



## RESEARCH ARTICLE

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# Estimating contributions from nursery areas to fish stocks in freshwater systems using otolith fingerprints: The case of the streaked prochilod in the La Plata Basin (South America)

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**Abstract**

Stock composition studies using otolith fingerprints have scarcely been developed in freshwater systems. In this context, the La Plata Basin is the second largest fluvial-marine system of South America, where *Prochilodus lineatus* is the most important fishery resource. Despite the basin importance, estimation of mixed stock composition has not been explored yet. In this paper, the contribution of the two main nursery areas to two fishing stocks (Uruguay and Paraná rivers) of *P. lineatus* was evaluated using otolith's core microchemistry, considering two groups: subadult (2 years) and adult (4 years). Estimates were made using two maximum likelihood methods. Chemical composition of young-of-year fish caught in nursery areas in 2010 was used as baseline of the models, whereas chemical composition of the core of subadult and adult otoliths was used as sample of unknown origin. Results suggest that the subadult stock from Paraná was not mixed (contribution ~ 100%), whereas the stock from Uruguay had a contribution from the Paraná nursery ( $1.5 \pm 1.2$ – $17.9 \pm 3.96\%$ ). For the adults, the degree of mixing increased and the contribution from both nursery areas to the Paraná and Uruguay stocks varied between  $14.8 \pm 4.18\%$  and  $85.2 \pm 4.18\%$ , respectively. The potential application of otolith fingerprints and maximum likelihood mixture models is here highlighted for determining the relative importance of recruitment sources of fish in the La Plata Basin. Because the contributions of the different nursery areas shared among several nations in turn affect the composition of internationally shared stocks, comprehensive management agreements at the basin level are necessary.

**KEYWORDS**

connectivity, LA-ICP-MS, maximum likelihood, otolith microchemistry, recruitment, stock composition

## 1 | INTRODUCTION

The efficiency of fisheries management depends on understanding population structure and stock mixing (Cadrin, Karr, & Mariani, 2013). Therefore, it is necessary to identify the nursery areas and fish stocks, and the estimation of contributions from nurseries to stocks. This type of information allows decisions to be made about which areas may or

may not be exploited and to evaluate if factors such as overfishing affect the composition of populations (Cadrin et al., 2013). In addition, stock composition studies give an idea about migrations and connectivity (Rooker et al., 2014), which is a useful information to generate comprehensive management policies among the different areas involved.

Over the past two decades, the chemical composition (trace elements and isotopes) of fish otoliths (ear stones) has been widely

used as a natural tag of habitat water (Avigliano & Volpedo, 2016; Cadrin et al., 2013; Campana, 1999). Because otolith calcium carbonate (aragonite) accretes daily and is not subsequently resorbed or otherwise altered (Campana & Neilson, 1985; Campana & Thorrold, 2001; Casselman, 1990; Elsdon et al., 2008), otolith fingerprints have allowed the study of stock composition and natural origins of various species, both marine and freshwater (Crook & Gillanders, 2006; Radigan, Carlson, Fincel, & Graeb, 2018; Rooker et al., 2008, 2014; Rooker, Wells, Itano, Thorrold, & Lee, 2016; Thorisson, Jónsdóttir, Marteinsdóttir, & Campana, 2011). In general, most studies of stock composition using otolith fingerprints have been developed in marine environments (Rooker et al., 2008; Schloesser, Neilson, Secor, & Rooker, 2010; Thorisson et al., 2011), being relatively scarce in freshwater systems (Crook & Gillanders, 2006; Radigan et al., 2018).

With 3,170,000 km<sup>2</sup>, the La Plata Basin is the second largest freshwater system of the South America. The main fishery resources of the Basin are *Prochilodus lineatus* (Valenciennes, 1836), *Pseudoplatystoma* sp. (Bleeker, 1862), *Salminus brasiliensis* (Cuvier, 1816), and *Hoplias* sp. (Bloch, 1794; MINAGRO, 2018). Despite the economic importance of these fisheries in the La Plata Basin, estimation of mixed stock composition has not been explored yet.

The streaked prochilod, *P. lineatus*, is by far the main fishery resource of the Basin, but there is a lack of statistics on catching of this species for consumption. It is common knowledge that some countries, such as Argentina, have exceeded 40,000 t/year of streaked prochilod, only captured in the lower region of the Basin (Baigún, Minotti, & Oldani, 2013; MINAGRO, 2018). The reproductive cycle of this species is correlated with the natural flood pulse regime, with migrations upstream and spawning in open river waters coupled to the flooding periods, as a mechanism of dispersion of eggs (Agostinho, Gomes, Veríssimo, & Okada, 2004; Baigún et al., 2013; Gubiani, Gomes, Agostinho, & Okada, 2007; Sverlij, Espinach Ros, & Ortí, 1993). Several works reported that the streaked prochilods migrate over 1,000 km to feed and reproduce (Avigliano, Pisonero, Dománico, Sánchez, & Volpedo, 2017; Bonetto, Canon Veron, & Roldán, 1981; Delfino & Baigun, 1985; Espinach Ros, Sverlij, Amestoy, & Spinetti, 1998). However, as the flood pulses are not regular, mass spawning and recruitment do not occur every year (Sverlij et al., 1993). This results in the existence of only one or two dominant cohorts in the Basin, which might last for more than a decade and on which fisheries are based (Baigún et al., 2013; Sverlij et al., 1993). In the last years, fishery has been based mainly on the currently dominant 2010 cohort, and on previously exploitable 2008 and 1997 cohorts. However, fisheries based on a single cohort might be especially sensitive to overexploitation in the medium or long term. Therefore, basic knowledge aspects, such as connectivity and degree of contribution of nursery areas to different stocks, are fundamental for management (Abaunza, Murta, & Stransky, 2013; Dudley & Waugh, 1980; Jennings, Kaiser, & Reynolds, 2009).

