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Mixed-stock and discriminant models use for assessing recruitment sources of estuarine fish populations in La Plata Basin (South America)

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Abstract

The objective of this study was to identify potential recruitment sources of *Prochilodus lineatus* from freshwater areas (Paraná and Uruguay rivers) to estuarine population of the Río de la Plata Estuary (La Plata Basin, South America), considering young (age-1) and adult (age-7) fish. LA-ICP-MS chemical analysis of the otolith core (nine element:Ca ratios) of an unknown mixed sample from Río de la Plata Estuary (2011 and 2017) was compared with a young-of-year baseline data set (same cohort) and classified into freshwater nurseries (Paraná or Uruguay river) by using maximum classification-likelihood models (MLE and MCL) and quadratic discriminant analysis (QDA). Considering the three models used, the Uruguay River was the most important contributor for both young and adult populations. The young population (2011) was highly mixed with contributions between 31.7 to 68.3%, while the degree of mixing was found to decrease in 2017 (adult fish) from 97.1 to 100% contributions. The three employed methods showed comparable estimates, however, the QDA showed a high similarity with the MCL model, suggesting sensitivity to evaluate small contributions, unlike the MLE method. Our results show the potential application of maximum likelihood mixture models and QDA for determining the relative importance of recruitment sources of fish in estuarine waters of the La Plata Basin.

Introduction

Otolith chemistry has been widely used to study stock composition or natal origin of several fish species (Crook & Gillanders, 2006; Rooker *et al.*, 2008, 2014, 2016; Thorisson *et al.*, 2011; Radigan *et al.*, 2018) because the otolith material (calcium carbonate and trace elements) is deposited constantly and it is not resorbed (Campana & Neilson, 1985; Casselman, 1990; Campana & Thorrold, 2001; Elsdon *et al.*, 2008). Most studies of natal origin using otolith chemistry have been developed for marine species (Rooker *et al.*, 2008; Schloesser *et al.*, 2010; Thorisson *et al.*, 2011), there being fewer for freshwater and estuarine fish (Crook & Gillanders, 2006; Radigan *et al.*, 2018).

Streaked prochilod (*Prochilodus lineatus*, Valenciennes, 1836) is the main fishery resource of the La Plata Basin and it is distributed throughout the basin (Argentina, Bolivia, Brazil, Paraguay and Uruguay). The management of *P. lineatus* is complex because it is a transboundary species and the sources of recruitment are not completely known. This species migrates to feed and spawn (over 1500 km), and currently the fishery is mainly based on the dominant 2010 cohort (Bonetto *et al.*, 1981; Delfino & Baigun, 1985; Espinach Ros *et al.*, 1998). The most important reproductive areas are located in the middle/low reaches of the Paraná and Uruguay rivers (Sverlij *et al.*, 1993), which are the two main tributaries of the Río de la Plata Estuary (Figure 1). Countries such as Argentina have exported more than 40,000 t year^{-1} of *P. lineatus* caught in these areas (Baigún *et al.*, 2013; MINAGRO, 2018). Particularly, the Río de la Plata Estuary has historically been an important capture area for both Argentina and Uruguay. In this sense, it is very important to know the degree of contribution from freshwater nursery areas to estuarine stocks to support the efficiency of fisheries management in the estuary.

Recently, Avigliano *et al.* (2018*b*) have developed a multi-elemental baseline data set based on Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) determination of nine *lapilli* otolith trace elements of young-of-year (YOY) *P. lineatus*, which represent the two main known nursery areas in La Plata Basin (Paraná and Uruguay rivers). This baseline could allow estimation of the natal origin of fish caught in the estuary in relation to the freshwater nurseries. Maximum likelihood-based methods and discriminant analysis (DA) have been widely used to study stock composition due to their high discriminatory power in mixed situations (Campana, 1999; Gillanders, 2002, 2005; Kerr & Campana, 2013).

Fig. 1. Sampling sites of *Prochilodus lineatus* (red hatched area). Arrows show the origin of the contributions from freshwater nursery areas to estuary. Green hatched areas show the freshwater nursery areas.

These models, based on otolith LA-ICP-MS microchemistry have demonstrated their potential to study natal origin of marine and freshwater fish (Crook & Gillanders, 2006; Thorisson *et al.*, 2011; Rooker *et al.*, 2014), which could contribute to understanding the recruitment sources of the Río de la Plata Estuary.

