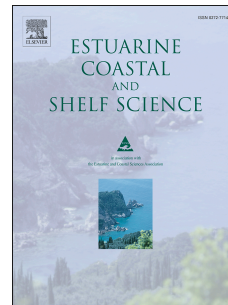# Journal Pre-proof

Population structure and habitat connectivity of *Genidens genidens* (Siluriformes) in tropical and subtropical coasts from Southwestern Atlantic

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Author Statement

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Esteban Avigliano: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - Review & Editing, Visualization.

Barbara Maichak de Carvalho: Investigation, Writing - Review & Editing.

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Marcelo Vianna: Conceptualization, Methodology, Resources, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

1   **Population structure and habitat connectivity of *Genidens genidens* (Siluriformes) in tropical**

2   **and subtropical coasts from Southwestern Atlantic**

3

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21

22 **Abstract**

23 We use otolith trace element-to-calcium element concentraton ratios (Ba:Ca, Cu:Ca, Mg:Ca, Mn:Ca,

24 Ni:Ca, Sr:Ca and Zn:Ca) and shape to evaluate the degree of segregation and presence of different

25 nursery areas and fish stocks of *Genidens genidens* in the Brazilian coastal area. Otolith core

26 (juvenile stage) and edge (adult stage) element:Ca fingerprints (N=89), as well as otolith elliptic

27 Fourier descriptors (N=147) were compared between four estuaries. Several element:Ca ratios were

28 significantly different between estuaries for otolith core and edge (p<0.05). MANOVA,

29 PERMANOVA, linear and quadratic discriminant analyses based on Fourier descriptors (12

30 harmonics) and microchemistry were effective for discriminating between all sampling sites

31 (p<0.05), except Itapanhaú River and Paranaguá Bay (p>0.05). The discriminant analyses showed

32 jackknifed classification from 54.1% to 100% considering young (core microchemistry) and adult

33 life stages (edge microchemistry and shape). When otolith microchemistry and shape were

34 combined, the jackknifed classification increased in relation to the separates methods, ranged from

35 89.5 to 100% (mean 95.2%). The high degree of segregation (low connectivity) in juvenile and adult

36 stages between the estuaries is consistent with the presence of isolated populations, and advocates for

37 estuary-specific management strategies.

38

39 **Key words:** catfish; estuaries; nursery grounds; segregation; stock; trace-elements.

1. Introduction

Estuaries play vital roles in the life cycle of several species of ichthyofaunal, making them one of the most important environments for the maintenance of world fisheries (Blaber, 2000). Here estuaries are defined as transitional waters under the influence of both freshwater and marine systems (Elliott et al., 2007). Numerous species of fish found in estuaries use these systems in different ways. For example, some fish species use estuaries intermittently for spawning or feeding, whereas other species complete their entire life cycle within a single estuary (Elliott et al., 2007; Potter et al., 2015). Many concepts have been proposed to categorize fish according to their habitat use patterns and applicability; such categorizations are challenging to establish for fish groups that exhibit high plasticity (Able, 2005; Blaber, 2000; Elliott et al., 2007; Potter et al., 2015).

Catfish of the Ariidae family are the dominant species in estuarine environments in the Southwestern Atlantic Ocean (Gomes et al., 2001). This family includes catfish from tropical and subtropical regions, occurring in marine, estuarine and freshwater environments, usually abundant in muddy and shallow bottom coastal waters (Schmidt et al., 2008; Silva-Junior et al., 2013). With catches that have exceeded 28,781 t in Brazil (Freire et al., 2015), they are among the most economically important resources for artisanal fishing, mainly off southeastern and south Brazil (Fischer et al., 2011; Gomes and Araújo, 2004; Maciel et al., 2018a). Ariids can exhibit seasonal movements between environments with different salinities related to different biological aspects, such as reproduction, feeding and ontogeny (Avigliano et al., 2017c; Schmidt et al., 2008). The estuarine and coastal regions are considered prime feeding, breeding and shelter areas for this group (Avigliano et al., 2017c; Schmidt et al., 2008; Silva-Junior et al., 2013). *Genidens genidens* (Cuvier, 1829) is one of the most abundant species in estuarine systems off Brazilian coast (Andrade-Tubino et al., 2008; Schmidt et al., 2008), occurring on the eastern coast of South America, from Bahia (Brazil) to Buenos Aires (Argentina), inhabiting shallow coastal waters, estuaries and lagoons (Fischer et al., 2011). This catfish has a peculiar reproductive biology, where females produce large oocytes and males carry out oral incubation of the offspring, with the reproductive period occurring once a year during the hot months, from late spring to early autumn (Maciel et al., 2018a; Wallace and Selman, 1981). Although *G. genidens* is an important commercial species for artisanal fishery (Silva-Junior et al., 2013), its population structure and life history are not well known, and the few existing management policies aimed at this resource are restricted only to a few regions (MMA, 2004; SUDEPE, 1984). Therefore, that knowledge is a necessary input to design adequate management strategies and guarantee the sustainability of the resource (Cadrin et al., 2014).

3

In addition to the relevance as a fishery, this species also has ecological significance. While Barbieri et al. (1992) and Hostim-Silva et al. (2009) suggested that *G. genidens* leaves the estuary after the reproductive period, other authors (Maciel et al., 2018a; Silva-Junior et al., 2013) suggested that it completes its entire life cycle within the estuarine environment. Then, Silva-Junior et al. (2013) have suggested that this catfish could be used a sentinel species for biomonitoring pollution in Guanabara Bay (Brazil). If this were the case, this species would have the potential to be used as a sentinel in other estuaries. However, knowledge gaps about the degree of connectivity between the populations and pattern of habitat use across their range of distribution still exist, which can interfere with its applicability as a potential sentinel species of these ecosystems.

Otolith chemical composition has been used as natural marker being a useful tool to resolve biological issues such as stock and nursery area delimitation (Kerr and Campana, 2014). Otolith are calcified structures present in the inner ear of teleostean fish (Campana, 1999; Panfili et al., 2002). Otoliths are acellular and metabolically inert, any elements deposited on their growing surface are permanently retained, with the predominant source of most of the inorganic elements being the surrounding water (Avigliano et al., 2019; Campana, 1999; Kerr and Campana, 2014). In this sense, the elements that are deposited in the core correspond to early stages of life, while those that are incorporated in the edge correspond to recent periods.

