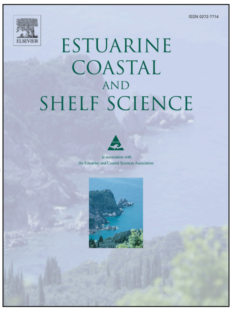

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Silversides (*Odontesthes bonariensis*) reside within freshwater and estuarine habitats, not marine environments

Esteban Avigliano[1*], Nathan Miller[2], Alejandra Vanina Volpedo[1]

[1]Instituto de Investigaciones en Producción Animal (INPA-CONICET-UBA), Facultad de Ciencias Veterinarias, Universidad de Buenos Aires (UBA), Av. Chorroarín 280 (C1427CWO), Ciudad Autónoma de Buenos Aires, Argentina.
[2]Jackson School of Geosciences, University of Texas at Austin, Austin, Texas 78712.
corresponding author: *estebanavigliano@conicet.gov.ar, Tel/Fax: +54-11-45248484

Abstract

Otolith core-to-edge Sr:Ca ratio was determined by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to analyze the salinity-habitat migration history of the silverside, *Odontesthes bonariensis*, within the Uruguay River (freshwater) and Río de la Plata Estuary (estuarine water) (Plata Basin, South America). Regular core-to-edge oscillations in Sr:Ca suggest that the silverside makes annual migrations between freshwater (<1 PSU) and brackish (>1 PSU) habitats, with no evidence of marine incursion or non-migratory individuals. Empirical equations that represent the relationship between conductivity/salinity and otolith Sr:Ca ratio were used to identify where in an otolith an individual transitioned between freshwater and brackish habitats. In most specimens, the first migration between habitats likely occurred within the first year of life. Average numbers of changes between stable Sr:Ca signatures (sites with different salinities) determined by Change-Point analysis were similar from Uruguay (8.9±3.7) River and Río de la Plata Estuary (7.5±2.5) for comparable age fish ($p<0.05$), suggesting that habitat use is similar in both collection sites.

Keywords: diadromy; microchemistry; migration; Laser ablation; residence; Pejerrey fish.

1. Introduction

Silverside *Odontesthes bonariensis* (Valenciennes, 1835) is a fish species native to Argentina, Uruguay, Brazil and Chile, and has been introduced to Europe and Asia (Avigliano and Volpedo, 2013b; Dyer, 2006). For Argentina and Uruguay, the silverside is the second economically most important fishery resource, with production marketed for local consumption as well as international export (Italy, Netherlands, Ukraine, Russia and the United States of America) (MINAGRO, 2018). Exploitation takes place mainly in the Plata Basin (especially in Uruguay River and Río de la Plata Estuary) between early spring and late autumn (April-September), coincident with upstream breeding migrations (Avigliano, and Volpedo, 2013a). The spatial distribution of the species during the rest of the year is unknown, with hypotheses ranging from permanence in delta lagoons to migration from fresh water towards brackish and/or marine waters. Avigliano and Volpedo (2013a) recently suggested that the species remains in relatively high salinity waters at the distal (southwest) margin of Plata Basin estuary and does not use the marine environment. In order to help manage production of this species, it is important to understand silverside migration and habitat use during their lifetimes.

Capture-tag-recapture studies could provide important information, but are cost prohibitive due to the vast size of the basin and the large number of tagged specimens for representative recaptures (Begg and Waldman, 1999). Over the past two decades, the chemical composition of fish otoliths (ear stones) has been widely used as a natural tag of habitat use. Since otolith calcium carbonate (aragonite) accretes regularly, and is not subsequently resorbed or otherwise altered (Campana and Neilson, 1985; Campana and Thorrold, 2001; Casselman, 1990; Elsdon et al., 2008), chemical time-series established from core-to-edge otolith transects can be used to reconstruct how aqueous environments (habitats) varied throughout ontogeny (Duponchelle et al., 2016; Halden and Friedrich, 2008). The Sr to Ca ratio (Sr:Ca) of otoliths and ambient water have been demonstrated to have a positive correlation with salinity, and thus otolith Sr:Ca is a useful indicator of habitat in environments with salinity gradients (Bath et al., 2000; Martin et al., 2004; Secor and Rooker, 2000; Sturrock et al., 2012). For silverside in the Plata Basin, empirical relationships between otolith and water Sr:Ca and conductivity (or salinity) and water Sr:Ca have previously been demonstrated (Avigliano, and Volpedo, 2013a; Avigliano et al., 2015).

