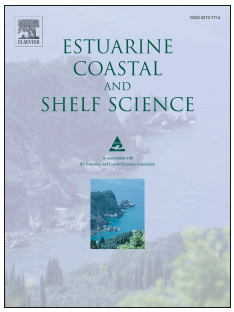


Integrated use of otolith shape and microchemistry to assess *Genidens barbus* fish stock structure

Thaís Rodrigues Maciel, Marcelo Vianna, Barbara Maichak de Carvalho, Nathan Miller, Esteban Avigliano

PII: S0272-7714(21)00410-8

DOI: https://doi.org/10.1016/j.ecss.2021.107560

Reference: YECSS 107560

To appear in: *Estuarine, Coastal and Shelf Science*

Received Date: 16 June 2021

Revised Date: 27 July 2021

Accepted Date: 15 August 2021

Please cite this article as: Maciel, Thaí.Rodrigues., Vianna, M., Maichak de Carvalho, B., Miller, N., Avigliano, E., Integrated use of otolith shape and microchemistry to assess *Genidens barbus* fish stock structure, *Estuarine, Coastal and Shelf Science* (2021), doi: https://doi.org/10.1016/j.ecss.2021.107560.

Integrated use of otolith shape and microchemistry to assess *Genidens barbus* fish stock structure

Thaís Rodrigues Maciel[a], Marcelo Vianna[a,b], Barbara Maichak de Carvalho[c], Nathan Miller[d], Esteban Avigliano[e] *

[a] Instituto de Biologia, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho, 373, Ilha do Fundão, (21941-541) Rio de Janeiro, RJ, Brazil.

[b] Instituto Museu Aquário Marinho do Rio de Janeiro-AquaRio (IMAM/AquaRio) – Rio de Janeiro Marine Aquarium Research Center, Rio de Janeiro, Brazil. E-mail address: mvianna@biologia.ufrj.br

[c] Programa de Pós-Graduação em Engenharia Ambiental, Departamento de Engenharia - Universidade Federal do Paraná, Centro Politécnico, (81531-970) Bairro Jardim das Américas, Curitiba, Paraná, Brazil. E-mail address: bmaicarvalho@gmail.com

[d] Jackson School of Geosciences, University of Texas at Austin, Austin, (78712) Texas, USA. E-mail address: nrmiller@jsg.utexas.edu

[e] Instituto de Investigaciones en Producción Animal (INPA-CONICET-UBA), Facultad de Ciencias Veterinarias, Universidad de Buenos Aires (UBA), Av. Chorroarín 280, (C1427CWO) Ciudad Autónoma de Buenos Aires, Argentina. E-mail address: estebanavigliano@conicet.gov.ar

* Corresponding author: estebanavigliano@conicet.gov.ar

Abstract

Otolith composition (edge vs core: Mg/Ca, Mn/Ca, Zn/Ca, Sr/Ca, Ba/Ca) by LA-ICP-MS and Elliptic Fourier analysis were integrated to evaluate spatial segregation of adult and juvenile stages of *Genidens barbus* from specimens collected from five coastal areas off Brazil (Paraíba do Sul River, Guanabara Bay, Itapanhaú River mouth, Paranaguá Bay), Argentina and Uruguay (La Plata Estuary). Fisheries of this diadromous catfish have largely collapsed in the southwest Atlantic coastal region due to overexploitation. An understanding of population structure is now critically needed for improved management strategies for this endangered species. PERMANOVA based on chemistry showed significant differences ($p<0.05$) between all sites, except Itapanhaú River and Paranaguá Bay (edge). Shape, by comparison, found significant differences between all sampling sites, except Guanabara Bay and Paranaguá Bay, and Itapanhaú River and Paranaguá Bay. Discriminant analysis cross-classification success based on chemistry ranged from 33.3 (Paranaguá Bay) to 100% (La Plata Estuary), and 66.7 (Paranaguá Bay) to 100% (La Plata Estuary) for otolith edges (mean=61.3%) and cores (mean=78.9%). For otolith shape, the jackknifed rate (mean=45.9%) was relatively low for all sites (32.1-44.7%) except La Plata Estuary (67.6%). Although we do not find otolith shape to be particularly useful; otolith microchemistry supports the presence of different management units. The results revealed that on a small geographic scale (~300 km) microchemistry might not be efficient to discriminate between some sampling sites.

Key words: catfish; estuaries; fish stock; segregation; trace elements.

1. Introduction

The diadromous catfish *Genidens barbus* (Lacépède 1803), family Ariidae, inhabits freshwater, estuarine and marine environments ranging from Bahía (Brazil) to Patagonia (Argentina) (Caille et al., 1995; López and Bellisio, 1965). This catfish presents a plastic and complex life cycle, including differing amphidrome migration patterns and resident freshwater specimens (Avigliano et al., 2021, 2017c, 2015b). Amphidromus specimens typically migrate from the sea/estuary to lower salinity waters during reproduction period (Avigliano et al., 2017c). Males perform oral incubation of offspring, releasing them in the mouth of the estuary, where they remain until complete development, and then migrate as far as the continental platform (Avigliano et al., 2017c, 2015b, 2015c; Reis, 1986a).

Formerly an important species for artisanal fishing, mainly in Southern Brazil (Gomes and Araújo, 2004; Reis, 1986b; Velasco et al., 2007), the catch rate has dropped significantly in recent decades. In response to dramatic declines in landings (e.g., from 30,000 in 1962 to to 10,000 in 2011; (Mendonça et al., 2017), *G. barbus* was added to the List of Endangered Brazilian Fauna, subsequently banning its capture, transport, and storage (MMA, 2014). Factors besides overfishing, such as pollution and habitat loss, also contribute to this decline (Barletta and Lima, 2019; Kime, 1995). Complex reproduction and high age of first maturity (8.5 years for females and 9 years for males, (Reis, 1986a) also hinder the recovery of this fishery species. Thus, effective management of *G. barbus* stocks is essential to their recovery. The term “fishing stock” refers to a commercially exploited resource that is included within fishery management strategies (Begg and Waldman, 1999). Fishing stocks are basic work units in which the state of fisheries is assessed and management policies applied to implement sustainable exploitation (Tanner, et al., 2015). Among the various techniques available for

identifying fish stocks, chemical fingerprinting of calcified structures, such as otoliths, has proven to be one of the most efficient. Otoliths are calcified structures present in the inner ear of teleost fish that form by continuous outward (radial) growth through life (Campana, 1999). Ambient water is the predominant source of most inorganic elements deposited in its matrix (Campana, 1999; Hüssy et al., 2020; Kerr and Campana, 2014), although diet, ontogeny and genetics can also affect its chemical composition (Clarke et al., 2011; Elsdon, and Gillanders, 2003; Morales-Nin, 2000). Because otoliths are metabolically inert structures, the elements deposited in their accreting surface layers are permanently retained (Campana, 1999), and the core-to-edge chemical composition can be used as a biological tracer to discriminate between fish groups that spend parts of their lives in different areas (Kerr and Campana, 2014). In fact, otolith core and edge elemental signatures have proven useful for identifying both nursery areas (Avigliano et al., 2016; Bailey et al., 2015; Patterson et al., 2008) and discriminating fish stocks (Avigliano, 2021; Kerr and Campana, 2014; Soeth et al., 2019; Tanner, et al., 2015).

In Southwestern Atlantic (South America) coastal waters, a relationship between the water and *G. barbus* otoliths has been reported for Ba:Ca and Mn:Ca ratios (Avigliano et al., 2019). Other microchemical studies have identified four *G. barbus* nursery areas using otolith core compositions (Avigliano et al., 2016) and five fish stocks through integration of dorsal fin spine and otolith edge compositions (Avigliano, et al., 2017; Avigliano et al., 2019). However, evidence supporting the existence of different stocks is fragmented because the studies have thus far been limited to widely separated geographic areas (e.g., 700-1000 km spacing) and have sampled limited portions of life histories (e.g., otolith edges). Critically needed are studies integrating sampling sites with differing spacings but covering the entire distribution area of this fishery.

Besides the life history information gained from otolith chemistry, otolith shape can provide complementary marker information for stock discrimination (Biolé et al., 2019; Hüssy et al., 2016; Ibáñez et al., 2017). Genetic and environmental factors (*e.g.* temperature, depth, salinity and food availability) can significantly impact fish growth rates and otolith growth patterns (Longmore, et al., 2010; Vignon and Morat, 2010a). Accordingly, fish that experience different environmental conditions may tend to present differences in otolith shape, and these differences can be statistically characterized. Otolith shape (morphometry) analysis is also much more economical than microchemistry and has proven useful for discerning habitat use (Avigliano et al., 2015d, 2014), sexual dimorphism (Maciel et al., 2019), species (Avigliano et al., 2018; Gut et al., 2020), relative growth (Maciel et al., 2019; Perin and Vaz-dos-Santos, 2014), stocks (Annabi et al., 2013; Biolé et al., 2019; Soeth et al., 2019) and nursery areas (Avigliano et al., 2017b). More efficient and accurate stock characterization may thus be obtained from integrated studies of otolith morphometry and chemistry (Biolé et al., 2019; Longmore, et al., 2010; Soeth et al., 2019).

Toward improved management strategies of this endangered species, we integrate otolith microchemistry and morphometry to discern *G. barbus* population structure in the main area of its distribution.in the Southwestern Atlantic coastal region.

