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Combined use of otolith microchemistry and morphometry as indicators of the habitat of the silverside (*Odontesthes bonariensis*) in a freshwater–estuarine environment

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ABSTRACT

The silverside *Odontesthes bonariensis* is a highly salt-tolerant freshwater–estuarine species found in the Southern Hemisphere. The objective of the present study was to perform a simultaneous evaluation of the morphometry and microchemistry (Sr/Ca and Ba/Ca ratios) of the otoliths of this species to provide information on its displacements and to identify fish stocks. The morphometry and microchemistry of fish otoliths from three regions of the lower section of the Plata Basin (Uruguay River, Paraná River Delta and De la Plata River estuary) were compared. The results showed that otoliths of fish from the southern lower section of the Plata Basin tended to be circular in shape and had a lower ratio of sulcus perimeter to total otolith perimeter than those fish collected in the north of the studied region. There is a trend to increase the otolith Sr/Ca ratio with increasing water conductivity along a latitudinal (north–south) gradient, while the Ba/Ca ratio decreased significantly along such gradient. Present results suggest that the silverside population may be geographically divided along the north–south axis of the lower section of the Plata Basin.

1. Introduction

Otoliths are complex polycrystalline structures composed of calcium carbonate (approximately 96%) and trace elements immersed in a protein matrix (Campana et al., 1997). These structures are located in the inner ear of fishes and have a role in hearing and maintenance of equilibrium (Popper and Zhongmin, 2000). In the last decade, the analysis of the otolith chemical composition has been increasingly used to study fish displacements and identify fish stocks (e.g. Kraus and Secor, 2004; Schuchert et al., 2010; Tabouret et al., 2010).

The trace metals accumulated in the otoliths come from water and food (Limburg, 1995; Milton and Chenery, 2001; Ranaldi and Gagnon, 2008), with strontium (Sr) and barium (Ba) being the most studied (Elsdon and Gillanders, 2005; Hamer et al., 2006; Tabouret et al., 2010). Otoliths have a variable Sr concentration, which is mainly due to the chemical composition of the water where fish lives, and secondarily to environmental temperature (Campana, 1999; Wells et al., 2003). The otolith Sr was found to be positively correlated with water salinity (Kraus and Secor, 2004), while the otolith Sr:Ca ratio was shown to be positively and negatively correlated with water temperature above and below 10 °C, respectively (Campana, 1999). On the contrary, the otolith Ba concentration is negatively associated with water salinity (Miller, 2011). The simultaneous use of the otolith Sr/Ca and Ba/Ca ratios has been recently applied to investigate the displacement of catadromous and diadromous species from a fluvio-marine environment (e.g. Tabouret et al., 2010; Feutry et al., 2012). Tabouret et al. (2010) determined movement patterns of European eel and Feutry et al. (2012) demonstrated this for three species of the genus *Kuhlia* between water masses of different salinity.

However, these ratios and the otolith morphometry have never been studied simultaneously for freshwater–estuarine species displacing along environments of different salinity.

The lower section of the Plata Basin includes the Uruguay River, Paraná River Delta and De la Plata River estuary. This environment is characterized by a pronounced salinity gradient (Avigliano and Volpedo, 2013) and great fish diversity (García et al., 2010). The De la Plata River estuary can be divided into three regions: inner, middle and outer (Fig. 1). The salinity is lower than 0.2 PSU in the inner section, while it ranges from 0.04 to 10 PSU and from 5 to