Analytical techniques used to decipher ontogenetic changes via otolith chemical time-series are typically spot or line scan analysis by electron probe micro-analysis (EPMA), micro proton-induced X-ray emission (micro-PIXE) (Daros et al., 2016; Hedger et al., 2008), or laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) (Fowler et al., 2016; Kissinger et al., 2016; Morales-Nin et al., 2014). Here we use LA-ICP-MS line scans on silverside otoliths collected from specimens captured in the most productive areas of the Plata Basin, ranging from its freshwater (Uruguay River) to estuarine (Río de la Plata Estuary) reaches, in order to assess the utility of Sr:Ca for tracking silverside migration. Through relationships established between Plata Basin conductivity/salinity and Sr:Ca, we use the derived otolith Sr:Ca ratio time series to estimate the migration frequency and displacement age of silverside.

2. Materials and Methods

2.1. Study area and sample collection

After the Amazon, the Plata Basin is the second largest fluvial-marine system in the Americas, with a drainage area of 3,170,000 km^2 that spans 19 degrees of latitude (17-36°S) and portions of five South American countries (Fig. 1). Basin outflow is south-southeast to the Paraná Delta and Atlantic Ocean, involving Paraná (4,000 km long) and Uruguay (1,800 km long) as major rivers (Guerrero et al., 1997). These rivers converge in the Río de la Plata Estuary (Fig. 1), with an area of 35,000 km^2 (Guerrero et al., 1997).

Plata River Estuary is divided into three zones (inner, middle, and outer), according to different characteristics such as water quality, geomorphology and ecology (Fig. 1) (Cortelezzi et al., 2007; Piola et al., 2003). The inner or freshwater zone (A in Fig. 1) has salinity below 0.2 Practical Salinity Unit (PSU) and the coarsest sediments while, the middle or transition zone (B in Fig. 1) has salinities ranging between 0.04 and 5 PSU and fine sediments (Cortelezzi et al., 2007; Guerrero et al., 2010). The outer zone (C in Fig. 1) has highest salinities between 5 and 30 PSU and is characterized by sediments bordering a large sandy body (Cortelezzi et al., 2007; Guerrero et al., 2010). This section extends from the continental coast to the 50 m isobath and includes a salt wedge (marine water intrudes beneath the freshwater mass and lies close to the bottom). The location of the salt wedge is mainly determined by bathymetry, but it is also influenced by increasing flow rate (Avigliano, and Volpedo, 2013a; Guerrero et al., 1997). The main forcing factors in the outer zone are the strong southeasterly winds, as they homogenize the water column (Guerrero et al., 2002, 1997; Guerrero and Piola, 1997).

Fish were collected using hooks between May and September 2011 and August 2012 in the freshwater Uruguay River (Salinity = 0 PSU; 32°28.352'S-58°12.282'W) and Río de la Plata Estuary (Salinity: 0.5-1 PSU; 34°42.762'S-57°48.881'W). Salinity was taked of Piola et al. (2003) and (Guerrero et al., 2002).

Fish were kept refrigerated at 4°C until reaching the laboratory (Institute of Animal Production Research, Argentina), total length (TL) was measured and *sagittae* otoliths extracted.

2.2. Age determination, otolith selection and preparation

Otoliths were washed with Milli-Q water and dried. The left otolith of each pair was embedded in epoxy resin and sectioned transversely through the core to a thickness of 700 µm using a low speed saw (Buehler Isomet, Hong Kong, China) equipped with twin diamond edge blades and spacers. Annual growth increments in each otolith section were counted under stereomicroscopic inspection (Leica EZ4-HD, Singapore) at 40X magnification with transmitted light, while otoliths were immersed in ultrapure water. This approach for age estimation has been previously verified by López Cazorla et al. (2011) for aging *sagittae* otoliths of *O. bonariensis*. Cazorla et al. (2011) have reported a maximum age of 6 years. Only fish between 3-5 years were selected for analysis (global mean: 4.0±0.4 yrs; N = 53; Table 1) to assure that differences in fish age not confound any geographic-specific differences in elemental composition. In preparation for LA-ICP-MS analysis, otoliths were fixed to glass slides using clear epoxy resin, polished using 9 µm-grit sandpaper to eliminate small marks produced by the saw, ultrasonically cleaned (3 minutes) in Milli-Q ultrapure water, and dried in a laminar flow hood.

2.3. Determination of Sr:Ca ratios by LA-ICP-MS

Elements concentrations (^{88}Sr and ^{43}Ca) were measured by LA-ICP-MS at the University of Texas at Austin in a single analytical session, using a New Wave Research UP 193-FX fast excimer (193 nm wavelength, 4-6 ns pulse width) laser system coupled to an Agilent 7500ce ICP-MS. Otolith Ba was not considered because previous analyzes showed no relationship between Ba:Ca and Sr:Ca nor between Ba:Ca and salinity, suggesting that this element is not suitable for the study of displacements of silverside in this particular saline gradient (unpublished data).