In recent decades, the integration of otolith chemistry with otolith shape has improved understanding of population structures for several groups of fish (Avigliano et al., 2014; Soeth et al., 2019). Because otoliths record geochemical time-series throughout the life histories of fish, otolith chemical fingerprints have been widely applied to delimit fish stocks and nursery areas (Avigliano et al., 2019, 2017a; Kerr and Campana, 2014; Longmore et al., 2010). Such studies contribute important information for the management and conservation of species with complex habitat use patterns (Albuquerque et al., 2012; Avigliano et al., 2017c; Tanner et al., 2016). Several otolith chemical markers have been useful for delineating population structures and life histories of congeners such as *G. barbus* from the Southwestern Atlantic Ocean (Avigliano et al., 2017c, 2017a, 2016). In particular, in many diadromous species, strontium-to-calcium ratio (Sr:Ca) has been very useful to describe movements between environments with different salinity, because this is positively related to salinity in some estuarine systems (Brown and Severin, 2009). In addition, otolith contour (shape) can also vary between different populations in relation to the environmental (depth, temperature, salinity), genetic or physiological characteristics, and thus, may be useful to delimit the populations and even closed species (Bani et al., 2013; Reichenbacher and Reichard, 2014; Vignon and Morat, 2010).

106   Here we compared element:Ca signatures of otolith cores (juvenile stage) and edges (adult

107   stage), as well as otolith shapes (using elliptic Fourier descriptors) between tropical and subtropical

108   estuaries from Southwestern Atlantic, to evaluate segregation degree, and presence of different

109   nurseries and fish stocks of *G. genidens*. In addition, a exploration of otolith edge Sr:Ca temporal

110   variations was carried out to evaluate its ability as a salinity proxy for future studies.

111

112   **2. Materials and methods**

113

114   *2.1. Study area and sampling*

115   The study was conducted from fish captured in four estuaries (Paraíba do Sul River,

116   Guanabara Bay, Itapanhaú River and Paranaguá Bay) along the southeastern and southern coast of

117   Brazil (Fig. 1).

118   Paraíba do Sul River has 1145 km, with a drainage basin of 55.400 km^2. It crosses three of

119   the most important and developed states of Brazil (Minas Gerais, São Paulo and Rio de Janeiro) and

120   suffers the impacts of industrial and urban discharges along its course (Carvalho et al., 1999). The

121   estuary of the Paraíba do Sul River, located at the north of the coast of the state of Rio de Janeiro,

122   near the frontier with the state of Espírito Santo, was classified as a delta with a salinity range

123   between 0-30 (Krüger et al., 2006). It is characterized by the development of beach ridges on the

124   sides of the channel, having an outlet called the main estuary (Atafona region), and other outlet, the

125   secondary estuary (near Gargaú) (21º36'23.9"S and 41º02'43.8"W) (Bernini and Rezende, 2004).

126   Guanabara Bay is located in the state of Rio de Janeiro (22°48'24.3"S and 43°09'14.7"W).

127   The bay area is 380 km^2, with a drainage basin of 4080 km^2, and is characterized as a semi-enclosed

128   coastal ecosystem of estuarine nature, with salinity range between 0-35 (Kjerfve et al., 1997;

129   Meniconi et al., 2012). Surrounded by the metropolitan region of the state, the bay is directly

130   affected by the high population density and the industrial park in its surroundings, impacting by

131   wastewater, agriculture and industry, being considered one of the most degraded estuarine systems of

132   the Brazilian coast (Meniconi et al., 2012; Silva-Junior et al., 2016).

133   The third sampling site, the Itapanhaú River estuary, is located at the eastern of state of São

134   Paulo coast, being connected to Bertioga Chanel (23º49'47.1"S and 46º09'12.8"W). It is an

135   anthropized environment, being close to the largest port terminal in Latin America and several

136   industrial poles (Ambrozevicius and Abessa, 2008). Itapanhaú River has a drainage basin area of

137   approximately 260 km^2, being the main responsible for the fluvial contribution to the chanel

138   (Bernardes and Miranda, 2001). This region is dominated by tidal waves, and the salinity in Bertioga

139 Chanel range between 20-33 (Eichler et al., 2006), while in the estuary of the Itapanhaú River
140 ranging from 0-35 SPU (Maciel, 2019, unpublished data).

141   Paranaguá Estuarine Complex, due to its continuous cover of the Atlantic Forest, is
142 considered a Natural Patrimony of Humanity (Diegues, 1995). However, the area suffers impacts of
143 ports and industries installed in the region (Santos et al., 2006). It is located on the coast of the state
144 of Paraná, occupying an area of 612 km^2, divided in two main axes, north-south (Laranjeira and
145 Pinheiro Bay) and east-west (Paranaguá Bay) (25°29'26.7"S and 48°29'55.6"W) (Lana et al., 2001).
146 This estuary is dominated by tidal waves, characterized as semi-diurnal tides with a salinity range
147 between 0-31 (Dias et al., 2016).

148   *Genidens genidens* specimens (total N=147) were obtained from artisanal fisheries landings
149 in the four study estuaries between January 2014 and December 2017. The specimens were identified
150 according to Marceniuk (2005) and Marceniuk and Menezes (2007) and measured (total length - TL,
151 ±1 mm). The *lapilli* otoliths were removed, washed with milli-Q water, air-dried, and subsequently
152 weighed individually with an electronic scale (±0.001g). Annual periodicity of annuli formation was
153 validated by Maciel et al. (2018b). To reduce the potential effect of age on chemical variables, the
154 analysis of otolith microchemistry was restricted to fish aged between 5 and 11 years, resulting in 22
155 fish for Paraíba do Sul River, 25 fish for Guanabara Bay, 23 fish for Itapanhaú River and 19 fish for
156 Paranaguá Bay (Table 2). Weights, lengths and ages of the study specimens are shown in Table 1.

157

158 *2.2. Otoliths morphometry*

159   For morphometric analysis, we photographed 147 right otoliths of *G. gendiens* (Paraíba do
160 Sul River N= 30; Guanabara Bay, N= 30; Itapanhaú River, N= 26; Paranaguá Bay, N= 61, Table 1)
161 on a black background, next to a scale, with digital camera (Nikon Coolpix L110), using the same
162 focal length.

163   Elliptic Fourier analysis (EFA) was performed to quantify differences in otolith contour
164 between estuaries. In this analysis, otolith curvature is modelled as a two dimensional closed curve
165 by applying a combination of harmonically related sine and cosine functions (descriptors), where
166 each function is composed of four Fourier coefficients (FC). According to Fourier power spectrum
167 (Crampton, 1995), the first 12 harmonics achieve 99.99 % of the cumulated power and 48 FCs can
168 represent the otoliths outline. The first harmonic was used to normalize the FCs, so they remain
169 invariant with respect to otolith size and rotation (Ferson et al., 1985). As this normalization
170 transforms the first three FCs into constants, these were excluded, resulting in 45 variables instead of
171 48. The FCs were calculated from Shape 1.3 software.