Therefore, the objective of this project was to estimate the contribution of *Prochilodus lineatus* from freshwater nursery areas to estuarine populations (young and adult fish) of the Río de la Plata Estuary (La Plata Basin), using otolith LA-ICP-MS chemistry analysis and discriminatory models such as maximum classification-likelihood and discriminant analysis.

Materials and methods

Study area and sampling

The La Plata Basin crosses through five countries (Argentina, Bolivia, Brazil, Paraguay and Uruguay) in South America and lies between latitudes 17° and 36°, where the current flows in a north–south direction (Figure 1). Fish were caught by using trammel nets in the Río de la Plata Estuary on the border between Argentina and Uruguay (Figure 1) in 2011 and 2017. Collection permits in Argentina were granted by Ministerio de Asuntos Agrarios de Buenos Aires and fish handling during sampling was performed following guidelines of the ethical committee of the Consejo Nacional de Investigaciones Cientícas y Técnicas (CONICET). Fish were measured (standard length = SL, in cm) and the *lapilli* otoliths were extracted. *Lapilli* otolith was used instead of *sagittae* or *asterisci* otolith because it is larger in Characiformes such as *P. lineatus* (Avigliano *et al.*, 2015; Volpedo *et al.*, 2017).

Sample preparation and chemical analysis

Left otoliths were weighed, decontaminated three times with 2% HNO_3 (Merck KGaA, Garmstadt, Germany) and rinsed five times in Milli-Q water (18.2 mOhm cm^{-1}). Decontaminated otoliths were embedded in epoxy, and then sectioned through the core to a thickness of 1000 µm using a low speed saw (Buehler Isomet, Hong Kong, China).

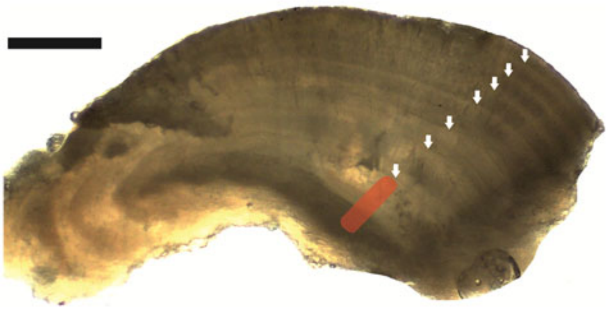

Fig. 2. *Lapillus* otolith section of *Prochilodus lineatus* (age-7) from Río de la Plata Estuary showing the core laser ablation area (red hatched area). The white arrows indicate the annuli. Scale bar: 250 µm.

Fish age was estimated by counting the *annuli* in the otolith (Espinach Ros *et al.*, 2008) sections using a stereomicroscope (Leica EZ4-HD, Singapore). After that, sections were fixed to glass slides, polished using 9 µm-grid sandpaper and ultrasonically cleaned for 5 min in Milli-Q water.

The isotope concentrations ^{138}Ba, ^{65}Cu, ^{43}Ca, ^{7}Li, ^{25}Mg, ^{55}Mn, ^{208}Pb, ^{85}Rb, ^{88}Sr and ^{66}Zn were determined by LA-ICP-MS at the Department of Physics from the University of Oviedo, Spain. A 193 nm ArF Excimer laser ablation system (Photon Machines Analyte G2) coupled to an ICP-QMS Agilent 7700 (Santa Clara, USA) was used for the analysis. Analytical conditions were configured according to Avigliano *et al.* (2018*a*, 2018*b*). Radial line-scans of ~190 µm length were carried out from core to the first *annuli* (Figure 2) using a circular aperture at scanning speed of 5 µm s^{-1} (spot diameter: 85 µm; crater depth: 160 µm). Intensity ratios ^{232}Th^{16}O/^{232}Th (<0.35%) and ^{238}U/^{232}Th (~1) were used for monitoring the plasma robustness and NIST 612 and NIST 610 reference materials (silicate glass) were used as external and secondary standard, respectively (Pearce *et al.*, 1997; Jochum *et al.*, 2011; NIST, 2012). Analyte recoveries for NIST 610 ranged from 97 to 104% for ^{7}Li, ^{25}Mg, ^{65}Cu, ^{85}Rb, ^{88}Sr, ^{138}Ba and ^{208}Pb and 70 to 85% for ^{66}Zn and ^{55}Mn. Isotope signals were normalized to the internal standard ^{43}Ca (38.8 wt.%) (Yoshinaga *et al.*, 2000; Hamer *et al.*, 2015) and the concentrations were expressed as molar ratios (element/Ca: µmol mol^{-1} and mmol mol^{-1}).