The laser system is equipped with a large format, two-volume laser cell, for direct sampling of the ablation plume with fast (<1 s) washout times to minimize spatial carryover. The large format cell accommodated all otolith thin section mounts and standards for coordinating sampling traverses. Otoliths were scanned from cores to edges (Fig. 2), and bracketed hourly by standard measurements (USGS MACS-3, NIST 612; made in triplicate for 60-seconds). Laser ablation parameters optimized from otolith test ablations were line scans at 10 µm/s using a 25 µm spot, 60% laser power, 10 Hz repetition rate, and a He cell flow of 800 mL/min. Prior to analysis, sample and standard traverses were preablated at 60% power using a 50 µm spot moving at 50 µm/s. Laser energy densities over the analytical session averaged 1.87±0.09 J/cm^2 with 4.85% variation. The ICP-MS operated at an RF power of 1600W with an Ar carrier flow of 950 mL/min. Oxide production rate as monitored by ThO/Th for NIST 612 was 0.180%. The quadrupole time-resolved method involved integration times between 25 and 50 ms. The analytical sampling period of 0.3002 s, equivalent to a reading every 3.002 µm, corresponded to 94.9% measurement time. Time-resolved intensities were converted to concentration (ppm) equivalents using Iolite software (Univ. Melbourne), with ^{43}Ca as the internal standard and a Ca index value of 38.3 weight %. Baselines were determined from 60-s gas blank intervals measured while the laser was off and all masses were scanned by the quadrupole. USGS MACS-3 was used as the primary reference standard. Analyte recoveries for secondary standard NIST 612 averaged 99±4% for Sr (N=12; vs. GeoREM preferred values; http://georem.mpch-mainz.gwdg.de). The limits of detection (LOD) are calculated from the standard deviation of the blank. The LOD, normalized to Ca, were 0.0017 mmol/mol for Sr:Ca. Concentrations of Sr were expressed as molar ratios (element:Ca = mmol/mol) (Bailey et al., 2015; Sinclair et al., 1998).

2.4. Life history and data analysis

2.4.1. Otolith Sr:Ca-basis for classification of conductivity/salinity-habitat

The use of otolith Sr:Ca ratio as a habitat marker for silverside in the Plata Basin has previously been validated (Avigliano, and Volpedo, 2013a; Avigliano et al., 2015), where a direct relation between the Sr:Ca for otoliths and ambient water, as well as salinity over the range of 0-30 PSU has been demonstrated (Avigliano, and Volpedo, 2013a).

Because relationships between salinity and otolith Sr:Ca are often unknown, studies that analyze otolith Sr:Ca profiles often designate transition thresholds between habitats (i.e. freshwater and brackish) in relatively arbitrary ways, for example, by designating different habitats among individuals if otolith edge Sr:Ca varies beyond one (Bradbury et al., 2008; Panfili et al., 2012), or two standard deviations (Avigliano et al., 2017b; Lin et al., 2014; Tabouret et al., 2010) from the mean. More accurate interpretations about environmental displacement can be obtained for study areas where salinity and otolith Sr:Ca have been systematically studied. In this paper, transition threshold between freshwater and brackish habitats in the Plata Basin system was estimated to facilitate the interpretation of environmental conductivity/salinity variations prospectively recorded by otolith Sr:Ca time-series. The otolith Sr:Ca transition threshold was calculated using the empirical equations published by Avigliano and Volpedo (2013a):

Equation 1 (R^2=0.97) represents the quadratic relationship between the water conductivity and the Sr:Ca ratio of water in the sampling area.

$$y = 11.81\mathrm{x}^2 - 24.3\mathrm{x} + 10.57 \text{ (Eq. 1)}$$

Where *y* is the conductivity and x the Sr:Ca ratio of water.

Equation 2 (R^2=0.98) represents the linear relationship between the Sr:Ca ratio of water and otoliths for *O. bonariensis*:

$$\mathrm{x} = 0.509\mathrm{X} + 1.055 \text{ (Eq. 2)}$$

Where X is the Sr:Ca ratio of otolith and x the Sr:Ca ratio of water.

If the variable x of equation 1 is replaced by equation 2, equation 3 is obtained, which represents the relationship between the conductivity and the Sr:Ca ratio of otolith as follows:

$$y = 11.81(0.509X + 1.055)^2 - 24.3(0.509X + 1.055) + 10.57 \text{ (Eq. 3)}$$

In this work, salinity of 1 PSU was considered the boundary between freshwater and brackish habitats. According to the available literature about hydrogeology of the study area (Carol et al., 2008; Guerrero et al., 2002, 1997; Guerrero and Piola, 1997), It is assumed that this salinity is conservative enough to represent the transition between freshwater and estuary. This salinity is equivalent to a conductivity of 1.6 mS/cm in the Río de la Plata Estuary (Carol et al., 2008). By substituting these value in equation 3, a reference otolith Sr:Ca threshold of 1.02 mmol/mol with 95% of confidence interval of 0.92-1.12 mmol/mol is obtained, marking the transition between freshwater (lower values) from brackish habitats (higher values). This threshold value was plotted with otolith Sr:Ca profiles in order to visualize and analyze ontogenetic displacement patterns. In order to facilitate the interpretation of the profiles, the location of the *annuli* was identified in the graphics by measuring the distance from the core to the edge of the annuli on the ablation transect. The distances were determined on images taken with a stereomicroscope (Leica EZ4-HD, Singapore) at 40X magnification using Image-Pro Plus 4.5 software.

