



BRIEF REPORT



Habitat use of the amphidromous catfish *Genidens barbus*: first insights at its southern distribution limit

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ABSTRACT

White sea catfish *Genidens barbus* is an endangered amphidromous species distributed between northeastern Brazil and northern Argentine Patagonia, whose migratory behaviour at the southern end of its range is unknown. Here, the migratory patterns of this species were studied including marine and freshwater (Patagonian stock) environments via otolith core-to-edge Sr/Ca and Ba/Ca profiles. Based on the Sr/Ca ratio, all specimens were classified as amphidromous with annual cycles of movement between waters of relatively low to high salinity occurring during spring. The core Sr/Ca ratio matched with the reference values for freshwater in all specimens. No evidence of resident freshwater or marine individuals was found. No evident antagonistic relationship was found between both Sr/Ca and Ba/Ca ratios. A Ba/Ca peak was observed followed by a drop coinciding with the core edge, highlighting the need to evaluate the potential transfer from the yolk, and to understand what factors are linked to the growth marks within the first year of life.

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Q6 Introduction

White sea catfish *Genidens barbus* (Lacépède, 1803) is an endangered (MMA 2014) amphidromous fish distributed between northeastern Brazil and northern Argentine Patagonia. Individuals make annual migrations between environments with different salinities from the first year of life (Araújo 1988; Avigliano et al. 2017) and reach sexual maturity between 8.5 and 9 years (Reis 1986a; Velasco et al. 2007). Their reproductive strategy involves the production of a few large eggs and male gastric incubation (Reis 1986a; Araújo 1988). Reproductive migration is associated with a great loss of energy due to the fact that the incubation and transport period of larvae lasts up to 4 months, during that time the individuals do not feed (Reis 1986a; Velasco and Reis 2004). This particular life cycle along with low reproduction rate and high age of first maturation, added to the

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lack of effective fisheries management policies, and has pushed the resource to its collapse, whose exploitation was prohibited since 2015 (MMA 2014). In the last five years, this situation has stimulated a series of studies on its population structure and life history, with the aim of contributing to the administration and recovery of the resource (Avigliano and Volpedo 2016; Di Benedetto et al. 2018). Studies based on otolith and dorsal fin spine chemistry revealed different stocks throughout their distribution, with a geographic dependence on reproductive estuaries or rivers (Avigliano et al. 2015a, 2016, 2019a). Otoliths are acellular calcareous concretions located in the inner ear of fish, which grow continuously and are considered metabolically inert. In many diadromous species, the otolith strontium (Sr) and barium (Ba) concentration is directly related to salinity and their availability in the surrounding water; therefore, they are considered good salinity proxies (Martin and Thorrold 2005; Brown and Severin 2009). Otolith core-to-edge Sr/Ca profiles allow describing four migration patterns in *G. barbatus* from Rio de Janeiro (Brazil) to the La Plata Basin (Argentina-Uruguay) (Avigliano et al. 2015b, 2017). Recently, two studies based on the dorsal spine and otolith chemistry have reported a *G. barbatus* stock in northern Patagonia at the southern end of its distribution (Avigliano et al. 2019, 2020a, 2020b); thus, information is urgently needed to formulate stock-specific conservation strategies (Avigliano et al. 2019).

Based on the recent findings, the goal of this study was to investigate the *G. barbatus* habitat use at its southern distribution limit including the Patagonian stock and the mixing area with the La Plata Basin stock, by using otolith core-to-edge Sr/Ca and Ba/Ca profiles.

Materials and methods

Fish were collected at the along Argentine coast (Mar de las Pampas-MDP, Argentina; 37°21'44"S- 57°02'5.27"W) and the Negro River 20 km from the river mouth (Patagonian stock, Río Negro, Argentina; 40°52'32"S - 62°54'34"W) between November 2017 and January 2018 with longlines and fishing rods. Mar de las Pampas (Figure 1(a)) is a coastal marine environment from the Pampean Plain, whose salinities vary from 27 to 34, while Negro River (Figure 1(a)) is a freshwater system that crosses the Patagonian steppe from the Andes to the sea. Fish from the Patagonian stock were caught in freshwater and upper estuary (conductivity: 120–8,060 $\mu\text{S}/\text{cm}$, Abrameto et al. 2013). Mean fish total length \pm SD and range were 31.6 \pm 5.9 cm (24.0–41.0 cm) for MDP (N=10), and 40.8 \pm 4.2 cm (32.3–49.1 cm) for Negro River (N=13). Lapillar otoliths were extracted, decontaminated with 2% ultrapure nitric acid (Ensure, Merk, Germany), rinsed three times with ultrapure water, and embedded in crystal epoxy resin (Avigliano et al. 2019). A slow-speed saw (Isomet, Hong Kong, China) was used to obtain section the otoliths to the core. These were fixed to glass slides with crystal epoxy resin, and polished using aluminum oxide sandpaper sheets (Avigliano et al. 2019). Finally, otolith sections were rinsed three times, and sonicated for 10 min with ultrapure water. Fish were aged by counting the growth rings in the otolith sections using a stereomicroscope (Leica EZ4-HD, Singapore). Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) was employed to measure ^{43}Ca , ^{88}Sr , and ^{138}Ba ion signals in core-to-edge transects (Figure 1(b)), using a 193 nm ArF Excimer laser (Photon Machines Analyte