In the streaked prochilod, it was demonstrated that there are two main nursery areas in the middle and lower regions of the La Plata Basin (latitude 27–35°), where there are no dams and the migratory corridor is continuous (Avigliano, Fortunato, et al., 2016; Avigliano, Domanico, Sánchez, & Volpedo, 2017; Sverlij et al., 1993). These

nursery areas correspond to the middle sections of the Paraná and Uruguay rivers, and it was reported that chemical signatures of otoliths make it possible to differentiate each other (Avigliano, Domanico, et al., 2017). In addition, a previous study has suggested that the contribution to the Basin of larvae (without considering the probability of recruitment) from the Paraná River nursery is significantly greater than the one from the Uruguay River (Fuentes, Gómez, Vegh Llamazares, Lozano, & Salva, 2015).

The principal goal of this paper was to estimate, for the first time, the fish contributions from nursery areas to fish stocks in the La Plata Basin, using simultaneously otolith fingerprints and maximum classification-likelihood (MCL) models. Streaked prochilod was used as a model species. Specifically, the contribution of the two main nursery areas to two fishing stocks of the dominant cohort (Uruguay and Paraná rivers) was evaluated, considering subadult (2 years) and adult (4 years) specimens separately. Estimates were made using two methods of stocks composition evaluation, direct maximum likelihood estimation (MLE) and MCL estimator. The chemical composition of young-of-year (YOY) fish caught in nursery areas in 2010 was used as a baseline for the models, whereas the chemical composition of the core of subadult and adult otoliths (same cohort) caught in the same areas is used as a sample of unknown origin.

## 2 | MATERIAL AND METHODS

### 2.1 | Sampling design

The La Plata Basin goes through five countries in South America and is located between latitudes 17° and 36°, with a north–south current direction (Figure 1). Fish were collected using trammel nets in the main courses of the Uruguay River (31°25.246'S–58°1.407'W, 31°59.105'S–58°9.599'W; Argentina–Uruguay international boundary), and in the Paraná River (27°25.467'S–58°48.133'W; Argentina–Paraguay international boundary; Figure 1). These two areas of the Paraná and Uruguay rivers correspond approximately to the two main nursery areas (Avigliano, Domanico, et al., 2017). Fish were killed with percussive stunning (Van De Vis et al., 2003), placed on ice, and transported to the laboratory, where they were measured (standard length = SL) and the *lapilli* otoliths were extracted. Otoliths were cleaned of any remaining tissue with a plastic toothbrush and rinsed with distilled water.

Young-of-year fish (age-0) were caught between February and August 2010, at both breeding sites ( $N = 34$  for Paraná River and  $N = 31$  for Uruguay River). To evaluate the mix of stocks, fish were caught at both sites in 2012 and 2014. The fish were caught exactly in the same locations for every year (2010, 2012, and 2014). Only age-2 fish caught in 2012 ( $N = 32$  for Paraná River and  $N = 27$  for Uruguay River) and age-4 caught in 2014 ( $N = 28$  for Paraná River and  $N = 30$  for Uruguay River) were used (Table 1). In this way, all the estimates of natal origin were made using fish corresponding to the 2010 cohort. The age-2 fish correspond to subadult specimens and the age-4 fish to adults of reproductive age (Sverlij et al., 1993). Sample sizes close to 30 were used for each year and site because in previous studies that amount was enough to estimate the proportion of “brackish-



**FIGURE 1** Sampling sites of *Prochilodus lineatus* (hatched area). Arrows show principal migration routes and connectivity for each nursery area and sampling year [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 1** Descriptive statistics (age in years and standard length in mm) of the individuals used for otoliths chemistry analysis

	N	Age	SL ± SD	Min	Max
Baseline (2010)					
Uruguay	31	0	19.1 ± 2.9	19.1	25.0
Paraná	34	0	20.4 ± 1.9	20.4	25.5
2012					
Uruguay	27	2	27.7 ± 3.7	21.0	36.0
Paraná	32	2	26.2 ± 4.2	26.2	36.0
2014					
Uruguay	30	4	32.0 ± 4.1	26.0	45.3
Paraná	28	4	31.7 ± 4.6	31.7	40.0

Note. N: number of samples; SL ± SD: standard length ± standard deviation.

freshwater migratory/freshwater migratory" individuals in the Uruguay and Paraná rivers (Avigliano, Pisonero, et al., 2017).