Sr:Ca ratio of the core was compared between study sites using t-test to assess and discuss whether the spawning areas of both groups are similar in conductivity/salinity. Data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests respectively.

2.4.2. Quantification of changes in the life history

Change-Point analysis (CPA) was used to quantify the number of changes between sites with different salinities (stable Sr:Ca signatures) recorded in otolith LA-ICP-MS transects (Avigliano et al., 2017b; Hegg et al., 2015; Shrimpton et al., 2014). We assume that Sr:Ca variations measured from core-to-edge in each transect represent different hydrochemical (salinity) areas encountered by the fish during its life (Freshwater et al., 2015; Hegg et al., 2015). These changes do not necessarily imply movements between freshwater and brackish habitats, because stable Sr:Ca signatures change can be found within these habitats (Avigliano et al., 2017a; Freshwater et al., 2015; Hegg et al., 2015). CPA determined whether there had been a change in the underlying process that generated the sequence of events and, if so, identified where the change occurred. The procedure used to perform a CPA comprises a combination of cumulative sum charts and bootstrapping to detect changes (Taylor, 2000). The analysis provides both confidence levels and confidence intervals for each change (95% confidence is used for all confidence intervals) and it is robust to issues of non-normality (Shrimpton et al., 2014). The Change-Point Analyzer 2.3 software package (Taylor, 2000) was used for CPA.

Following Walther et al. (2011) and Shrimpton et al. (2014), the variable "number of changes" was compared between locations using the non-parametric Kruskal Wallis test because the response was not normally distributed with heterogeneous variance (Shapiro-Wilk, $p < 0.05$; Levene, $p < 0.05$), even after transformation log (x+1). Prior to Kruskal Wallis test, we evaluated the association between the number of Sr:Ca changes and the fish age using ANCOVA (number of changes as a variable and age as covariate) and Spearman correlation. The existence of a co-variation with the age, could affect the interpretation of the variable number of changes of the Sr:Ca ratio.

3. Results

3.1. Otolith core-to-edge variations in Sr:Ca

Otolith cores had Sr:Ca values of 1.25±0.45 (0.50-2.81) and 1.24±0.30 (0.69-1.79) mmol/mol for the Uruguay River and Río de la Plata Estuary specimens, respectively, with no significant differences between sites ($T_{df=51}$=-0.1; p=0.9). The percentage of individuals showing a Sr:Ca ratio in the core higher to the threshold was 71 and 70% for the Uruguay River and Río de la Plata Estuary, suggesting permanence in areas with salinity>1 PSU.

The range and average of otolith core-to-edge Sr:Ca ratios were similar between Uruguay River silverside specimens (range: 0.21 to 3.64 mmol/mol; mean ± SD: 1.35 ± 0.40 mmol/mol) and Río de la Plata Estuary silverside specimens (range: 0.23 to 3.54 mmol/mol; mean ± SD: 1.32 ± 0.35 mmol/mol).

Core-to-edge profiles from both collection localities exhibit cyclical Sr:Ca oscillations (Fig. 3 a-l), which appeared to coincide with otolith annuli, suggesting seasonal migrations between areas with different salinity (Fig. 3). No individuals showed a stable Sr:Ca throughout core-to-edge transects, which would potentially indicate sedentary behaviour related to the same salinity (non-migrant) throughout its entire life.

For silverside from both collection sites, most core-to-edge transects were dominated by intervals with elevated Sr:Ca levels consistent with brackish habitats (Fig. 3), suggesting that the species spends a greater proportion of time in this environment. Proportion of the core-to-edge transects that was above the threshold value (brackish / freshwater *100) was 81% for Uruguay River and 82% for Río de la Plata Estuary. Migratory cycles occasionally involve both freshwater and brackish habitats (see stable Sr:Ca signatures in Fig. 3 a-d and g-j), although in some cases large oscillations observed in the Sr:Ca ratio correspond only to brackish values (see stable Sr:Ca signatures in Fig. 3 e-f and k-l).

According to stable Sr:Ca signatures identified by the CPA, in ~80% of silverside specimens (N=42/53), the first migration between freshwater and brackish habitats occurred within the first year of life (Fig. 3 a, b, f, g and h); later migrations occurred in four individuals from the Río de la Plata Estuary (~21%, N=4/19) (Fig. 3 c-f) and four from the Uruguay River (~11.7 %, N=4/34) (Fig. 3 i-l).

