Brazil, suggesting the existence of at least two nursery areas. For Uruguay, the correctly classified individual rate using core microchemistry was relatively low (48%), although significant multivariate differences were observed (PERMANOVA, $p<0.05$). Moreover, this jackknifed classification rate was nonetheless higher (0.48) than the prior probability (0.29), suggesting a non-negligible segregation behavior during the early stage. This relatively high misclassification rate could be due to limitations in the discriminant power of the model used or to the presence of connectivity between stocks. Considering both approaches simultaneously (core and edge chemistry), the results suggest a high segregation through life between the three studied sites.

Factors affecting the incorporation of trace elements into the otolith calcium carbonate matrix are element-species-specific and can be related to environmental factors such as salinity and water composition (Bouchard et al., 2015; Brown and Severin, 2009; Elsdon, and Gillanders, 2003; Martin et al., 2004). Furthermore, in several marine species, temperature, physiological, and genetic factors can also affect the incorporation rate of specific elements (Brown and Severin, 2009; Limburg et al., 2015; Martin and Wuenschel, 2006). For example, because the salinity of marine environments is relatively homogeneous, the otolith Sr:Ca and Ba:Ca ratios can be relatively constant in several marine fishes (Brown and Severin, 2009). In these cases, Sr:Ca and Ba:Ca can be strongly influenced by genetic factors and may not be a good habitat indicator (Brown and Severin, 2009).

On the other hand, in several euryhaline species the otolith Sr:Ca ratio is positively correlated with the water Sr:Ca and salinity, whereas the otolith Ba:Ca ratio may be negatively related to salinity (Avigliano et al., 2018a; Tabouret et al., 2010). Thus, these ratios can be useful habitat indicators in environments with salinity gradients (Avigliano et al., 2018a; Daros et al., 2016; Tabouret et al., 2010). Acuña Plavan and Sellanes (2007) and Acuña Plavan and Viana (2000) have reported that *U. brasiliensis* can inhabit estuarine waters (it is found in salinities higher than 18), and migrates among coastal areas and the open sea, according to reproductive purposes, salinity and temperature changes. Our results show that both the Sr:Ca and Ba:Ca ratios tended to be higher in the core than in the edge (Figure 4), therefore, the typical antagonistic relationship reported in euryhaline fish was not observed. Based on our data, we cannot recommend the use of the Sr:Ca and Ba:Ca ratios as markers of displacement among environments with different salinities. Nevertheless, additional studies are needed to confirm the usefulness of these elements as salinity indicators.

The combination of otolith shape and chemistry has been widely used to discriminate stocks because it allows to obtain more robust information on the stock structure (Avigliano et al., 2014; Callicó

Fortunato et al., 2017; Soeth et al., 2019). In this study, the EFA allowed us to discriminate the samples caught at the border of Brazil and Argentina (Table 3). However, the shape analysis did not allowed us to discriminate the Uruguayan population from the other two areas (Table 3 and Figure 5), which could be due to a connectivity between stocks or a weakness of the method to separate certain groups. Brazilian codling otolith are very irregular (highly scalloped edges, Figure 2), which could affect the discrimination power of EFA. Environmental factors such as depth, salinity, water composition, and temperature − as well as genetics−can be responsible for inter-stock differences in the otolith shape (Avigliano et al., 2017b; Campana and Casselman, 1993; Cañás et al., 2012; Sea et al., 2008; Tuset et al., 2003). Vignon and Morat (2010) have indicated that both genetic and environmental factors play a significant role in determining the otolith shape of the snapper *Lutjanus kasmira*. In that species, environment and nuclear and mitochondrial DNA have a synergistic influences that control the otolith shape (Vignon and Morat, 2010). Nevertheless, in some cases where there are no intraspecific genetic differences, environmental factors (i.e., temperature, salinity, and feeding) are the main parameters that control the otolith shape variations (Vignon and Morat, 2010). In addition, a strong correlation between the otolith morphometric and genetic components have been reported in several species such as killifish (Reichenbacher and Reichard, 2014).