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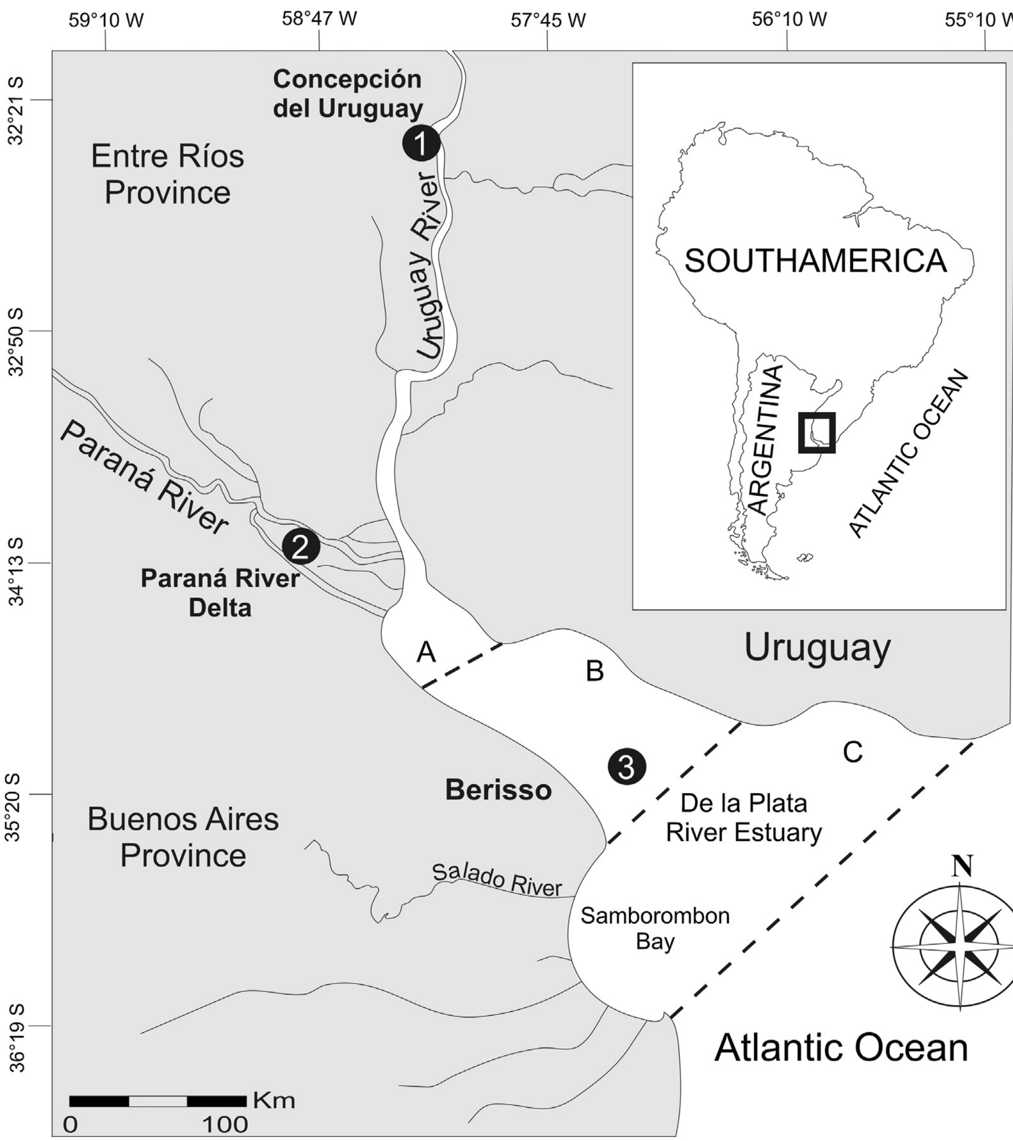

Fig. 1. Lower section of the Plata Basin. Solid black circles indicate the sampling sites: 1, Uruguay River; 2, Paraná River Delta; and 3, De la Plata River Estuary. (A) Inner region; (B) middle region; and (C) outer region of the De la Plata River.

25 PSU in the middle and outer regions, respectively (Urien, 1966, 1967).

The silverside *Odontesthes bonariensis* (Valenciennes, 1835) is one of the most important species for commercial and sport fishing in the region. It is very tolerant to high salinity concentrations (Solari et al., 2009; Kopprio et al., 2010). During the reproductive season, between April and September, it moves upstream from the De la Plata River estuary to the lower basins of the Paraná and Uruguay Rivers (freshwater environments). The species possibly remains in the De la Plata River estuary during the rest of the year (Avigliano and Volpedo, 2013). The first sexual maturation coincides with the first year of life (total length between 13 and 16.5 cm)(Grosman et al., 2001; Barros et al., 2004). This fish is generally zooplanktivorous and has thermolabile sex determination (Kopprio et al., 2010).

The objective of the present study is to perform a simultaneous evaluation of the morphometry and microchemistry (Sr/Ca and Ba/Ca ratios) of the saccular otolith of the silverside (*O. bonariensis*), a highly salt-tolerant species, to provide information on its displacements and to identify potential fish stocks.