3.2. Quantification of changes in the life history

The average number of significant Sr:Ca shifts in otolith core-to-edge transects was 8.88±3.72 (range: 3-16) for Uruguay River, and 7.45±2.52 (range: 8-13) for Río de la Plata Estuary (global mean: 8.15±3.72). Because ANCOVA indicates no co-variation between specimen age and number of Sr:Ca changes (F=1.91; p> 0.05), it was not necessary to make age corrections in variables number of changes. The Mann-Whitney test also shows no significant differences in the number of changes between the two sites (W=448, p=0.1).

4. Discussion

Otolith microchemistry has been widely used to study the migration of teleost fish (Avigliano, et al., 2018; Avigliano, and Volpedo, 2016; Panfili et al., 2012; Tzeng et al., 2002). Several diadromous species have been studied in Latin America (Avigliano, and Volpedo, 2016; Condini et al., 2016; Daros et al., 2016), and the relationship between water Sr:Ca ratios and salinity in large estuaries has been reported (Albuquerque et al., 2012; Avigliano, and Volpedo, 2013a). In this study, otolith Sr:Ca variations have been useful for documenting cyclic migratory patterns between freshwater and estuarine water for *O. bonariensis* silverside.

Otolith elemental concentrations can be influenced by a diversity of factors, ranging from environment (salinity, temperature) (Brown, and Severin, 2009; Elsdon, and Gillanders, 2003; Martin et al., 2004), genetic coding (Barnes and Gillanders, 2013) and physiology (growth rates, metabolic changes) (Kalish, 1991; Radtke and Shafer, 1992; Sturrock et al., 2014). With some exceptions, otoliths for diadromous species generally demonstrate a positive relationship between Sr concentration and salinity, although the magnitude of Sr incorporation may differ between species (Brown, and Severin, 2009). While incorporation of Sr in otoliths for certain species such as *Argyrosomus japonicus* may be genetically influenced, the relationship between Sr:Ca and salinity remains positive (Barnes and Gillanders, 2013). In species where the larvae depends on the yolk sac for a relatively long time, for example in salmonids such as *Oncorhynchus mykiss* and *Salmo trutta*, it has been shown that the Sr:Ca ratio of the otolith is not only influenced by salinity, but could be derived from the yolk sac and should reflect the mother's environment (Kalish, 1990; Liberoff et al., 2014; Rohtla et al., 2012).

There are few studies that related salinity with the levels of Sr:Ca of otolith (Avigliano, and Volpedo, 2013a; Kanai et al., 2014; Secor et al., 1995; Shrimpton et al., 2014). For example, Kanai et al. (2014) experimentally estimated the relationship between Sr:Ca of otolith and salinity for *Zenarchopterus dunckeri* and have subsequently used this function to study displacements of wild individuals. Secor et al. (1995) performed similar approximations on the striped bass, *Morone suxntilis*, through laboratory tests and later making inferences about wild specimens. The present study benefits from previous documentation of otolith Sr:Ca in *O. bonariensis* and its relationship to habitat salinity in the Plata Basin (Avigliano, and Volpedo, 2013a). These relationships enable us to make some inferences about the silverside life history.

We are aware that our freshwater-brackish water threshold of 1 PSU is subjective and that its modification could influence the interpretation of some results. Because the confidence interval was relatively narrow, the results should not change significantly. However, setting a fixed threshold allows a common basis for comparison that facilitates the interpretation of silverside core-to-edge otolith profiles. If the transition threshold had been calculated as the mean of the Sr:Ca ratio of the edge of otolith of fish caught in freshwater (Uruguay River) by an addition of 1 or 2 standard deviations (Avigliano et al., 2017a,b; Bradbury et al., 2008; Lin et al., 2014; Panfili et al., 2012; Tabouret et al., 2010), values of 0.98 and 1.21 mmol/mol would be obtained respectively. It is important to clarify that the estimations on Sr:Ca ratio of the edge of otolith of fish caught in freshwater were performed using the last 10 μm of the transect and made only for comparative and discussion purposes. These values include the threshold estimated in this work of 1.02 mmol/mol and have a span similar to the confidence interval obtained in our study (0.92-1.12 mmol/mol). Despite the similarity in thresholds obtained between these two approaches, only the former is directly linked to salinity and thus is a superior proxy of salinity habitat.

Using the better-constrained otolith Sr:Ca proxy of salinity habitat, we results suggest that silverside typically migrate twice annually between areas with different salinity (Fig. 3). None of the studied individuals had otolith Sr:Ca values consistently above or below the 1 PSU threshold, arguing against a non-migratory lifestyle for the silveside. Using the categorizations suggested by Elliott et al. (2007) for migrating fish, the silverside would fit in the "estuarine migrant" or "freshwater migrant" classifications, because both options refer to possible reproductive events and displacements between both environments. Although it should be noted that the data suggest that the use of the estuarine environment (> 80%) predominates over the freshwater environment.