## 2.2 | Otolith preparation and trace element analysis

Otolith preparation and trace element analysis were performed according to Avigliano, Pisonero, et al. (2017). Left otoliths were weighed using a Sartorius AG ED 2242 (Göttingen, Germany) analytical balance, decontaminated three times with 2% HNO<sub>3</sub> (Merck KGaA, Garmstadt, Germany; Arslan & Secor, 2008), rinsed five times with Milli-Q water (resistivity of 18.2 mΩ/cm), and dried overnight in a laminar flow hood. Otoliths were embedded in epoxy resin and sectioned transversely through the core to a thickness of 1,000 μm, using a Buehler IsoMet low-speed saw (Hong Kong,

China) with diamond edge blades. In order to estimate the fish age, the number of rings in the otoliths section was counted with the piece immersed in ultrapure water, using a stereomicroscope (Leica EZ4-HD, Singapore; Figure 2). Age estimation by counting the *annuli* in *lapilli* otoliths of *P. lineatus* was validated by Espinach Ros et al. (2008).

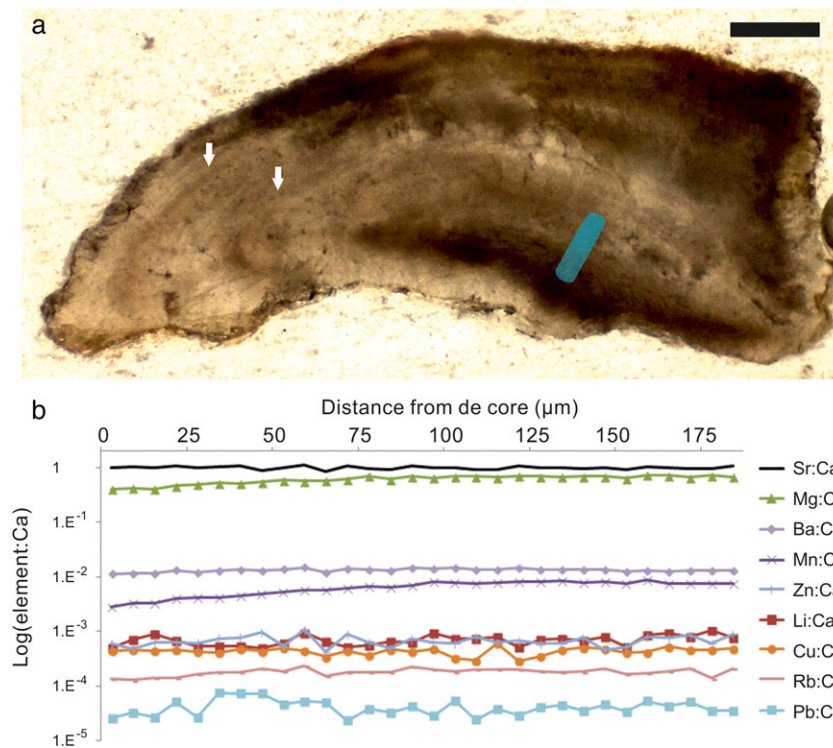
In preparation for laser ablation–inductively coupled plasma–mass spectrometry (LA–ICP–MS) analysis, otoliths were fixed to glass slides using clear epoxy resin, polished using 9-μm grit sandpaper, ultrasonically cleaned (3 min) in Milli-Q ultrapure water, and dried in a laminar flow hood.

The abundances of isotopes <sup>7</sup>Li, <sup>25</sup>Mg, <sup>43</sup>Ca, <sup>55</sup>Mn, <sup>65</sup>Cu, <sup>66</sup>Zn, <sup>85</sup>Rb, <sup>88</sup>Sr, <sup>138</sup>Ba, and <sup>208</sup>Pb were determined by LA–ICP–MS, using a 193-nm ArF\* Excimer laser ablation system (Photon Machines Analyte G2) coupled to an ICP–quadrupole MS Agilent 7700 (Agilent Technologies, Santa Clara). Radial line-scans of 190-μm length were carried out from core (Figure 2) at a scanning speed of 5 μm/s. This length represents approximately the entire core region. LA–ICP–quadrupole MS operating conditions are shown in Table 2.

Helium was used as the carrier gas in the ablation cell, and argon was added before entering the ICP–MS. Ion optics were adjusted to yield maximum sensitivity and balanced mass response while ablating National Institute of Standards and Technology (NIST) standard reference material (SRM) 612 glass. The optimization was carried out monitoring <sup>7</sup>Li<sup>+</sup>, <sup>133</sup>Cs<sup>+</sup>, <sup>232</sup>Th<sup>+</sup>, <sup>238</sup>U<sup>+</sup>, and <sup>232</sup>Th<sup>16</sup>O<sup>+</sup> ion signal intensities. Plasma robustness was monitored via the <sup>232</sup>Th<sup>16</sup>O/<sup>232</sup>Th and <sup>238</sup>U/<sup>232</sup>Th intensity ratios. In particular, ThO<sup>+</sup>/Th<sup>+</sup> intensity ratios were always below 0.35%, and <sup>238</sup>U<sup>+</sup>/<sup>232</sup>Th<sup>+</sup> intensity ratio was ~1.05. Additionally, the cross-calibration (PA-factor) of the pulse and analogue modes of the secondary electron multiplier detector in the ICP–MS was carried out daily, to ensure a linear response of the instrument of >8 orders of magnitude for the isotopes of interest.