The study area has a decreasing thermal gradient from north to south with different climatic and oceanographic features, depths, and several tropical and temperate estuaries (Avigliano et al., 2016). These factors could imprint a distinctive shape and chemistry in the otoliths, which could explain some multivariate differences found between the *U. brasiliensis* stocks. This is supported by several studies which have reported different stocks of species such as *Genidens barbus* (Avigliano, et al., 2019; Avigliano et al., 2015b, 2017b), *Percophis brasiliensis* (Avigliano et al., 2015a), *Micropogonias furnieri* and *Cynoscion guatucupa* (Volpedo and Cirelli, 2006) from the same study area by using otolith microchemistry. In addition, Spalding et al. (2007) divided the SAO into three main marine ecoregions (Southeastern Brazil, Rio Grande and Uruguay–Buenos Aires Shelf, Figure 1) based on oceanographic and faunal characteristics. The *U. brasiliensis* stocks from Brazil and

Argentina found in our work seem to reflect the Atlantic biogeographic regions described by Spalding et al. (2007), which is in agreement with the stock delimitations of other species such as *P. brasiliensis* (Braicovich and Timi, 2008) and *G. barbus* (Avigliano, et al., 2019).

On the other hand, the collection areas from Uruguay and Argentina are within the same ecoregion (Figure 1), which could also explain the relative low percentages of classification found for Uruguay (shape and microchemistry). Again, this could be due to: 1) high connectivity, 2) relatively homogeneous environment, 3) discriminant power of the variables used, or 4) a combination of these factors. The environmental homogeneity is an unlikely factor because the sampling sites from Argentina and Uruguay have a different salinity, temperature, and depth (Guerrero et al., 2010). Regardless, it is clear that the relationship between the otolith chemical composition and the environment must be further tested.

The delimitation of stocks found in this work is consistent with those reported by Pereira et al. (2014) that suggested the presence of 3 stocks in the SAO based on the analysis of parasite assemblages. Unlike our study, Pereira et al. (2014) collected samples from the three different ecoregions (greater areas and more marked environmental differences), which could contribute to a better discrimination of the stocks.

Our results not only indicate the presence of different stocks, as previously reported by Pereira et al. (2014), but also suggest a strong spatial segregation during ontogeny. Additional studies should incorporate samples from the Rio Grande ecoregion, which we infer could correspond to the Uruguayan stock. Moreover, the incorporation of other methods such as genetics and otolith stable isotopes could contribute to better evaluate the connectivity between these three areas and define more appropriate stock management policies.

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Table 1: Summary of fish descriptive statistics (mean and range) for each sampling site. N, sample size; TL, total length (cm); W, weight (g); SD, standard deviation.

	N	TL ± SD	W ± SD
		Otolith chemistry	
Argentina	30	46.0 ± 3.4 (37.0-52.0)	904 ± 221 (445-1365)
Uruguay	25	46.7 ± 6.9 (31.5-54.0)	1025 ± 459 (232-1658)
Brazil	30	48.7 ± 4.9 (30.2-54.0)	1004 ± 304 (193-1500)
		Fourier descriptors	
Argentina	86	44.6 ± 4.1 (32.0-52.0)	844 ± 212 (315-1365)
Uruguay	28	44.6 ± 9.0 (26.0-54.0)	931 ± 512 (142-1658)
Brazil	61	51.4 ± 5.5 (29.2-54)	1139 ± 333 (179-1630)

Table 2: Statistics and p-values of the ANOVA (F, Fisher) and Kruskal–Wallis (H) tests used to evaluate univariate differences between sites. df=degrees of freedom.

	Core chemistry (df=82)		Edge chemistry (df=82)	
Element:Ca ratio	Statistic	*p*	Statistic	*p*
Li:Ca	H = 2.12	0.3	F = 17.5	0.0001
Mg:Ca	H = 2.39	0.3	F= 2.54	0.08
Mn:Ca	H = 0.02	0.9	H = 34.9	0.0001
Fe:Ca	H = 19.9	0.0001	H = 23.9	0.0001
Zn:Ca	H = 17.5	0.0002	H = 2.25	0.3
Rb:Ca	H = 22.6	0.0001	H = 19.5	0.0001
Sr:Ca	F = 5.59	0.005	H = 21.4	0.0001
Ba:Ca	H = 22.2	0.0001	H = 22.3	0.0001

Table 3: Cross-classification matrix of the discriminant analysis. The numbers represent the classification percentage. N: sample size. Percentage of correctly reclassified individuals were indicates in bold numbers.