Recruitment sources estimates

Because the otolith weight and standard length could be associated with the incorporation of elements into the otolith (Campana, 2013), the effect of these variables on the elemental ratios was assessed by Spearman correlation.

Natal origin of fish caught in 2011 and 2017 in the Río de la Plata Estuary was predicted using three different methods: direct maximum-likelihood-estimation (MLE), maximum classification-likelihood estimator (MCL), and quadratic discriminant analysis (QDA). MLE and MCL were carried out using HISEA program (Millar, 1990), while QDA was performed using Systat 13.

Otolith core fingerprints of YOY fish sampled in 2010 (same cohort) were used as baseline data (Avigliano *et al.*, 2018*b*). The baseline was comprised of *lapilli* otolith core ratio values (Ba:Ca, Cu:Ca, Li:Ca, Mg:Ca, Mn:Ca, Rb:Ca, Sr:Ca, Pb:Ca and Zn:Ca) of YOY *P. lineatus* from Paraná and Uruguay nurseries. This baseline data set has shown high ability in detecting

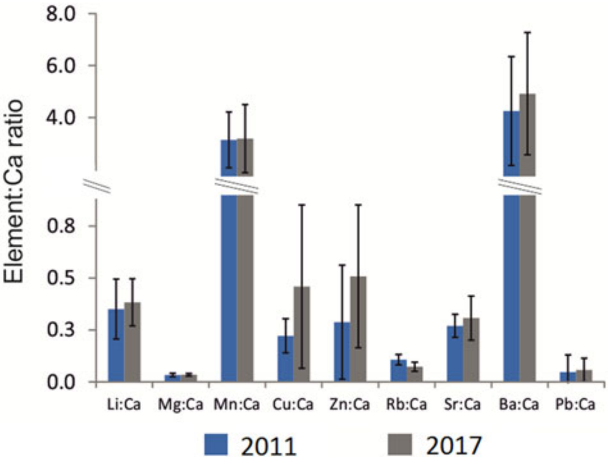

Fig. 3. Mean ± standard deviation of elemental ratios in µmol mol^{-1} (Sr:Ca and Mg:Ca in mmol mol^{-1}) in *lapilli* otoliths core from Río de la Plata Estuary for sampling year.

differences between Uruguay (96.6%) and Paraná (100%) rivers (average = 98.3%) fingerprints (Avigliano *et al.*, 2018*b*).

Prior to the QDA, multicollinearity of the baseline was tested by calculating the tolerance value, which was calculated as 1 minus R^2 of the respective variable with all other variables included in the current model (Hair *et al.*, 2014). Moreover, homogeneity of variance-co-variance matrices was verified using Box test. The prior probabilities of classification were estimated on sample sizes and group numbers (White & Ruttenberg, 2007) and the classification accuracy was evaluated by leave-one-out cross-validation. The mean discriminant coefficient (Backhaus *et al.*, 2016) was used to estimate the weight of each elemental ratio that contributed most to the separation among the two freshwater sources.

Standard deviations of the maximum likelihood methods estimates were calculated by running the HISEA program in simulation mode (bootstrap) using 1000 simulations (Millar, 1990; DeVries *et al.*, 2002; Rooker *et al.*, 2008, 2014, 2016; Avigliano *et al.*, 2018*b*).

Results

Age-1 fish caught in 2011 (N = 29, SL ± SD = 23.0 ± 1.8 cm) and age-7 caught in 2017 (N = 24, SL ± SD = 44.5 ± 1.4 cm) were used, and the estimates of origin were made using fish corresponding to the baseline cohort (2010 dominant cohort).

No significant correlations were found between otolith weight or standard length and elemental ratios ($P > 0.05$).

