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Spatial segregation and connectivity in young and adult stages of *Megaleporinus obtusidens* inferred from otolith elemental signatures: Implications for management

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ABSTRACT

Boga *Megaleporinus obtusidens* is a teleost fish of economical and sport importance from Río de la Plata Basin (South America). Otolith core and edge elemental ratios (Ba:Ca, Cu:Ca, Li:Ca, Mg:Ca, Mn:Ca, Pb:Ca, Rb:Ca and Sr:Ca) were compared among three sampling areas from the Río de la Plata Basin (Paraná and Uruguay Rivers and Río de la Plata Estuary) to evaluate the applicability of the fingerprint to study segregation and connectivity in young and adult stages. Several ratios were significantly different among sites for otolith core and edge ($p < 0.05$). PERMANOVA ($p < 0.05$) and quadratic discriminant function analysis (classification rates: 86.8% and 82.5% for otolith core and edge, respectively) were found to be highly effective in detecting differences in otolith core and edge fingerprints between sampling sites suggesting the existence of spatial segregation in young and adult life stages, respectively. The presence of relatively isolated groups may require the need to manage the stocks separately.

1. Introduction

Boga *Megaleporinus obtusidens* (Valenciennes, 1846) is a teleost fish of economical and sport importance that is distributed in temperate and subtropical areas from South America (Ramirez et al., 2017). Tagging studies showed that *M. obtusidens* is a migrating fish that can swim more than 500 km (Bonetto et al., 1981; Espinach Ros, 1999). The temporary pattern of recaptures of tagged fish in the Uruguay River (Fig. 1), showed that migration happens at the beginning of autumn (April), when fish migrate to the Middle Paraná or upstream of the Uruguay River in a few days (Espinach Ros, 1999). It has been suggested that these upstream displacements are related to the low temperatures recorded in autumn and winter (April-August) in the southern portion of the basin (Espinach Ros, 1999).

Fish probably reproduce upstream during the winter, and return to their summer feeding areas when temperatures have raised within the range of preference of the species (Espinach Ros, 1999).

Despite the socio-economic relevance of the species, reliable fishery statistics are not available in the region and very little is known about the structure of populations and the delimitation of fish stocks. In Argentina, captures are regulated exclusively with the export quota, without taking into account the population structure or connectivity. Information on nursery areas and population structure helps fishery management and contributes to rational use and conservation of the species (Cadrin et al., 2013).

Fish otolith chemistry has contributed to study the life history, segregation, connectivity and nursery identification of several fish species (Avigliano and Volpedo, 2016; Campana, 2013; Tanner et al., 2015; Vasconcelos et al., 2008). Otoliths are calcified structures composed of calcium carbonate deposited mainly as aragonite (Campana et al., 1997) and they are located in the inner ear of fish and have a role in hearing and maintaining equilibrium (Popper and Lu, 2000). Trace elements (e.g. Ba, Li, Mg, Mn, Sr) and heavy metals (e.g. Pb, Cu) are acquired by fish during the life history and preserved within the otolith

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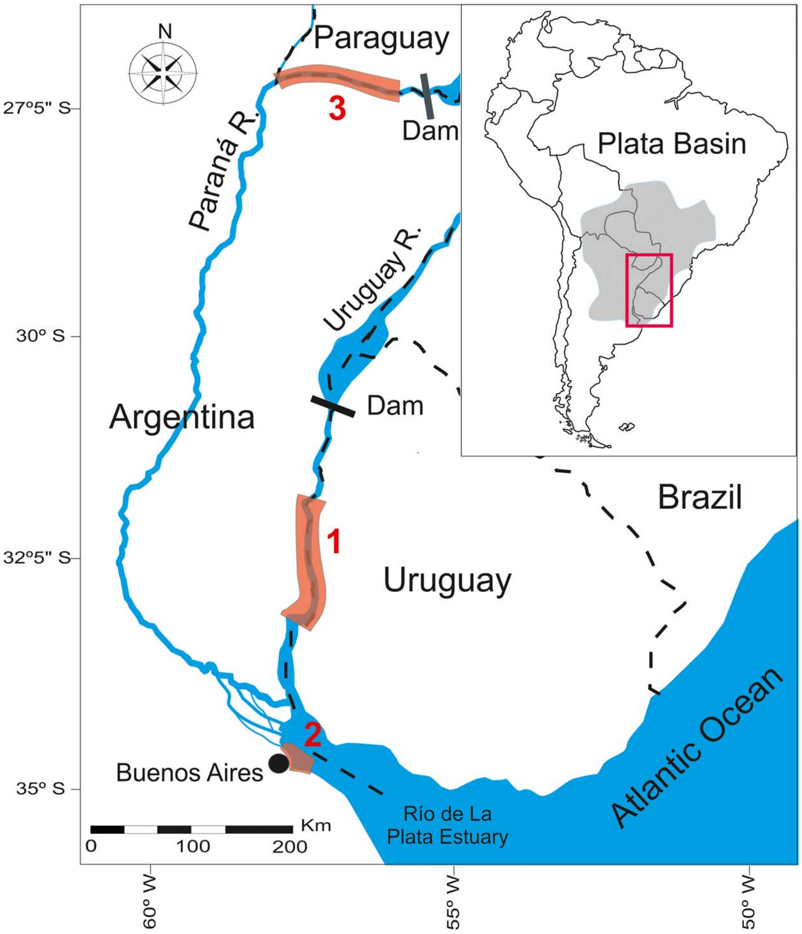