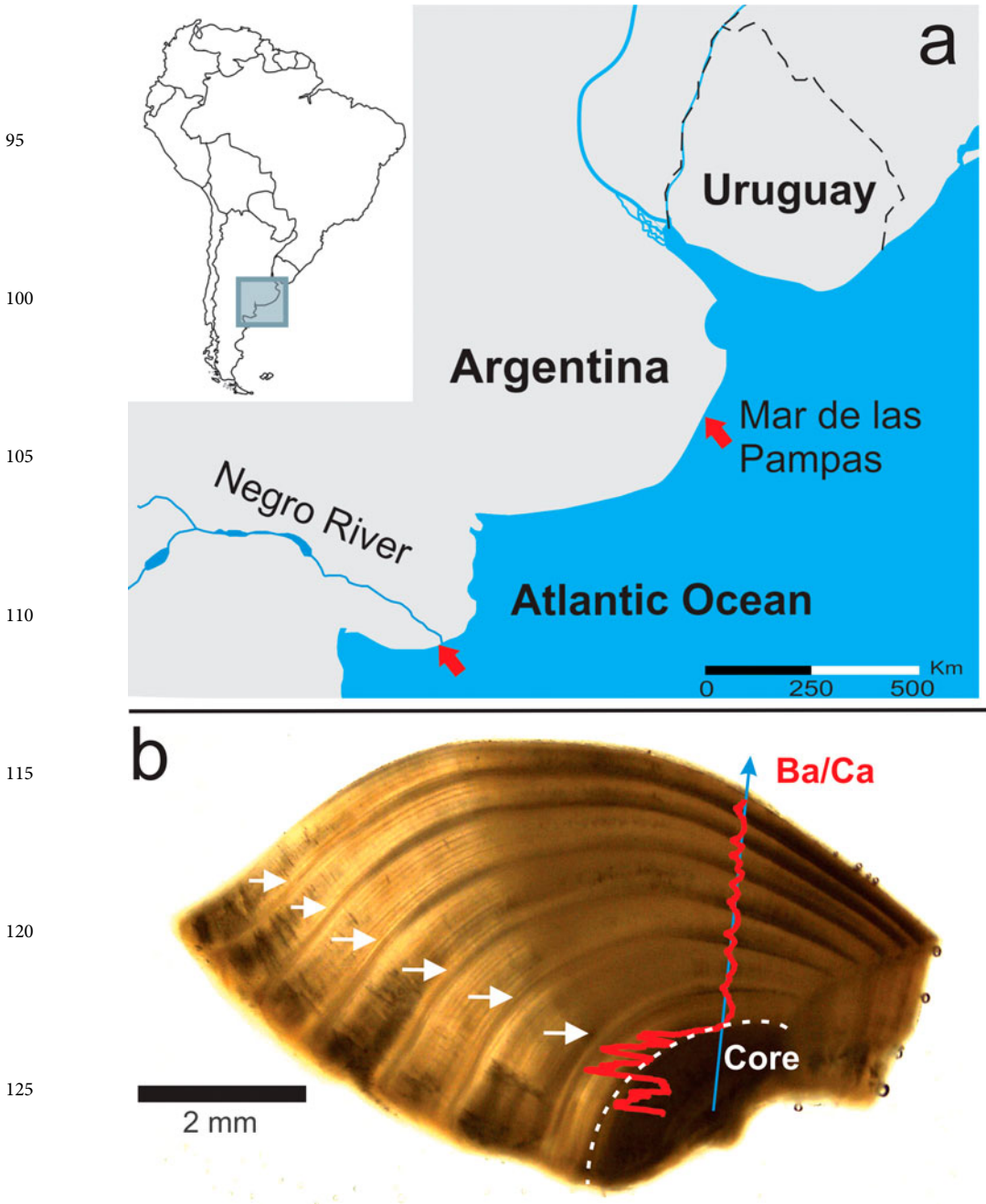


Figure 1. Study area map and *Genidens barbatus* otolith section at the core level. (a) Sampling sites (red arrows). (b) *Lapilli* otolith sectioned at the core level showing the core-to-edge-laser ablation transects (blue arrow). The red line represents the Ba/Ca ratio and the white arrows indicate the *annuli* position.