172

173 *2.3. Otolith chemistry*

174   The left *lapilli* otoliths were decontaminated with 3% hydrogen peroxide, 2% HNO_3 (Merck

175 KGaA, Garmstadt, Germany) (Avigliano et al., 2017b) and rinsed with Milli-Q water (resistivity of

176 18.2 mOhm/cm). Otoliths were embedded in epoxy resin and sectioned transversely using a Buehler

177 Isomet low speed saw (Hong Kong, China) to obtain 700µm-thick central sections. Sections were

178 fixed to glass slides using epoxy resin, polished using 10 µm-grit sandpaper and rinsed with Milli-Q

179 water. In order to account for the possible effect of fish age on last-growth compositions, otolith

180 annuli were counted using a stereomicroscope (Leica EZ4-HD, Singapore) at 40X magnification, and

181 only fish aged between 5 and 11 years were selected (N=89), as previously explained.

182   To assess the population structure, ^{138}Ba, ^{63}Cu, ^{43}Ca, ^{24}Mg, ^{55}Mn, ^{60}Ni, ^{88}Sr and ^{66}Zn were

183 analyzed in otolith core (~first year) and edge (~last two years) by Laser Ablation Inductively

184 Coupled Plasma Mass Spectrometry (LA-ICP-MS) at the University of Texas at Austin Department

185 of Geosciences (USA) (Fig. 2). The laser system consisted in an ESI NWR193-UC excimer laser

186 ablation system (193nm, 4ns pulse width) coupled to an Agilent 7500ce ICP-MS. A rectangular

187 aperture of 25x100 µm was used at 8 µm s^{-1} (repetition rate/ of 10 Hz, energy densities/ 3.8 Jcm$^{2-1}$).

188 To minimize the temporal sampling alias, the long axis of the aperture was maintained parallel to

189 growth banding in all the transects. Prior to measurements, the surface contaminants were removed

190 by pre-ablation, using a spot size of 25x125 µm at 50 m/s. The ICP-MS was operated at power of

191 1600 W with using argon as carrier gas (flow/ 800 mL min^{-1}). Ratios $^{232}Th^{16}O/^{232}Th$ (<0.35%) and

192 $^{238}U/^{232}Th$ (~1) were used during tuning to monitor plasma robustness. NIST 612 and USGS MACS-

193 3 reference materials, analyzed in replicate each hour, were used as external and primary standards,

194 respectively (Jochum et al., 2011; Pearce et al., 1997).

195   Intensities were converted to concentrations (mg/kg) by using Iolite software (Paton et al.,

196 2011), with ^{43}Ca (38.3 weight %) as the internal standard (Yoshinaga et al., 2000).

197   Reference materials analysis relative to GeoREM preferred values (Jochum et al., 2011)

198 showed acceptable recoveries, namely: 98 % for ^{138}Ba, 106 % for ^{63}Cu, 96 % for ^{24}Mg, 103 % for

199 ^{55}Mn, 102 % for ^{60}Ni, 100 % for ^{88}Sr and 93 % for ^{66}Zn. Detection limits (LOD, mg/kg) based on

200 three times the standard deviation of the estimated baseline intensity during bracketing gas blank

201 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

202 and 0.28 for ^{66}Zn. Concentrations were expressed as molar ratios in relation to Ca (element/Ca,

203 mmol/mol).

204

205 *2.4. Data analysis*

7

206   FCs and elemental ratios were tested for normality and homogeneity of variance using the
207 Shapiro-Wilk and Levene's tests. Only FCs (29) that displayed a normal distribution and
208 homogeneity of variance after transformation were used for the statistical analysis. Because total
209 length (TL) can confound shape variation among sampling sites, the effect of these variables on FCs
210 was tested by using analysis of covariance (ANCOVA). Seven variables co-varied with TL and were
211 corrected by subtracting the common slope of the ANCOVAs. After that, FCs were normalized to
212 TL to avert potential allometric effects using the allometric relationship and standardized to a fish
213 length of 30.9 cm (mean TL for all fish) (Lleonart et al., 2000). Multivariate analysis of variance
214 (MANOVA) was used to assess differences in the otolith morphology between sampling sites. After
215 testing the homogeneity of variances-co-variances matrices by using Box test (p>0.05), linear
216 discriminant function analysis (LDA) were used to test the ability of otolith contour to classify
217 samples into specific catch areas and prior probabilities was calculated.

218   No elemental ratios fulfilled the assumptions of normality and homogeneity simultaneously,
219 even after being Log (x+1) transformed. Then, nonparametric statistics was performed to assess the
220 special segregation in the young and adult stages between estuaries. The effect of age, TL and otolith
221 weight on the element:Ca ratios were tested using Spearman correlation and ANCOVA (Kerr and
222 Campana, 2014; Longmore et al., 2010). ANCOVA is robust to violations of the assumption of
223 normality and homogeneity (Olejnik and Algina, 1984). No significant covariance and correlation
224 were found between age, TL or otolith weight and element:Ca ratios for otolith core and edge
225 (p>0.05). Kruskal-Wallis test was used to make univariate comparisons between sampling sites for
226 otolith core and edge separately. Permutational multivariate analysis of variance (PERMANOVA)
227 was used to assess multi-elemental differences in otolith core and edge chemistry between sampling
228 sites. These analyses were performed based on Mahalanobis distances (Anderson, 2006) with 9999
229 permutations. Because the assumption of homogeneity of variances-covariances was not met (Box
230 test, p<0.001), quadratic discriminant function analysis (QDA) was used instead of LDA to assess
231 the ability of elemental ratios (core and edge separately) to sort fish into specific fishing sites.

232   An additional LDA was carried out integrating morphometry and chemistry data to evaluate
233 if classification rates improve to those obtained by using each method separately. For both LDA
234 (morphometric analysis) and QDA (microchemistry analysis), multicollinearity was tested by
235 obtaining the tolerance value, which was calculated as 1 minus R^2 of the respective variable with all
236 other variables included in the model (Hair et al., 2010). The prior probabilities of classification were
237 estimated on sample sizes and group numbers (White and Ruttenberg, 2007) and the classification
238 accuracy was evaluated by leave-one-out cross-validation (jackknifed procedure).

239   The Sr:Ca transects of the last otolith portion (~last two years) were compared with the

240   *annuli* position to evaluate its ability as a salinity proxy and reveal potential temporal patterns of

241   displacement (Avigliano et al., 2017c). Statistical tests were performed using the Systat 13 and SPSS

242   19 programs.

243

244   **3. Results**

245

246   *3.1. Otolith morphometry*

247   MANOVA was effective for discriminating between all sampling sites (F= 6.41, p<0.0001),

248   except Itapanhaú River and Paranaguá Bay (p= 0.095). According to the prior probabilities (0.20 for

249   Paraíba do Sul River, 0.20 for Guanabara Bay, 0.17 for Itapanhaú River and 0.41 for Paranaguá Bay)

250   based on sample sizes and group numbers, mean rates of LDA were relatively high (>70%) for

251   Paraíba do Sul River, Guanabara Bay and Paranaguá Bay and low for Itapanhaú River (54%) (Table

252   2 and Fig. 5).