2. Materials and methods

2.1. Study area

Genidens barbus specimens were collected from five Southwestern Atlantic coastal river mouth estuaries, four off Brazil: 1) Paraíba do Sul River, 2) Guanabara Bay, 3) Itapanhaú River, 4) Paranaguá Bay, and one off Argentina-Uruguay: 5) La Plata (Figure 1).

Paraíba do Sul River mouth is a delta (Krüger et al., 2006) located in Rio de Janeiro state (21º36'23.9"S, 41º02'43.8"W), near the frontier with Espírito Santo state (Brazil). The delta is characterized by beach ridges, that separate a main estuary in the Altafona region from a secondary estuary in the Gargaú region (Bernini and Rezende, 2004). Paraíba do Sul River has a length of 1145 km, a drainage basin of 55.400 km^2 and salinities ranging from 0 to 30‰ (Krüger et al., 2006). Guanabara Bay, southeast of Rio de Janeiro State (Brazil) (22°48'24.3"S, 43°09'14.7"W), is a semi-closed estuarine coastal ecosystem (Meniconi et al., 2012). It has an area of 380 km^2, a contributing drainage basin of 4080 km^2 and salinities ranging from 0 to 35‰ (Kjerfve et al., 1997). This bay is currently considered to be one of the most degraded estuarine systems off the Brazilian coast (Meniconi et al., 2012; Valentin et al., 1999). Itapanhaú River estuary, east of São Paulo state (Brazil) (23º49'47.1"S, 46º09'12.8"W), contributes to the Bertioga Channel, adjacent to Santos Bay, the largest port terminal of Latin America (Ambrozevicius and Abessa, 2008). It has a drainage basin of 260 km^2 (Bernardes and Miranda, 2001) and salinities ranging from 0 to 35‰ (Maciel, unpublished data). Paranaguá Estuarine Complex, located in coastal Paraná state (Brazil) (25°29'26.7"S, 48°29'55.6"W), is divided into into north-south (Laranjeira and Pinheiro) and east-west (Paranaguá Bay) regions, with an area of 612 km^2 (Lana et al., 2001) and salinities ranging from 0 to 31‰ (Dias et al., 2016). La Plata Estuary is formed by the convergence of the Paraná and Uruguay rivers (Guerrero et al. 1997), between Argentina and Uruguay (34°30'S e 58°10'W). With an area of 35,000 km^2, the estuary is the second largest fluvio-marine system in the Americas, with a contributing drainage basin area of 3,170,000 km^2. Salinities range from 0 to 32‰ (Acha et al., 2008; Avigliano et al., 2017c).

155 **2.2. Sampling**

156 All sample specimens were obtained from small scale artisanal landings, except those
157 from La Plata Estuary, Argentina (Paraná Guazú and Sauce rivers), which were
158 collected by scientific collections using hook. Fishes were identified based on
159 Marceniuk (2005) and Marceniuk and Menezes (2007) keys, measured (total length -
160 TL, ± 0.1 cm) and the *lapillus* otoliths were removed, washed with ultrapure water, air
161 dried and weighed (± 0.001g). Table 1 shows the biometrics information.

162

163 **2.3. Otolith chemistry**

164 *Genidens barbus* left otoliths (Table 1) were decontaminated with 3% H_2O_2, 2% HNO_3
165 (Merck KGaA, Garmstadt, Germany) and washed with Milli-Q water (resistivity 18.2
166 mOhm/cm), and dried (Avigliano et al., 2017d). Otoliths were inserted into epoxy resin,
167 and 700 µm thick central sections were obtained from cured mounts using a Buehler
168 Isomet low speed saw (Hong Kong, China). The sections were fixed onto glass slides
169 using epoxy resin, and the upper otolith surface polished with 10 µm grit sandpaper and
170 then thoroughly rinsed with Milli-Q water. As age may influence the chemical
171 composition of calcified structures, annual otolith growth rings (Reis 1985b) were
172 counted under magnification (40x) using a Leica EZ4-HD stereomicroscope. Laser
173 Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) was used to
174 measure ^{43}Ca, ^{24}Mg, ^{51}V, ^{55}Mn, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{107}Ag, ^{111}Cd, ^{138}Ba, ^{202}Hg
175 and ^{208}Pb in otolith cores and edges at the University of Texas at Austin Department of
176 Geosciences (USA). The LA-ICP-MS system consisted in an ESI NWR193-UC
177 excimer laser ablation system (193 nm, 4ns pulse width) coupled to an Agilent 7500ce
178 ICP-MS. A rectangular aperture of 25x100 µm was used at 8 µm s^{-1} (repetition rate/ of

10 Hz, energy densities/ 3.8 Jcm^{2-1}). To minimize the temporal sampling alias, the long axis of the aperture was maintained parallel to growth banding in all the transects.

Based on the growth ring chronologies, the sampled core and edge regions correspond to within the first year (inner 1,000-1,500 µm) and last two years (outer 300 µm) of life, respectively. Prior to measurements, the surface contaminants were removed by pre-ablation, using a spot size of 25x125 µm at 50 m s^{-1}. The ICP-MS was operated at power of 1600 W with using argon as carrier gas (flow/800 mL min^{-1}). Ratios $^{232}Th^{16}O/^{232}Th$ (<0.35%) and $^{238}U/^{232}Th$ (~1) were used during tuning to monitor the oxide production and mass fractionation plasma robustness. NIST 612 and USGS MACS-3 reference materials, analyzed in replicate each hour, were used as external and primary standards, respectively (Jochum et al., 2011).

Analyte intensities were converted to elemental concentrations (mg kg^{-1}) using Iolite software (Paton et al., 2011), with ^{43}Ca (38.3 weight %) as the internal standard (Yoshinaga et al. 2000).

Reference materials recoveries averaged and were within 7% of to GeoREM preferred values (Jochum et al. 2011): 98% for ^{138}Ba, 106% for ^{63}Cu, 96% for ^{24}Mg, 103% for ^{55}Mn, 102% for ^{60}Ni, 100% for ^{88}Sr and 93% for ^{66}Zn. Detection limits (LOD, mg kg^{-1}) based on three times the standard deviation of the estimated baseline intensity during bracketing gas blank intervals were 0.01 for ^{138}Ba, 0.21 for ^{63}Cu, 0.12 for ^{24}Mg, 0.16 for ^{55}Mn, 0.07 for ^{60}Ni, 0.01 for ^{88}Sr and 0.28 for ^{66}Zn. Concentrations were expressed as molar ratios in relation to Ca (element/Ca, mmol mol^{-1}). Otolith ^{51}V, ^{60}Ni, ^{63}Cu, ^{85}Rb, ^{107}Ag, ^{111}Cd, ^{202}Hg and ^{208}Pb levels were below LOD, thus, further data analysis is based on concentrations of Mg, Mn, Zn, Sr and Ba.

2.4. Otolith morphometry

Digital images of the distal face of *G. barbus* right *lapilli* otoliths (Table 1) were recorded (Nikon Coolpix L110) at the same magnification against a black background with a scale.

Differences in otolith contour between estuaries were calculated using Elliptic Fourier Analysis (EFA), where otolith curvature was modeled as a two-dimensional closed curve applying a combination of harmonically related sine and cosine functions (descriptors), with each function composed of four Fourier Coefficients (FCs). The first 12 harmonics obtaind 99.99% of the accumulated power, according to the Fourier Power Spectrum (Crampton, 1995) (Figure 2). The first three descriptors were used to normalize the FCs, being converted into constants, totaling 45 FCs instead of 48. The FCs were calculated using the Shape 1.3 software.

2.5. Statistical analysis

A covariance analysis (ANCOVA) was used to verify the effect of total length (TL) on FCs. One FC (b8) co-varied with TL ($F=4.0$, $p=0.045$) and were corrected by subtracting the common slope of the ANCOVAs. To avoid potential allometric effects, FCs were normalized to TL using the allometric ratio and standardized to a fish length of 58.5 cm (mean TL for all fish) (Lleonart et al. 2000). A principal component analysis (PCA) was performed to reduce the dimension of the morphometric data matrix (Tuset et al., 2020) and convert the 45 FCs into 11 independent principal components (PCs), created through the orthogonal combination of the original variables, which accumulated 99.9% of the variability. This process also reduces multi-collinearity between the FCs.

Shapiro-Wilk and Levene tests were performed to test elemental ratios for normality and homogeneity of variance, respectively. Spearman and ANCOVA correlation were

229 used to test the effect of age, TL and otolith weight on the element:Ca ratios (Longmore
230 et al. 2010; Kerr and Campana 2014). Only Mg:Ca, Mn:Ca, Zn:Ca and Sr:Ca in the core
231 co-varied with age ($7.0<F<37$ $0.0001<p<0.01$) and this trend was removed by
232 subtracting the common slope of the ANCOVAs. Since core Mg:Ca, Zn:Ca and Sr:Ca
233 ratios fulfilled the assumptions of normality and homogeneity (Shapiro-Wilk and
234 Levene tests, $p>0.05$), ANOVA and Tukey tests were used to make univariate
235 comparisons between sampling sites. Core Mn:Ca and Ba:Ca ratios and edge
236 elemental:Ca ratios did not meet the assumptions simultaneously (Shapiro-Wilk and
237 Levene tests, $p<0.05$), so Kruskal Wallis was used for the same purpose.
238 Permutational multivariate analysis of variance (PERMANOVA) based on the distances
239 of Mahalanobis with 9999 permutations (Anderson 2006) was used to evaluate otolith
240 morphometric (PCs) and multi-elemental (core and edge separately) differences for each
241 sampling site.
242 Discriminant analyses were performed to assess the ability of the data to be classified
243 in their respective capture areas. Because the variance–covariance matrices were not
244 homogeneous (Box test, $p <0.05$), quadratic models (QDA) instead of linear were
245 applied to assess classification by otolith shape (PCs), otolith core and edge chemistry.
246 An additional discriminant analysis (lineal model, LDA) was conducted using the
247 otolith shape variables (PCs) and the edge elemental ratios together to determine if the
248 integrated use of these two methods of stock assessment can improve discriminatory
249 ability in relation to their independent use. LDA was used because co-variance matrices
250 were homogeneous (Box test, $p>0.05$).
251 Prior to discriminant analyses, multi-collinearity was verified by using the tolerance and
252 F-to-remove values (Hair et al., 2013). The classification accuracy of the QDA and
253 LDA were tested by leave-one-out cross-validation (stepwise jackknifed procedure).

