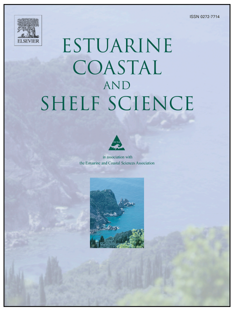

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Otolith edge fingerprints as approach for stock identification of *Genidens barbus*.

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Abstract

The purpose of this paper is to assess the use of multi-elemental otolith fingerprints as a tool to delimit catfish *Genidens barbus* fish stocks in four estuaries from the southwestern Atlantic Ocean. Barium:Calcium (Ca), Magnesium:Ca, Manganese:Ca, Sodium:Ca and Strontium:Ca ratios in the otolith edge were determined by LA-ICPMS. PERMANOVA analysis reveal significant differences in the multi-element signatures among estuaries (p=0.0001-0.002). Reclassification rates of quadratic discriminant analysis are high, averaging 89.9 % (78-100%). The new data presented here show that the otolith chemistry is a potential tool for stock identification, and indicates the presence of at least four stocks which should probably be handled independently.

Keywords: Catfish; multi-elemental fingerprints; stock delimitation; South-western Atlantic estuaries

1. Introduction

Among the major fisheries in South America that have strongly declined in recent decades is the catfish *Genidens barbus* (Lacépède 1803), a species of commercial importance that is distributed in subtropical and temperate zones of the southwestern Atlantic Ocean (López and Bellisio, 1965; Velasco et al., 2007). The biology of catfish is highly complex, due to the presence of different migratory patterns such as cyclic semi-amphidromy, amphidromy, anadromy and freshwater residency (Avigliano et al., 2017b, 2015a). Reproductive events occur in spring and summer in relatively low salinity environments (rivers and estuaries) (Araújo, 1988; Reis, 1986a, 1986b). Parental care by males is observed in this species, where eggs or juveniles are carried in the oropharingeal cavity up to 3 months from the mating area to the external estuary (moderate-high salinity) (Reis, 1986a). Recently, the species was included in the Red List of endangered species in Brazil and several studies are being carried out for the recovery of the fisheries (Avigliano and Volpedo, 2016; Di Dario et al., 2015).

The identification of fish stocks is a prerequisite to study the dynamics and structure of the fishery management units. In this respect, the efficiency of the management of a given fishery depends on the correct delimitation of stocks (Cadrin et al., 2013). Several methodologies have been historically used for stock delimitation as meristic, morphometric landmark, parasites, fatty acid profiles, allozymes, mitochondrial DNA, external an internal tags, otolith morphometry and microchemistry (Cadrin et al., 2013).

Multi-elemental fingerprints in the otolith edge has contributed to stock identification of several fish species in the world (Avigliano and Volpedo, 2016; Campana, 2013; Tanner et al., 2015). Fish otoliths are apposition structures composed of calcium carbonate deposited in a protein matrix, with small quantities of certain elements such as Ba, Li, Mg, Mn, Na, Sr, and Zn (Campana et al., 1997). These trace elements are acquired by an individual fish during the ontogenetic period and preserved within the otolith microstructure. Hence, multi-elemental fingerprints in the edge may reflect geographic groups or stocks (Campana, 2013; Tanner et al., 2015).

The trace element composition of catfish *lapillus* otolith has proved to be a useful tool as a natural tag and has allowed the identification of nursery areas, population structure and life history (Avigliano et al., 2017b, 2016, 2015a, 2015b). Recently, Avigliano et al. (2015b) have suggested the presence of two fish stocks south of the species known distribution. However, the delimitation of fish stocks remains poorly constrained in the rest of the species distribution area.

The purpose of this study was to evaluate the multi-elemental fingerprints of the *lapillus* otolith edge as a tool to delimit *G. barbus* fish stocks in the southwestern Atlantic Ocean. Here, we determined the Ba:Ca, Mg:Ca, Mn:Ca, Na:Ca and Sr:Ca ratios of otolith edge by LA-ICPMS in catfish caught in four different estuaries (Guanabara Bay, Paranaguá Bay, Patos Lagoon in Brazil, and Plata River estuary in Argentina). Multi-elemental otolith fingerprints were compared among fish collected from each estuary in order to establish patterns in the data that may be used to identify and evaluate the presence of stocks. This information is important for the sustainable exploitation of the resource, the development of assessment and management models.

2. Materials and Methods

2.1 Sample collection and preparation

Adult catfish were caught in Guanabara Bay (GB), Paranaguá Bay (PB), Patos Lagoon (PL), and Plata River estuary (PR) (Fig. 1) with gillnets, hooks and longlines between November 2010 and May 2015. The total fish length (in cm) was recorded and both *lapilli* otoliths were removed and rinsed with ultrapure water (18.2 MΩ/cm) (Millipore, São Paulo, Brazil).