253

254   *3.2. Otolith chemistry*

255   All element:Ca ratios in otolith cores showed significant differences between sampling sites

256   (Fig. 3). Otolith core Mg:Ca were highest in Itapanhaú River, intermediate in Guanabara Bay and

257   lowest in Paraíba do Sul River and Paranaguá Bay (H= 12.2; p= 0.0068). Mn:Ca and Ni:Ca were

258   highest in Paraíba do Sul River, intermediate in Guanabara Bay and lowest in the Itapanhaú River

259   and in the Paranaguá Bay (H= 46.5 and H= 62.2; p<0.0001). Core Cu:Ca were highest in Paraíba do

260   Sul and Itapanhaú River and lowest in Guanabara and Paranaguá Bays (H= 17.5; p= 0.0006), while

261   Zn:Ca and Sr:Ca were lowest in the Paraíba do Sul River (H= 8.80; p= 0.0321 and H= 31.5;

262   p<0.0001, restrictively), and no significant differences were found between the rest of the sites

263   (p>0.05). Core Ba:Ca was significantly highest in Paraíba do Sul River, intermediate in Guanabara

264   and Paranaguá Bays and lowest in Itapanhaú River (H= 64.4; p<0.0001).

265   Otolith edge Mn:Ca was significantly lowest in Guanabara Bay (H= 19.0; p= 0.0003). Edge

266   Ni:Ca was highest in Paraíba do Sul River, intermediate in Guanabara Bay and lowest in Itapanhaú

267   River and Paranaguá Bay (H= 51.8; p<0.0001), whereas edge Zn:Ca was highest in Paranaguá Bay

268   (H= 11.6; p= 0.0087). Edge Sr:Ca was highest in Guanabara Bay (H= 35.17; p<0.0001). Edge Ba:Ca

269   was significantly highest in Paraíba do Sul River, intermediate in Guanabara Bay and lowest in

270   Itapanhaú River and in Paranaguá Bay (H= 63.8; p<0.0001). No significant differences were found

271   for the Mg:Ca and Cu:Ca ratios at the otolith edge (p> 0.05).

272   All edge Sr:Ca profiles (~up to 930 µm) seem to show around two cyclical oscillations per

273   year in all estuaries with a maximum range of variation from 2.4 to 9.0 mmol/mol (mean±SD:

274   3.9±0.54 for core and 3.9±0.51 for edge) (Fig. 4).

275   For both edge and core, multivariate analyses of fingerprints were effective for discriminating

276   between sampling sites. PERMANOVA analysis demonstrates that there were significant

277   multivariate differences among all comparisons between sampling sites (expect between Itapanhaú

278   River and Paranaguá Bay for edge, p=0.08) for otolith edge (F= 6.2; p<0.0001) and core (F= 6.9;

279   0.0001<p<0.022).

280   According to QDA, the jackknifed classifications were higher than the expected prior

281   probability of classification (0.25 for Paraíba do Sul River, 0.28 for Guanabara Bay, 0.26 for

282   Itapanhaú River and 0.21 for Paranaguá Bay) for both, edge (mean= 75.8%) and core (mean= 86.2%)

283   (Table 2, Fig. 5). For edges, the percentage of well classified individuals obtained was high for

284   Paraíba do Sul River, Guanabara Bay and Itapanhaú River (86.4-87.0%) and low for Paranaguá Bay

285   (42.1%). For cores, the percentage of correctly classified individuals was moderate-to-high and

286   ranged from 73.7 to 100%.

287

288   *3.3. Integration of morphometry with element:Ca chemistry*

289   Integration of the two methods, morphometric and element:Ca signatures, improved the

290   percentage of correctly classified sites (mean of 95.2%). The cross-classification matrix (Table 2,

291   Fig. 5d) demonstrates a high percentage of correctly classified individuals ranging from 89.5 to

292   100%. It is particularly noteworthy that correct classifications for Paranaguá Bay increased

293   significantly (from 42.1–73.7 to 89.5%) (Table 2, Fig. 5d). In this analysis, the prior probabilities of

294   classification were 0.25, 0.26, 0.28 and 0.21 for Paraíba do Sul River, Guanabara Bay, Itapanhaú

295   River and Paranaguá Bay, respectively.

296

297   **4. Discussion**

298

299   The population estructure, connectivity and habitat use of *G. genidens* is not yet well defined

300   and controversial, because some authors suggested that this catfish leaves the estuary after the

301   reproductive period (Barbieri et al., 1992; Hostim-Silva et al., 2009), while other authors supported

302   estuarine resident behavior (Maciel et al., 2018a; Silva-Junior et al., 2013).

303   In this study, we used different otolith natural markers to reveal different aspects of its

304   biology, such as population structure, and potential displacements. The incorporation rate of these

305   markes into the otolith can be affect for the surrounding water chemistry, as well as environmental,

genetic, and physiological (Clarke et al., 2011; Elsdon and Gillanders, 2003; Morales-Nin, 2000). Even a differential fishing pressure among catch areas could alter fish mortality, growth and reproduction rates, which could affect otolith microchemical patterns (Catalán et al., 2018). However, the relative importance of each factor is species-dependent. For example, Mg incorporation into the otolith can be related with environmental factors such as salinity and temperature, and ontogenetic alterations may also occur in several fish species (Avigliano et al., 2015b; Bouchard et al., 2015; Gaetani and Cohen, 2006). Otolith Mn:Ca could be associated to the exposure to hypoxic environments, but also can vary with salinity and temperature (Altenritter et al., 2018; Elsdon and Gillanders, 2003; Limburg et al., 2015, 2011). In addition, diet and ontogenetic changes could also affect the incorporation of Mn into the otolith (Elsdon and Gillanders, 2003; Limburg et al., 2015, 2011). Moreover, freshwater and estuarine areas have great chemical heterogeneity due to fluvial and anthropogenic inputs. Contamination of the environment with different heavy metals such as Zn, Ni and Cu could have some influence on otolith element:Ca signatures (Avigliano et al., 2018b, 2015a). The chemical composition of the diet could have a direct effect on the incorporation of Zn in the otolith of some euryhaline species like *Pagrus auratus* (Ranaldi and Gagnon, 2008). For several diadromous and euryhaline fish, the otolith Sr:Ca and Ba:Ca ratios have respectively positive and negative related with salinity (Avigliano et al., 2018a; Kerr and Campana, 2014), though Bouchard et al. (2015) recorded an increase of Ba:Ca ratio with the salinity in *Boreogadus saida*. Because any of these factors may influence otolith element:Ca compositions, specific factors responsible for particular element:Ca signatures are difficult to constrain in *G. genidens* at the present time. Nevertheless, in other congener species (*G. barbus*), it has been shown that water chemical composition is a major factor regulating the incorporation of some elements in calcified structures such as otolith and fin spines (Avigliano et al., 2019).