Finally, the expected prior probability classification (random rate) was estimated on sample sizes and group numbers, and then, proportion tests were performed to assess the difference between random and the percentage of correctly classified individuals.

Statistical analyses were performed using Mystat 13 and SPSS 17.0 software.

3. Results

3.1. Otolith chemistry

Three element:Ca ratios (Mg:Ca, Sr:Ca, Ba:Ca) were significantly different ($p<0.05$) between several sampling sites for otolith edge measurements (Figure 3), whereas no differences were observed for Mn:Ca ($H= 2.98$; $p= 0.5613$) and Zn:Ca ($H= 2.78$; $p= 0.5950$). Otolith edge Mg:Ca was higher in Paranaguá Bay and lower in Paraíba do Sul River ($H= 13.40$; $p= 0.0095$). Edge Sr:Ca was higher in Paraíba do Sul River, Itapanhaú River and Paranaguá Bay, and lower in Guanabara Bay. Edge Ba:Ca ratio was high in La Plata Estuary and low in Guanabara Bay.

Multivariate analysis (PERMANOVA) based on otolith edge chemistry reveals significant differences between all sites ($2.4<F<6.1$; $0.003<p<0.22$), except for Itapanhaú River-Paranaguá Bay ($F=1.4$, $p=0.22$) and Paraíba do Sul River-Itapanhaú River ($F=1.8$, $p=0.09$). The first two axes of the QDA (Wilks's Lambda=0.041, F=22.7, $p<0.0001$) explained 97% of the variability, with the first axis being the most representative (93%). Based on means of standardized coefficients, the most relevant variables for classification were Ba/Ca (-1.1) and Sr/Ca (0.34) for the first function and Sr/Ca (0.93) and Zn/Ca (-0.29) for the second one.

Cross-classification matrix indicates that 61.3% of individuals were correctly classified. For the chemical analyses, expected prior probabilities of classification (in %) were 18, 24, 31, 8 and 7 for Paraíba do Sul River, Guanabara Bay, Itapanhaú River,

Paranaguá Bay and La Plata Estuary, respectively (Table 2). The percentage of classification was perfect (100%) for the La Plata Estuary and relatively high for Guanabara Bay (86.4%), indicating they are non-random outcomes (Table 2). For the other sites, low/moderate (33.3-55.5%) and no significant differences in classification rate were observed with respect to random (Table 2). However, misclassified individuals were assigned to geographically proximal sampling sites, except for La Plata Estuary.

Otolith core Mg:Ca was higher in Paraíba do Sul River and Itapanhaú River, intermediate in Guanabara and Paranaguá Bays and lower in La Plata Estuary ($F= 13.0$; $p= 0.01$). Core Mn:Ca ratio was higher in La Plata Estuary and lower in the other sampling sites ($H= 18.1$; $p= 0.001$). Core Zn:Ca was higher in Paranaguá Bay and Paraíba do Sul River, intermediate in Guanabara Bay and Itapanhaú River and lower in La Plata Estuary ($H= 11.2$; $p= 0.02$). Sr:Ca was higher in Itapanhaú River and Paranaguá Bay, intermediate in Paraíba do Sul River and Guanabara Bay, and lower in La Plata Estuary ($H= 27.0$; $p<0.0001$). The highest core Ba:Ca values were found in specimens collected from Paraíba do Sul River and La Plata Estuary ($H= 46.9$; $p<0.0001$).

PERMANOVA based on otolith core chemistry demonstrates significant differences between all sites ($2.6<F<6.2$; $0.0001<p<0.01$). The first two function of the QDA (Wilks's Lambda=0.088, F=14.9, p<0.0001) explained 83 and 7% of the variability, respectively (accumulate=90%). Ba/Ca (first function=0.69, second function=0.86) and Mn/Ca (first function=0.51, second function=-0.77) were the most important variables for the classification. The mean jackknifed classification percentage was 78.9% and like otolith edge, the classification percentage was 100% for the La Plata Estuary. The jackknifed percentages for the other sites ranged from 66.7 to 82.4%. All

304 jackknifed classification percentages were significantly higher than random (Table 2).
305 Except for Paranaguá and Paraiba do Sul capture sites, misclassified individuals from
306 other sites were classified into geographically close sampling sites.
307
308 **3.2. Otolith morphometry**
309 PERMANOVA showed significant difference in the otolith shape between all sampling
310 sites (F=3.0; $0.0001<p<0.02$), except between Guanabara and Paranaguá bays, and
311 between Itapanhaú River and Paranaguá Bay ($0.051<p<0.12$).
312 According to the LDA (Lambda de Wilks=0.39, $p<0.0001$) the first two discriminant
313 functions explained 51.3% and 21.9% (accumulate: 73.2%) of the variability,
314 respectively.
315 The percentage of well-classified individuals (mean=45.9%) was relatively low for all
316 sites (32.1-67.6%), except La Plata Estuary (67.6%). The prior probabilities of
317 classification (random, in %) were 18 for Paraíba do Sul River, 26 for Guanabara Bay;
318 19 for Itapanhaú River, 18 for Paranaguá Bay and 21 for La Plata Estuary. Re-
319 classification rates were significantly higher than random for Paraíba do Sul River and
320 La Plata estuary (Table 2).
321
322 **3.3. Simultaneous use of chemistry and morphometry**
323 According to LDA (Wilks's Lambda=0.05, F=11.7, $p<0.0001$) based on the concomitant
324 use of the two techniques , the mean classification percentage (58.2%) was intermediate
325 in relation to that obtained when both methods were applied separately (Table 2). The
326 LDA analysis had high jackknifed classification success for La Plata Estuary (100%),
327 moderate success for Guanabara Bay (66.7%) and relatively low success for the other

328 sites (16.7-54.5%). Only Guanabara Bay and La Plata Estuary showed higher
329 classification rates than chance (Table 2).
330
331 **4. Discussion**
332 This study evaluated otolith microchemistry and shape for the first time as a potential
333 integrated tool for discrimination of catfish stocks in Southwestern Atlantic estuarine
334 settings, including two (Paraíba do Sul River and Itapanhaú River) estuaries that have
335 not been considered in previous population structure evaluations. Multivariate analysis
336 based on otolith chemistry proved effective for delimiting several capture sites. QDA
337 based on edge chemistry was particularly effective for delimiting samples from La Plata
338 Estuary and Guanabara Bay, while the other sites had low classification rates. The high
339 classification rates obtained for La Plata Estuary (100%) and Guanabara Bay (86.4%)
340 are consistent with previous studies, which reported values of 100% for Guanabara Bay
341 (Avigliano et al., 2017d) and between 81.3% (Avigliano et al., 2019) and 100%
342 (Avigliano et al., 2017d, 2015c) for La Plata Estuary. Although classification rates are
343 sensitive to the variables used and the number of groups, the available evidence
344 supports that different stocks reside within these two coastal systems.
345 Based on morphological and chemical analyzes, Paraíba do Sul, the northernmost site
346 studied to date, showed a high overlap with the sites located to the south (Guanabara
347 Bay, 500 km) and Paranaguá Bay (1,000 km), while high segregation was observed in
348 relation to the more southern and remote (2,400 km). Itapanhaú River, one of the sites
349 studied for the first time, had a high overlap with sites located to the north (Paraíba do
350 Sul and Guanabara Bay), but showed high segregation in relation to the southernmost
351 localities (Paranaguá Bay and La Plata Estuary). *G. barbus* specimens from Paranaguá
352 Bay (N=6) were mostly classified (misclassification=66.7%) in Itapanhaú River, but we

353 acknowledge that this could be a statistical artifact due to the relatively small sample
354 size compared to other capture sites. Previous studies have reported classification
355 percentages of 82-100% using otoliths (Avigliano et al., 2017d, 2019a) and 100% using
356 spines (Avigliano et al., 2019c, 2020) for Paranaguá Bay. However, consistent with
357 previous otolith and spine studies we observe no overlap with La Plata Estuary
358 (Avigliano et al., 2020, 2019, 2017d) and relatively low (18%) overlap with Guanabara
359 Bay (Avigliano et al., 2017d), which supports that the chemical signature of Paranaguá
360 Bay can be distinguished from Guanabara Bay and La Plata Estuary.