	N	**Argentina**	**Uruguay**	**Brazil**
		Otolith core chemistry		
Argentina	30	**60.0**	20.0	20.0
Uruguay	25	28.0	**48.0**	24.0
Brazil	30	3.3	3.3	**93.3**
Mean				68.2
		Otolith edge chemistry		
Argentina	30	**83.3**	6.7	10.0
Uruguay	25	12.0	**68.0**	20.0
Brazil	30	6.7	6.7	**86.7**
Mean				80.0
		Fourier descriptors		
Argentina	86	**70.0**	16.7	13.3
Uruguay	28	16.0	**48.0**	36.0
Brazil	61	10.0	23.3	**66.7**
Mean				61.5

Table S1: Mean concentration and standard deviation (SD) obtained for the NIST SRM 612 (analyzed as unknown) in each analytical session. Rb is not validated for NIST (Relative standard deviation obtained <3.8%).

Replicates for NIST SRM 612	Concentration (mg/kg)	Li	Mg	Ca	Mn	Fe	Cu	Zn	Sr	Ba
A	Mean	36	56	84000	36	101	35	32	76	37
	SD	1	2	2446	1	15	2	2	3	1
B	Mean	36	55	82436	35	91	34	35	76	37
	SD	1	2	2162	1	12	2	4	2	1
C	Mean	36	56	83848	36	101	35	32	76	37
	SD	1	2	2357	1	15	2	2	3	1
D	Mean	38	60	84848	38	91	37	33	77	37
	SD	1	2	2801	1	12	2	2	2	1
E	Mean	36	56	85062	36	102	34	32	78	36
	SD	2	2	2542	1	12	2	2	3	1
F	Mean	37	59	87156	37	101	34	35	79	38
	SD	1	2	2799	1	13	2	2	2	1
G	Mean	36	56	85151	36	87	33	35	73	37
	SD	1	2	2583	1	12	2	2	2	1
H	Mean	38	59	86934	38	94	36	38	79	40
	SD	1	2	2691	1	13	1	3	2	1
I	Mean	38	59	84857	37	94	35	34	76	39
	SD	1	3	3448	2	12	2	2	3	2
J	Mean	37	57	86283	37	95	35	33	76	38
	SD	1	2	2998	1	14	2	2	3	5
K	Mean	36	55	86716	35	86	32	35	75	36
	SD	1	2	2579	1	10	2	2	2	1
L	Mean	35	54	85602	35	91	32	34	75	36
	SD	1	2	2392	1	12	2	2	2	1
M	Mean	35	55	83713	35	99	33	36	76	37
	SD	1	2	2116	1	13	2	2	2	1

N	Mean	37	61	84716	38	102	37	38	78	40
	SD	1	2	2611	2	13	2	2	2	2
O	Mean	39	62	85053	39	100	38	37	81	40
	SD	1	2	2579	1	9	2	2	3	1
P	Mean	35	53	83776	34	95	32	33	74	36
	SD	1	2	2239	1	11	2	3	2	1
Q	Mean	38	57	86369	36	109	34	36	76	38
	SD	2	3	3926	1	13	2	2	3	2
R	Mean	39	62	85053	39	100	38	37	81	40
	SD	1	2	2579	1	9	2	2	3	1
	Average of all the NIST 612 analysed	37	57	85089	36	96	35	34	77	37
	SD	1	3	1306	1	6	2	2	2	1
	Relative difference (%) eith respect to Jochum et al. (2011)	8	16	1	4	In the range	9	14	1	4

