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Otolith elemental fingerprint and scale and otolith morphometry in *Prochilodus lineatus* provide identification of natal nurseries

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ABSTRACT

The dentification of nursery areas is a basic requirement for fishing management in large rivers. Morphometry (circularity, ellipticity, form factor, rectangularity and roundness indices) and chemistry (Sr:Ca, Ba:Ca and Zn:Ca ratios) of *lapilli* otolith, and geometric morphometry of scales of juveniles *Prochilodus lineatus*, were compared in three sites in the Plata Basin, in order to evaluate their applicability to identify possible nursery areas. Otolith microchemistry based on ICP-OES found significant differences in the Ba:Ca and Zn:Ca ratios among sampling sites. When all the combined techniques were considered, the quadratic discriminant analysis (QDFA) showed the highest classification success (89.5–92.9%), in relation to separate techniques classification. Otolith microchemistry, individually considered, appears to be a good and effective tool to identify individual fish from different locations (77.8%–84.2%). Otolith morphometry found significant differences in the ellipticity, circularity and form factor indices between sites. Otolith morphological indices supported results from the elemental study with a success in the allocation of 63.2–78.6%. When considering all variables for scale geometric morphometry, discriminant analysis showed a good percentage of the classification of the individuals (58.3–82.8%). These results indicate that the otolith microchemisty and morphometry and scale morphometry are acceptable markers of habitat and represent a potential tool (in combination or individually) for the identification of streaked prochilod nursery areas.

1. Introduction

The streaked prochilod (*Prochilodus lineatus* Valenciennes, 1836) is one of the most important commercial freshwater species in South America. This species, distributed in the Plata Basin, is exploited by Argentina, Brazil, Bolivia, Paraguay and Uruguay (Espinach Ros and Fuentes, 2000). The mentioned basin has a natural flooding pulse regime (Neiff and Malvárez, 1999) associated to the reproductive cycle of the streaked prochilod, involving migrations upstream, followed by spawning in open river waters coupled to the flooding periods as a mechanism of dispersion of eggs mechanism (Espinach Ros and Sánchez, 2006; Sverlij et al., 1993). The streaked prochilod migrations for food or reproduction have a distance of more than 1000 km (Bayley, 1973; Espinach Ros et al., 2008). Even though there is a lack of statistics on catching of this species for consumption, it is of common knowledge that some countries, such as Argentina, have exported 36,000 t/year of streaked prochilod captured only in the lower region of the basin (Minagro, 2013, 2004).

In the last decades, studies on the species have been intensified, but most research efforts were made in the middle and low section of the Paraná River in Argentina (Fig. 1) (Espinach Ros and Sánchez, 2006; Espinach Ros, 2008; Espinach Ros et al., 2012; Sverlij et al., 1993). It is suggested that this region represents the primary

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Fig. 1. Study area. The circles indicate the streaked prochilod (*Prochilodus lineatus*) collection sites.

breeding area and the most important catching region of the Plata Basin. However, in spite of the socio-economical importance of the resources of the region, othersub-basins such as Uruguay River (Argentina, Brazil and Uruguay border) (Fig. 1) are scarcely studied and managed (Baigún et al., 2012; Bayley, 1973; CARU, 2014, 2010). In recent years, the presence of eggs and larvae of this species has been found in Uruguay River, downwards and upwards of the Salto Grande hydroelectric dam (Argentina-Uruguay), suggesting both areas as a breeding region (CARU, 2014, 2010).