361 The performance of both techniques applied simultaneously was lower than that of the
362 chemistry used alone. This is probably due to the relatively low efficiency of
363 morphometry to discriminate between some of the *G. barbus* stocks (Table 2).
364 However, when both techniques are moderately efficient to discriminate between
365 stocks, the simultaneous use of these tends to increase the classification percentages
366 (Biolé et al., 2019; Longmore, et al., 2010; Soeth et al., 2019), since the variables can
367 respond to different drivers, for example environmental, physiological, genetic, or
368 evolutionary (Avigliano, 2021; Tanner, et al., 2015).

369 The high overlap obtained here among the closest sites (e.g. Itapanhaú River and
370 Paranaguá Bay, and Paraíba do Sul, Guanabara Bay and Paranaguá Bay) could be due
371 to a high connectivity between these sampling sites, however, similar environmental or
372 endogenous pressures (e.g. physiological factors) on these sites could contribute to
373 similar chemical signatures in the region (Avigliano, 2021). A recent *G. genidens*
374 otolith edge microchemical study found high misclassification rates (up to 52.6%)
375 between Itapanhaú River and Paranaguá Bay (Maciel et al., 2020). These authors
376 suggested that *G. genidens* presents low connectivity between the studied estuaries, so
377 similar chemical fingerprints in the present study may indicate similar environmental

pressures rather than high connectivity.

Maciel et al. (2020) also found that otolith core-based classification rates (78.9%) for *G. barbus* were somewhat higher than edge-based classification rates (61.3%), suggesting some segregation between study sites. However, classification of adult fish based on the otolith cores should be taken with caution because the environment used by juveniles does not necessarily correspond to that in capture sites. Assuming that there is high segregation throughout ontogeny, differences in classification success between core and edge could be due to spatio-temporal environmental variations. For example, Avigliano et al. (2017a) demonstrated that temporal variation in the chemical signature of the adult catfish otolith core can affect the percentages of spatial classification.

Ba/Ca, Sr/Ca, Mn/Ca and Zn/Ca were the most important variables for delimiting groups. In several diadromous species, Ba/Ca and Sr/Ca can negatively and positively proxy salinity, rather than to other exogenous or endogenous factors (Brown and Severin, 2009), and thus have been widely used to track migratory routes in saline gradients (Araya et al., 2014; Hermann et al., 2016). Particularly in *G. barbus*, these element:Ca ratios in calcified structures were associated with salinity and surrounding water concentration (Avigliano et al., 2019), and have been useful in describing the life history patterns (Avigliano et al., 2017c, 2015b). The incorporation of Mn into the otolith can be regulated by both endogenous and exogenous factors, and is species-dependent (Hüssy et al., 2020; Sturrock et al., 2015). In some species such as *Micropogonias furnieri* (Avigliano, et al., 2021), *Micropogonias undulatus* (Altenritter et al., 2018; Dorval et al., 2007) and *Morone saxatilis* (Mohan et al., 2012), Mn/Ca has been linked with environmental concentration (Dorval et al., 2007; Mohan et al., 2012), while in *Gadus morhua*, *Platichthys flesus*, *Pseudopleuronectes americanus*, and *M. undulates*, it has been negatively associated to dissolved oxygen. Other factors such as

403 temperature, salinity, food composition and/or growth have also been identified as
404 potential Mn drivers (Hüssy et al., 2020; Thomas and Swearer, 2019). For *G. barbus*,
405 Mn/Ca values are several times higher in the core than in the edge, as previously
406 reported by (Avigliano et al., 2017d, 2017a), and similarly reported for other species
407 (Limburg et al., 2015; Miller, 2009; Rogers et al., 2019), including *G. genidens* (Maciel
408 et al., 2020) and other ariids like *Cathorops spixii* (Maichak de Carvalho et al., 2020).
409 Mn enrichment in otolith cores has been linked to maternal transfer, transition to free-
410 embryo or juvenile or growth (Hüssy et al., 2020). Literature on otolith Zn
411 incorporation is scare. Otolith Zn levels seem to be independent of environmental
412 concentrations (Ranaldi and Gagnon, 2008), but may be influenced by diet (Ranaldi and
413 Gagnon, 2008), growth (Hüssy et al., 2020; Sturrock et al., 2015), ontogeny (Avigliano
414 et al., 2015a; Ranaldi and Gagnon, 2008) and gonadosomatic activity (Sturrock et al.,
415 2015). In the present study, Zn/Ca values were comparable between core and and
416 spatially within the edge, and controlling factor are unclear.

417 Otolith shape is also affected for endogenous and exogenous factors (Reichenbacher et
418 al., 2009; Vignon and Morat, 2010b), which may be expressed as inter and intra-specific
419 morphometric differences. Quantitative characterization of these differences have been
420 exploited in recent decades to study stock compositions (Biolé et al., 2019; Longmore,
421 et al., 2010) and species delimitation (Avigliano et al., 2018; Tuset et al., 2013). Among
422 the main environmental morphologic drivers are temperature, salinity, depth and diet
423 (Lombarte and Lleonart, 1993; Sea et al., 2008; Tuset et al., 2003; Vignon, 2018),
424 however, genetics (Annabi et al., 2013; Reichenbacher and Reichard, 2014; Vignon and
425 Morat, 2010b), growth rate and ontogeny (Vignon, 2012) can also affect morphology.
426 In this study, multivariate analyses of otolith contours allowed partial site
427 discrimination, particularly La Plata Estuary and marginally for Paraíba do Sul.

However, the otolith shape showed low discriminatory power and high overlap in both PERMANOVA and discriminant analysis, therefore, this methodology is not recommended for delimiting *G. barbus* stocks, at least among the sampling sites from Brazil. Interestingly, EFA provide efficient for delimiting *G. genidens* catfish population between Paraíba do Sul River, Guanabara Bay and Paranaguá Bay (Maciel et al., 2020), suggesting that factors that regulate otolith shape are species-dependent within the genus. On the other hand, *G. genidens* is considered to be a less migratory species than *G. barbus* (Maciel et al., 2020), remaining within estuaries throughout most of its life. This would not only reduce the chance of connectivity, but could reduce the variability of environments to which they are exposed, impacting on the otolith morphometry.

In summary, this work complements the evaluation of catfish population structure in the Southeastern Atlantic margin between latitude 21° and 34° S, by integrating otolith shape and microchemistry in previously studied and two new study sites. Otolith shape was not particularly useful for classification, whereas otolith microchemistry supported the site specific differences reported by previous studies, and shed light on the regional population structure as a whole. The results revealed on a smaller geographical scale (~300 km), microchemistry may confuse between some sampling sites, which could be revealing the limits of the method's discriminatory capacity or events of high connectivity. Synthesis of all findings supports the presence of different management units, corresponding to the La Plata Estuary, Lagoa dos Patos, Paranaguá-Itapanhaú River, and Paraíba do Sul-Guanabará. Future studies may improve population assessments of connectivity and distribution limits by including other methodologies, such as genetics, capture and recapture assessments.

5. Acknowledgements

We thank the CNPq, FAPERJ (MV) and CAPES (TM and BMC) for the research grants and studentships. Funding was provided by National Council for Scientific and Technological Development (CNPq), Ministry of Science, Technology, Innovations and Communications (MCTIC) and Secretary of Aquaculture and Fisheries, Ministry of Agriculture, Livestock and Food Supply (SAP-MAPA) (Call MCTI/MPA/CNPq Nº 22/2015 - Process: 445782/2015-3), Agencia Nacional de Promoción Científica y Tecnológica (Grant PICT 2015-1823), and Universidad de Buenos Aires (Grant UBACyT 160 20020150100052BA).

6. References

Acha, E.M., Mianzan, H., Guerrero, R., Carreto, J., Giberto, D., Montoya, N., Carignan, M., Marcelo Acha, E., Mianzan, H., Guerrero, R., Carreto, J., Giberto, D., Montoya, N., Carignan, M., 2008. An overview of physical and ecological processes in the Rio de la Plata Estuary. Cont. Shelf Res. 28, 1579–1588. https://doi.org/10.1016/j.csr.2007.01.031

Altenritter, M.E., Cohuo, A., Walther, B.D., 2018. Proportions of demersal fish exposed to sublethal hypoxia revealed by otolith chemistry. Mar. Ecol. Prog. Ser. 589, 193–208. https://doi.org/10.3354/meps12469

Ambrozevicius, A.P., Abessa, D.M.S., 2008. Acute toxicity of waters from the urban drainage channels of Santos (São Paulo, Brazil). Panam. J. Aquat. Sci. 3, 108–115.

Annabi, A., Said, K., Reichenbacher, B., 2013. Inter-population differences in otolith morphology are genetically encoded in the killifish *Aphanius fasciatus* (Cyprinodontiformes). Sci. Mar. 77, 269–279. https://doi.org/10.3989/scimar.03763.02A

Araya, M., Niklitschek, E.J., Secor, D.H., Piccoli, P.M., 2014. Partial migration in introduced wild chinook salmon (*Oncorhynchus tshawytscha*) of southern Chile. Estuar. Coast. Shelf Sci. 149, 1–9. https://doi.org/10.1016/j.ecss.2014.07.011

Avigliano, E., Alves, N.., Rico, R., Ruarte, C., D'Atri, L., Méndez, A., Pisonero, J., Volpedo, A., Borstelmann, C., 2021. Population structure and ontogenetic habitat use of *Micropogonias furnieri* in the southwestern Atlantic Ocean inferred by otolith chemistry. Fish. Res. 204, 105953.