Using the microchemistry of whole otolith, Avigliano et al. (2014) found significant differences in the Ba:Ca ratio of otolith (but not Sr:Ca) among the same collection sites of this study and detected some significant differences in same otolith morphometric variables. The Ba:Ca differences could be related to upwelling of deeper Ba enriched water to the estuary location

(Ferguson et al., 2011; Webb et al., 2012). Based on these results, Avigliano et al. (2014) suggested that habitat use could differ between study sites, with predominantly migratory individuals in Uruguay River and residents in the Río de la Plata Estuary. In concordance, we also find comparable ranges and average values of Sr:Ca ratios between sites. However, we find no significant differences between the number of changes, consistent with similar use of habitat for both collection sites. Whole otolith analysis would average a lifetime of biomineralization and thus only possibly reflect the salinity of the dominant habitat experienced during the majority of growth. This fact could explain the differences of between sites reported by Avigliano et al. (2014).

The reference value of Sr:Ca for the transition between estuary and sea also varies among species, although it ranges typically from ~4.3 to ≥8 mmol/mol (Avigliano et al., 2017b; Bradbury et al., 2008; Daros et al., 2016). Considering the highest otolith Sr:Ca ratios (3.48 and 3.64 mmol/mol for Río de la Plata Estuary and Uruguay River, respectively), the results suggest that species does not use the marine environment. This finding is consistent with the summer distribution of the species in brackish waters, as suggested by Avigliano and Volpedo (2013a). On the other hand, there is a population that inhabits in an endorheic lake from Patagonia (Argentina) with salinity that varies between 27-35 PSU, where otolith Sr:Ca values exceeds 7 mmol/mol (Avigliano et al., 2015), being consistent with the previous statements.

It is important to highlight that, especially on the sea front (outer section of the Río de la Plata Estuary), the distribution of salinities may suffer seasonal variations that may in turn be influenced by the wind, tides and pulses of freshwater flooding (Guerrero et al., 1997; Guerrero and Piola, 1997). These variations could hinder the interpretation of the results if it is attempted to relate the salinity to the geographical location in these areas. However, the results of this work and the previous reports (Avigliano, and Volpedo, 2013a) do not support the hypothesis of the use of high salinity environments, which correspond, due to hydrographic conditions, to the less stable area of the estuary in terms of influences of tidals, wind, etc. (Guerrero et al., 1997; Guerrero and Piola, 1997).

Based on otolith age estimates from *annuli* counting and distinct core-to-edge Sr:Ca variations, the first migration between freshwater and brackish habitats occurs within the first year of life in most silverside (~80%). Sexual maturation of silverside may occur before the first year of life (total length: 100-140 mm) (Burbidge et al., 1974; Calvo and Dadone, 1972). Thus, the first migration may be linked to the first reproductive event. However, additional studies are required to evaluate possible roles of seasonal changes in diet or other factors such as wintering controlling the first migration.

In most collected fish, Sr:Ca values indicative of brackish use were predominately observed in the first part of their life (70%), which may suggest spawning or hatching in this habitat. Because the eggs of the silverside are adherent (stick to the riverbed or vegetation), drift downstream caused by the current of the basin could be discarded. Nevertheless, even though this is true, it has been observed that in a Pampean lagoon (Chascomús, Argentina), the silverside can spawn in shallow waters and with very compact sediments where there are no aquatic plants (Ringulet, 1943). If this also happens in the Plata Basin, it is possible that a proportion of eggs laid in non-vegetated fresh water environments will move towards the estuary due to the current. Furthermore, it is necessary to discard first the existence of an effect of the maternal habitat in the core of the otoliths (Kalish, 1990). Because fish migrate upstream to spawn (Avigliano, and Volpedo, 2013a) it is possible that females could retain estuarine values of Sr:Ca that they transfer to their offspring and consequently their otoliths, as it happens in other groups such as salmonids (Kalish, 1990; Liberoff et al., 2014). Once again, additional studies are required to determine if the Sr:Ca ratio of the first portion of the otolith core is a safe indicator of the spawning or hatching site.

In conclusion, the integration of water chemistry (conductivity/salinity) with otolith aging and core-to-edge Sr:Ca reveals several new aspects of silverside life history. According to previous investigations that report the presence of the species in fresh water only in the cold months of the year (Avigliano, and Volpedo, 2013a; Avigliano and Volpedo, 2013b), complemented by the results of this work, it can be suggested that silversides make annual migrations between areas with different salinities. It does not necessarily imply annual changes between freshwater and estuarine habitats, being able to migrate cyclically within the estuarine environment. Apparently, the silverside would not occupy marine habitats and the first migration between freshwater and estuarine habitats would occurs within the first year of life in most fish. These advances in understaning ontogenetic habitat use by this species may contribute to new management and administrative policies that will ensure the sustainability of fisheries.