Identification of nursery areas is a very important tool to generate strategies that may ensure the efficiency of sustainable management of fisheries (Beck et al., 2001; Colloca et al., 2009). In this sense, preservation and management of nursery areas promote the maintenance of fishery resources, avoiding their decay to irreversible values. This decline could not only compromise the fisheries continuity, but also affect the productive socioeconomic sector that depends on them (Beck et al., 2001). The chemical composition and morphometry of fish otoliths are valuable natural tags of habitual use, due to specific otolith properties. Even though physiological factors can affect the incorporation of trace elements in the otolith, salinity and temperature are among the most relevant environmental factors regarding the incorporation of elements in this structure (Bouchard et al., 2015; Campana, 1999; Elsdon and Gillanders, 2002; Martin and Thorrold, 2005; Secor and Rooker, 2000; Sturrock et al., 2012). The predominant source of several elements as Sr and Ba to otoliths is the surrounding water (Kerr and Campana, 2013). Likewise, otolith morphometry is also related to environmental factors including salinity, temperature, depth, among others (Avigliano et al., 2014; Lombarte, 1992; Lombarte et al., 2010; Reichenbacher and Reichard, 2014). Lately, due to the strong relationship between chemistry and morphometry of fish otolith with different environmental features these tools have been widely used to identify nursery areas of different commercially important species (Avigliano and Volpedo, 2016; Avigliano et al., 2015a; Bailey et al., 2015; Bouchard et al., 2015; Gillanders et al., 2003; Rooker et al., 2001; Tanner et al., 2013; Tournois et al., 2013; Vasconcelos et al., 2008, 2007). On the other hand, the use of geometrical morphometry of scales has been recently performed to identify populations (Staszny et al., 2012) and could also be a good tool to discriminate nursery areas.

Based on the above considerations, the present study tests the applicability of the otolith elemental fingerprint and scale and otolith morphometry in *Prochilodus lineatus* to identify possible nursery areas. For this purpose, morphometry (ellipticity, circularity, form factor, rectangularity, and roundness indices) and microchemistry (Sr:Ca, Ba:Ca and Zn:Ca ratios) of *lapilli* otolith, and geometric morphometry of scales were compared between three sampling sites from the Plata Basin.

2. Material and methods

2.1. Study area and sample collection

The Plata Basin, with an area of 3,170,000 km^2, is among the largest basins in the world. The most important rivers are the Paraná (4000 km long) and the Uruguay (1800 km long) (Fig. 1) (Guerrero et al., 1997). The Plata Basin goes through 5 South-American countries (Argentina, Bolivia, Brazil, Uruguay and Paraguay). Paraná and Uruguay rivers headwaters are the mountains of the Atlantic forest of southeast Brazil, then becoming the international boundary between Argentina, Brazil, Paraguay and Uruguay (Fig. 1). The Plata Basin discharges into the Río de la Plata estuary (30,362 km^2) with an average discharge of 23,000 m^3/s towards the Atlantic Ocean (Guerrero et al., 1997).

Fish samples were collected between April 2010 and November 2010 by using multifilament three-layer nets in the Uruguay River, upstream (UpUR) and downstream (DoUR) of the hydroelectric dam of Salto Grande (Corrientes and Entre Ríos provinces, Argentina-Brazil international boundary), and in the Paraná River (Corrientes province, Argentina-Paraguay international boundary) (Fig. 1). Fish were transported to the laboratory at 4 °C where they were measured (standard length = SL) and the *lapilli* otoliths were extracted. We preferred to use lapillus otoliths rather than sagittal or asteriscus otoliths because they were larger and allowed less measurement error (Assis, 2005; Avigliano et al., 2015e). Scales were removed from the shoulder region in front of the 1st dorsal fin ray above the lateral line and stored dry in paper envelopes, according to Ibañez et al. (2007).

2.2. Selection of samples

Fish of 0+ years old were selected for the study. The otoliths were washed with ultrapure water and dried. The left otolith of each pair was sectioned transversely through the core by using a rotary saw equipped with a diamond blade (Dremel® 250 and 300) and they were burned directly onto a Bunsen burner (Christensen, 1964). The number of rings in the otolith section was counted through the use of a stereomicroscope (Leica® EZ4-HD, Singapore) at 30× magnification. Age determination by counting the ring number in *lapillus* otoliths of *P. lineatus* was validated by Espinach Ros et al. (2008). In total, 19 individuals from UpUR site (SL mean ± SD and range: 15.9 ± 2.4; 11.0–19.1 cm), 20 individuals from DoUR (15.9 ± 3.0; 11.5–20.0 cm) and 29 from Paraná River (18.9 ± 2.4; 12.0–20.2 cm) were selected for the analysis.

2.3. Otolith microchemistry

The right otoliths (age – 0+) were washed in Milli-Q water and once dry, they were transferred to a sterile centrifuge tube and weighed by using a Sartorius AG® ED2242 (Göttingen, Germany) microbalance to the nearest 0.0001 g. Then, otoliths were decontaminated 3 times with 1.7% HNO_3 and finally rinsed 5 times with