Avigliano, E., Carvalho, B.M., Leisen, M., Romero, R., Velasco, G., Vianna, M., Barra, F., Volpedo, A.V., 2017. Otolith edge fingerprints as approach for stock identification of *Genidens barbus*. Estuar. Coast. Shelf Sci. 194, 92–96. https://doi.org/10.1016/j.ecss.2017.06.008

Avigliano, E., 2021. Optimizing the methodological design in fish stock delineation from otolith chemistry: review of spatio-temporal analysis scales. Rev. Fish. Sci. Aquac. in press.

Avigliano, E., Carvalho, B., Velasco, G., Tripodi, P., Vianna, M., Volpedo, A.V., 2016. Nursery areas and connectivity of the adults anadromous catfish (*Genidens barbus*) revealed by otolith-core microchemistry in the south-western Atlantic Ocean. Mar.

Freshw. Res. 68, 931–940. https://doi.org/10.1071/MF16058
Avigliano, E., Carvalho, B., Velasco, G., Tripodi, P., Volpedo, A.V., 2017a. Inter-annual variability in otolith chemistry of catfish *Genidens barbus* from South-western Atlantic estuaries. J. Mar. Biol. Assoc. United Kingdom. https://doi.org/10.1017/S0025315417000212
Avigliano, E., Carvalho, B.M., Miller, N., Gironde, S.C., Tombari, A., Limburg, K.E., Volpedo, A.V., 2019. Fin spine chemistry as a non-lethal alternative to otoliths for stock discrimination in an endangered catfish. Mar. Ecol. Prog. Ser. 614, 147–157. https://doi.org/10.3354/meps12895
Avigliano, E., Domanico, A., Sánchez, S., Volpedo, A. V., 2017b. Otolith elemental fingerprint and scale and otolith morphometry in *Prochilodus lineatus* provide identification of natal nurseries. Fish. Res. 186, 1–10. https://doi.org/10.1016/j.fishres.2016.07.026
Avigliano, E., Leisen, M., Romero, R., Carvalho, B., Velasco, G., Vianna, M., Barra, F., Volpedo, A.V., 2017c. Fluvio-marine travelers from South America: Cyclic amphidromy and freshwater residency, typical behaviors in *Genidens barbus* inferred by otolith chemistry. Fish. Res. 193, 184–194. https://doi.org/10.1016/j.fishres.2017.04.011
Avigliano, E., Maichak de Carvalho, B., Leisen, M., Romero, R., Velasco, G., Vianna, M., Barra, F., Volpedo, A.V., 2017d. Otolith edge fingerprints as approach for stock identification of *Genidens barbus.* Estuar. Coast. Shelf Sci. 194, 92–96. https://doi.org/10.1016/j.ecss.2017.06.008
Avigliano, E., Martinez, C.F.R., Volpedo, A.V., 2014. Combined use of otolith microchemistry and morphometry as indicators of the habitat of the silverside (*Odontesthes bonariensis*) in a freshwater-estuarine environment. Fish. Res. 149, 55–60. https://doi.org/10.1016/j.fishres.2013.09.013
Avigliano, E., Miller, N., Maichak fr Carvalho, B., Córdoba, S., Tombari, A., Volpedo, V.A., 2020. Fin spine metals by LA-ICP-MS as a method for fish stock discrimination of *Genidens barbus* in anthropized estuaries. Fish. Res. 230, 105625. https://doi.org/10.1016/j.fishres.2020.105625
Avigliano, E., Pisonero, J., Méndez, A., Tombari, A., Volpedo, A. V., 2021. Habitat use of the amphidromous catfish *Genidens barbus*: first insights at its southern distribution limit. New Zeal. J. Mar. Freshw. Res. 0, 1–7. https://doi.org/10.1080/00288330.2021.1879178
Avigliano, E., Rolón, M.E., Rosso, J.J., Mabragaña, E., Volpedo, A.V., 2018. Using otolith morphometry for the identification of three sympatric and morphologically similar species of *Astyanax* from the Atlantic Rain Forest (Argentina). Environ. Biol. Fishes. https://doi.org/10.1007/s10641-018-0779-2
Avigliano, E., Saez, M.B., Rico, R., Volpedo, A. V., 2015a. Use of otolith strontium:calcium and zinc:calcium ratios as an indicator of the habitat of *Percophis brasiliensis* Quoy and Gaimard, 1825 in the southwestern Atlantic Ocean. Neotrop. Ichthyol. 13, 187–194. https://doi.org/10.1590/1982-0224-20130235
Avigliano, E., Velasco, G., Volpedo, A.V., 2015b. Assessing the use of two southwestern Atlantic estuaries by different life cycle stages of the anadromous catfish *Genidens barbus* (Lacépède, 1803) as revealed by Sr:Ca and Ba:Ca ratios in otoliths. J. Appl. Ichthyol. 31, 740–743. https://doi.org/10.1111/jai.12766
Avigliano, E., Velasco, G., Volpedo, A.V., 2015c. Use of lapillus otolith microchemistry as an indicator of the habitat of *Genidens barbus* from different estuarine environments in the southwestern Atlantic Ocean. Environ. Biol. Fishes

98, 1623–1632. https://doi.org/10.1007/s10641-015-0387-3
Avigliano, E., Villatarco, P., Volpedo, A.V., 2015d. Otolith Sr:Ca ratio and morphometry as indicators of habitat of a euryhaline species: The case of the silverside *Odontesthes bonariensis*. Ciencias Mar. 41, 189–202. https://doi.org/10.7773/cm.v41i3.2464
Bailey, D.S., Fairchild, E., Kalnejais, L.H., 2015. Microchemical signatures in juvenile winter flounder otoliths provide identification of natal nurseries. Trans. Am. Fish. Soc. 144, 173–183. https://doi.org/10.1080/00028487.2014.982259
Barletta, M., Lima, A.R.A., 2019. Systematic review of fish ecology and anthropogenic impacts in South American estuaries: Setting priorities for ecosystem conservation. Front. Mar. Sci. 6, 1–29. https://doi.org/10.3389/fmars.2019.00237
Begg, G.A., Waldman, J.R., 1999. An holistic approach to fish stock identification. Fish. Res. 43, 35–44. https://doi.org/10.1016/S0165-7836(99)00065-X
Bernardes, M.E.C., Miranda, L.B., 2001. Circulação estacionária e estratificação de sal em canais estuarinos: simulação com modelos analíticos. Rev. Bras. Oceanogr. 49, 115–132. https://doi.org/10.1590/S1413-77392001000100010
Bernini, E., Rezende, C.E., 2004. Estrutura da vegetação em florestas de mangue do estuário do rio Paraíba do Sul, Estado do Rio de Janeiro, Brasil. Acta Bot. Brasilica 18, 491–502. https://doi.org/10.1590/S0102-33062004000300009
Biolé, F.G., Thompson, G.A., Vargas, C. V., Leisen, M., Barra, F., Volpedo, A.V., Avigliano, E., 2019. Fish stocks of *Urophycis brasiliensis* revealed by otolith fingerprint and shape in the Southwestern Atlantic Ocean. Estuar. Coast. Shelf Sci. 229, 106406. https://doi.org/10.1016/j.ecss.2019.106406
Brown, R.J., Severin, K.P., 2009. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. Can. J. Fish. Aquat. Sci. 66, 1790–1808. https://doi.org/10.1139/F09-112
Caille, G.M., Ferrari, S., Albrieu, C., 1995. Los peces de la Ría de Gallegos, Santa Cruz, Argentina. Nat. Patagónica, Ciencias Biológicas 3, 191–194.
Campana, S.E., 1999. Chemistry and composition of fish otoliths: Pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. 188, 263–297. https://doi.org/10.3354/meps188263
Clarke, L.M., Conover, D.O., Thorrold, S.R., 2011. Population differences in otolith chemistry have a genetic basis in menidia menidia. Can. J. Fish. Aquat. Sci. 68, 105–114. https://doi.org/10.1139/F10-147
Crampton, J.S., 1995. Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. Lethaia 28, 179–186. https://doi.org/10.1111/j.1502-3931.1995.tb01611.x
Dias, T.H., Oliveira, J., Sanders, C.J., Carvalho, F., Sanders, L.M., Machado, E.C., Sá, F., 2016. Radium isotope (^{223}Ra, ^{224}Ra, ^{226}Ra and ^{228}Ra) distribution near Brazil's largest port, Paranaguá Bay, Brazil. Mar. Pollut. Bull. 111, 443–448. https://doi.org/10.1016/j.marpolbul.2016.07.004
Dorval, E., Jones, C.M., Hannigan, R., Montfrans, J. van, 2007. Relating otolith chemistry to surface water chemistry in a coastal plain estuary. Can. J. Fish. Aquat. Sci. 64, 411–424. https://doi.org/10.1139/f07-015
Elsdon, T.S., Gillanders, B.M., 2003. Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri.* Mar. Ecol. Prog. Ser. 260, 263–272. https://doi.org/10.3354/meps260263
Gomes, I.D., Araújo, F.G., 2004. Reproductive biology of two marine catfishes (Siluriformes, Ariidae) in the Sepetiba Bay, Brazil. Rev. Biol. Trop. 52, 143–156.