In this study, otolith Ba:Ca, Mg:Ca and Mn:Ca ratios were up to 10 times higher in the core (Fig. 3) than in the edge. Avigliano et al. (2017c, 2015b) found higher levels of Ba:Ca and Mg:Ca in otolith core of *G. barbus* caught in Brazilian estuaries. These authors suggested that high levels of Ba:Ca and Mg:Ca in *G. barbus* otolith core could be due to different factors, for example, changes in the rate of incorporation during the ontogeny. On the other hand, high Mn:Ca in cores could indicate use of hypoxic environments (Limburg et al., 2015, 2011) in the juvenile stage. Otolith Mn:Ca ratio showed an increase in the summer (hypoxic period) and decline in winter (normoxia period) in cod *Gadus morhua* while, a general decrease in otolith Mn through ontogeny has also been observed (Limburg et al., 2015). Barbieri et al. (1992) and Maciel et al. (2018a) reported that the spawning period of *G. genidens* spans from October to March (warm months), coinciding with high surface water temperatures (hypoxic period). Furthermore, there is evidence that the juveniles are released

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340 by males after oral incubation in the inner region of estuaries (Araújo, 1988; Gomes and Araújo,

341 2004), where, in general, the water quality is poor, due to discharges of anthropogenic origin (*e.g.* in

342 Guanabara Bay, <0.2 mg/l of dissolved oxygen was recorded in regions of inner estuary; Silva-

343 Junior et al., 2016), which may also generate a hypoxia condition. Additionally, the low variation

344 observed among Ni:Ca, Cu:Ca and Zn:Ca levels between the otolith core and edge could suggest that

345 both juveniles and adults experience comparable levels of water pollution throughout their lives.

346   Otolith edges have mean Sr:Ca between 3.92 and 3.97 mmol/mol, according to the values

347 reported for estuarine use in the sympatric species *G. barbus* (3.7-5.9 mmol/mol) (Avigliano et al.,

348 2017c). Nevertheless, we found minimum and maximum Sr:Ca of 2.4 and 9.0 mmol/mol in *G.*

349 *genidens*. For *G. barbus*, these values (2.4 and 9.0) correspond to the use of freshwater and marine

350 environments, respectively (Avigliano et al., 2017c). In this sense, if both species have comparable

351 migration thresholds of Sr:Ca, *G. genidens* otoliths chemical composition suggest that the species

352 completes its life cycle within the estuaries, as was previously described (Maciel et al., 2018a; Silva-

353 Junior et al., 2013), but could venture into freshwater and saltwater. This hypothesis is supported by

354 occasional collections reported on the maritime platform, near the mouth of estuaries (Denadai et al.,

355 2012; Santos et al., 2016).

356   Cyclical Sr:Ca oscillations in edge transects (~up to 930 µm or two years) (Fig. 4) are

357 consistent with regular migrations between different salinity environments, as previously reported for

358 *G. barbus* (Avigliano et al., 2017c). However, unlike *G. barbus*, these movements seem to be

359 biannual, not annual (Fig. 4). Nevertheless, although it is generally assumed that salinity is the main

360 factor influencing Sr:Ca variations in otoliths of diadromous or euryhaline fish (Brown and Severin,

361 2009), it has not yet been empirically established for *G. genidens*. Therefore, the migratory

362 inferences based on oscillatory Sr:Ca should be taken with caution. Further investigations are

363 required to verify if otolith Sr:Ca follow natural salinity variations, as well as to determine factors

364 affecting other element:Ca otolith signatures in *Genidens* spp, and to assess if them are similar

365 among sympatric species of the same genus.

366   The multielemental analyzes were efficient for discriminating catfish populations between the

367 four estuaries with potential for delimiting stock and nursery areas. Chemical element:Ca signatures

368 of otolith cores are usually used as nursery habitat markers (Avigliano et al., 2016; Campana, 2005;

369 Kerr and Campana, 2014), whereas element:Ca signatures of otolith edges and otolith shape can be

370 used as stock indicators (Avigliano et al., 2014; Campana, 2005; Kerr and Campana, 2014; Soeth et

371 al., 2019). Our results showed a high segregation (low connectivity) in juvenile and adult stages

372 among estuaries, suggesting that populations remain relatively isolated throughout the life cycle.

To complement the information generated for element:Ca signatures, we also evaluated otolith shape. The spatial morphometric differences found are strong evidence that these groups present a high segregation between estuaries. Genetics can influence otolith shape (Reichenbacher and Reichard, 2014; Vignon and Morat, 2010), however, environmental factors (*e.g.* temperature, depth, salinity and food availability) can also have a significant impact on fish growth rates, producing variation in the otolith growth patterns, which in turn affects the otolith morphometry (Longmore et al., 2010).

Herein, the individual use of otolith shape and microchemistry proved to be efficient for discriminating between all sampling sites, with the exception of Itapanhaú River (shape) and Paranaguá Bay (edge microchemistry) (Table 2). The lowest classification rates observed for the Itapanhaú River and Paranaguá, could be related to a relatively low discriminating power of the employed variables when the methodologies were applied separately. Nonetheless, the effectiveness was enhanced with simultaneous use of the two techniques (Table 2). This integrated approach may be an effective tool for further studies of catfish population structure. The ability to differentiate stocks and nursery areas through integrated otolith chemistry and shape analysis has been demonstrated for several Southwestern Atlantic species, such as *Odontesthes bonariensis* (Avigliano et al., 2014), *Prochilodus lineatus* (Avigliano et al., 2017b), *Mugil liza* (Fortunato et al., 2017b), *Mugil cephalus* (Fortunato et al., 2017a).

The results allow suggesting specific management strategies for each estuary, given the strong evidence that they support four independent stocks and nurseries, which probably have distinct population parameters. Considering the complex reproductive biology of this species, which makes it extremely vulnerable to fishing pressure (Maciel et al., 2018a), we recommend for each nursery area described here the implementation of minimum catch size and closed catch seasons, based on the spawning and incubation period.

Our results also have important implications for the management of these estuarine ecosystems, which are essentials for the maintenance of fisheries. If the anthropogenic interferences remain in their course and speed, the natural resources and services provided by the estuaries will face the most severe degradation in the near future (Barletta and Lima, 2019). Thus, considering that *G. genidens* could complete its life cycle within these environments, it would be possible to use it as a sentinel species, as was previously proposed for Guanabara Bay (Silva-Junior et al., 2013). Finally, this paper highlights the importance of extending this study to other estuaries, with the aim of contributing to fisheries management of *G. genidens*, as well as to generate knowledge bases for their potential use for the biomonitoring of these habitats.