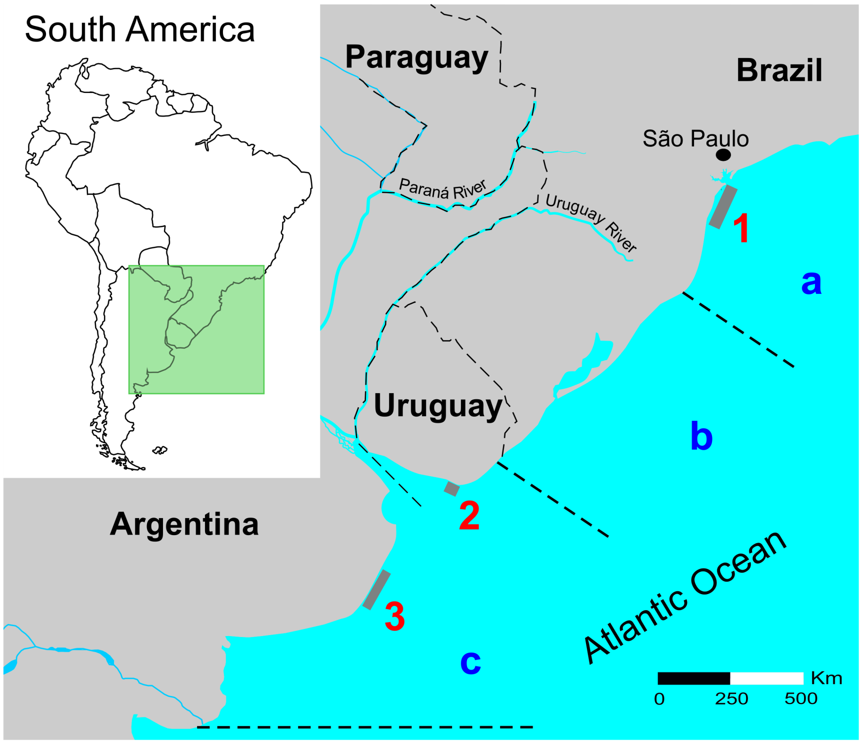
Figure 1: Study area map. Red areas show the sampling sites of *Urophysis brasiliensis*. 1, Brazil; 2, Uruguay; and 3, Argentina. Dashed lines delimit the ecoregions according to Spalding et al. (2007): a, Southeastern Brazil; b, Rio Grande and c, Uruguay–Buenos Aires Shelf.

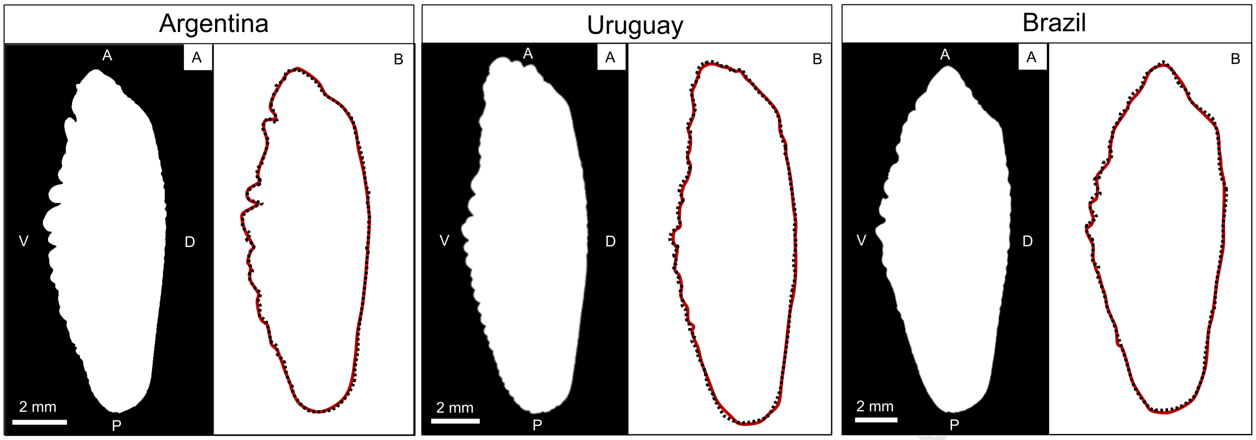
Figure 2: Otolith of *Urophycis brasiliensis* from each sampling site. A: Right sagittal otolith. D: dorsal, V: ventral, A: anterior and P: posterior. B: Brazilian codling otolith shape outlines reconstruction for successive cumulative contribution of the first 28 harmonics of the elliptical Fourier analysis (Fourier power spectrum = 99.9999%). Dotted line: original otolith outline; solid line: the cumulative contribution of harmonics.