NIST SRM 612 silicate glass reference material was employed as an external reference material (Jochum et al., 2011; NIST, 2012; Pearce et al., 1997), whereas silicate glass NIST SRM 610 was analysed as a secondary standard. Although the use of a reference material corresponding to bicarbonate would be opportune, silicate glasses (NIST 610 and 612) as external and secondary reference materials have given reliable results to determine trace elements in otoliths and, therefore, are widely established in the literature (Avigliano et al., 2018; Callicó Fortunato et al., 2017; Crook & Gillanders, 2006; Elsdon & Gillanders, 2005; Fowler, Smith, Booth, & Stewart, 2016).

Ion signals were normalized to the internal standard (<sup>43</sup>Ca<sup>+</sup>), and concentrations of elements were calculated using a standard bracketing method (Jackson, Longerich, Dunning, & Fryer, 1992). Calcium concentration of the otolith matrix was assumed to be 38.8 wt.% (Hamer et al., 2015; Yoshinaga, Nakama, Morita, & Edmonds, 2000). Results showed a marked stability of the otolith calcium signal along the scan lines (relative standard deviation < 0.05%; Figure 2). Ion signals were collected before the ablation process to determine their background level and during the ablation process. 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 each analysis session to monitor and correct for any signal drift. The maximum deviation observed during one analytical session was about 4%.



**FIGURE 2** Otolith section of *Prochilodus lineatus* (age-2) from Uruguay River showing the core laser ablation area (a) and element:Ca results (logarithmic scale for better visualization) of the first 190  $\mu\text{m}$  from the core (b). The white arrows indicate the *annuli*. Magnification,  $\times 40$ . Bar: 250  $\mu\text{m}$  [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 2** LA-ICP-MS operating conditions

Instrument	Parameter	Value
ArF 193-nm laser ablation	Laser fluence	12 J/cm <sup>2</sup>
	Repetition rate	10 Hz
	Pitsize diameter	50 $\mu\text{m}$
	Ablation cell	Two-volume HelEx cell
	Cell gas flow	He, 0.8 l/min
	Scan speed	5 $\mu\text{m}/\text{s}$
	ICP-MS	Acquisition mode
Nebulizer gas flow		Ar, 0.9 l/min
Isotopes measured		<sup>43</sup> Ca, <sup>7</sup> Li, <sup>25</sup> Mg, <sup>55</sup> Mn, <sup>65</sup> Cu, <sup>66</sup> Zn, <sup>85</sup> Rb, <sup>88</sup> Sr, <sup>138</sup> Ba, <sup>208</sup> Pb and <sup>88</sup> Sr
Integration time		210 ms/isotope

Note. LA-ICP-MS: laser ablation-inductively coupled plasma-mass spectrometry.

Analysis of NIST 610 showed good agreement with the following element recovery rates: 100.4% for <sup>7</sup>Li, 104.3% for <sup>25</sup>Mg, 85.7% for <sup>55</sup>Mn, 97.4% for <sup>65</sup>Cu, 70.0% for <sup>66</sup>Zn, 99.9% for <sup>85</sup>Rb, 101.1% for <sup>88</sup>Sr, 104% for <sup>138</sup>Ba, and 107% for <sup>208</sup>Pb. Although the percentages of recovery of <sup>55</sup>Mn and <sup>66</sup>Zn were relatively moderate, they were stable during all the analyses with a relative standard deviation lower than 3%. Therefore, <sup>55</sup>Mn and <sup>66</sup>Zn were considered for the estimates. In addition, previous studies showed the potential of these elements in the otolith as natural markers, as they have reported differences between the Paraná and Uruguay rivers, both for streaked prochilod and for other species such as Boga, *Megaleporinus obtusidens* (Avigliano et al., 2018; Avigliano, Domanico, et al., 2017; Avigliano, Fortunato, et al., 2016). All elements were consistently above detection limits, calculated as three times the standard deviation of the mean background gas values divided by the sensitivity. Element concentrations were expressed as molar ratios (element:Ca = mmol/mol). After the laser ablation, the

presence of vateritic inclusions was discarded by observing the otolith sections under reflected light with ethylenediaminetetraacetic acid (Tzeng et al., 2007).

### 2.3 | Data analysis

Individuals of age-0 were used as a baseline to estimate stock composition; it is important to verify that there are differences in the chemical signature of the cores prior to stock mixture analysis. Univariate and multivariate statistics were used to test differences in otolith microchemistry of yearling streaked prochilod from Paraná and Uruguay nurseries. Ratios were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. All ratios were  $\log_{10}(x + 1)$  transformed to meet assumptions of normality and homogeneity of variance. However, nonparametric statistics were used to compare Li:Ca and Cu:Ca ratios between sampling sites because these ratios did not meet the assumptions (Shapiro-Wilk,



$p < 0.05$ ; Levene,  $p < 0.05$ ), even after logarithmic transformation. The Mg:Ca, Mn:Ca, Zn:Ca, Rb:Ca, Sr:Ca, Ba:Ca, and Pb:Ca ratios were compared between nursery areas with  $t$  tests, whereas Cu:Ca and Li:Ca were compared using Mann–Whitney tests.