2. Materials and methods

2.1. Fish sample collection

A total of 348 fish were collected with sport fishing gear between May and September 2010, 2011 and 2012: 45 in the Uruguay River (near the locality of Concepción del Uruguay, Entre Ríos Province), 183 in the Paraná River Delta and 120 in the middle section of De la Plata River (estuary environment, Buenos Aires Province) (Fig. 1). The animals were measured (total length in cm) and stored at −4 °C until the removal of the saccular otoliths.

2.2. Otolith morphometry

To avoid possible year-class effects on trace element composition of saccular otoliths, fish with a total length of 25–35 cm were selected (Uruguay River, $N = 45$; Paraná River Delta, $N = 72$; De la Plata River estuary, $N = 33$). The internal face of the right saccular otoliths were photographed with a digital camera attached to a stereoscopic microscope. The following morphometrical variables

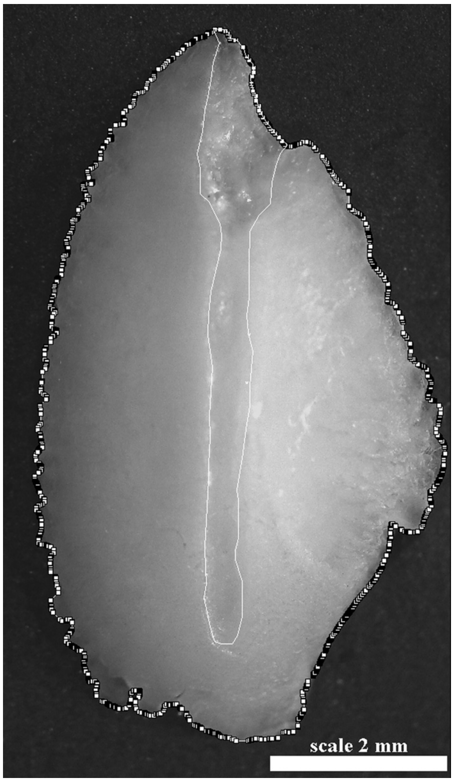

Fig. 2. Saccular otolith of *Odontesthes bonariensis* from Paraná River Delta. Solid line, sulcus perimeter (SP) and dotted line, otolith perimeter (OP).

of the right otoliths were recorded with an image processor (Image ProPlus 4.5®): otolith length (OL), otolith width (OW), otolith perimeter (OP), and sulcus perimeter (SP) in mm; otolith surface (OS), and sulcus surface (SS) in mm^2. The morphometric indexes OW/OL, SS/OS and SP/OP were calculated. The OW/OL index was proposed by Volpedo and Echeverría (2003) and indicates the tendency in the otolith shape (circular or elongate). The SS/OS index was proposed by Volpedo et al. (2008) and estimates the percentage of the otolith surface occupied by the sulcus, an area connecting the otolith with the saccular macula, through which information is sent to the brain (Kaufman et al., 1993). The SP/OP index was used for the first time in this work and indicates the smoothness of the sulcus margin relative to that of the otolith margin (Fig. 2).

2.3. Elemental otolith chemistry

A total of 75 right saccular otoliths of silversides (total length of 25–35 cm) from the Uruguay River (N = 25), Paraná River Delta (N = 25) and De la Plata River estuary (N = 25) were randomly selected.

The otoliths were weighed in an analytical balance (precision: 0.001 g) and then digested with 50% nitric acid. Sr (407.771 nm) and Ba (233.527 nm) concentrations were determined using inductively coupled plasma-atomic emission spectrometry (ICP-OES, Perkin Elmer Optima 2000 DV optical emission spectrometer) (detection limits: 10 and 8 ppb for Sr and Ba, respectively). Ca concentration was estimated by the EDTA volumetric method (APHA, 1993). All measurements were performed in triplicate and the otolith Sr/Ca and Ba/Ca ratios were then calculated.