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Acknowledgments

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672   **Figure captions**

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674   **Fig. 1.** Map showing the sampling sites (red icons) of *Genidens genidens* in tropical and subtropical

675   coasts from Southwestern Atlantic.

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677   **Fig. 2.** *Genidens genidens* otolith morphometry and chemistry. **a.** Left *lapillus* otolith; dorsal (D),

678   ventral (V), posterior (P), anterior (A) positions relative to the fish. **b.** Otolith contour; black line is

679   the real otolith contour and red line is the contour generated by using 12 harmonics. **c.** Cross-section

680   of left *lapillus* otolith through the core (age/ 5+ years); showing dorsal (D), ventral (V), internal

681   lateral face (IL), external lateral face (EL) positions relative to the fish; red lines indicate the LA-

682   ICP-MS measurement transects. **d.** Example of element:Ca ratios for an otolith core transect (log

683   scale used to better show respective element:Ca signatures).

684

685   **Fig. 3.** Mean ± SD of the elemental ratios (mmol/mol) for otolith core and edge from the four

686   sampling sites. Different letters indicate statistically significant differences between sampling sites

687   ($p<0.05$). PSR, Paraíba do Sul River; GB, Guanabara Bay; IR, Itapanhaú River; PB, Paranaguá Bay.

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689   **Fig. 4.** Otolith edge Sr:Ca ratio profiles (~two years) of *Genidens genidens* from the four estuaries

690   (core-to-edge direction is to the right). Gray and white areas correspond to opaque and hyaline

691   bands, respectively (one year equals to two complete *annuli*). Around two cyclical oscillations per

692   year were observed.

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694   **Fig. 5.** Graphic representation of the discriminant analysis of *Genidens genidens* otoliths from the

695   four sampling sites. **a.** LDA for otolith shape; **b.** QDA for otolith core chemistry; **c.** QDA for otolith

696   edge chemistry; **d.** LDA for otolith shape and edge chemistry together.

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725   **Table 1** Summary of fish descriptive statistics (mean and range) for each sampling site. N, sample

726   size; TL, total length (cm); W, weight (g); SD, standard deviation; age in years.

	N	TL ± SD	W ± SD	Age ± SD
Otolith chemistry				
Paraíba do Sul River	22	34.4 ± 1.2	303 ± 54.9	6.1 ± 1.6
		(32.3-36.5)	(220-400)	(5.0-11.0)
Guanabara Bay	25	28.6 ± 1.2	192 ± 39.4	6.9 ± 1.0
		(28.0-32.0)	(149-315)	(6.0-9.0)
Itapanhaú River	23	30.7 ± 1.8	282 ± 66.9	7.3 ± 1.6
		(28.0-34.5)	(200-410)	(5.0-11.0)
Paranaguá Bay	19	32.7 ± 1.5	292 ± 56.3	8.6 ± 2.0
		(29.2-35.6)	(192-409)	(5.0-11.0)
Fourier descriptors				
Paraíba do Sul River	30	35.4 ± 2.4	351 ± 111	-
		(32.3-42.3)	(220-680)	-
Guanabara Bay	30	29.0 ± 1.4	200 ± 42.8	-
		(28.0-32.0)	(149-315)	-
Itapanhaú River	26	30.9 ± 2.1	286 ± 71.0	-
		(28.0-36.0)	(200-420)	-
Paranaguá Bay	61	29.7 ± 5.4	225 ± 113	-
		(15.7-40.2)	(22.5-409)	-

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744 **Table 2** Cross-classification matrices of the discriminant analyses. The numbers represent the

745 classification percentage. N: sample size.

|	N	Paraíba do Sul River	Guanabara Bay	Itapanhaú River	Paranaguá Bay
Otolith morphometry (LDA)					
Paraíba do Sul River	30	**96.2**	0	3.8	0
Guanabara Bay	30	13.3	**70.1**	10.3	6.7
Itapanhaú River	26	0	18	**54.1**	17.9
Paranaguá Bay	61	0	3.3	16.7	**80.1**
Mean					**75.1**
Otolith core chemistry (QDA)					
Paraíba do Sul River	22	**100**	0	0	0
Guanabara Bay	25	0	**80**	16	4
Itapanhaú River	23	0	8.7	**91.3**	0
Paranaguá Bay	19	0	5.3	21.1	**73.7**
Mean					**86.2**
Otolith edge chemistry (QDA)					
Paraíba do Sul River	22	**86.4**	4.5	9.1	0
Guanabara Bay	25	0	**88**	8	4
Itapanhaú River	23	0	0	**87**	13
Paranaguá Bay	19	0	5.3	52.6	**42.1**
Mean					**75.9**
Otolith morphometry and edge chemistry (LDA)					
Paraíba do Sul River	22	**100**	0	0	0
Guanabara Bay	25	0	**100**	0	0
Itapanhaú River	23	0	0	**91.3**	8.7
Paranaguá Bay	19	0	0	10.5	**89.5**
Mean					**95.2**