Elemental ratios of the unknown mixed sample from Río de la Plata Estuary (Age-1 and Age-7) are shown in Figure 3. The ratios that showed highest mean levels for both Age-1 (young) and Age-7 (adult) fish were Sr:Ca, Mg:Ca and Ba:Ca (>0.42 µmmol mol^{-1}), whereas the lowest were Mn:Ca, Li:Ca, Zn:Ca, Cu:Ca, Rb:Ca and Pb:Ca (<0.31 µmmol mol^{-1}). The tendency Sr:Ca>Mg:Ca>Ba:Ca>Mn:Ca>Li:Ca>Zn:Ca>Cu:Ca>Rb:Ca>Pb:Ca was observed for Age-1 fish, while Sr:Ca>Mg:Ca>Ba:Ca>Mn:Ca>Zn:Ca>Cu:Ca>Li:Ca>Rb:Ca>Pb:Ca was obtained for Age-7 fish. These data were compared with YOY baseline and classified into freshwater nurseries (Paraná and Uruguay rivers) using QDA and maximum likelihood models. Results of stock composition estimates are shown in Figure 4. MLE suggested that the age-1 (2011) population was mixed with contributions of 68.3 ± 4.7 and 31.7 ± 4.7% for Uruguay and Paraná nurseries, respectively. Estimates using MCL indicated a slightly higher degree of mixing with percentages of 54 ± 4.9 and 46 ± 4.9% for Uruguay and Paraná Rivers, respectively. QDA also indicated that the population was highly mixed, with 51.7% of the young fish originating from the Uruguay River, while the remaining young fish were classified as Paraná fingerprint (48.3%).

In relation to age-7 fish (2017), the degree of mixing was found to decrease with respect to 2011 (Figure 4). MLE showed that the population of adult fish from Río de la Plata Estuary was not mixed, with 100 ± 0.7% of the fish originating from the Uruguay River. A similar result was observed by MCL, where 91.7 ± 3.3% of recruits originated from Uruguay waters, suggesting a limited contribution from the Paraná River. Like the MCL model, QDA showed a low proportion from the Paraná River (8.3%), the Uruguay River being the highest contributor (91.7%).

Based on the QDA discriminant coefficients, Rb:Ca (−0.81), Zn:Ca (−0.42) and Sr:Ca (0.33) ratios were identified as the most important variables among the two freshwater sources.

The estimates based on bootstrap mode showed standard deviations lower than 5% (Figure 4), suggesting a good degree of certainty of the estimations.

Discussion

It is necessary to know the recruitment sources from freshwater nursery areas to estuarine stocks to support the efficiency of fisheries management in the La Plata Basin. For this, it is mandatory to test the efficiency of different estimation methods. In this paper, maximum likelihood mixture models and QDA showed comparable estimates, nevertheless, QDA and MCL showed a high sensitivity to assess small contributions. Thus, MCL and QDA seem to be the most appropriate estimators for studying the recruitment sources of *P. lineatus* from Río de la Plata Estuary. According to the three models, the Uruguay River was the most important contributor for both young (age-1) and adult (age-7) populations. These results show the potential of these methods for studying the recruitment sources of *P. lineatus* from freshwater to estuarine environments of the La Plata Basin.

Maximum likelihood mixture models and QDA based on otolith LA-ICP-MS microchemistry have shown to be a potential tool for studying recruitment sources in several continents (Crook & Gillanders, 2006; Thorisson *et al.*, 2011; Rooker *et al.*, 2014). Nevertheless, the application of these methods has been poorly implemented in Latin America (Niklitschek *et al.*, 2014; Avigliano, & Volpedo, 2016; Avigliano *et al.*, 2018*b*), this paper being the first one using otolith microchemistry to study contributions from freshwater nursery areas to estuarine fish populations.

Several authors have employed both MLE and MLC methods (Fraile *et al.*, 2014; Rooker *et al.*, 2014; Avigliano *et al.*, 2018*b*), while others have used MLE (Crook & Gillanders, 2006) or MCL (Lazartigues *et al.*, 2016, 2017), separately. Discriminant analysis models have been less used because it is necessary to know the prior probabilities of classification to each group, these being rarely known in nature (Millar, 1990; Gillanders, 2005). For example, to assume equal probabilities of classification could tend to under-estimate the misclassification probabilities, especially when the size of the baseline is very small (Millar, 1990). In this regard, recent authors have preferred to used maximum likelihood estimators (Fraile *et al.*, 2014; Rooker *et al.*, 2014; Lazartigues *et al.*, 2017; Avigliano *et al.*, 2018*b*), although these methods in combination with discriminant analysis have also been used (Fraile *et al.*, 2014). Avigliano *et al.* (2018*b*) have assessed the contribution from nursery areas to freshwater stocks of *P. lineatus* in the La Plata Basin using otolith chemistry and maximum classification-likelihood models (MLE and MLC methods) simultaneously. In that study, the degree of mixing of adults was higher than in young fish from a freshwater environment.