Otoliths (N=46) were weighed using an analytical balance (Sartorius AG ED 2242, Göttingen, Germany), washed with ultrapure water and dried. The left otolith of each pair was embedded in epoxy resin and sectioned transversely through the core to a thickness of 700 µm using a Buehler Isomet low speed saw (Hong Kong, China) equipped with twin diamond edge blade. Only fishes between 8–12 years were used (randomly selected) for analysis to avoid possible effects caused by the age of the fish on the data interpretation (Avigliano et al., 2017a). Mean age±standard deviation (in years) were 10.3±1.75, 10.2±1.56, 8.64±0.92 and 8.73±1.27 for PR (N=15), PL (N=9), PB (N=11) and GB (N=11). Annual periodicity of ring formation was validated by Reis (1986a). Mean total length±standard deviation and range (in cm) were 63.2±7.05 (52.0–74.2), 60.7±6.32 (54.7–71.2), 64.9±6.02 (58.0–81.9), 59.2±9.16 (45.9–75.4) for PR, PL, PB and GB.

2.2 Determination of elements by LA-ICP-MS and data analysis

Elemental concentrations in otolith sections were measured using a 193nm ArF laser ablation system (Photon Machines Analyte G2) coupled to an ICP-MS iCapQ ThermoFisher at the Andean Geothermal Center of Excellence (CEGA), Universidad de Chile, Santiago, Chile.

The abundances of isotopes ^{23}Na, ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr, ^{138}Ba was determined by laser ablation on fifty µm line-scans performed on the outermost 300 microns of the otolith edge, which represents approximately the last year of life. NIST SRM 612 silicate glass reference material was used as an external standard (Jochum et al., 2011), whereas the USGS synthetic calcium carbonate MACS-3 (Jochum et al., 2012) and silicate glass NIST SRM 610 were analyzed as secondary standards. The two external standards measurements allowed us to calculate the precision and the accuracy of the analysis. Precision was less than 10% for all elements analyzed. Accuracy was less than 1%, except for Mg (~13%). This higher value for Mg can be explained by the uncertainty of the Mg concentration in the NIST SRM 612 standard (Jochum et al., 2011).

Prior to recording transect measurements; the otolith surface was pre-ablated using a spot size of 85 µm and a scan speed of 30 µm/s. Then, the ablation scans were performed using a 50 µm spot size, a scan speed of 10 µm/s, an energy density of 5 mJ/cm^2 and a repetition rate of 10 Hz. Element concentrations are expressed as molar ratios (element:Ca = mmol/mol). Limit of detection (LOD) was calculated from the standard deviation of the blank and normalized to Ca (0.0011, 0.00075, 8.99, 0.067 and 0.028 mmol/mol for Sr:Ca, Ba:Ca, Na:Ca, Mg:Ca and Mn:Ca). Uncertainties were determined for each the analyzed elements (i.e., Na: 5.8%; Ba: 8%; Sr: 8%; Mn: 8.5%; and Mg: 13.1%).

Nonparametric statistics were used to compare the elemental ratios between sampling sites because the ratios did not fit the normal distribution and homogeneity of variance (Shapiro-Wilk, $p < 0.05$; Levene, $p < 0.05$) even after logarithmic transformation. To ensure that differences in fish age did not confound spatial patterns in elemental composition, the effect of age on the elemental ratios was examined using analysis of covariance (ANCOVA) with age as co-variate (Campana, 2013; Longmore et al., 2010). Only the Sr:Ca ratio showed a correlation with age ($p < 0.05$). This age effect was corrected by subtracting the common slope ($b = 0.11$) in ANCOVA (Campana, 2013; Longmore et al., 2010).

Univariate and multivariate statistics were used to evaluate the presence of different stocks (Campana, 2013). Each ratio was compared among sampling sites using Kruskal-Wallis. Because the data did not fit the multi-dimensional non-normality assumptions; permutational multivariate analysis of variance (PERMANOVA) was used to evaluate geographical differences in the multi-elemental fingerprints of the otolith edge. After testing the multicollinearity between variables, quadratic discriminant function analysis (QDA) was used to assess the ability of elemental ratios to sort fish into specific catch areas. The calculation of the expected prior probability classification was based on sample sizes and group numbers (White and Ruttenberg, 2007). A randomization test was used to determine if the classification success rate was significantly different from random data (White and Ruttenberg, 2007). Statistical tests were performed using the Ginkgo 1.7 and SPSS 19 programs.