Fig. 1. *Megaleporinus obtusidens* sampling areas within the Río de la Plata Basin (hatched area). 1, Uruguay River; 2, inner section of the Río de la Plata Estuary (freshwater-estuarine environment); and 3, Paraná River.

structure (Herrera-Reveles et al., 2013; Miller, 2011; Reis-Santos et al., 2013; Sturrock et al., 2014). The predominant source of most elements for the otoliths is the surrounding water. These trace elements are incorporated from the water to the fish's blood plasma via gills or intestines, then into the endolymph, and finally into the crystallizing otolith (Bouchard et al., 2015; Campana, 2013; Sturrock et al., 2012). Trace elements in combination with growth rings result in a file that records environment information frequented by fish and exposure to pollutants throughout ontogeny (Halden and Friedrich, 2008). In this sense, otolith chemical signature in the core (young stage) may reflect nurseries areas, while the edge (adult stage) could indicate the presence of geographic management units (Campana, 2013; Reis-Santos et al., 2015).

The present study tests the applicability of the otolith multi-elemental fingerprint to identify possible spatial segregation and connectivity in young and adult stages of *Megaleporinus obtusidens*. For this purpose, otolith core and edge elemental ratios (Ba:Ca, Cu:Ca, Li:Ca, Mg:Ca, Mn:Ca, Pb:Ca, Rb:Ca and Sr:Ca) were compared among three sampling areas from the Río de la Plata Basin (Paraná and Uruguay Rivers and Río de la Plata Estuary).

2. Materials and methods

2.1. Study locations and collection

The Río de la Plata Basin is the second largest fluvial-marine system in the Americas, following the Amazonian, with a drainage area of 3,170,000 km^2 (latitude from 17 to 36°S) (Fig. 1). The outflow of the basin is south-southeast to the Paraná Delta and the Atlantic Ocean, involving Paraná, Paraguay, Uruguay and Pilcomayo as major rivers (> 4500 km long). These rivers converge in the Río de la Plata Estuary (Fig. 1) and discharge on average 23,000 m3/s of freshwater into the sea (Guerrero et al., 1997).

Fish were collected using trammel nets between February 2010 and November 2011 in the Paraná and Uruguay Rivers and Río de la Plata Estuary. Fish were kept refrigerated at 4 °C until reaching the laboratory, where they were measured (standard length = SL) and dissected to extract *lapilli* otoliths. *Lapilli* otoliths were used rather than *sagittae* otoliths because they were larger and allowed less measurement error".

2.2. Sample preparation

Otoliths were weighed, washed with Milli-Q water (resistivity: 18.2 mOhm/cm) and dried. The left otolith of each pair was embedded in epoxy resin and sectioned transversely through the core to a thickness of 750 μm using a low speed saw (Buehler Isomet, Hong Kong, China).