G2, USA) coupled to an ICP-QMS (Agilent 7700, Japan). Core-to-edge transects were performed at a scanning speed $15 \mu\text{m/s}$ with a spot size of $85 \mu\text{m}$ (fluence= 3.75 J/cm^2 ; pulse repetition rate= 10 Hz). Helium was used as carrier gas while argon was added

before entering the ICP, which was operated at 1600 W. NIST612 (trace elements in silicate glass) was used to monitor plasma robustness ($^{238}\text{U}/^{232}\text{Th}\sim 1.2$) and the oxide production rates ($^{232}\text{Th}^{16}\text{O}/^{232}\text{Th}$, <0.4%). NIST612 and MACS-3 (trace elements in synthetic calcium carbonate) were measured in triplicate at the beginning and at the end of the analytical session and every 10 otoliths to monitor the elemental fractionation caused by sensitivity drift (Lin et al. 2016). MACS-3 and NIST612 were employed as calibration and secondary standards (Avigliano et al. 2019), respectively, while ^{43}Ca Q7 was used as an internal standard (38.3% weight, (Yoshinaga et al. 2000). Recovery rates ranged from 101 to 105%, and the precision based on quadruplicate secondary standard analysis was below 5%. Sr and Ba concentrations were expressed in relation to Ca. Migratory patterns were classified in amphidromous type a-c and freshwater resident according to (Avigliano et al. 2017). Type 'a' was defined as fish whose Sr/Ca signature at the beginning of life is compatible with freshwater use and move at least once to a marine environment; type 'b' corresponded to individuals whose Sr/Ca signal at the beginning of life matches with freshwater and then migrate to estuarine water; and type 'c' was defined as fish whose initial Sr/Ca ratio is compatible with estuarine water and move to freshwater, marine or both. Sr/Ca values above 5.98 mmol/mol were considered as seawater use and those below 3.75 mmol/mol as incursions to freshwater (Avigliano et al. 2017).

Results

Otolith Sr/Ca ratio varied between 2.01 and 7.74 mmol/mol for MDP, and from 2.31 to 7.18 mmol/mol for Negro River. For both sampling sites, the Sr/Ca transects suggested annual cycles of movement between waters of relatively low to high salinity (Figure 2). The core Sr/Ca ratio matched with the reference values for freshwater in all specimens, so they were classified as amphidromous type 'a' except one fish from Negro River (type 'b') (Figure 2(d)). The resident freshwater pattern was not found in the Negro River.

Otolith Ba/Ca ratio ranged from 0.0016 to 0.15 $\mu\text{mol/mol}$ for MDP and from 0.0030 to 0.14 $\mu\text{mol/mol}$ for Negro River. In all fish analysed, a Ba/Ca peak was observed in the otolith core followed by a strong drop, coinciding with the edge of the core (Figures 1 (b) and 2). No evident antagonistic relationship was found between both Sr/Ca and Ba/Ca ratios (Figure 2).

Discussion

This study reports the first insights about *G. barbuis* life history at the southern end of its distribution range, revealing the predominance of the amphidromous type 'a' pattern (fish whose Sr/Ca signature at the beginning of life is compatible with freshwater use and move at least once to a marine environment). The predominance of the type 'a' pattern was reported for estuaries in central Brazil, while in southern Brazil and Argentina, populations exhibited a mixture of patterns (Avigliano et al. 2015b, 2017). The resident freshwater pattern was reported only in Lagoa dos Patos (Brazil), representing up to 33% of the sampled fish (Avigliano et al. 2017). However, the range of life-history patterns is sample size-dependent, so other patterns are expected to be found when more otoliths are analysed.