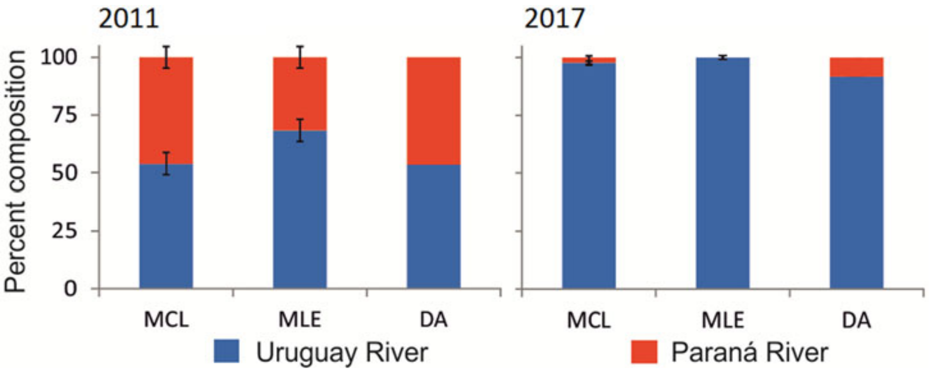

Fig. 4. Results of maximum-likelihood-estimation (MLE), maximum-classification-likelihood (MCL) and quadratic discriminant analysis (DA). Per cent composition estimates indicate the recruitment source of young (age-1, N = 29) and adult (age-7, N = 24) *Prochilodus lineatus* from Río de la Plata Estuary.

Avigliano *et al.* (2018*b*) have not found remarkable differences for the estimates and standard deviations obtained using MLC and MLE methods. However, they have suggested that the MCL method could be more useful than MLE for *P. lineatus*, considering that MCL has a better performance in the estimates when there are very low contributions (Millar, 1990).

Elemental incorporation into the otolith may be related to the surrounding environment, physiology and genetics, among other factors (Ranaldi & Gagnon, 2008; Brown & Fuentes, 2010; Webb *et al.*, 2012; Barnes & Gillanders, 2013). In this paper, Rb:Ca, Zn:Ca and Sr:Ca ratios were identified as the variables that contributed most to the separation among the two freshwater sources. Interestingly, Rb:Ca incorporation pathways have not yet been explored. Nevertheless, Rb:Ca has previously been detected in *lapilli* otolith of *Megaleporinus obtusidens* from the La Plata Basin (same study area) (Avigliano *et al.*, 2018*a*), which suggests that this element could be available in the environment. Otolith Sr:Ca ratio is generally positively related to salinity (Campana, 1999; Avigliano & Volpedo, 2013; Bouchard *et al.*, 2015), while otolith Zn:Ca incorporation may be influenced by diet (Ranaldi & Gagnon, 2008).

All models showed that there is a temporal variability in the stock composition (Figure 4). The temporal variation in the composition of stocks could be related to the active migratory behaviour of the species and environmental factors (floods, water flow), which could affect the displacements. Due to the large size of the estuary (35,000 km^2, Guerrero *et al.*, 1997), it is possible that the stock composition also varies spatially. Thus, it is strongly recommended to assess the mixing of stocks for all age classes and cohorts considering different sampling sites in the estuary. In the case of this work, it is evident that for the 2010 cohort, the sources of recruitment from the Uruguay River were fundamental for Río de la Plata Estuary populations. On the other hand, the areas with the potential to generate alternative sites of spawning in the migratory corridor should be monitored, because the existence of new or small contributions from unknown nursery areas could modify the estimates made. In addition, the monitoring may contribute to the management of the predominant nursery areas, which should be properly conserved.

The results of this work allow recommending the use of similar approaches with other commercially important species that inhabit the estuary and could have nurseries in freshwater such as *Pseudoplatystoma corruscans*, *P. fasciatum*, *Megaleporinus obtusidens*, *Luciopimelodus pati* and *Salminus brasiliensis*. In this regard, new species-specific baselines should be generated using YOY fish of the different species of the basin.

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