3. Results

Considering all sampling sites, element:Ca levels (mean±standard deviation and range) were 0.007±0.01 (0.001-0.03), 0.04±0.03 (0.02-0.2), 0.002±0.003 (0.0003-0.02), 9.9±1.8 (7.0-15.1), 4.6±0.9 (2.0-6.1) mmol/mol for Ba:Ca, Mg:Ca, Mn:Ca, Na:Ca and Sr:Ca, respectively (Fig. 2). Ba:Ca ratio was found to be high in PR and PB and low for PL ($p=0.0001-0.04$). The otolith edge Mg:Ca ratio was high for PR, intermediate for PL and low for GB ($P<0.02$). Na:Ca ratio was higher for PR, low for PB and GB, and intermediate for PL ($P<0.04$). Sr:Ca was high for PB and low for PL, and intermediate for PR and GB. No significant differences ($p>0.05$) were found between sites for Mn:Ca ratios.

Multivariate methods were highly effective at detecting different otolith fingerprints among sites, indicating the existence of four stocks. Specifically, PERMANOVA analysis revealed significant differences in the multi-element signatures of the otolith edge between all sampling sites ($p=0.0001-0.002$). Reclassification rates of QDA were generally high, averaging 89.9% (Table 1). Percentages of correctly classified individuals were 100% for PR, 78% for PL, 82% for PB and 100% GB. These values are significantly different from random data (prior probabilities for groups: 0.32 for PR, 0.20 for PL, 0.24 for PB and 0.24 for GB) (randomization test: $p< 0.05$). Hence, the multi-elemental signatures appear to be a powerful tool to discriminate populations.

4. Discussion

According to Campana (2013), the presence of different fingerprints among groups of fish with comparable age implies different life histories and that the otolith elemental composition can be used as a stock delimitation indicator. Univariate analysis showed significant elemental variations between some estuaries, with the sole exception of the Mn:Ca ratio. These variations were maximized with the multivariate analysis of PERMANOVA and QDA. Significant differences and high classification percentages were observed for every studied site. The two multivariate methods show that the otolith edge chemical signatures are an efficient approach to discriminate catfish stocks among the studied estuaries. Our results also indicate the presence of at least four fish stocks for the species associated with each estuary. In addition, the reclassification rates obtained for PL and PB (≥78%) suggest a relatively low connectivity among nearby estuaries, which is consistent with the chemical signature of the core of adult specimens (Avigliano et al., 2016). However, reclassification rates of 100% have been obtained for the extremes of the distribution (PR and GB populations), indicating that these groups tend to be closed. The presence of relatively closed populations could be linked to the homing behavior of the species suggested by Avigliano et al. (2016).

Previous works have suggested the existence of different fish stocks between PL and PR using the Sr:Ca, Ba:Ca and Mg:Ca ratios (Avigliano et al., 2015b). Nevertheless, these authors have used the micromilling technique averaging the last four years of life. In the present work, the LA-ICPMS technique and the possibility to measure several additional trace elements allowed us to increase the percentage of PL classification from 63.6% to 78%, while the percentage obtained for PR was not modified (100%) (Avigliano et al., 2015b).

Avigliano et al. (2017b, 2015a) have reported different migratory patterns for the species. The most common is the amphidromous types (amphidromy and semi-amphidromy), although freshwater specimens can reside in PL. Variability in migratory behaviors should be taken into account for future studies of stock identification, as the inclusion of resident specimens could affect the classification percentages when studying the connectivity of migrating individuals.

The incorporation of elements in the otolith is species-dependent and could also be influenced by environmental (salinity, temperature) (Bouchard et al., 2015; Brown and Severin, 2009; Elsdon and Gillanders, 2003; Martin et al., 2004), genetic (Barnes and Gillanders, 2013) and physiological factors (growth rates, metabolic changes) (Kalish, 1991; Radtke and Shafer, 1992; Sturrock et al., 2014). The specific factors involved in the incorporation of elements in catfish *G. barbus* remains largely unknown, as are the possible differential effects among populations.The determination of these factors and their impacts could contribute to a better stock identification and assessment of connectivity between different sites or environments. The fish age can also be a factor, however in this study only fishes between 8–12 years were selected for analysis, and hence the influence of age can be dismissed. So far, only the Sr:Ca and Ba:Ca ratios of catfish otolith have been related to factors undoubtedly affect the characteristics of the water and could have printed distinctive salinity (Avigliano et al., 2017b, 2015a, 2015b). The relationship between Sr:Ca and Ba:Ca with salinity has been useful to define the range of habitat of the species (from freshwater to ocean), as well as to describe different migratory patterns (freshwater residence or migration between freshwater, estuary and ocean) (Avigliano et al., 2017b, 2015a).