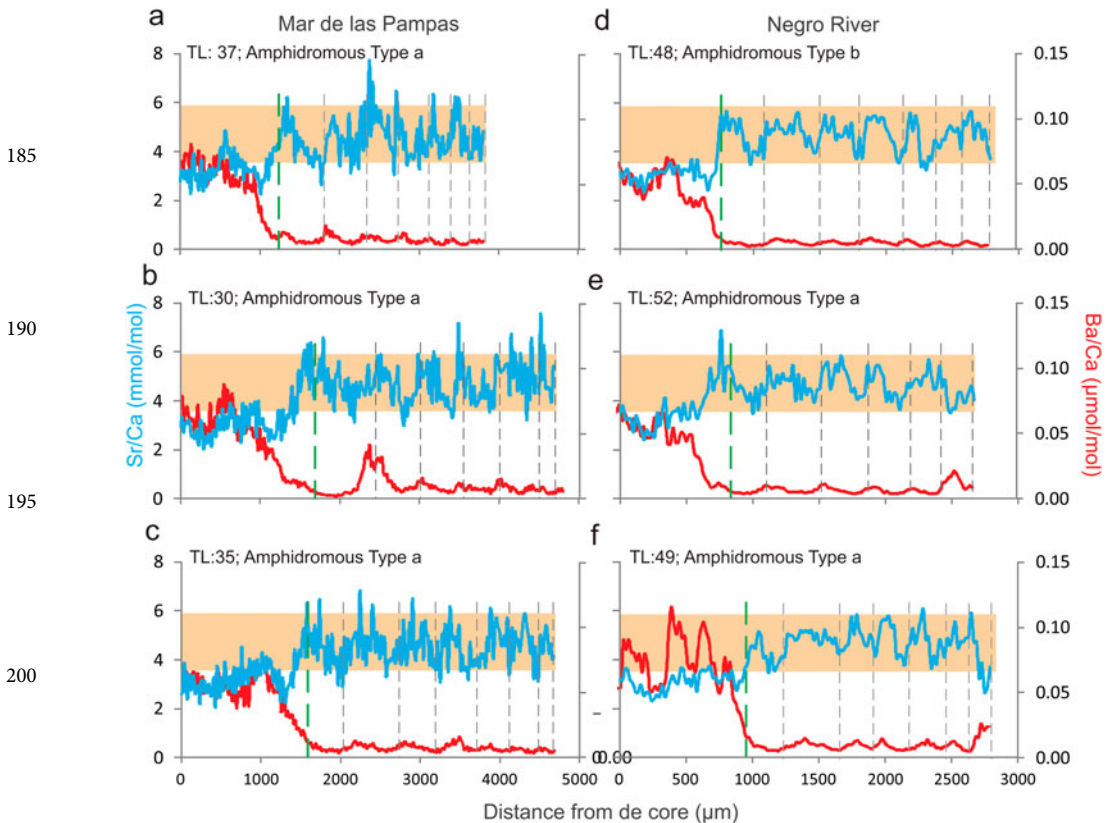


Figure 2. Representative *Genidens barbus* otolith core-to-edge Sr/Ca and Ba/Ca profiles. The vertical gray dotted lines indicate the annual marks, while the green line indicates the location of the core edge. The light orange horizontal indicate estuarine environment use. TL = total length (cm).

According to the annual growth marks which are formed during the warm months (December and January) (Reis 1986b and Avigliano et al. 2020a) and the annual Sr/Ca variations found, migrations toward lower salinity environments would occur during the austral spring. This observation is consistent with the annual migratory runs upstream reported for the species, which occur cyclically from the first year in spring (Avigliano et al. 2017; 2020a), suggesting amphidromous migration for feeding, rather than for reproduction.

High Ba/Ca values were also reported in the core of the ancient *G. barbatus* from Patagonia (Avigliano et al. 2020a), and even in other species of the same family such as *G. genidens* Cuvier, 1829 (Maciel et al. 2020) and *Cathorops spixii* Agassiz, 1829 (Maichak de Carvalho et al. 2020), and in other fish taxa (Ruttenberg et al. 2005). It has been suggested that potential physiological changes (e.g. hatching, flexion, transition to free-embryo, etc.) and maternal effect associated to prolonged yolk-feeding can affect the Ba incorporation into the otolith core (Kalish 1990; Ruttenberg et al. 2005; Liberoff et al. 2014). Because the reproductive strategy of *G. barbatus* involves a prolonged yolk-sac period, these hypotheses could be plausible.

Conclusions

230 All specimens were classified as amphidromous with annual cycles of movements between environments with different salinity during spring and no evidence of resident freshwater or resident marine individuals was found. This report raises new unknowns that need to be resolved to improve the potential of the otolith chemistry as a habitat proxy for *G. barbatus*. It is necessary to evaluate the potential transfer of trace element from the yolk to the otolith core and to understand what factors are linked to the core mark. Is it possible that this mark corresponds to the transition to free-embryo stage? If so, could the strong Ba drop be an indication of this event? Since this species has such large eggs, it should be possible to (1) analyse their chemistry, and (2) dissect otoliths out of the embryos to perform chemistry on.

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Disclosure statement

245 No potential conflict of interest was reported by the author(s).

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Data availability statement

255 The data are available upon request to the corresponding author.

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