Growth *annuli* in each otolith section were counted under stereomicroscopic loupe inspection with transmitted light, while otoliths were immersed in ultrapure water to increase the clarity when reading. To avoid the effect that the fish age or standard length could have on the interpretation of the elemental ratios, only fish with 2^+ or 3 *annuli* were used (age not validated). Mean standard length ± standard deviation (cm) were 34.1 ± 0.8, 30.0 ± 4.6, 33.1 ± 5.9 for the Uruguay River (N = 14), Río de la Plata Estuary (N = 17) and Paraná River (N = 22), respectively.

Otoliths sections were fixed to glass slides using clear epoxy resin, polished using 9 μm-grit sandpaper and ultrasonically cleaned (3 min)

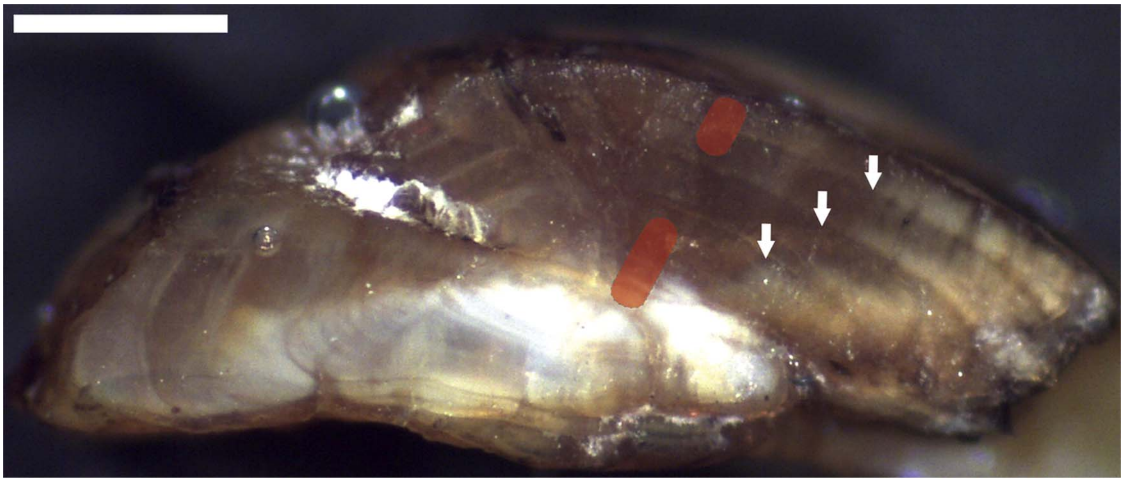

Fig. 2. Otolith x-section of *Megaleporinus obtusidens* from Uruguay River showing the core and edge laser ablation areas. Barr: 500 µm.

in Milli-Q ultrapure water.

2.3. Elements analysis by LA-ICP-MS

The elements concentration ^{43}Ca, ^{7}Li, ^{25}Mg, ^{55}Mn, ^{65}Cu, ^{85}Rb, ^{88}Sr, ^{138}Ba and ^{208}Pb were measured by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) in a single analytical session (22/09/2017), using a 193 nm ArF* Excimer laser ablation system (Photon Machines Analyte G2) coupled to an ICP-MS Agilent 7700 (Agilent Technologies, Santa Clara, USA).

Two radial line-scans of 187 µm were carried out separately in the otolith core and edge (Fig. 2). This distance represents approximately the entire core area and the last complete *annuli*.

Helium was used as the carrier gas in the ablation cell and Argon was added before entering the ICP-MS. The ion optics were adjusted to yield maximum sensitivity and balanced mass response ablating standard reference material NIST 612 glass (National Institute of Standards and Technology). The optimisation was carried out monitoring $^{7}\text{Li}^{+}$, $^{133}\text{Cs}^{+}$, $^{232}\text{Th}^{+}$, $^{238}\text{U}^{+}$ and $^{232}\text{Th}^{16}\text{O}^{+}$ ion signal intensities. Plasma robustness was monitored via the $^{232}\text{Th}^{16}\text{O}/^{232}\text{Th}$ and the $^{238}\text{U}/^{232}\text{Th}$ intensity ratios. $\text{ThO}^{+}/\text{Th}^{+}$ intensity ratios were always below 0.4% and $^{238}\text{U}^{+}/^{232}\text{Th}^{+}$ intensity ratio was ~ 1.01. The cross calibration of the pulse and analogue stages of the scanning electron microscope detector ("PA-factor") was conducted to ensure a linear response of the instrument of > 8 orders of magnitude for the isotopes of interest. Laser ablation parameters optimized from otolith test ablations were line scans at 5 µm/s using a 85 µm spot, 12 J/cm^2 Laser fluence, 10 Hz repetition rate, and a He cell flow of 800 mL/min. The ICP-MS operated at an RF power of 1600 W with an Ar carrier flow of 900 mL/min.