https://doi.org/10.15517/rbt.v52i1.14763
Gut, C., Vukić, J., Šanda, R., Moritz, T., Reichenbacher, B., 2020. Identification of past and present gobies: distinguishing *Gobius* and *Pomatoschistus* (Teleostei: Gobioidei) species using characters of otoliths, meristics and body morphometry. Contrib. to Zool. 89, 282–323. https://doi.org/10.1163/18759866-BJA10002
Hair, J.F., Black, W.C., Babin, B.J., Anderson, R.E., 2013. Multivariate Data Analysis: Pearson New International Edition. Pearson new international edition, Harlow, United Kingdom. https://doi.org/10.1016/j.ijpharm.2011.02.019
Hermann, T.W., Stewart, D.J., Limburg, K.E., Castello, L., 2016. Unravelling the life history of Amazonian fishes through otolith microchemistry. R. Soc. Open Sci. 3, 160206. https://doi.org/10.1098/rsos.160206
Hüssy, K., Limburg, K.E., Pontual, H. De, Thomas, O.R.B., Cook, K., Heimbrand, Y., Blass, M., Sturrock, A.M., 2020. Element patterns in otoliths: the role of biomineralization. Rev. Fish. Sci. Aquac. 1–33. https://doi.org/10.1080/23308249.2020.1760204
Hüssy, K., Mosegaard, H., Albertsen, C.M., Nielsen, E.E., Hemmer-Hansen, J., Eero, M., 2016. Evaluation of otolith shape as a tool for stock discrimination in marine fishes using Baltic Sea cod as a case study. Fish. Res. 174, 210–218. https://doi.org/10.1016/J.FISHRES.2015.10.010
Ibáñez, A.L., Hernández-Fraga, K., Alvarez-Hernández, S., 2017. Discrimination analysis of phenotypic stocks comparing fish otolith and scale shapes. Fish. Res. 185, 6–13. https://doi.org/10.1016/j.fishres.2016.09.025
Jochum, K.P., Weis, U., Stoll, B., Kuzmin, D., Yang, Q., Raczek, I., Jacob, D.E., Stracke, A., Birbaum, K., Frick, D.A., Günther, D., Enzweiler, J., 2011. Determination of reference values for NIST SRM 610-617 glasses following ISO guidelines. Geostand. Geoanalytical Res. 35, 397–429. https://doi.org/10.1111/j.1751-908X.2011.00120.x
Kerr, L.A., Campana, S.E., 2014. Chemical composition of fish hard parts as anatural marker of fish stocks, in: Stock Identification Methods: Applications in Fishery Science: Second Edition. Academic Press., San Diego, USA, pp. 205–234. https://doi.org/10.1016/B978-0-12-397003-9.00011-4
Kime, D.E., 1995. The effects of pollution on reproduction in fish. Rev. Fish Biol. Fish. 5, 52–95. https://doi.org/10.1007/BF01103366
Kjerfve, B., Ribeiro, C.H.A., Dias, G.T.M., Filippo, A.M., Quaresma, V.S., 1997. Oceanographic characteristics of an impacted coastal bay: Baía de Guanabara, Rio de Janeiro, Brazil. Cont. Shelf Res. 17, 1609–1643.
Krüger, G.C.T., Carvalho, C.E.V., Suzuki, M.S., 2006. Dissolved nutrient, chlorophyll-a and DOC dynamic under distinct riverine discharges and tidal cycles regimes at the Paraíba do Sul River estuary, RJ, Brazil. J. Coast. Res. II, 724–730.
Lana, P.C., Marone, E., Lopes, R.M., Machado, E.C., 2001. The subtropical estuarine complex of Paranaguá Bay, Brazil. Ecol. Stud. 144, 131–145. https://doi.org/10.1007/978-3-662-04482-7_11
Limburg, K.E., Walther, B.D., Lu, Z., Jackman, G., Mohan, J., Walther, Y., Nissling, A., Weber, P.K., Schmitt, A.K., 2015. In search of the dead zone: Use of otoliths for tracking fish exposure to hypoxia. J. Mar. Syst. 141, 167–178. https://doi.org/10.1016/j.jmarsys.2014.02.014
Lombarte, A., Lleonart, J., 1993. Otolith size changes related with body growth, habitat depth and temperature. Environ. Biol. Fishes 37, 297–306. https://doi.org/10.1007/BF00004637
Longmore, C., Fogarty, K., Neat, F., Brophy, D., Trueman, C., Milton, A., Mariani, S.,

2010. A comparison of otolith microchemistry and otolith shape analysis for the study of spatial variation in a deep-sea teleost, *Coryphaenoides rupestris*. Environ. Biol. Fishes 89, 591–605. https://doi.org/10.1007/s10641-010-9674-1

López, R.B., Bellisio, N.B., 1965. Contribuición al conocimiento del *Tachysurus barbus* (Lacépède), bagre del Mar argentino (Pisces, Ariidae), in: Anuario Del II Congreso Latinoamericano Zoología. São Paulo, pp. 145–153.

Maciel, T.R., Avigliano, E., Maichak de Carvalhoc, B., Miller, N., Viannaa, M., 2020. Population structure and habitat connectivity of *Genidens genidens* (Siluriformes) in tropical and subtropical coasts from Southwestern Atlantic. Estuar. Coast. Shelf Sci. 242, 106839.

Maciel, T.R., Vaz-dos-Santos, A.M., Barradas, J.R.S., Vianna, M., 2019. Sexual dimorphism in the catfish *Genidens genidens* (Siluriformes: Ariidae) based on otolith morphometry and relative growth. Neotrop. Ichthyol. 17, e180101[1]-e180101[8]. https://doi.org/10.1590/1982-0224-20180101

Maichak de Carvalho, B., Pisonero, J., Mendez, A., Volpedo, A. V., Avigliano, E., 2020. Spatial environmental variability of natural markers and habitat use of *Cathorops spixii* in a neotropical estuary from otolith chemistry. J. Mar. Biol. Assoc. UK 100, 783–793.

Marceniuk, A.P., 2005. Chave para identificação das espécies de bagres marinhos (Siluriformes, Ariidae) da costa brasileira. Bol. do Inst. Pesca 31, 89–101.

Marceniuk, A.P., Menezes, N.A., 2007. Systematics of the family Ariidae (Ostariophysi, Siluriformes), with a redefinition of the genera, Zootaxa. https://doi.org/10.11646/zootaxa.1416.1.1

Mendonça, J.T., Quito, L., Jankowsky, M., Balanin, S., Neto, D.G., 2017. Diagnóstico da pesca do bagre-branco (*Genidens barbus* e *G . planifrons*) NO LITORAL Sudeste-sul do Nrasil: subsídios para o ordenamento. Inst. Pesca, São Paulo. 77.

Meniconi, M.F.G., Silva, T.A., Fonseca, M.L., Lima, S.O.F., Lima, E.F.A., Lavrado, H.P., Figueiredo-Jr., A.G., 2012. Baía de Guanabara: Síntese do conhecimento ambiental. Biodiversidade. Petrobras, Rio de janeiro.

Miller, J.A., 2009. The effects of temperature and water concentration on the otolith incorporation of barium and manganese in black rockfish *Sebastes melanops*. J. Fish Biol. 75, 39–60. https://doi.org/10.1111/j.1095-8649.2009.02262.x

MMA, 2014. Portaria MMA Número 445, DOU: Brasília. Brazil.

Mohan, J.A., Rulifson, R.A., Reide Corbett, D., Halden, N.M., 2012. Validation of oligohaline elemental otolith signatures of striped bass by use of *in situ* caging experiments and water chemistry. Mar. Coast. Fish. 4, 57–70. https://doi.org/10.1080/19425120.2012.656533

Morales-Nin, B., 2000. Review of the growth regulation processes of otolith daily increment formation. Fish. Res. 46, 53–67. https://doi.org/10.1016/S0165-7836(00)00133-8

Patterson, W.F., Cowan, J.H., Wilson, C.A., Chen, Z., 2008. Temporal and spatial variability in juvenile red snapper otolith elemental signatures in the Northern Gulf of Mexico. Trans. Am. Fish. Soc. 137, 521–532. https://doi.org/10.1577/T06-264.1

Perin, S., Vaz-dos-Santos, A.M., 2014. Morphometry and relative growth of the Brazilian sardine, *Sardinella brasiliensis* (Steindachner, 1879) in the southeastern Brazilian bight. Arq. Zool. 45, 63–72.

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. Bio. Ecol. 360, 56–62. https://doi.org/10.1016/j.jembe.2008.03.013

Reichenbacher, B., Feulner, G.R., Tanja, S.M., 2009. Geographic variation in otolith morphology among freshwater populations of *Aphanius dispar* (Teleostei, Cyprinodontiformes) from the Southeastern Arabian Peninsula. J. Morphol. 270, 469–484. https://doi.org/10.1002/jmor.10702

Reichenbacher, B., Reichard, M., 2014. Otoliths of five extant species of the annual killifish *Nothobranchius* from the east African Savannah. PLoS One 9, e112459. https://doi.org/10.1371/journal.pone.0112459

Reis, E.G., 1986a. Reproduction and feeding habits of the marine catfish, *Netuma barba* (Siluriformes, Ariidae), in the estuary of the Patos Lagoon (Brazil). Atlântica 8, 35–55.

Reis, E.G., 1986b. A pesca artesanal de bagres marinhos (Siluriformes: Ariidae) no estuário da Lagoa dos Patos, RS, Brasil. Rio Grande.