2.4. Elemental water chemistry

Water samples were collected on August 2011 in the Uruguay River, between April 2010 and October 2011 in the Paraná River Delta and between September 2011 and May 2012 in De la Plata River estuary (Buenos Aires Province) (Fig. 1). Conductivity was determined in situ using a Hanna HI9033W portable meter. Samples were preserved by the addition of 2 ml of nitric acid per liter of water (APHA, 1993; 3010 B method) and maintained at 4 °C until further analysis. Sr and Ba concentrations were determined using ICP-OES (EPA, 1994; 200.7 Method), and Ca concentration was estimated by the EDTA volumetric method (APHA, 1993). Water Sr/Ca and Ba/Ca ratios were then calculated.

2.5. Statistical analysis

Analysis of covariance (ANCOVA) was used to determine the effect of otolith weight on the magnitude of the following variables: OW/OL, SS/OS and SP/OP indexes, and otolith Sr/Ca and Ba/Ca ratios. These variables were significantly correlated to otolith weight and were corrected for the effect of otolith weight, using the common within-group slope (b) for the variable on otolith weight (e.g. Cardinale et al., 2004; Galley et al., 2006; Burke et al., 2008). They were then compared among sampling sites using one-way analysis of variance (ANOVA), followed by the Bonferroni test (Sokal and Rohlf, 1995). The data related to otolith morphometry and microchemistry were subjected to discriminant analysis and dispersion analysis, respectively. Data were analyzed with the statistical software SPSS® and InfoStat®.

3. Results

3.1. Otolith morphometry

The OW/OL index differed significantly between fish collected in the Uruguay River and those from the De la Plata River estuary (p = 0.0001), while it was intermediate for fish collected in the Paraná River Delta (Table 1). The SS/OS and SP/OP indexes were similar between silversides from the Paraná River Delta and De la Plata River estuary (p = 0.18), but differed significantly between these fish groups and fish from the Uruguay River (p = 0.0001) (Table 1). Discriminant analysis showed a separation of values corresponding to the Uruguay River, which were positioned on the positive part of the first canonical axis, from those corresponding to the other sampling sites, which tended to cluster toward the negative direction (Fig. 3).

3.2. Elemental otolith chemistry

There is a slight trend to augment the otolith Sr/Ca ratio with increasing water conductivity along a latitudinal (north–south) gradient (Fig. 4A). The otolith Ba/Ca ratio of fish collected in De la Plata River estuary differed significantly from that of silversides

Table 1
Otolith morphometric indexes (mean ± standard deviation) for the studied sampling sites. OL (otolith length), OW (otolith width), OP (otolith perimeter), SP (sulcus perimeter), OS (otolith surface), SS (sulcus surface), N (sample number). Different letters indicate statistically significant differences (p < 0.001).

	N	OW/OL	SS/OS	SP/OP
Uruguay River	45	0.58 ± 0.03^{a}	0.18 ± 0.01^{a}	0.73 ± 0.03^{a}
Paraná River Delta	72	0.59 ± 0.02^{ab}	0.16 ± 0.01^{b}	0.66 ± 0.02^{b}
De la Plata River estuary	33	0.60 ± 0.03^{b}	0.16 ± 0.02^{b}	0.65 ± 0.03^{b}

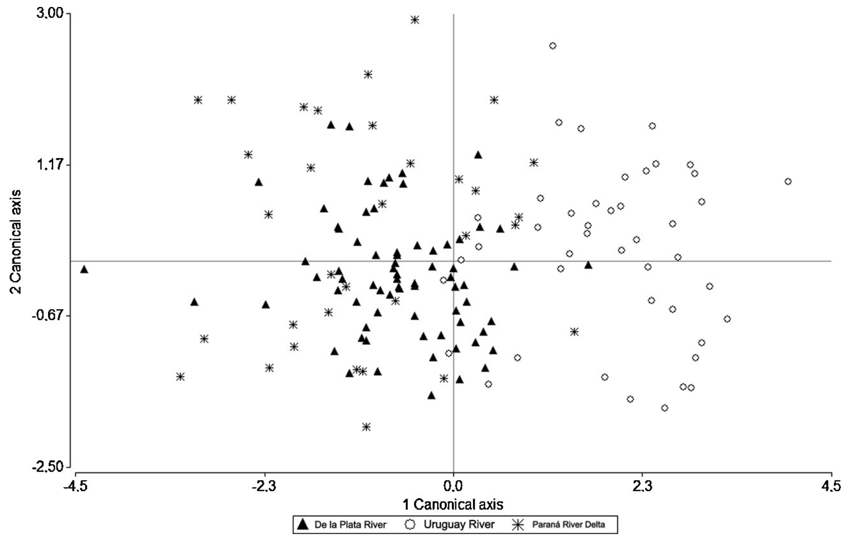

Fig. 3. Discriminant analysis of the OW/OL, SP/OP and SS/SP indexes for the studied sampling sites. Uruguay River ($N = 45$); Paraná River Delta ($N = 72$); De la Plata River estuary ($N = 33$).