Each 8 otolith samples, NIST 612 silicate glass reference material was employed as an external reference material (Jochum et al., 2011; NIST, 2012; Pearce et al., 1997), while silicate glass NIST 610 was analysed as a secondary standard. The concentrations of elements were normalized to the internal standard (^{43}Ca). Net ion signals were employed in the bracketing quantification method, where the reference material (NIST 612) is analysed at the beginning and at the end of analysis session to monitor and correct for any signal drift (deviation < 4%). Time-resolved intensities were converted to concentration (ppm) equivalents, with ^{43}Ca as the internal standard and a Ca index value of 38.3 weight% (Hamer et al., 2015; Yoshinaga et al., 2000).

Analysis of NIST 610 showed good agreement, with the following element recovery rates: 95.7% for ^{7}Li, 91.4% for ^{25}Mg, 100.3% for ^{55}Mn, 100.5% for ^{65}Cu, 94.5% for ^{85}Rb, 95.9% for ^{88}Sr, 93.8% for ^{138}Ba and 103.03% for ^{208}Pb. All elements were consistently above detection limits (3 standard deviation of the mean background gas values). Elements were expressed as molar ratios (element:Ca = mmol/mol).

2.4. Data analysis

Elemental ratios were tested for normality and homogeneity of variance using the Shapiro Wilk and Levene's tests respectively. To ensure that differences in otolith mass did not confound spatial patterns in otolith edge and core elemental composition, the effect of otolith mass (otolith weight as covariate) on the elemental ratios was examined using analysis of covariance (ANCOVA) (Campana, 2013). Only otolith edge Ba:Ca varied significantly with otolith weight (ANCOVA, $p < 0.05$) and it was corrected using the common within-group slope ($b = -0.00036$) on otolith weight.

Univariate tests were used to compare the elemental ratios (otolith core and edge) between sampling sites. After testing normality and homoscedasticity assumptions (Shapiro Wilk and Levene tests, $p > 0.05$), original data series of otolith core Li:Ca, Mg:Ca and Sr:Ca were compared with ANOVA, followed by the Bonferroni test. Otolith core Mn:Ca, Cu:Ca, Rb:Ca, Ba:Ca and Pb:Ca ratios did not fit the normal distribution and homogeneity of variance (Shapiro–Wilk, $p < 0.05$; Levene, $p < 0.05$), even after transformation $\log(x + 1)$. Therefore, Kruskal Wallis tests were used to compare ratios between study sites.

Otolith edge Mn:Ca and Sr:Ca ratios met the normal distribution and homogeneity of variance (Shapiro–Wilk, $p > 0.05$; Levene, $p > 0.05$) and were compared with ANOVA, followed by the Bonferroni test. However, otolith edge Ba:Ca, Li:Ca, Mg:Ca, Cu:Ca and Rb:Ca ratios were compared using Kruskal Wallis tests because they did not met the assumptions (Shapiro–Wilk, $p < 0.05$; Levene, $p < 0.05$).

Multivariate statistics were used to evaluate spatial differences in otolith core and edge multi-elemental fingerprints. Permutational multivariate analysis of variance (PERMANOVA) was used to detect differences in otolith core and edge between sampling sites, while quadratic discriminant function analysis (QDA) was used to assess the ability of elemental ratios to classify fish to their catch area. Based on number of groups and sample sizes, the expected prior probability classification was calculated and a randomization test was performed to determine if the classification rates were significantly different from random (White and Ruttenberg, 2007). Statistical tests were performed using the SPSS 19 and Ginkgo 1.7 programs.

3. Results

Element:Ca ratios are shown in Table 1. The otolith core Li:Ca ratio was high for the Río de la Plata Estuary, intermediate for the Uruguay River and low for the Paraná River ($F = 14.6$, $P = 0.0007$). Otolith core Mg:Ca ratio was high for the Paraná River, low for the Uruguay River, and intermediate for the Río de la Plata Estuary ($F = 5.6$, $p = 0.002$). Rb:Ca in the core was significantly higher for the Paraná and Uruguay

Table 1
Elemental ratios (in mmol/mol, means ± standard deviation) in the otolith core and edge for each study site. Different letters indicate statistically significant differences ($p < 0.05$). N: sample size.