To ensure that differences in otolith mass or total length did not confound spatial patterns in elemental composition, the effect of otolith mass and total length on the elemental ratios was examined using Spearman correlation and analysis of covariance (Campana, 2013). No correlation or covariation was found between otolith mass or total length and elemental ratios ( $p > 0.05$ ).

The Hotelling  $T^2$  test was used to evaluate differences in the multielemental composition between nurseries. This test is robust to violations of the assumptions of normality and homogeneity of variance (Jørgensen & Rajeswaran, 2005).

Quadratic discriminant function analyses (QDAs) were used to test the ability of baseline to sort fish into specific nursery areas. In order to identify the most discriminant elements and to evaluate the gain of synergetic contribution of all the elements, two QDAs were performed using (a) the elements with the greatest discriminatory power and (b) all (nine) elements. To determine the value of each elemental ratio that contributed most to the separation of the two groups, the mean discriminant coefficient was calculated using the mean discriminant coefficient (Backhaus, Erichson, Plinke, & Weiber, 2016):

$$bj = \sum |bj_k| * EA_k \quad (k = 1, k = \dots), \quad (1)$$

where  $b_{jk}$  is the standardized discriminant function coefficient for the variable  $j$  with respect to the discriminant function  $k$ , and  $EA_k$  is the proportion of the eigenvalue of the discriminant function  $k$  in relation to the sum of all eigenvalues.

Previously, the multicollinearity between variables was tested. Expected prior probability classification was calculated on the basis of number of groups and sample sizes. A randomization test was performed to determine if the classification rates were significantly

different from random (White & Ruttenberg, 2007). Statistical analyses were performed using the SPSS 19 and Ginkgo 1.7 programs.

Origin of fish caught in 2012 and 2014 (unknown samples) was predicted with two stock mixture analysis methods using the otolith fingerprints and HISEA program (Millar, 1990): direct MLE and MCL estimator, respectively. Unlike discriminant analysis, stock composition analysis using maximum likelihood-based methods provides maximal discriminatory power in mixed stock situations (Campana, 1999; Gillanders, 2002; Kerr & Campana, 2013). Moreover, discriminant analysis requires prior knowledge of stock proportions in the mixture (Campana, 2005; Kerr & Campana, 2013). In general, MLE has a better performance in the estimates than does the MCL method, except in some cases when there are very low or no contributions. In these cases, the MCL could be a more robust estimator (Millar, 1990). In this work, the two estimators were used for comparative purposes. Otolith fingerprints of YOY fish sampled in 2010 (same year classes) were used as baseline data (known sample). Error terms (variability of the estimator) of estimated proportion were generated using the baseline data by running the HISEA program in bootstrap mode with 1,000 simulations (DeVries, Grimes, & Prager, 2002; Millar, 1990; Rooker et al., 2008, 2014, 2016).

The maximum likelihood estimates were performed using the combination of variables that showed the highest percentage of classification in the QDAs.

### 3 | RESULTS

Element:Ca levels of baseline and unknown mixed sample from the Paraná and Uruguay rivers (2012 and 2014) are given in Table 3.

Considering baseline data, Mn:Ca and Rb:Ca ratios (Table 3) were significantly higher in Paraná River compared with Uruguay River ( $p < 0.003$ ). However, the opposite pattern was observed for the Cu:Ca and Zn:Ca ratios ( $p < 0.05$ ; Table 3). No significant differences ( $p > 0.05$ ) were found between nurseries for Li:Ca, Mg:Ca, Rb:Ca, Sr:Ca, and Pb:Ca ratios.

**TABLE 3** Mean  $\pm$  standard deviation of elemental ratios (mmol/mol) in cores of otoliths from different locations for sampling year