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Core   **Edge**

Sampling Site
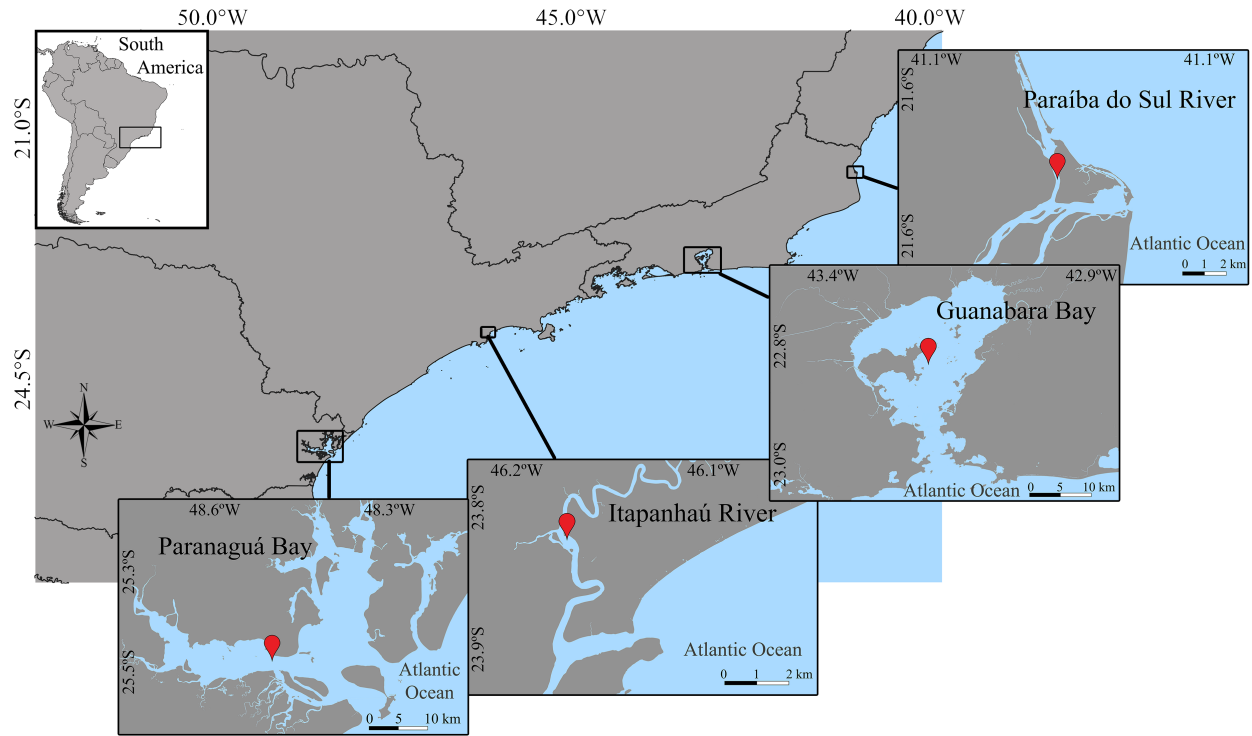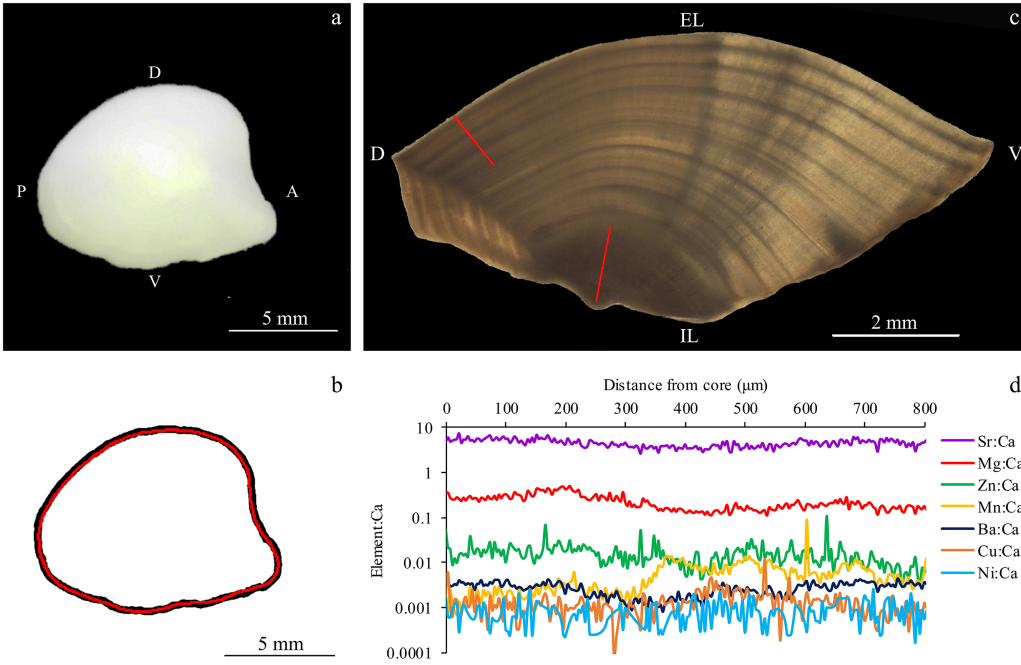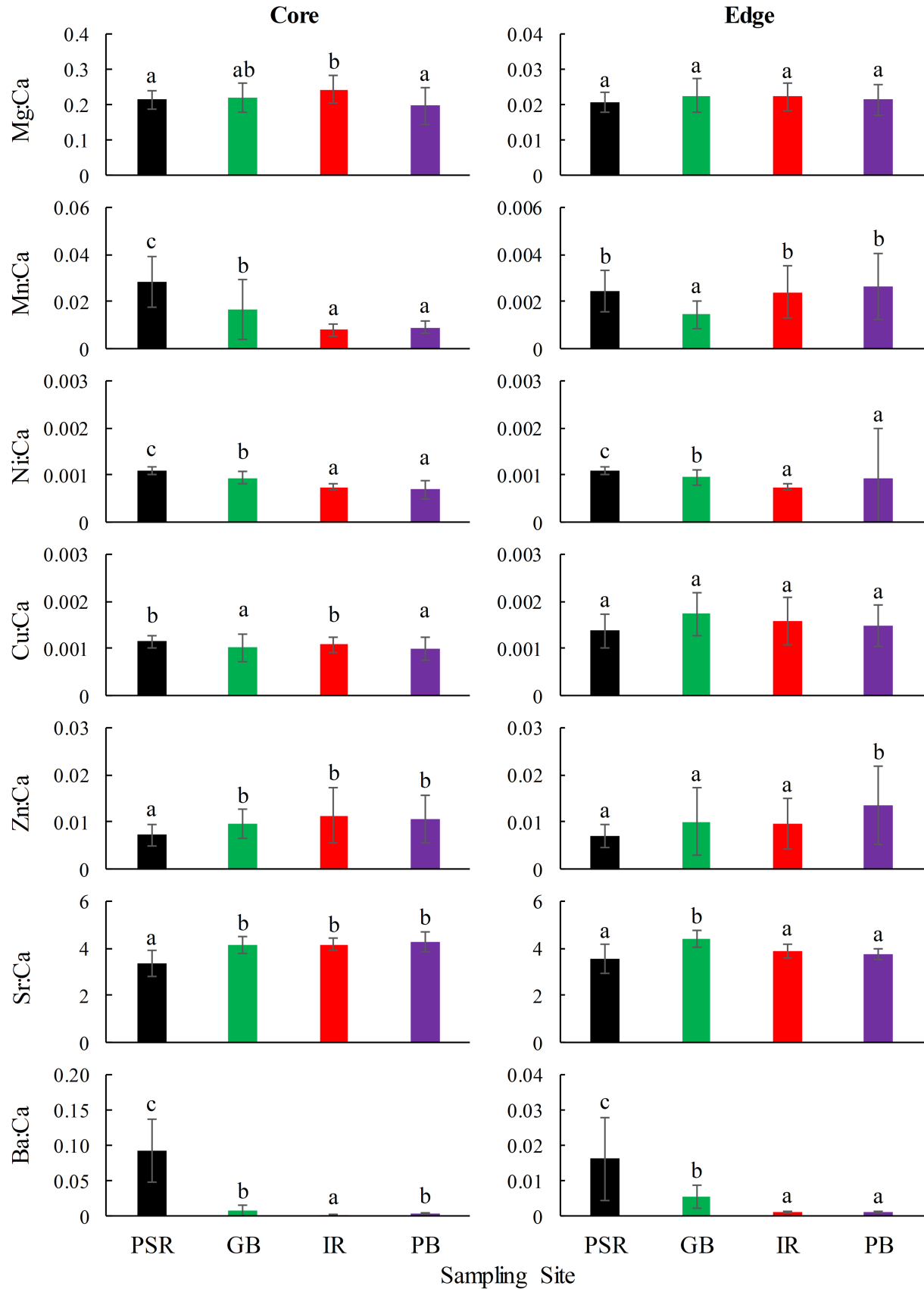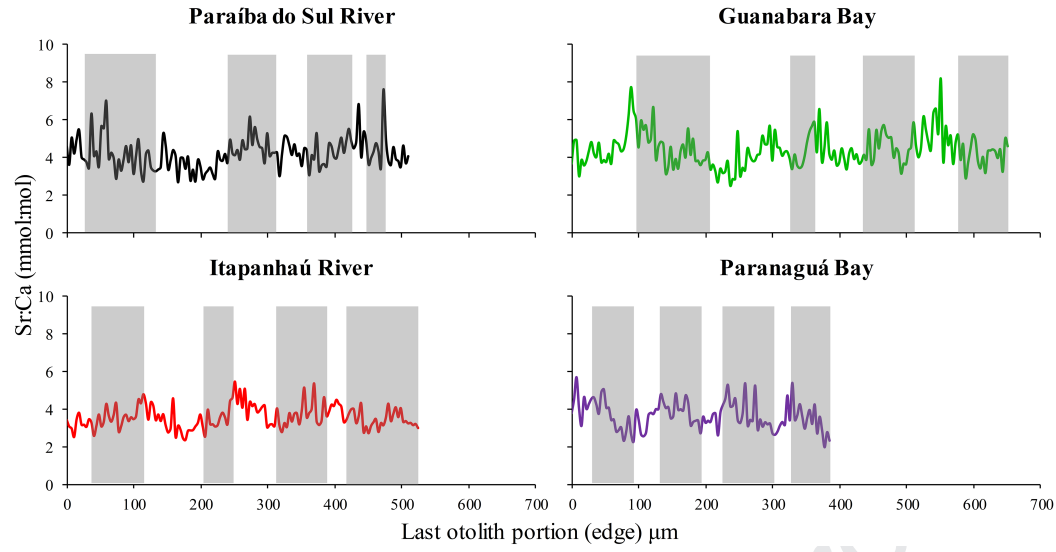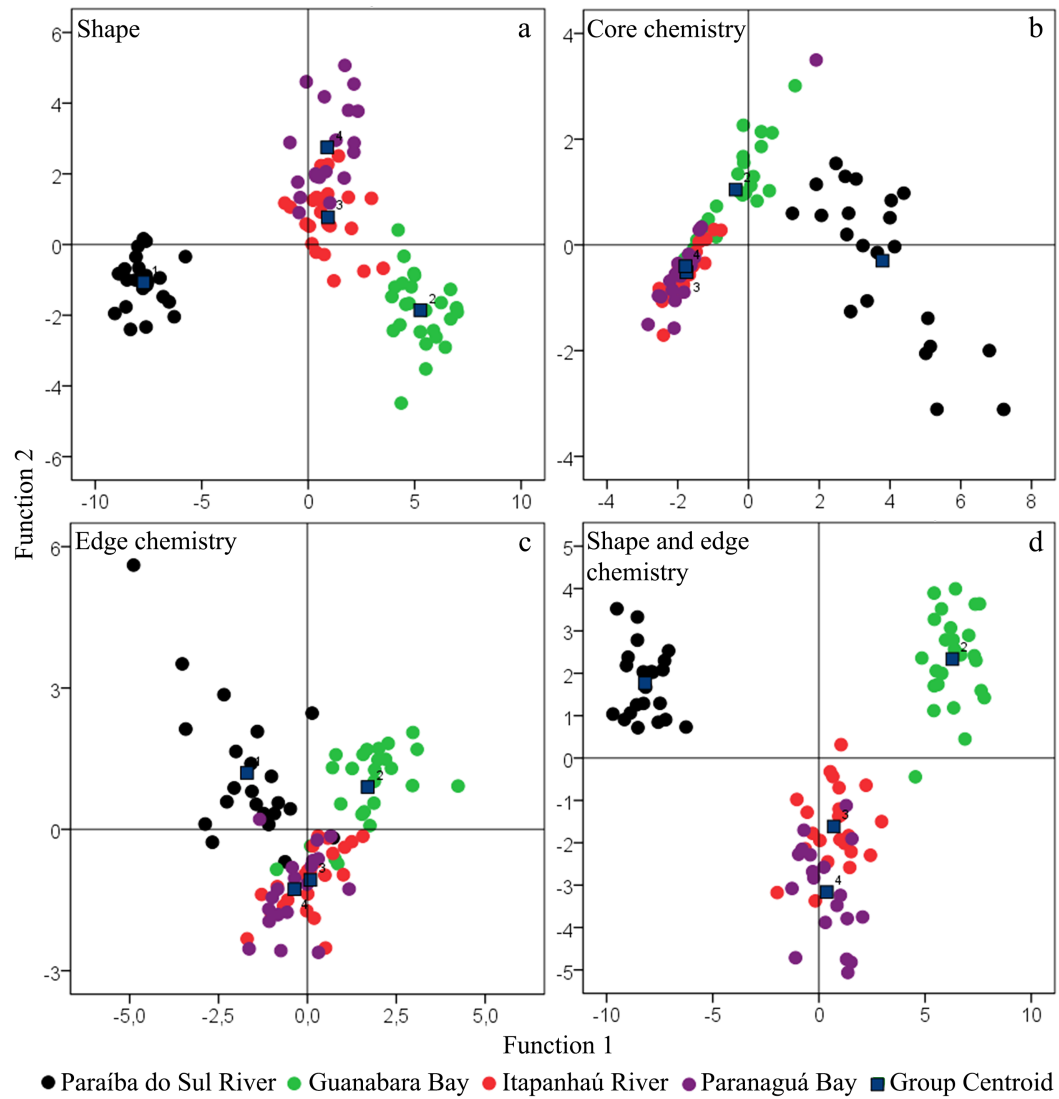
Highlights:

Otolith shape and chemistry are good tools for stock identification.

Geniens genidens is estuarine-resident, but could make oceanic incursions.

The results suggest four different populations and nursery areas.

There is low connectivity between catfish populations from the studied estuaries.

The populations should be managed as separate groups.

Conflict of Interest

Dear Editor Mike Elliot,

The authors declare that there are no conflicts of interest that could improperly influence our work.

Your sincerely,

Thaís Rodrigues Maciel

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Prof. Dr. Marcelo Vianna