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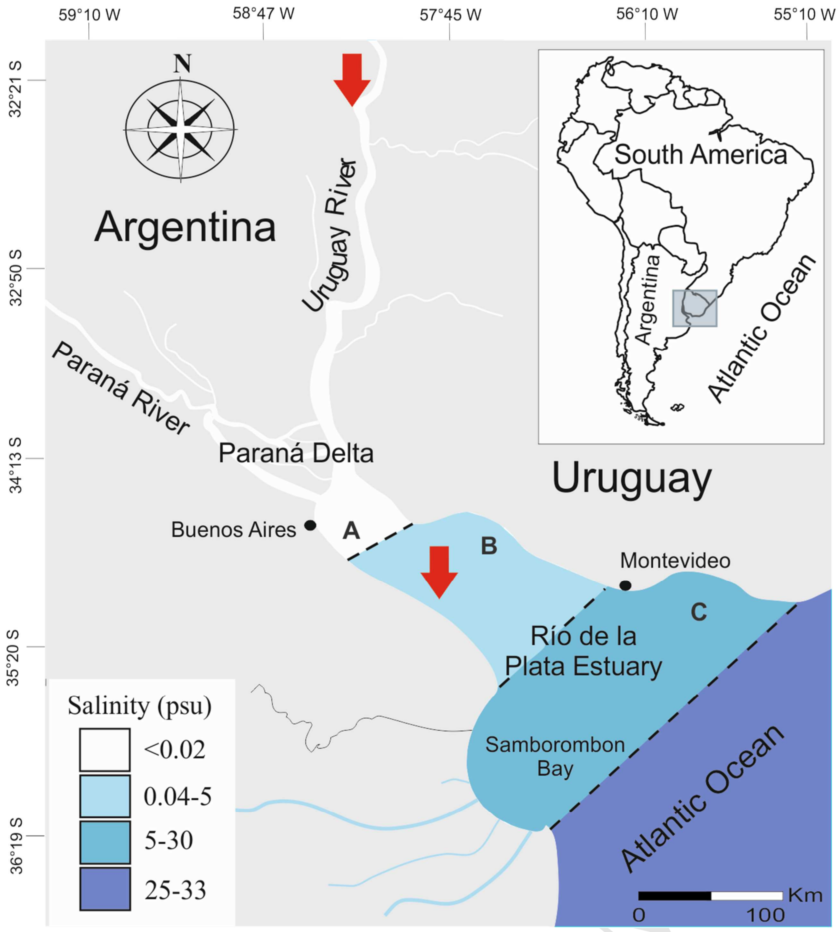
Fig. 1 Silverside *Odontesthes bonariensis* sampling sites within the Uruguay River and Río de la Plata Estuary (red arrows). A, inner section (freshwater habitat); B, middle section (brackish habitat); and C, outer section of the Río de la Plata Estuary (estuarine–marine habitat).