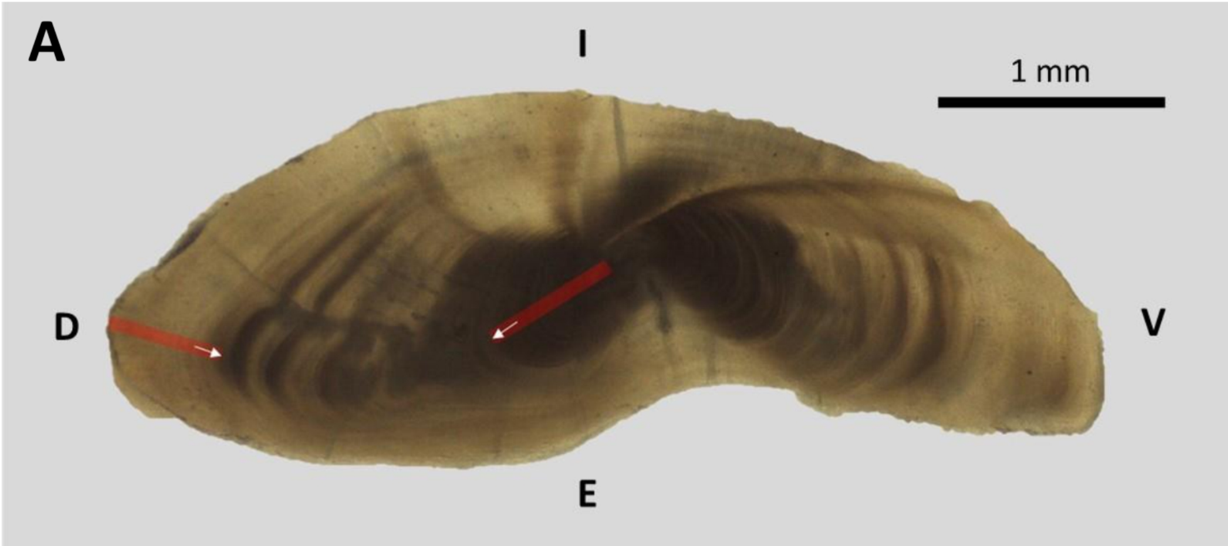
Figure 3: A: Otolith section of *Urophycis brasiliensis* from Argentinian coast showing the core and edge laser ablation area. The white arrows indicate the direction of ablation. D: dorsal, V: ventral, I: internal face, E: external face. B: element:Ca results (logarithmic scale for better visualization) of the otolith edge.

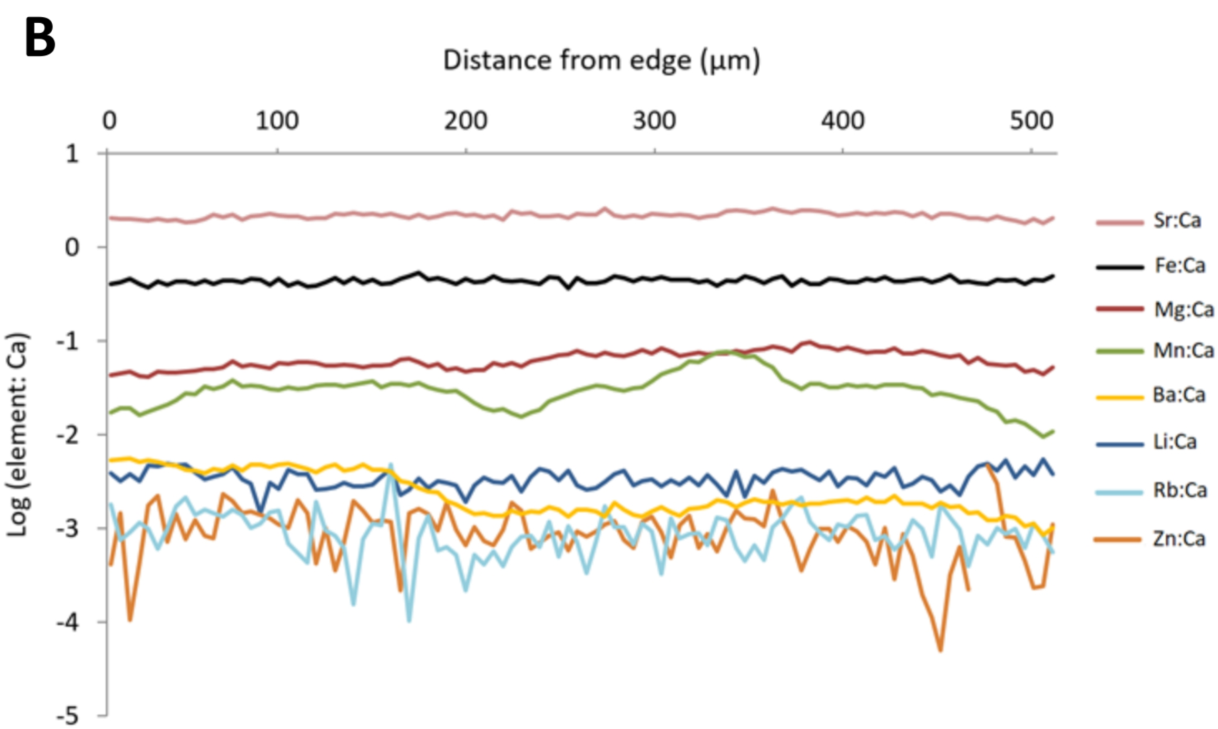
Figure 4: Box plot showing the distribution of the elemental ratios (mmol/mol) for otolith edge and core of *Urophycis brasiliensis* from different sampling sites, including: median (midline); mean (dot); 25th and 75th percentiles for Mn:Ca; Fe:Ca; Zn:Ca; Rb:Ca and Ba:Ca ratios or standard error for Li:Ca, Mg:Ca and Sr:Ca ratios (box); and the range (bars). Different letters show significant difference between sampling sites ($p < 0.05$, Table 3).