	N	Ba:Ca	Cu:Ca	Li:Ca	Mg:Ca	Mn:Ca	Sr:Ca	Rb:Ca	Pb:Ca
		Otolith core							
Uruguay River	14	$1.4E^{-02} \pm 8.0E^{-03}$	$3.6E^{-04} \pm 1.5E^{-04}$	$7.5E^{-04} \pm 2.0E^{-04}$ab	$7.2E^{-02} \pm 2.1E^{-02}$b	$7.6E^{-03} \pm 2.7E^{-03}$	1.01 ± 0.20	$5.1E^{-04} \pm 1.6E^{-04}$a	$6.7E^{-05} \pm 7.1E^{-05}$
Río de la Plata Estuary	17	$1.8E^{-02} \pm 8.3E^{-03}$	$3.7E^{-04} \pm 2.5E^{-04}$	$9.6E^{-04} \pm 2.8E^{-04}$b	$8.9E^{-02} \pm 2.0E^{-02}$ab	$1.2E^{-02} \pm 7.2E^{-03}$	1.14 ± 0.23	$2.3E^{-04} \pm 3.4E^{-05}$b	$3.8E^{-05} \pm 2.9E^{-05}$
Paraná River	22	$1.7E^{-02} \pm 1.1E^{-02}$	$3.7E^{-04} \pm 1.7E^{-04}$	$6.0E^{-04} \pm 3.7E^{-04}$a	$9.8E^{-02} \pm 2.5E^{-02}$a	$1.3E^{-02} \pm 6.7E^{-03}$	1.15 ± 0.29	$5.8E^{-04} \pm 2.4E^{-04}$a	$6.0E^{-05} \pm 6.3E^{-05}$
		Otolith edge							
Uruguay River	14	$4.4E^{-03} \pm 1.3E^{-03}$a	$3.9E^{-04} \pm 1.7E^{-04}$a	$3.5E^{-04} \pm 8.2E^{-05}$ab	$3.2E^{-02} \pm 1.1E^{-02}$a	$1.2E^{-02} \pm 3.6E^{-03}$	0.69 ± 0.13c	$3.3E^{-04} \pm 1.6E^{-04}$a	$1.1E^{-04} \pm 1.2E^{-04}$
Río de la Plata Estuary	17	$6.2E^{-03} \pm 2.1E^{-03}$a	$1.1E^{-03} \pm 9.9E^{-04}$b	$5.2E^{-04} \pm 2.7E^{-04}$b	$5.1E^{-02} \pm 3.1E^{-02}$b	$1.5E^{-02} \pm 5.1E^{-03}$	1.11 ± 0.13b	$1.1E^{-04} \pm 3.6E^{-05}$b	$2.1E^{-04} \pm 2.1E^{-04}$
Paraná River	22	$6.9E^{-03} \pm 2.9E^{-03}$b	$4.6E^{-04} \pm 3.8E^{-04}$a	$3.7E^{-04} \pm 3.0E^{-04}$a	$3.2E^{-02} \pm 1.1E^{-02}$ a	$1.4E^{-02} \pm 4.1E^{-03}$	0.97 ± 0.18a	$3.1E^{-04} \pm 1.2E^{-04}$a	$9.8E^{-05} \pm 8.1E^{-05}$

Rivers than for the Río de la Plata Estuary (H = 32.8, p = 0.0001). No significant differences were found between sites for otolith core Ba:Ca, Cu:Ca, Mn:Ca, Sr:Ca and Pb:Ca ratios ($p > 0.05$).

