

Nursery areas and connectivity of the adults anadromous catfish (*Genidens barbatus*) revealed by otolith-core microchemistry in the south-western Atlantic Ocean

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Abstract. The anadromous catfish, *Genidens barbatus*, is a commercial and vulnerable species from South America. The aims of the present study were to assess whether the nursery areas can be discriminated by using microchemical signatures of *lapillus* otoliths, to assess the accuracy of classifying fish in relation to natal nursery area and to discuss the possibility of existence of homing behaviour. Thus, the otolith-core chemical signatures (barium (Ba) : calcium (Ca), lithium (Li) : Ca, magnesium (Mg) : Ca, manganese (Mn) : Ca, strontium (Sr) : Ca, and zinc (Zn) : Ca ratios) of adult fish were compared among different estuaries (De La Plata River in Argentina, and Patos Lagoon, Paranaguá Bay and Guanabara Bay in Brazil). PERMANOVA analysis showed significant differences in the multi-element signatures of the otolith core among sampling sites for all cohorts (2002, 2003, 2006 and 2007), indicating that the technique has considerable potential for use in future assessments of population connectivity and nursery areas of *G. barbatus*. Via quadratic discriminant function analysis, fish were classified to natal nursery areas with 80–100% cross-validation classification accuracies. These results suggested that a high level of spatial segregation exists in adult catfish life and homing behaviour could not be ruled out on the basis of our data.

Additional keywords: discriminant analysis, endangered species, homing behaviour, micromilling, otolith microchemical signatures.

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Introduction

The catfish, *Genidens barbatus* (Lacépède, 1803), is an anadromous species, considered historically important for commercial and recreational fisheries (Reis 1986; Tavares and Luque 2004; Velasco *et al.* 2007; Minagro 2016). The species is distributed along the south-western Atlantic Ocean from Bahía, in Brazil, to San Blas, in Argentina (17°00'S–40°32'S) (López and Bellisio 1965; Avigliano and Volpedo 2015), and it is caught mainly in estuarine or coastal lagoon systems.

Fishery statistics on the anadromous catfish fishery are incomplete and generally are underestimated. However, there has been a steady and marked decrease in the catches in

the major fishing area, Patos Lagoon (Brazil). In this site, the capture dropped from 9000 Mg year⁻¹ in 1972–1973 to less than 200 Mg year⁻¹ since 1996 (Velasco *et al.* 2007; IBAMA 2009). Annual captures in Argentina and Uruguay ranged from 296 to 144 Mg between 2006 and 2013 (DINARA, see <http://www.dinara.gub.uy>, accessed 20 January 2016). All the same, these statistics are restricted to from the Argentine–Uruguayan common fishing zone (AUCFZ; Fig. 1) fisheries, from the shelf area shared by both countries. No fishery statistics are available for estuarine and freshwater environments, where the species is captured by the artisanal fleet during the reproductive migration (Avigliano *et al.* 2015a).