	Li:Ca	Mg:Ca	Mn:Ca	Cu:Ca	Zn:Ca
Baseline (2010)					
Uruguay	$8.34E^{-04} \pm 4.05E^{-04}$	$0.69 \pm 0.15$	$8.04E^{-03} \pm 4.20E^{-03}$	$1.40E^{-03} \pm 1.09E^{-03}$	$2.00E^{-03} \pm 2.13E^{-03}$
Paraná	$6.41E^{-04} \pm 3.69E^{-04}$	$0.62 \pm 0.13$	$1.41E^{-02} \pm 2.70E^{-02}$	$6.81E^{-04} \pm 4.60E^{-04}$	$8.69E^{-04} \pm 7.80E^{-04}$
2012					
Uruguay	$8.48E^{-04} \pm 2.27E^{-04}$	$0.61 \pm 0.19$	$6.14E^{-03} \pm 2.88E^{-03}$	$1.47E^{-03} \pm 2.51E^{-03}$	$1.31E^{-03} \pm 8.92E^{-04}$
Paraná	$5.95E^{-04} \pm 3.65E^{-04}$	$0.59 \pm 0.17$	$8.61E^{-03} \pm 4.46E^{-03}$	$9.48E^{-04} \pm 4.84E^{-04}$	$2.44E^{-03} \pm 3.23E^{-03}$
2014					
Uruguay	$7.90E^{-04} \pm 4.01E^{-04}$	$0.52 \pm 0.16$	$6.52E^{-03} \pm 3.72E^{-03}$	$1.17E^{-03} \pm 5.00E^{-04}$	$9.65E^{-04} \pm 5.32E^{-04}$
Paraná	$8.09E^{-04} \pm 4.42E^{-04}$	$0.56 \pm 0.14$	$6.98E^{-03} \pm 2.94E^{-03}$	$7.19E^{-04} \pm 3.76E^{-04}$	$2.15E^{-03} \pm 2.60E^{-03}$
	Rb:Ca	Sr:Ca	Ba:Ca	Pb:Ca	
Baseline (2010)					
Uruguay	$3.55E^{-04} \pm 1.30E^{-04}$	$1.00 \pm 0.18$	$1.21E^{-02} \pm 6.63E^{-03}$	$6.49E^{-05} \pm 4.55E^{-05}$	
Paraná	$6.99E^{-04} \pm 2.86E^{-04}$	$0.96 \pm 0.20$	$1.58E^{-02} \pm 8.51E^{-03}$	$1.89E^{-05} \pm 1.26E^{-05}$	
2012					
Uruguay	$3.24E^{-04} \pm 1.38E^{-04}$	$1.06 \pm 0.31$	$1.01E^{-02} \pm 6.66E^{-03}$	$9.07E^{-05} \pm 1.37E^{-04}$	
Paraná	$6.21E^{-04} \pm 2.36E^{-04}$	$1.05 \pm 0.26$	$2.38E^{-02} \pm 3.56E^{-02}$	$6.88E^{-05} \pm 7.39E^{-05}$	
2014					
Uruguay	$3.11E^{-04} \pm 7.72E^{-05}$	$0.99 \pm 0.47$	$1.44E^{-02} \pm 5.15E^{-02}$	$7.31E^{-05} \pm 1.35E^{-04}$	
Paraná	$6.23E^{-04} \pm 2.32E^{-04}$	$1.09 \pm 0.31$	$1.43E^{-02} \pm 8.32E^{-03}$	$4.26E^{-05} \pm 3.44E^{-05}$	

Multivariate analyses of baseline data were found to be highly effective in detecting differences in otolith fingerprints of YOY fish between nursery areas. Results from the Hotelling  $T^2$  test revealed significant differences in the otolith fingerprints between Paraná and Uruguay nurseries ( $p = 0.0001$ ). Based on the mean discriminant coefficients, the Rb:Ca ratio ( $b_j = -0.81$ ) was identified as the most important variable followed by the Zn:Ca ( $b_j = -0.42$ ) and Sr:Ca ( $b_j = 0.33$ ) ratios ( $b_j$  coefficients ranged between  $|0.14|$  and  $|0.30|$  for Ba:Ca, Cu:Ca, Li:Ca, Mg:Ca, Mn:Ca, and Pb:Ca). Reclassification rate of QDA based on Rb:Ca, Zn:Ca, and Sr:Ca ratios of the baseline averaged 84.5%, whereas the cross-classification matrix integrating the nine variables revealed the highest percentage of correctly classified individuals, 98.3%. When the nine variables were used, the percentages of correctly classified individuals were 96.6% for Uruguay and 100% for Paraná. Additionally, percentages of classification were significantly different from random ( $p < 0.0001$ ; prior probabilities for groups: 0.48 for Paraná and 0.52 for Uruguay nurseries).

To carry out the estimates of origin using the maximum likelihood methods, all (nine) variables were used due to the higher percentages of classification obtained in comparison with the model that only includes the most explanatory variables.

The otolith fingerprints of the fish caught in 2012 and 2014 were used to estimate the degree of mixing of the stocks from Paraná and Uruguay nurseries. Figure 1 displays principal migration routes and connectivity for each nursery area and sampling year, according to the estimation maximum likelihood methods.

For the fish caught in 2012 (age-2), MLE suggested that the stock from Paraná was not mixed (contribution = 100%), whereas the stock from Uruguay had a very low contribution from Paraná nursery ( $1.5 \pm 1.2\%$ ; Table 4). Estimates using MCL were similar for the Paraná group (contribution from Paraná stock to Paraná nursery =  $99 \pm 5.09\%$ ), whereas for the Uruguay River group, they indicated a higher degree of mix (contribution from Paraná stock to Uruguay nursery =  $17.9 \pm 3.96\%$ ).

For the 2014 samples (age-4), the degree of mixing was found to increase in both sites. According to the MLE,  $14.8 \pm 4.18\%$  of the adult streaked prochilod caught in the Uruguay River was originally from the Paraná nursery (MCL =  $34.3 \pm 3.92\%$ ). Estimated contribution of the streaked prochilod from the Uruguay nursery to the Paraná River fishery was  $41.4 \pm 4.9\%$  for MLE and  $38.7 \pm 4.2\%$  for MCL (Table 4).

The estimates based on 1,000 simulations (bootstrap mode) of the baseline data showed relatively low errors (less than 5.09%; Table 4),

indicating an acceptable degree of certainty associated with the estimations of the contributions.