collected in the Uruguay River and Paraná River Delta ($p = 0.0001$), while it was similar between these fish groups ($p = 0.1$) (Fig. 4B). However, the ratio tended to decrease from the Uruguay River toward the estuary. The dispersion analysis showed overlapping values of the otolith Sr/Ca and Ba/Ca ratios corresponding to the Paraná River Delta and Uruguay River, while values corresponding to De la Plata River estuary were grouped toward the lowest values of the horizontal axis (Fig. 5).

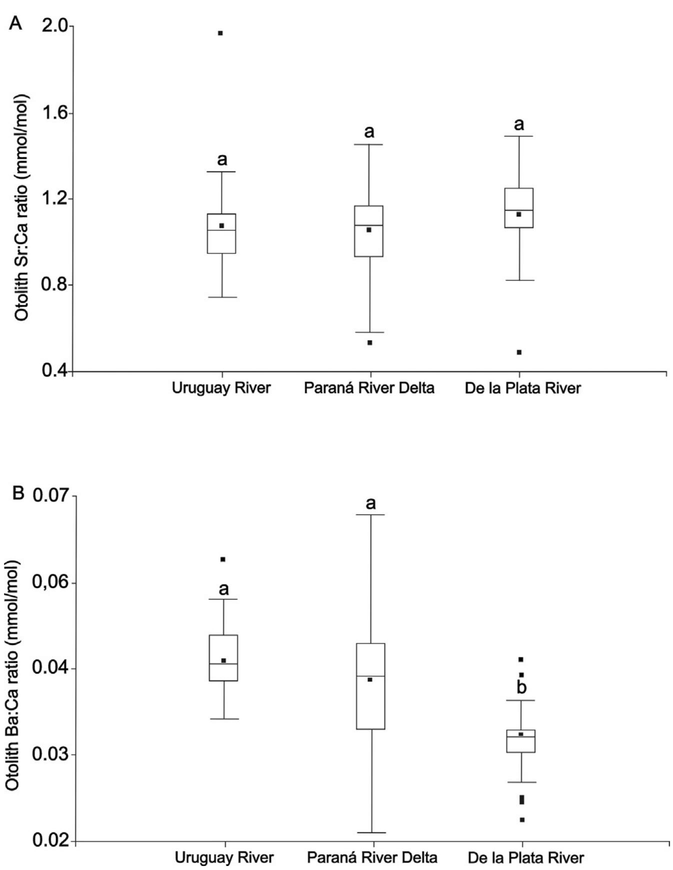

Fig. 4. Otolith Sr/Ca (a) and Ba/Ca (b) ratios for the studied sampling sites. Different letters indicate statistically significant differences ($p < 0.05$). Bars indicate the standard deviation.

3.3. Elemental water chemistry

Water Sr/Ca ratio and water conductivity from Uruguay River, Paraná River Delta and the De la Plata River estuary is reported in Table 2. The concentration of Ba was below the detection limit of the equipment used (8 ppb).

4. Discussion

The otoliths from De la Plata River estuary were elongated along the anterior-posterior axis, while those from Uruguay River tended to be circular in shape (Table 1). The tendency toward otolith circularity (higher OW/OL index) in association with increased water conductivity and salinity has been previously reported for *O. bonariensis* by Avigliano et al. (2012). Sulcus area and perimeter (SS/OS and SP/OP) were higher in otoliths of fishes of the Uruguay River (Table 1). Silverside migrates every year for reproduction from Samborombon Bay (estuarine environment) to estuarine and freshwater areas (Avigliano et al., 2012). In this aspect, fishes in Uruguay River migrate longer distances. Taking into account that sulcus area connects directly to the saccular macula of the otolith (Kaufman et al., 1993), this could explain the high development of the sulcus in relation to the otolith.