Rogers, T.A., Fowler, A.J., Steer, M.A., Gillanders, B.M., 2019. Discriminating natal source populations of a temperate marine fish using larval otolith chemistry. Front. Mar. Sci. 6, 1–17. https://doi.org/10.3389/fmars.2019.00711

Sea, I., Brophy, D., King, P.A., 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, 1670–1675.

Soeth, M., Spach, H.L., Daros, F.A., Adelir-Alves, J., de Almeida, A.C.O., Correia, A.T., 2019. Stock structure of Atlantic spadefish *Chaetodipterus faber* from Southwest Atlantic Ocean inferred from otolith elemental and shape signatures. Fish. Res. 211, 81–90. https://doi.org/10.1016/j.fishres.2018.11.003

Sturrock, A.M., Hunter, E., Milton, J.A., Johnson, R.C., Waring, C.P., Trueman, C.N., EIMF, 2015. Quantifying physiological influences on otolith microchemistry. Methods Ecol. Evol. 6, 806–816. https://doi.org/10.1111/2041-210X.12381

Tanner, S., Reis-Santos, P., Cabral, H.N., 2015. Otolith chemistry in stock delineation: A brief overview, current challenges and future prospects. Fish. Res. 173, 206–213. https://doi.org/10.1016/j.fishres.2015.07.019

Thomas, O.R.B., Swearer, S.E., 2019. Otolith Biochemistry—A Review. Rev. Fish. Sci. Aquac. 27, 458–489. https://doi.org/10.1080/23308249.2019.1627285

Tuset, V.M., Galimany, E., Farrés, A., Marco-Herrero, E., Otero-Ferrer, J.L., Lombarte, A., Ramón, M., 2020. Recognising mollusc shell contours with enlarged spines: Wavelet vs Elliptic Fourier analyses. Zoology 140, 125778. https://doi.org/10.1016/j.zool.2020.125778

Tuset, V.M., Lozano, I.J., GonzÍez, J.A., Pertusa, J.F., García-Díaz, M.M., 2003. Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). J. Appl. Ichthyol. 19, 88–93. https://doi.org/10.1046/j.1439-0426.2003.00344.x

Tuset, V.M., Parisi-Baradad, V., Lombarte, A., 2013. Application of otolith mass and shape for discriminating scabbardfishes *Aphanopus* spp. in the north-eastern Atlantic Ocean. J. Fish Biol. 82, 1746–1752. https://doi.org/10.1111/jfb.12101

Valentin, J.L., Tenenbaum, D.R., Bonecker, A.C.T., Bonecker, S.L.C., Nogueira, C.R., Villac, M.C., 1999. O sistema planctônico da Baía de Guanabara: Síntese do conhecimento, in: Silva, S.H.G., Lavrado, H.P. (Eds.), Ecologia dos ambientes costeiros do estado do Rio de Janeiro. Oecologia Brasiliensis, Rio de Janeiro, pp. 35–59. https://doi.org/10.4257/oeco.1999.0701.02

Velasco, G., Reis, E.G., Vieira, J.P., 2007. Calculating growth parameters of *Genidens barbus* (Siluriformes, Ariidae) using length composition and age data. J. Appl. Ichthyol. 23, 64–69. https://doi.org/10.1111/j.1439-0426.2006.00793.x

Vignon, M., 2018. Short-term stress for long-lasting otolith morphology — brief

745 embryological stress disturbance can reorient otolith ontogenetic trajectory. Can. J.
746 Fish. Aquat. Sci. 75, 10. https://doi.org/10.1139/cjfas-2017-0110
747 Vignon, M., 2012. Ontogenetic trajectories of otolith shape during shift in habitat use:
748 Interaction between otolith growth and environment. J. Exp. Mar. Bio. Ecol. 420–
749 421, 26–32. https://doi.org/10.1016/j.jembe.2012.03.021
750 Vignon, M., Morat, F., 2010a. Environmental and genetic determinant of otolith shape
751 revealed by a non-indigenous tropical fish. Mar. Ecol. Prog. Ser. 411, 231–241.
752 https://doi.org/10.3354/meps08651
753 Vignon, M., Morat, F., 2010b. Environmental and genetic determinant of otolith shape
754 revealed by a non-indigenous tropical fish. Mar. Ecol. Prog. Ser. 411, 231–241.
755 https://doi.org/10.3354/meps08651
756
757

758 **Figure 1:** Map of the study area in the Southwest Atlantic Ocean. Red arrows indicate
759 the approximate sampling region of *Genidens barbus*.
760
761 **Figure 2**: Relationship between the descriptors and the accumulated Fourier Power
762 Spectrum for *Genidens barbus* otoliths. The first 12 harmonics reached 99.99% of the
763 accumulated power.
764
765 **Figure 3:** *Genidens barbus* otolith edge and core elemental ratios mean ± SD
766 (mmol/mol) for sampling sites. Distinct letters show significant differences between
767 sites (p<0.05). PSR- Paraíba do Sul River; GB- Guanabara Bay; IR- Itapanhaú River;
768 PB- Paranaguá Bay; PE- La Plata Estuary.
769

Table 1: Descriptive statistics of *Genidens barbus* study specimens (mean and SD) used for chemical (N, sample size=72) and morphometric (N=159) analysis. The specimens used for the chemical analyzes were subsampled at random from the total sample pool (N=159). TL= total length (cm); W = weight (g); SD =standard deviation; Age = age in years.

Site	N	TL ± SD	W ± SD	Age ± SD
		Chemistry		
Paraíba do Sul River	17	61.1 ± 7.3	2265 ± 950	13.4 ± 2.2
Guanabara Bay	22	62.2 ± 8.7	2870 ± 1445	14.0 ± 2.0
Itapanhaú River	22	61.5 ± 5.4	2371 ± 699	14.2 ± 1.9
Paranaguá Bay	6	49.9 ± 9.1	2058 ± 1201	9.8 ± 1.0
La Plata Estuary	5	65.0 ± 4.5	2861 ± 714	11.8 ± 1.1
		Elliptic Fourier Analysis		
Paraíba do Sul River	28	62.8 ± 11.9	2742 ± 1720	
Guanabara Bay	38	62.2 ± 11.4	2925 ± 1677	
Itapanhaú River	31	61.7 ± 7.0	2544 ± 805	
Paranaguá Bay	28	48.8 ± 7.8	2295 ± 1291	
La Plata Estuary	34	55.8 ± 6.1	1700 ± 722	

777 **Table 2** Cross-classification matrices of the discriminant analyzes based on for *Genidens barbus* otolith morphometry and microchemistry. The
778 numbers represent the classification percentage. N: sample size; PSR: Paraíba do Sul; GB: Guanabara; IR: Itapanhaú; PG: Itapanhaú; RDP: Río
779 de la Plata.

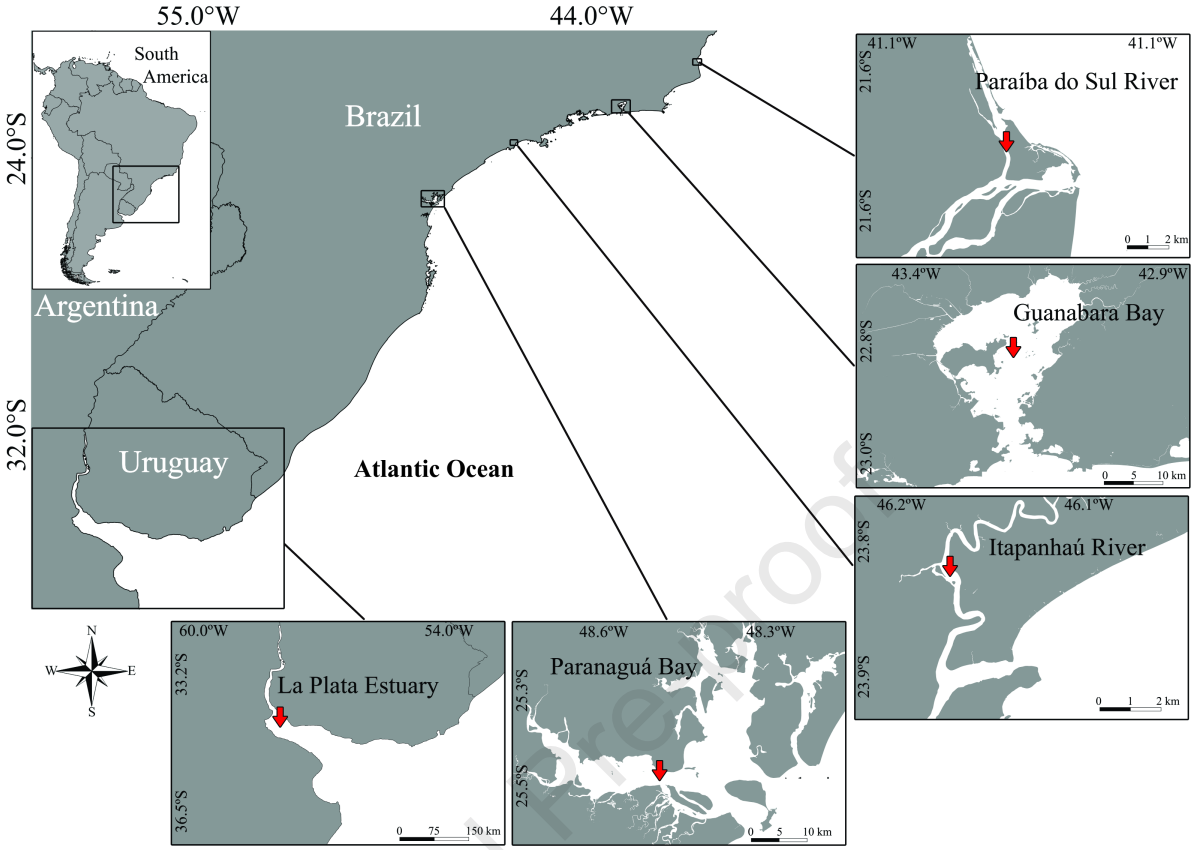
	Paraíba do Sul	Guanabara	Itapanhaú	Itapanhaú	Río de la Plata	N	Random (%)	p
Shape (QDA)								
PSR	**42.9**	17.9	17.9	7.1	14.3	28	18	0.04*
GB	18.4	**44.7**	5.3	15.8	15.8	38	24	0.09
IR	12.9	16.1	**38.7**	19.4	12.9	31	19	0.17
PG	7.1	21.4	21.4	**32.1**	17.9	28	18	0.23
RDP	5.9	8.8	5.9	11.8	**67.6**	34	21	<0.0001*
Mean					*45.2*			
Edge (QDA)								
PSR	**41.2**	23.5	29.4	5.9	0.0	17	24	0.3
GB	4.5	**86.4**	9.1	0.0	0.0	22	31	0.003*
IR	18.2	36.4	**45.5**	0.0	0.0	22	31	0.37
PG	0.0	0.0	66.7	**33.3**	0.0	6	8	0.22
RDP	0.0	0.0	0.0	0.0	**100**	5	7	0.003*
Mean					*61.3*			
Core (QDA)								
PSR	**82.4**	11.8	0.0	5.9	0.0	17	24	0.003*
GB	4.5	**68.2**	22.7	4.5	0.0	22	31	0.003*
IR	0.0	18.2	**77.3**	4.5	0.0	22	31	0.003*
PG	0.0	33.3	0.0	**66.7**	0.0	6	8	0.03*
RDP	0.0	0.0	0.0	0.0	**100**	5	7	0.003*
Mean					*78.9*			
Edge + Shape (LDA)								