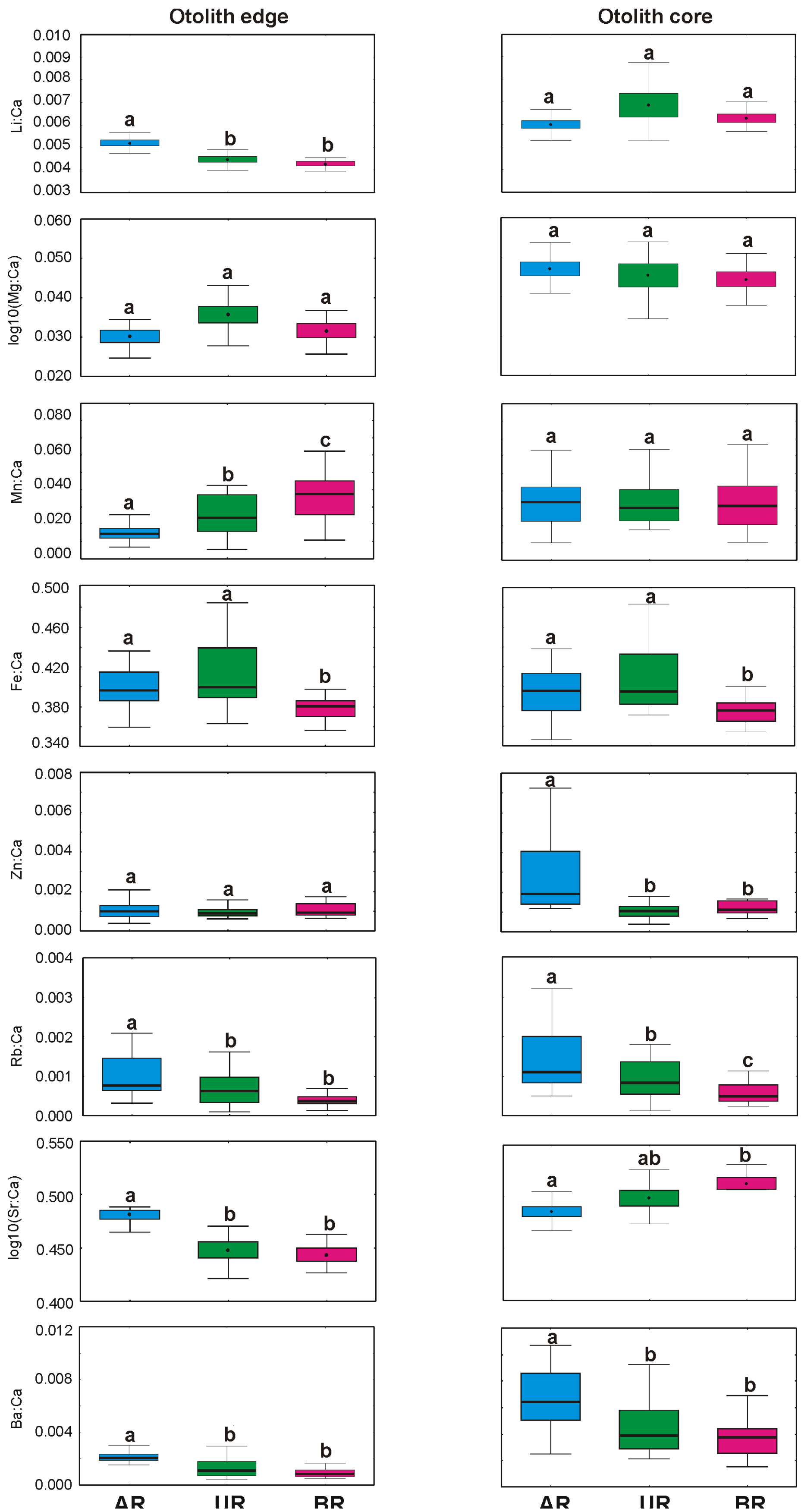
Figure 5: Quadratic discriminant analysis of *Urophycis brasiliensis* otolith. AR, Argentina; UR, Uruguay; BR, Brazil.