On this basis, the differences observed in the morphometric indexes of fish collected in the Uruguay River and in the De la Plata River estuary, may be related to differences in water chemistry and salinity of these areas, and their migratory behavior.

Present results indicate an increase in water conductivity and Sr/Ca ratio from the Uruguay River toward the De la Plata River estuary (Table 2). The otolith Sr/Ca ratio (Fig. 4A) followed the same north–south gradient as observed for water Sr/Ca ratio (Table 2). On the other hand, otolith Ba/Ca ratio tended to decrease with increasing water salinity (Fig. 5). The results concerning the otolith Sr/Ca and Ba/Ca ratios are consistent with those found in otoliths of fishes from the Northern Hemisphere, such as the estuarine species *Morone americana* (Kraus and Secor, 2004) and the catadromous species *Anguilla anguilla* (Tabouret et al., 2010).

Trace elements incorporated within the otolith matrix reflect the characteristics of water masses when salinity or conductivity and the Ba/Ca and Sr/Ca ratios in the environment remain relatively

Table 2
Water Sr/Ca ratio and conductivity for the Paraná River Delta and De la Plata River estuary (Buenos Aires Province, Argentina), ordered by date. X ± SD (mean ± standard deviation).

	Water Sr/Ca ratio (mmol mol^{-1})			Water conductivity (μS m^{-1})		
	Uruguay River	Paraná River Delta	De la Plata River estuary	Uruguay River	Paraná River Delta	De la Plata River estuary
April 10	–	1.01	–	–	106	–
June 10	–	1.42	–	–	94	–
July 10	–	1.02	–	–	40	–
Aug. 10	–	1.37	–	–	127	–
Sept. 10	–	0.66	–	–	165	–
Nov. 10	–	0.7	–	–	173	–
Aug. 11	0.51;0.70*	–	–	56.10;57.11*	–	–
Sept. 11	–	–	1.62	–	–	512
Oct. 11	–	1.41	–	–	116	–
Dec. 11	–	–	1.85	–	–	452
May. 12	–	–	2.85	–	–	331
Mean	0.60	1.08	2.11	56.60	117.63	431.67
SD	0.13	0.33	0.65	0.72	44.66	92.20

* In this simple site two water samples were taken, one in Argentinian and the other in Uruguayan coasts.

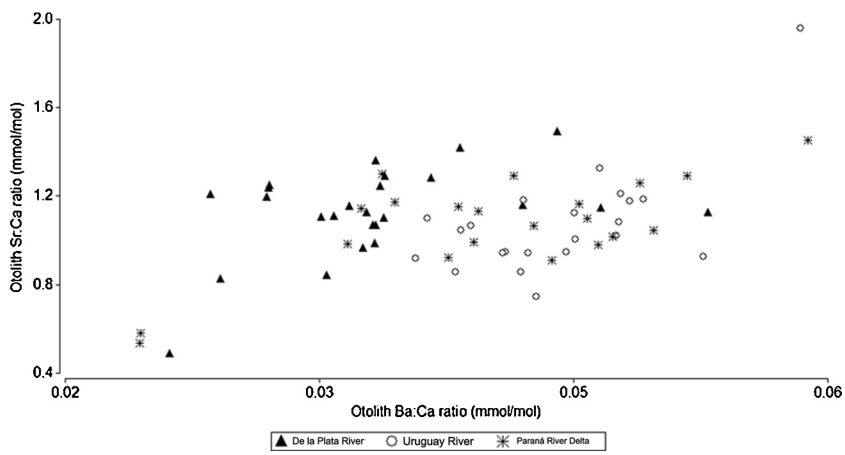

Fig. 5. Association between otolith Sr/Ca and Ba/Ca ratios for the studied sampling sites. Uruguay River (N = 25), Paraná River Delta (N = 25) and De la Plata River estuary (N = 25).

constant over time and the time spent by fish in a given area is long enough (Secor et al., 1995; Avigliano et al., 2012). During April and May some silversides undergo displacements from the Samborombón Bay, an estuarine environment with high salinity levels. They remain in the bay throughout the summer (Avigliano and Volpedo, 2013), from where some move until November to freshwater environments, namely, the middle and upper sections of the De la Plata River estuary, and Uruguay and Paraná Rivers (Fig. 1). The time spent by fish in the different regions of the Plata Basin seems to be enough for the Sr/Ca and Ba/Ca concentrations incorporated within the otolith matrix to reflect the characteristics of the water masses. The otolith Sr/Ca and Ba/Ca values corresponding to the De la Plata River estuary tended to be separated from those corresponding to the other studied sampling sites, while values corresponding to the Paraná River Delta tended to be highly dispersed (Fig. 5).