In relation to otolith edge, Cu:Ca and Mg:Ca ratios were found to be higher in the Río de la Plata Estuary than in the Uruguay and Paraná Rivers (Cu: H = 13.6, p = 0.001; Mg:Ca: H = 7.2; p = 0.02). Otolith edge Ba:Ca was significantly higher for the Paraná River than for the Uruguay River and the Río de la Plata Estuary (H = 11.2, p = 0.003). Li:Ca ratio in the edge was higher for the Río de la Plata Estuary, intermediate for the Uruguay River, and low for the Paraná River (H = 10.0, p = 0.006). Sr:Ca was significantly different between all sampling sites, where it was higher for the Río de la Plata Estuary than for the Uruguay and Paraná Rivers (F = 30.9, p = 0.0001). Rb:Ca was significantly higher for the Paraná and Uruguay Rivers compared to the Río de la Plata Estuary (H = 31.9, p = 0.0001). Otolith edge Mn:Ca and Pb:Ca ratios were similar between sites.

PERMANOVA and QDA analyzes were found to be highly effective in detecting differences in otolith core and edge fingerprints between sampling sites, suggesting the existence of spatial segregation in young and adult life stages, respectively. PERMANOVA analysis revealed multivariate significant differences of the otolith core (F = 6.1, p = 0.0001–0.0002) and edge (F = 9.9, p = 0.0001–0.001) between the three sampling sites. Classification rates of QDA were high, averaging 86.8% and 82.5% for otolith core and edge, respectively (Fig. 3, Table 2). The prior probabilities for groups were 0.26 for Uruguay River, 0.32 for the Río de la Plata Estuary and 0.41 for Paraná River. Percentages of correctly classified individuals were significantly different from random for otolith core and edge (randomization tests: $p < 0.05$), still provides acceptable discriminatory power.

4. Discussion

The existence of different multi-elemental chemical signatures suggests that the three groups studied remain with an important degree of geographical segregation, both in early and adult stages.

The concentration of different elements in the water or environmental factors such as temperature, salinity, diet and genetics may affect the incorporation of trace elements in the otoliths (Bouchard et al., 2015; Brown and Severin, 2009; Campana, 1999; Reis-Santos et al., 2013). In the Río de la Plata Basin, temperature decreases and salinity increases from North to South (Avigliano et al., 2015; Avigliano and Schenone, 2015; Avigliano and Volpedo, 2013). Moreover, the Paraná and Uruguay Rivers and the Río de la Plata Estuary have different degrees of contamination, topographic features, salinity ranges and hydrographic dynamics (Acha et al., 2008; Avigliano et al., 2015; Avigliano and Schenone, 2015). These factors together with a relatively gregarious behaviour of *Megaleporinus obtusidens* could reflect distinctive fingerprint in the otoliths, which explains the multi-elemental differences found among sampling sites. In several species, the Sr:Ca ratio of otoliths and ambient water are positively correlated with salinity, while otolith Ba:Ca ratio may be negatively related to salinity, thus these ratios are useful indicators of habitat in environments with salinity gradients (Bath et al., 2000; Secor and Rooker, 2000; Martin et al., 2004; Sturrock et al., 2012). In the specific case of the Plata Basin, a quadratic relationship between conductivity/salinity and water Sr:Ca has been demonstrated (Avigliano and Volpedo, 2013). This is partially reflected in our data, where a significantly higher Sr:Ca ratio was found at the edge of the otoliths of the Río de la Plata Estuary. However, the sampling area of the Río de la Plata Estuary presents fluctuating and very low salinities (< 0.2), which may not have an important effect on the incorporation of Sr:Ca or Ba:Ca. In addition, Cu levels were higher for the edge of the otolith in the Río de la Plata Estuary. This could be related to an anthropogenic effect because in the Río de la Plata Estuary are the two large capitals (Montevideo and Buenos Aires) that contribute to the increase of the heavy metal levels in this body of water (Avigliano et al., 2015; Avigliano and Schenone, 2015).