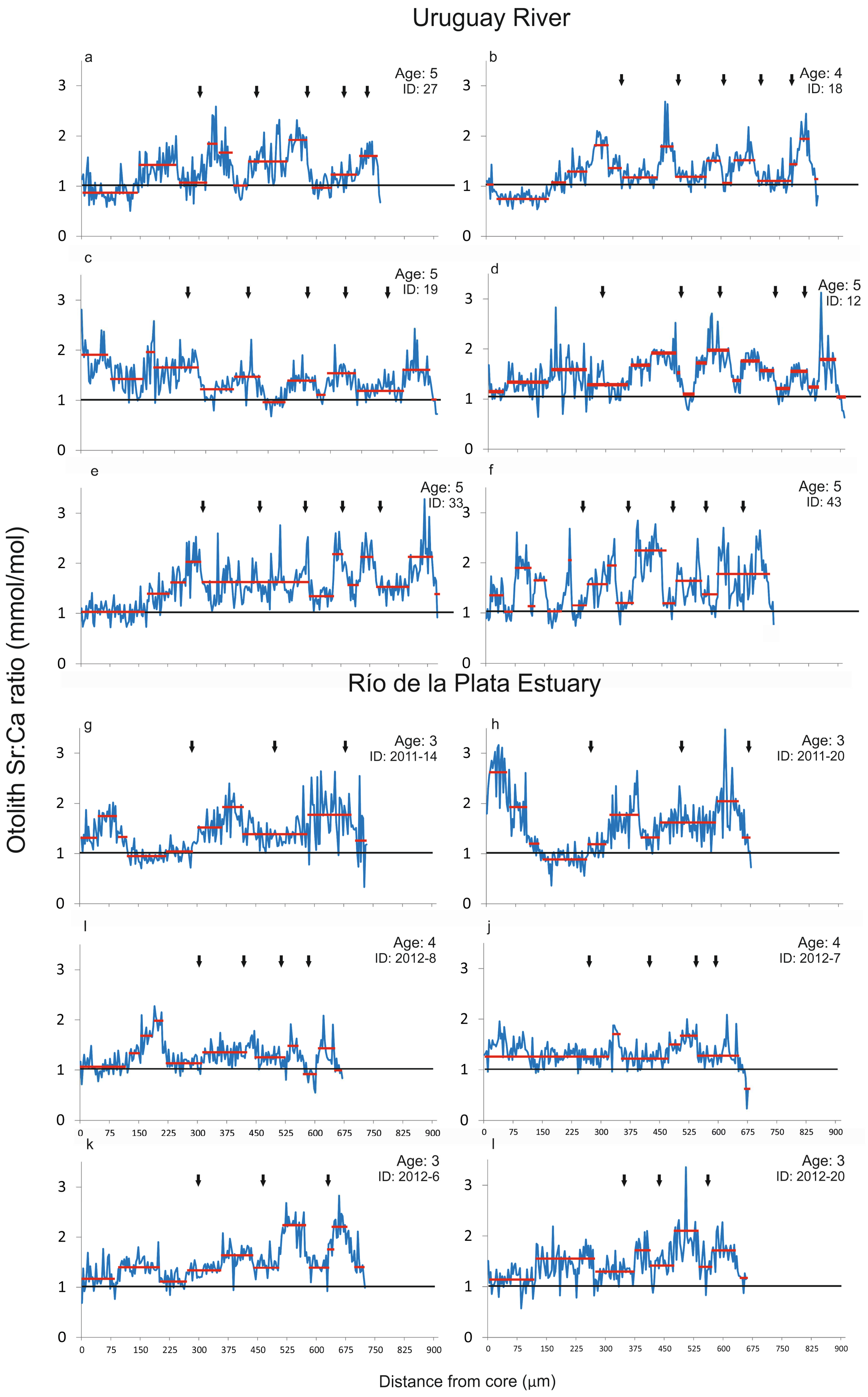
Fig. 2 Otolith section of a 5-year old *Odontesthes bonariensis* from Uruguay River showing the core-to-edge laser ablation transect. The white arrows indicate the *annuli*, while dotted rectangle indicates the analysis transect. Magnification=40X.

Fig. 3 Core-to-edge otolith Sr:Ca profiles for representative individuals of *Odontesthes bonariensis* from Uruguay River (a-f) and Río de la Plata Estuary (g-l) (age: 3-5). Black vertical arrows mark otolith *annuli* (age) and black horizontal indicate interpreted transition between freshwater and brackish habitat (~1 PSU). Horizontal lines stable signatures identified by change-point analysis.

Table 1 Descriptive statistics of individuals from each sampling site. N: sample size; SD: standard deviation.

	Age (year)		Total length (cm)		
	mean±SD	range	mean±SD	range	N
Uruguay River	4.8±0.36	4-5	32.7±1.81	30.9-36.2	34
Río de la Plata Estuary	3.2±0.43	3-5	30.5±1.46	27.1-33.6	19
Total					


59°10 W
58°47 W
57°45 W
56°10 W
55°10 W
32°21 S
32°50 S
34°13 S
35°20 S
36°19 S
N
Argentina
Uruguay River
Paraná River
Paraná Delta
Buenos Aires
A
B
C
Uruguay
Montevideo
Río de la Plata Estuary
Samborombon Bay
Atlantic Ocean
South America
Argentina
Atlantic Ocean
Salinity (psu)
<0.02
0.04-5
5-30
25-33
Km
0
100


Uruguay River
a
Age: 5
ID: 27
b
Age: 4
ID: 18
c
Age: 5
ID: 19
d
Age: 5
ID: 12
e
Age: 5
ID: 33
f
Age: 5
ID: 43
Río de la Plata Estuary
g
Age: 3
ID: 2011-14
h
Age: 3
ID: 2011-20
I
Age: 4
ID: 2012-8
j
Age: 4
ID: 2012-7
k
Age: 3
ID: 2012-6
l
Age: 3
ID: 2012-20
0
1
2
3
0
75
150
225
300
375
450
525
600
675
750
825
900
Otolith Sr:Ca ratio (mmol/mol)
Distance from core (µm)


100 µm
175 µm

Highlights

Sr:Ca transition threshold between habitats was 1.02 mmol/mol

First migration likely occurred within the first year of life

Silverside makes annual migrations between contrasting salinity environments

Silverside not uses marine habitats

Silverside uses freshwater and brackish habitats