## 4 | DISCUSSION

Methodologies such as tagging and mark-recapture have been used to study connectivity, migration, natal philopatry, and stock composition (Avigliano et al., 2018; Fritsch, Morizur, Lambert, Bonhomme, & Guinand, 2007; Pawson, Brown, Leballeur, & Pickett, 2008; Thorrold, Latkoczy, Swart, & Jones, 2001). However, in South America, tagging studies are extremely expensive due to the large surface of the basins and the number of specimens needed to be tagged. Alternatively, stock composition studies using otolith microchemistry and maximum likelihood mixture models are relatively common in other continents (Crook & Gillanders, 2006; Rooker et al., 2014; Thorisson et al., 2011). These methods have proven to be a good tool for recruitment, segregation, and connectivity studies of marine and freshwater species (Crook & Gillanders, 2006; Rooker et al., 2014; Thorisson et al., 2011). However, the application of these techniques is somewhat lagging in Latin America (Avigliano & Volpedo, 2016). In particular, this study is, to our knowledge, the first one using otolith microchemistry to study the stock composition in freshwater environments in the region.

In relation to streaked prochilod, the results of this work confirm that the nursery areas of the Paraná and Uruguay rivers can be discriminated using the multielemental signature of the otolith's core from juveniles under 1 year. Avigliano, Domanico, et al. (2017) have also obtained high percentages of classification (90.8%) for the same cohort integrating three techniques to discriminate nursery areas of streaked prochilod, including otolith microchemistry and morphometry, and scale geometric morphometry. However, here, a higher average classification percentage (98.6%) was obtained, possibly due to the incorporation of more elements (nine instead of three) in the data matrix and the use of more precise equipment.

Otolith core elemental incorporation might be influenced by a diversity of factors such as environment (salinity, temperature; Brown & Severin, 2009; Elsdon & Gillanders, 2003; Martin, Thorrold, & Jones, 2004), genetic coding (Barnes & Gillanders, 2013), physiology (growth rates, metabolic changes; Kalish, 1991; Radtke & Shafer, 1992; Sturrock et al., 2014), and diet and water composition (Ranaldi & Gagnon, 2008; Walther & Thorrold, 2006; Webb, Woodcock, & Gillanders, 2012). In the La Plata Basin, there is a decreasing temperature as well as an increasing salinity gradient from the north-south direction (Avigliano & Schenone, 2015; Avigliano & Volpedo, 2013). Moreover, sampling sites have different topographic features, depths, and hydrographic dynamics. All these factors undoubtedly affect the characteristics of the water and could print distinctive fingerprints in the otoliths, which explains some of the multielemental differences found in this paper. Levels of trace elements in water such as Mn, Cu, Zn, Pb, Sr, Ba, and Pb were reported for sampling points located between 200 and 400 km upstream in the Paraná and Uruguay rivers, respectively (Avigliano & Schenone, 2015). The levels of these elements are similar between the Paraná and Uruguay rivers, with the exception of Mn and Ba, whose concentrations are higher in the Paraná River (Avigliano & Schenone, 2015). The same trend was

**TABLE 4** Results of maximum likelihood (MLE) and maximum classification-likelihood (MCL) analysis of natal origins of *Prochilodus lineatus*

	N	MLE			MCL		
		% Uruguay	% Paraná	% error	% Uruguay	% Paraná	% error
2012							
Uruguay	27	98.5	0.15	1.20	82.1	17.9	3.96
Paraná	32	0	100	0.93	0	99.9	5.09
2014							
Uruguay	30	85.2	14.8	4.18	65.7	34.3	3.92
Paraná	28	41.4	58.6	4.90	38.7	61.3	4.20

observed in otoliths, only for Mn. Laboratory tests and specific field studies are needed to understand what factors that influence the incorporation of elements in the otolith of streaked prochilod, in order to enrich the interpretations of the microchemistry studies.

The use of a single cohort (dominant) reduces possible effects caused by large environmental-temporal variations on the incorporation of the elements during the first life stage of the fish. In some species, it was proved that the chemistry of the core, especially in the region corresponding to the prehatching period or even prior to the reabsorption of the yolk, is strongly influenced by the environment frequented by the mother rather than by the environment where the larva lives (Kalish, 1990; Liberoff et al., 2014). Moreover, no evidence suggesting that the development of eggs occurs in environments with different characteristics than the nursery areas found. Any potential and significant effect of maternal habitat on elementary determinations was here dismissed because the measured core area comprises a 190- $\mu\text{m}$  radius. In this species, the larva hatches with a *lapillus* otolith of  $\sim 7\text{-}\mu\text{m}$  radius (Brown & Fuentes, 2010), which is a size practically negligible in relation to the total length of the transect. An age of at least 60–75 days was estimated considering the length of the fish used. This age is considerably longer than the yolk period (yolk is reabsorbed relatively quickly, within the first 10 days posthatching; Brown & Fuentes, 2010).