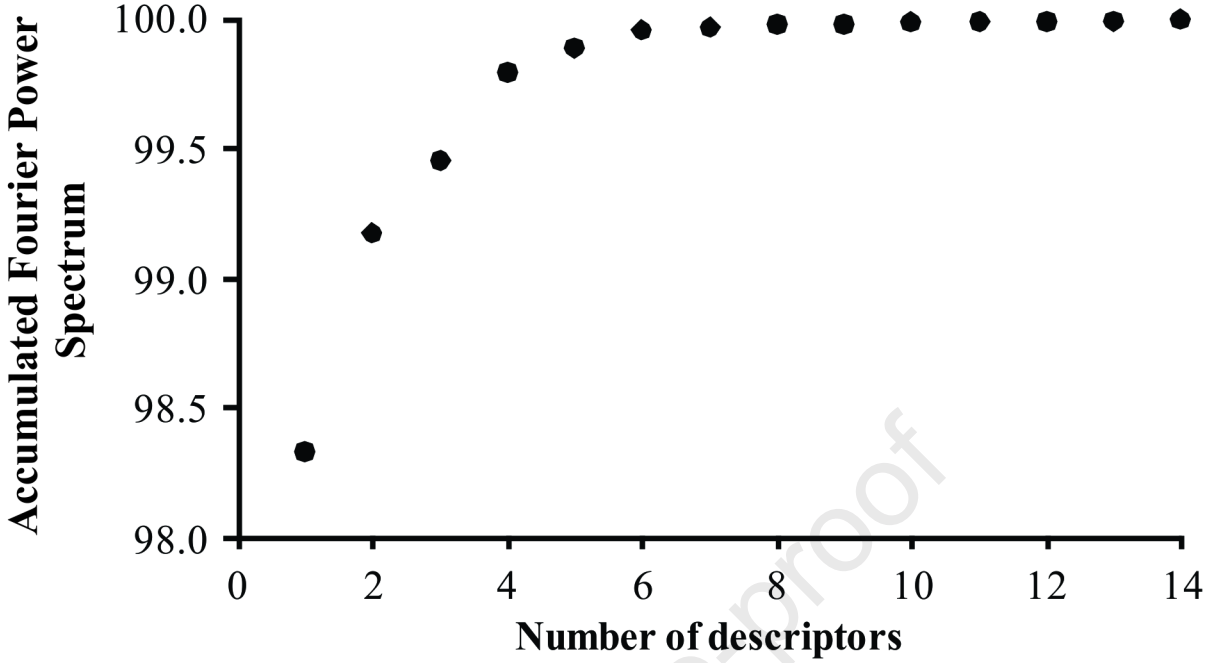
PSR	**52.9**	11.8	29.4	5.9	0.0	17	24	0.09
GB	14.3	**66.7**	14.3	4.8	0.0	22	31	0.02*
IR	13.6	27.3	**54.5**	4.5	0.0	22	31	0.14
PG	33.3	16.7	33.3	**16.7**	0.0	6	8	0.5
RDP	0.0	0.0	0.0	0.0	**100**	5	7	0.003*
Mean					*58.2*			

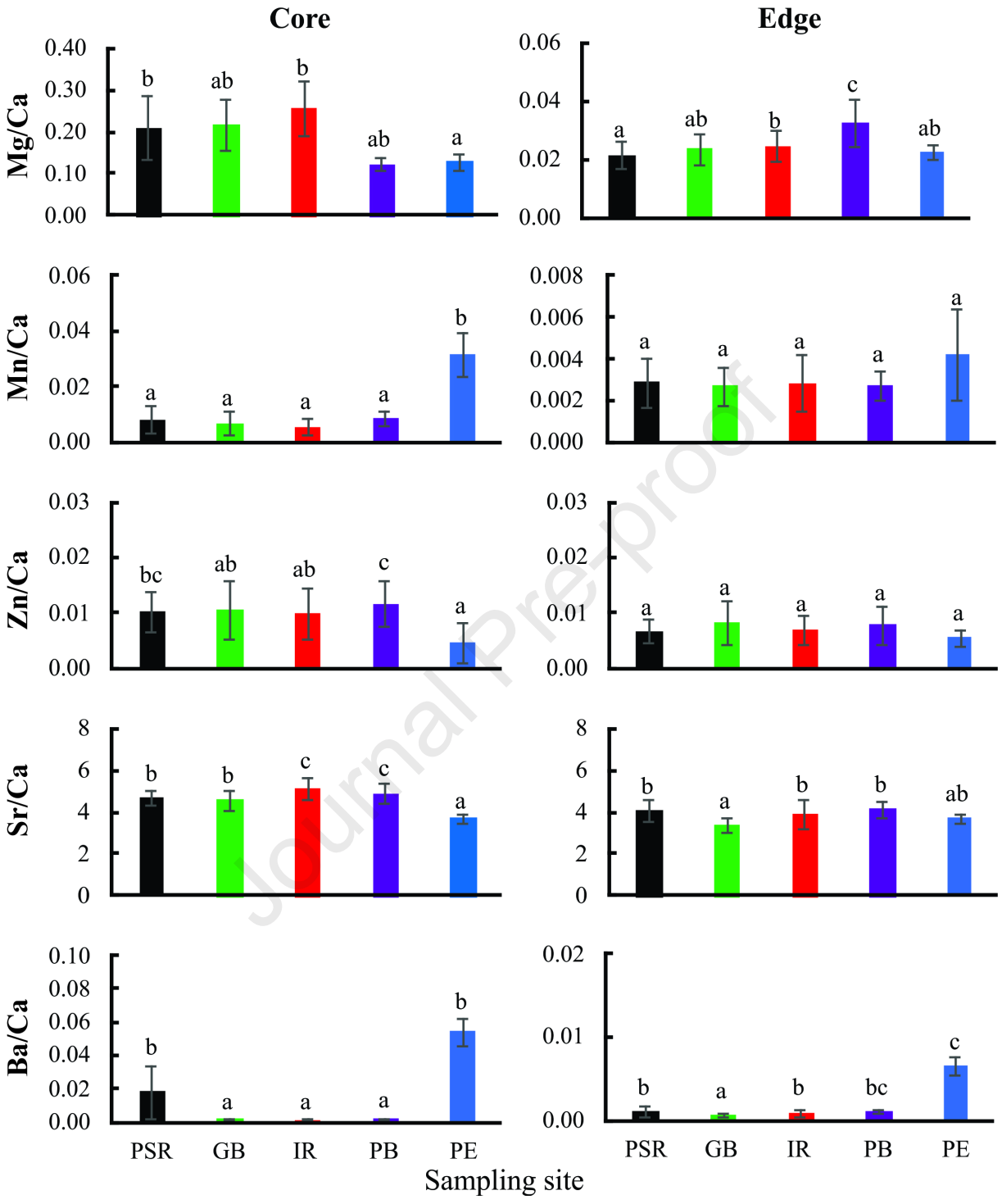
780

781

782







Spatial segregation in young and adult stages of *Genidens barbus* was studied.

Otolith microchemistry and shape are potential tools for stock identification.

Results suggest the presence of different management units.

High percentages of classification suggest low connectivity between some populations.

The populations should be managed as separate groups.

Declaration of interests

x The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

We have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.