The study area includes four estuaries distributed in a tropical, subtropical and temperate region and there is a decreasing temperature gradient from north–south direction. Estuaries had different climatic and topographic features, depths, salinity ranges, oceanographic patterns and hydrographic dynamics (Avigliano et al., 2016). These signatures in the otoliths, which explains the multi-elemental differences found in this paper. Moreover, it is possible that different oceanographic patterns or climatic regimes (El Niño occurrences) have affected the level of closed populations, the metapopulations or the connectivity (Bakun and Broad, 2003; Mann, 1993; Selkoe et al., 2007).

On the other hand, it has been reported that there could be inter-annual variation in the concentration of some elements of the otolith, probably due to temporary climatic variations, therefore future stock evaluations should be performed by limiting the age and cohorts (Avigliano et al., 2017a).

Microchemistry of the otoliths is a useful tool to delimitate stocks and is of potential interest for catfish because it requires fewer resources in comparison to other methodologies such as external tags. Methods such as the geometric morphometry of the body are not recommended because fish presents a relative flaccidity out of the water making difficult the positioning of landmarks. However, a holistic approach is recommended, and it is advisable to apply other approaches such as genetics, otolith morphometry or parasites (Avigliano and Volpedo, 2016; Begg and Waldman, 1999; Cadrin et al., 2013).

Considering the results obtained, we propose the use of Ba:Ca, Mg:Ca, Mn:Ca, Na:Ca and Sr:Ca ratios to delimit stocks. The Sr:Ca and Ba:Ca ratios are essential because they are directly related to the salinity which varies between the different estuaries (Avigliano et al., 2017b). In addition, other elemental ratios such as the Li:Ca ratio are of potential use to discriminate between different catfish groups (Avigliano et al., 2016).

Even though the relative low sampled size used in this work could be a limitation, the results indicate that the methodology used is effective to delimit fish stocks. In addition, the presence of at least four fish stocks suggests that they should probably be handled independently. Therefore, it is recommended to include the determination of multi-elemental signatures in future otoliths studies. This work is a baseline for future projects with a view to the correct delimitation of management units and the subsequent administration of the resource.