Estimates of stock composition showed that the degree of mixing was higher for adult fish (2014) compared with the subadults (2012), considering the estimations made with MLE and MCL (Figure 1). Avigliano, Pisonero, et al. (2017) reported that between 5% and 37% of the fish caught in the Uruguay and Paraná rivers use the estuary once in their lives, migrating at least 1,000 km from the capture areas (next to nursery areas here studied). These large displacements were observed in individuals older than 3 years (Avigliano, Pisonero, et al., 2017). Other authors indicated the dominance of specimens between <1 and 3 years in some environments such as streams, associated floodplains, and lagoons, whereas older specimens (4 or 5 years and older) were dominant in the main course of Paraná (Sverlij et al., 1993). This suggests that fish start to make large migrations between the subadult and adult periods, and this could explain, at least in part, the increase in the degree of mixing in 2014 compared with 2012. The dominance of juveniles, especially of a few weeks of life, outside the main course has been related to the breeding period (protection, feeding), whereas migrations of subadult or adult fish were related to food or reproductive purposes (Sverlij et al., 1993).

According to the large number of larvae observed in the Paraná River in relation to the Uruguay River (Fuentes et al., 2015), a greater contribution from the Paraná nursery area to the stock of the same river was expected. Nevertheless, the existence of small contributions of unknown secondary nursery areas (temporary or not) that could modify the estimates made here should be evaluated, and regions with the potential to support small foci of spawning in the migratory corridor should be explored. In this respect, the drift of eggs or larvae originated upstream of the dams, which limits the migratory corridor, could have some contribution of juveniles to the nursery areas studied (Fuentes, Gómez, Brown, Arcelus, & Espinach Ros, 2016). It was also documented that the nursery area located upstream of Salto Grande Dam (Uruguay River) has a different chemical signature to that located

downstream (Avigliano, Fortunato, et al., 2016). On the other hand, the segregation of fish of the same origin could affect the estimates (Lazartigues et al., 2017).

When the two methods of estimation were compared, there were no remarkable differences for the populations of known origin of the Paraná, where the maximum difference between both methods for the years 2012 and 2014 was 4.4%. Nevertheless, for the stock of the Uruguay River, estimations performed with MCL indicated a greater mix for the years 2012 and 2014 in relation to MLE (difference between methods, 13–23%). In any case, in both evaluated years, the largest contribution corresponds to the site of origin of the Uruguay River.

In general terms, when the degree of contribution is low (as in the case of the estimates obtained for the year 2012 by MLE), the MCL method could be more robust than MLE (Millar, 1990; Rooker et al., 2014). Then, considering that there could be low or no contributions for some stocks or years, the MCL method could be more useful than MLE for streaked prochilod. Nevertheless, particularly for the stock of the Uruguay River (2012), the estimation error was relatively low for both methods, although higher for MCL (MLE = 1.20; MCL = 3.96). Errors obtained for the same stock in 2014 were similar and acceptable between methods (MLE = 4.18; MCL = 3.92). At this point, defining which method is most suitable for the base matrix could be questionable. Some authors used both methods (Rooker et al., 2014), whereas others directly used MLE (Crook & Gillanders, 2006) or MCL (Lazartigues et al., 2016, 2017). Both estimators show an increase in the mix for 2014 and a greater contribution to each stock from the nursery areas of the same region.

These results showed that there is a significant connectivity among fish stocks and that there are important contributions from one group to the other. Moreover, the baseline constructed in this work showed good aptitude to be implemented for the study of stock composition of the streaked prochilod dominant cohort of the La Plata Basin. This foundation could even be improved by increasing the sample size or incorporating, for example, isotopes. In the future, baselines should be created with the next dominant cohorts, in order to contribute to the long-term management of this resource. In this sense, knowing the evolution of stock composition, in terms of time, will be an important input for the design of management schemes. For this reason, it is strongly recommended to evaluate the mixing of stocks for a higher age range (e.g., age 1–10). The surface area through which fish migrate includes at least five countries (Argentina, Bolivia, Brazil, Paraguay, and Uruguay). Therefore, management policies should be regional and not local or national in order to guarantee the sustainability of fisheries. Specifically in Argentina, there are at least six provinces that make use of the same resource and the regulations, management, control, and inspection policies such as size and gear restrictions or seasonal limits and licence requirements differ between provinces. Because it has been seen that the species carries out cross-border migrations and that the contributions of the different nursery areas shared among several nations in turn affect the composition of internationally shared stocks, comprehensive management agreements at the basin level are necessary. In addition, it is required to generate reliable fishery statistics in all the countries that share the fishery in order to monitor the volumes of captures together and

understand if the fishing pressure of the different nations may have a negative effect on the compositions of the populations. In this last point, the combination of good fishing statistics together with the stock composition evaluation method applied in this work will provide useful information for the regulation of the fishing effort in accordance with the variation of the composition of the fishable stocks.

Otolith chemistry was applied here for the first time in combination with maximum likelihood methods to study the stock composition in an important freshwater fishery from Latin America. In general, these methodologies are scarcely used in extensive freshwater systems, because most of the world's large basins are distributed in developing countries (Avigliano & Volpedo, 2016). The application in the case study has been successful; therefore, it is expected that similar methodologies will be applied with other species of commercial interest such as *M. obtusidens*, *Luciopermelodus pati*, *Pseudoplatystoma corruscans*, *P. fasciatum*, and *S. brasiliensis*.

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