Present results suggest a potential division of the silverside population from the lower section of the Plata Basin into two groups: One of these groups may spent more time in the estuarine area while the other may move to higher latitudes (sufficient time for morphometric differences or trends in the otolith Sr/Ca and Ba/Ca ratios to be detected).

Moreover, some outliers were detected in some of the De la Plata estuary specimens (Fig. 4). These results may indicate connectivity among the Paraná River Delta fish with the other sampling sites.

In summary, the simultaneous use of otolith morphometry and microchemistry appears as a potentially useful tool for studying fish displacements in freshwater and estuarine environments with a conductivity gradient. However, further research involving structure size or molecular genetics is needed for a better understanding of the interaction among the diverse silverside groups inhabiting the main De la Plata River tributaries.

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References

APHA, 1993. Standard Methods for the Examination of Water and Wastes. American Public Health Association, Washington, DC.
Avigliano, E., Tombari, A., Volpedo, A.V., 2012. El otolito de pejerrey (*Odontesthes bonariensis*), refleja el estrés ambiental. Biol. Acuat. 27, 9–15.
Avigliano, E., Volpedo, A., 2013. Use of otolith strontium:calcium ratio as indicator of seasonal displacements of the silverside (*Odontesthes bonariensis*) in a freshwater–marine environment. Mar. Freshwater Res. 64 (8), 746–751.
Barros, S.E., Regidor, H., Iwaszkiw, J., 2004. Biología pesquera del Pejerrey Odontesthes bonariensis (Cuvier y Valenciennes, 1835) en el subtrópico de Argentina. Aquatic 20, 32–37.

Burke, N., Brophy, D., King, P., 2008. Otolith shape analysis: its application for discriminating between stocks of Irish Sea and Celtic Sea herring (*Clupea harengus*) in the Irish Sea. ICES J. Mar. Sci. 65 (9), 1670–1675.

Campana, S., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. 188, 26–297.

Campana, S.E., Thorrold, S.R., Jones, C.M., Gunther, D., Tubrett, M., Longerich, H., Jackson, S., Halden, N., Kalish, J.M., Piccoli, P., De Pontual, H., Troadec, H., Panfili, J., Secor, G.H., Severin, K.P., Sie, S.H., Thresher, R., Teesdale, W.J., Cambell, J.L., 1997. Comparison of accuracy, precision and sensitivity in elemental assays of fish otoliths using the electron microprobe, PIXE and laser ablation ICPMS. Can. J. Fish Aquat. Sci. 54, 2068–2079.

Cardinale, M., Doering-Arjes, P., Kastowsky, M., Mosegaard, H., 2004. Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. Can. J. Fish Aquat. Sci. 61, 158–167.

Elsdon, T.S., Gillanders, B.M., 2005. Alternative life-history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. Can. J. Fish Aquat. Sci. 62, 1143–1152.

EPA, 1994. Methods for the Determination of Metals in Environmental Samples, Supplement 1. EPA, Columbus, OH.

Feutry, P., Tabouret, H., Maeda, K., Pécheyran, C., Keith, P., 2012. Diadromous life cycle and behavioural plasticity in freshwater and estuarine Kuhliidae species (Teleostei) revealed by otolith microchemistry. Aquat. Biol. 15, 195–204.

Galley, E., Wright, P., Gibb, F., 2006. Combined methods of otolith shape analysis improve identification of spawning areas of Atlantic cod. ICES J. Mar. Sci. 63, 1710–1717.

García, M., Jaureguizar, A., Protogino, L., 2010. From fresh water to the slope: fish community ecology in the Río de la Plata and the sea beyond. LAJAR 38, 81–94.

Grosman, F., Sanzano, P., Agüeria, P., González, G., Sergueña, S., 2001. Ecología reproductiva, edad, crecimiento, condición y alimentación del pejerrey (*Odontesthes bonariensis*) en un ambiente del SO de la provincia de Buenos Aires, Argentina. Aquatic 12, 1–24.