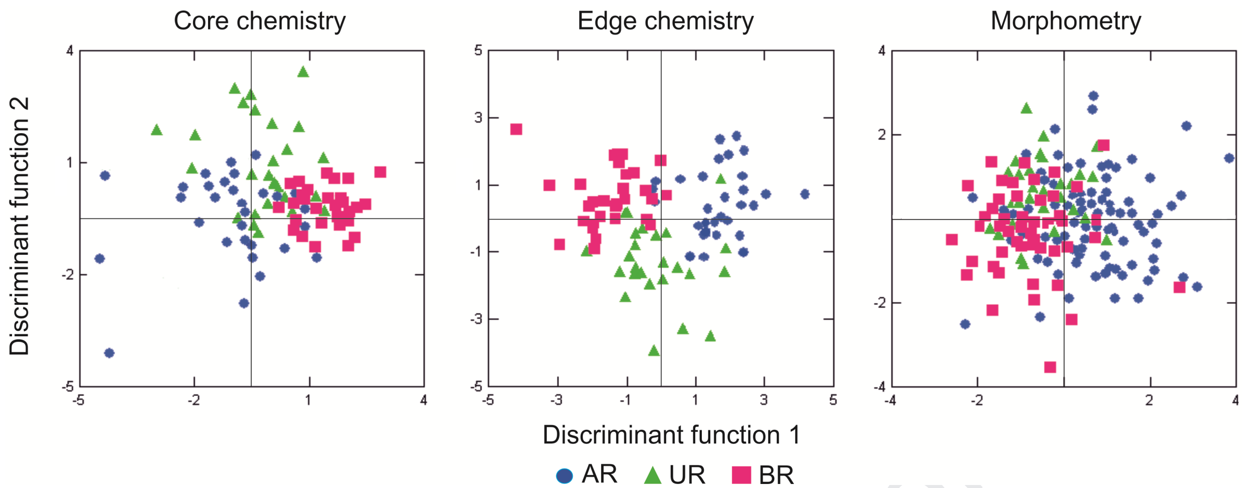

South America
Paraguay
Brazil
São Paulo
Paraná River
Uruguay River
1
a
Uruguay
b
2
Argentina
3
c
Atlantic Ocean
Km
0
250
500


Argentina
Uruguay
Brazil
A
B
V
D
P
2 mm


A
I
1 mm
D
V
E


B
Distance from edge (µm)
0
100
200
300
400
500
1
0
-1
-2
-3
-4
-5
Log (element: Ca)
Sr:Ca
Fe:Ca
Mg:Ca
Mn:Ca
Ba:Ca
Li:Ca
Rb:Ca
Zn:Ca


Otolith edge
Otolith core
Li:Ca
log10(Mg:Ca)
Mn:Ca
Fe:Ca
Zn:Ca
Rb:Ca
log10(Sr:Ca)
Ba:Ca
AR
UR
BR


Core chemistry
Edge chemistry
Morphometry
Discriminant function 2
Discriminant function 1
AR
UR
BR

Spatial segregation in young and adult stages of *Urophycis brasiliensis* was studied.

Otolith microchemisty and shape are potential tools for stock identification.

Results suggest the presence of at least 2 fish stocks and nursery areas.

High percentages of classification suggest low connectivity between populations.

The populations should be managed as separate groups.

UBA
Universidad de Buenos Aires

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Dear Editor in Chief, Dr. Mike Elliott

There is no conflict of interest between the authors of the manuscript.

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