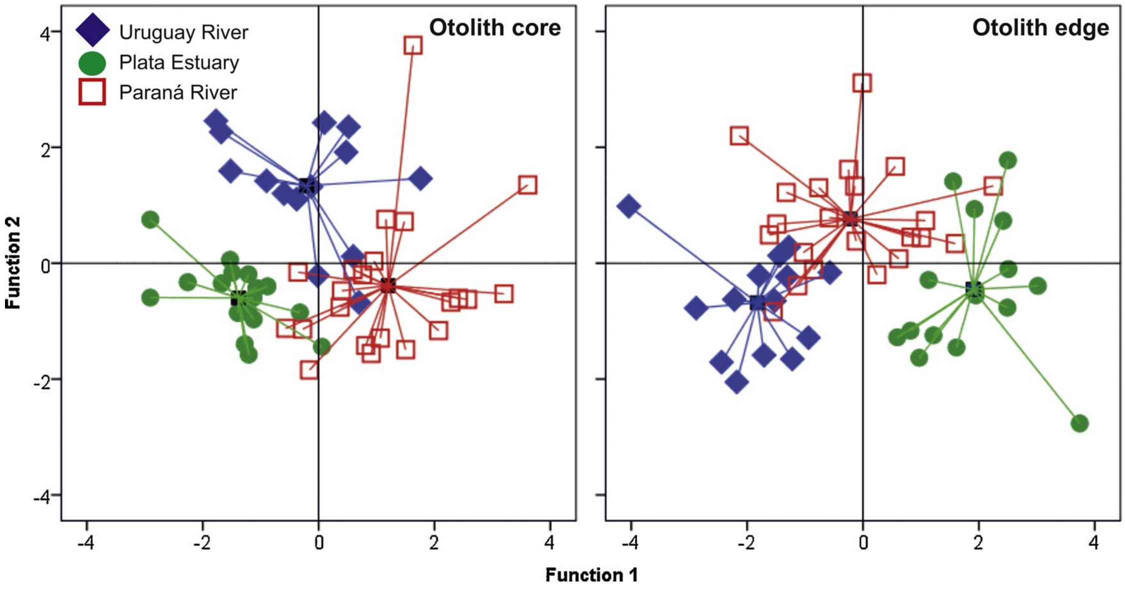

Fig. 3. Discriminant analysis of the otolith element:Ca ratios. (a) otolith core, (b) otolith edge.

Table 2
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	Uruguay River	Río de la Plata Estuary	Paraná River
		Otolith core		
Uruguay River	14	**71.4**	0.0	28.6
Río de la Plata Estuary	17	0.0	**94.1**	5.9
Paraná River	22	0.0	9.1	**90.9**
		Otolith edge		
Uruguay River	14	**78.6**	0.0	14.3
Río de la Plata Estuary	17	0.0	**100**	6.3
Paraná River	22	9.1	9.1	**81.8**

Other authors who have performed tagging and recapture studies have described it like a migratory species (Bonetto et al., 1981; Espinach Ros, 1999). Our results are not against such observations, but rather are fully compatible. There may be at least three groups with a significant degree of segregation but with a relative connectivity between them. On the other hand, the distances between the studied sites (250–1500 km) exceed, at least in part, the migratory distance reported so far for the species (300–540 km) (Bonetto et al., 1981; Espinach Ros, 1999), which could explain the high percentages of classification obtained.

Although it has been indicated that upstream migration could be triggered by the decrease in the temperature, no specific studies have been developed in this regard (Bonetto et al., 1981; Espinach Ros, 1999). As far as it is known, reproduction occurs in the spring, coinciding with the culmination of the upstream migration previously reported (Bonetto et al., 1981; Espinach Ros, 1999). At this point, the need to study the relationship between displacements and the life cycle is evident, as well as to estimate the proportion of gregarious and migrating fish, in order to contribute to the management of the fishery.

The presence of different management units or highly segregated groups has important implications for fishery management. In this sense, the presence of relatively isolated groups may require the need to manage the stocks separately. Now it is needed to identify possible nursery areas and small stocks in the basin. In this respect, it is advisable to use other methodologies to confirm the stable presence of different groups (nurseries and stocks). For that, other approaches such as parasitology, otolith and scale morphometry, should be applied to contribute to the correct delimitation of management units (Cadrin et al., 2013). For example, the combination of techniques such as otolith morphometry and microchemistry and scale morphometry has contributed to the delimitation of nursery areas of the migratory fish *Prochilodus lineatus* in the Río de la Plata Basin (Avigliano et al., 2017). On the other hand, it is recommended to incorporate a greater number of sampling sites into the assessments of fishery, in order to determine more accurately the spatial resolution of the segregation groups and the degree of connectivity between them (Bailey et al., 2015).

The results also call attention to the inter-jurisdictional management because the three groups studied are resources shared between Argentina-Uruguay and Argentina-Paraguay. In this sense, evaluation and management plans should consider not only the existence of different management units and the relative connectivity between them, but also the shared use of the resources.

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