Hamer, P.A., Jenkins, G.P., Coutin, P., 2006. Barium variation in *Pagrus auratus* (Sparidae) otoliths: a potential indicator of migration between an embayment and ocean waters in south-eastern Australia. Estuarine Coastal Shelf Sci. 68, 686–702.

Kaufman, G.D., Anderson, J.H., Beitz, A.J., 1993. Otolith-brain stem connectivity: evidence for differential neural activation by vestibular hair cells based on quantification of FOS expression in unilateral labyrinthectomized rats. J. Neurophysiol. 70, 117–127.

Kopprio, G.A., Freije, R.H., Strüssmann, C.A., Kattner, G., Hoffmeyer, M.S., Popovich, C.A., 2010. Vulnerability of pejerrey *Odontesthes bonariensis* populations to climate change in pampean lakes of Argentina. J. Fish Biol. 77, 1856–1866.

Kraus, R.T., Secor, E.R., 2004. Incorporation of strontium into otoliths of an estuarine fish. J. Exp. Mar. Biol. Ecol. 302, 85–106.

Limburg, K.E., 1995. Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima*. Mar. Ecol. Prog. Ser. 119, 25–35.

Miller, J.A., 2011. Effects of water temperature and barium concentration on otolith composition along a salinity gradient: implications for migratory reconstructions. Exp. Mar. Biol. Ecol. 40, 42–52.

Milton, D.A., Chenery, S.R., 2001. Sources of uptake of trace metals in otoliths of juvenile barramundi (*Lates calcarifer*). J. Exp. Mar. Biol. Ecol. 264, 47–65.

Popper, A.N., Zhongmin, L., 2000. Structure–function relationship in fish otolith organs. Fish. Res. 46, 15–25.

Ranaldi, M.M., Gagnon, M.M., 2008. Zinc incorporation in the otoliths of juvenile pink snapper (*Pagrus auratus* Forster): the influence of dietary versus waterborne sources. J. Exp. Mar. Biol. Ecol. 360, 56–62.

Schuchert, P.C., Arkhipkin, A.I., Koenig, A.E., 2010. Traveling around Cape Horn: otolith chemistry reveals a mixed stock of Patagonian hoki with separate Atlantic and Pacific spawning grounds. Fish. Res. 102, 80–86.

Secor, D.H., Henderson-Arzapalob, A., Piccoli, P.M., 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? J. Exp. Mar. Biol. Ecol. 192, 15–33.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, third ed. W.H. Freeman and Company, New York.

Solari, A.L., Mirta, L., García, L., Jaureguizar, A., 2009. Fish fauna from the Ajó river in Campos del Tuyú National Park, province of Buenos Aires, Argentina. Check List 5, 807–811.

Tabouret, H., Bareille, G., Clverie, F., Pecheyran, C., Prouzet, P., Donard, O.F., 2010. Simultaneous use of strontium:calcium and barium:calcium ratios in otoliths as markers of habitat: application to the European eel (*Anguilla anguilla*) in Adour Basin, South West France. Mar. Environ. Res. 70, 35–45.

Urien, C.M., 1966. Distribución de los sedimentos modernos del Río de la Plata superior. Bol. Serv. Hidrogr. Naval. 3, 197–203.

Urien, C.M., 1967. Los sedimentos modernos del Río de la Plata Exterior. Bol. Serv. Hidrogr. Naval. 4, 113–313.

Volpedo, A.V., Echeverría, D.D., 2003. Ecomorphological patterns of the sagitta in fish associated with bottom marine shelf in the Mar Argentino. Fish. Res. 60, 551–560.

Volpedo, A.V., Tombari, A.D., Echeverría, D.D., 2008. Eco-morphological patterns of the sagitta of Antarctic Fish. Polar Biol. 31, 635–640.

Wells, B.K., Rieman, B.E., Clayton, J.L., Horan, D.L., Jones, C.M., 2003. Relationships between water, otoliths, and scale chemistries of Westslope cutthroat trout from the Coeur d'Alene River, Idaho: the potential application of hard-part chemistry to describe movements in freshwater. Trans. Am. Fish Soc. 132, 409–424.