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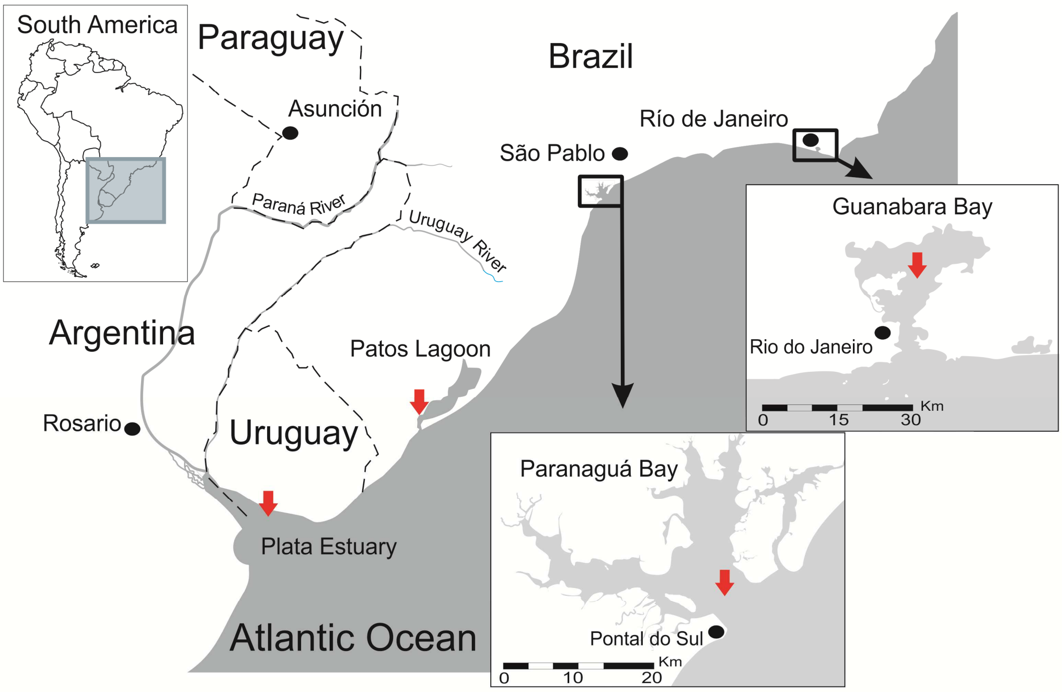
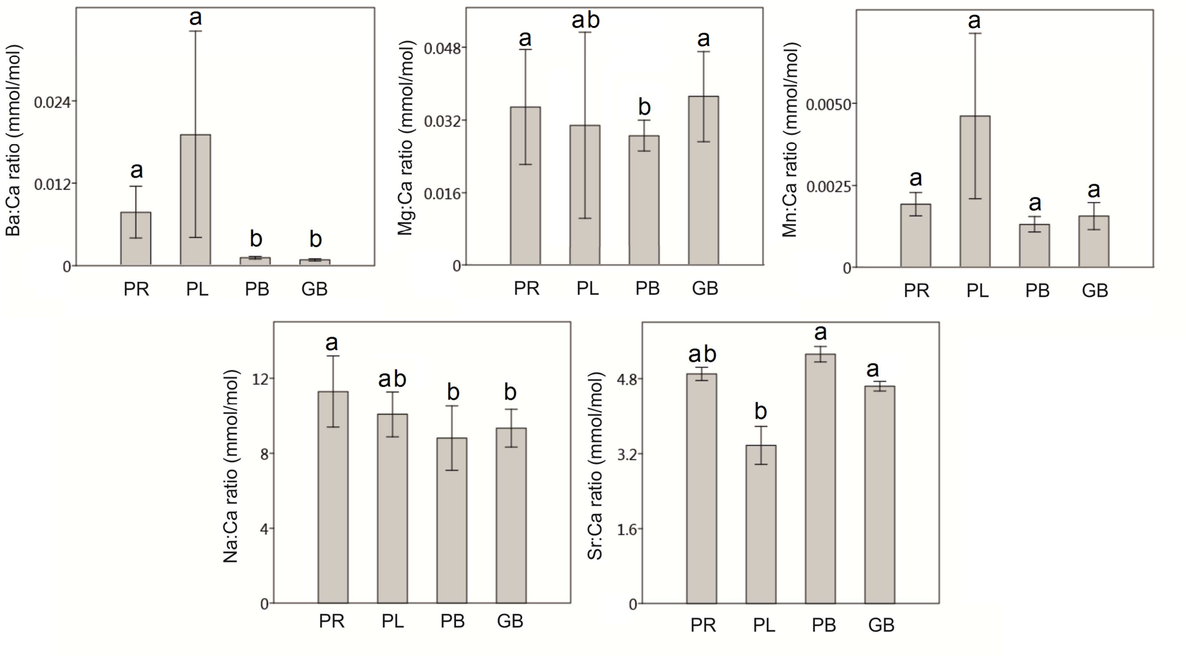
Figure 1: Fishing sites of the *Genidens barbus* (arrows).

Figure 2: Mean ± SE elemental ratio (mmol/mol) in otolith edge from different sampling locations. Different letters indicate statistically significant differences.

Table 1: Confusion matrix of quadratic discriminant analyses based on multi-elemental signature (Ba:Ca, Mg:Ca, Mn:Ca, Na:Ca and Sr:Ca) of otolith edge. PR, Plata River; PL, Patos Lagoon; PB, Paranagua Bay and GB, Guanabara Bay. Percentage of correctly reclassified individuals are indicated in bold numbers.

	PR	PL	PB	GB
PR	**100**	0	0	0
PL	22	**78**	0	0
PB	0	0	**82**	18
GB	0	0	0	**100**


South America
Paraguay
Brazil
Asunción
Río de Janeiro
São Pablo
Paraná River
Uruguay River
Guanabara Bay
Rio do Janeiro
0
15
30
Km
Argentina
Patos Lagoon
Rosario
Uruguay
Paranaguá Bay
Plata Estuary
Pontal do Sul
Atlantic Ocean
0
10
20
Km


Ba:Ca ratio (mmol/mol)
0
0.012
0.024
a
a
b
b
PR
PL
PB
GB
Mg:Ca ratio (mmol/mol)
0
0.016
0.032
0.048
a
ab
b
a
PR
PL
PB
GB
Mn:Ca ratio (mmol/mol)
0
0.0025
0.0050
a
a
a
a
PR
PL
PB
GB
Na:Ca ratio (mmol/mol)
0
4
8
12
a
ab
b
b
PR
PL
PB
GB
Sr:Ca ratio (mmol/mol)
0
1.6
3.2
4.8
ab
b
a
a
PR
PL
PB
GB

Otolith microchemisty is a potential tool for stock identification.

Results suggest the presence of at least 4 fish stocks of catfish.

High percentages of classification suggest low connectivity.

The stocks should be managed as separate groups.