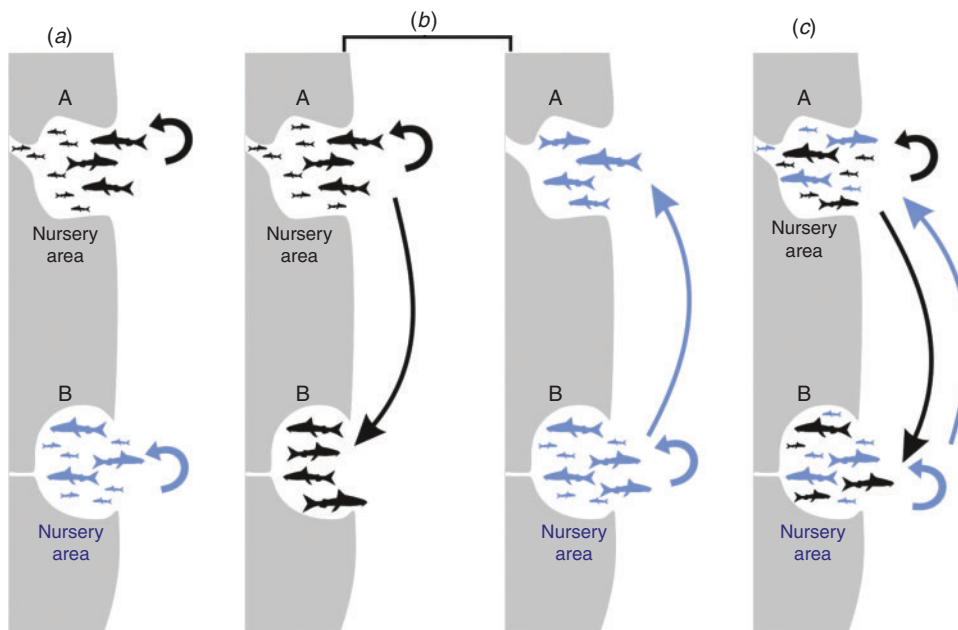


Fig. 1. Scheme of possible scenarios of migration or population structure for sexually mature adults (age >8 years) of anadromous catfish, *Genidens barbatus*. (a) Fish breed in places where they were born (homing behaviour); (b) some estuaries (A) function as nursery area and adults distribute themselves in different estuaries (A and B); and (c) adults breed in different estuaries no matter where they were born. Bold numbers indicate the percentage of correctly classified individuals.

Some ecological aspects of the life cycle are known (Reis 1986; Velasco and Reis 2004; Denadai *et al.* 2012; Avigliano *et al.* 2015a, 2015b). The adults migrate to the estuaries from an age of 7–9 years to breed (Reis 1986; Velasco and Reis 2004; Avigliano *et al.* 2015b). After females spawn in estuarine or freshwater environments, males return to the estuarine waters with the eggs inside their oropharyngeal cavity (Reis 1986; Velasco and Reis 2004). Juveniles are released into the lower-estuary waters, after which the adult males migrate to the continental shelf (Reis 1986; Velasco and Reis 2004). Juveniles live in the estuary until the age of 1–4 years, at which time they migrate to the open sea (Velasco *et al.* 2007; Avigliano *et al.* 2015b).

Recently, this species has been included in the Red List of endangered species in Brazil, because of its complex life cycle (low fecundity, oral incubation), its restricted distribution in fresh or estuarine waters during the reproductive period and the critical status of the species fishery. Thus, the capture, transport and marketing of the species has been prohibited since 2015 (Di Dario *et al.* 2015; MMA 2014). In Argentina, Baigún *et al.* (2012) classified the species as vulnerable.

Avigliano *et al.* (2015c) suggested the existence of different nursery areas and the presence of homing behaviour in Patos Lagoon (Brazil) and De La Plata River (Argentina–Uruguay). In this sense, the quality of the nursery areas is critical and the understanding of the connectivity between nursery sources (or estuarine systems in this case) and adult populations can lead to a more effective management and rebuilding of the fishery in the south-western Atlantic Ocean (Avigliano and Volpedo 2016). Nevertheless, it is difficult to determine nursery sources in the marine and estuarine environments by using

conventional tagging techniques. These techniques suffer from multiple drawbacks, including high costs, high mortality rates and low tag-return rates (Fairchild *et al.* 2013; Bailey *et al.* 2015). Even so, mapping connectivity from inshore *G. barbatus* nurseries to adult offshore populations may be possible via natural chemical tags incorporated in the otoliths. Fish otoliths are complex polycrystalline apatite structures, composed of calcium carbonate (96%) deposited as aragonite crystals in a protein matrix, and small quantities of other minerals (Campana *et al.* 1997). Because the trace elements acquired by an individual fish are preserved within the otolith microstructure formed during the corresponding ontogenetic period, their concentrations may reflect the passage of the fish through chemically different environments (Schuchert *et al.* 2010; Mai *et al.* 2014; Avigliano *et al.* 2015b; Phung *et al.* 2015). In this regard, the presence of different chemical fingerprints among groups of fish of similar age implies different environmental histories (Kerr and Campana 2013). Therefore, the analysis of elemental fingerprints of the otolith marginal area has been used to discriminate fish stocks (Campana *et al.* 2000; Ferguson *et al.* 2011; Avigliano *et al.* 2015a), whereas core area has been used for nursery identification (Brown 2006; Rooker *et al.* 2008; Schuchert *et al.* 2010; Geffen *et al.* 2011; Ibañez *et al.* 2012; Mariani and Bekkevold 2013; Bailey *et al.* 2015). Otolith core consists of all the material deposited during the larval and juvenile growth stages, before the formation of the first annulus.

In short, clarifying different aspects of the population structure of catfish, such as, for example, connectivity among nursery areas and homing behaviour, would allow the development of conservation and management strategies to recover its long-living populations. For this purpose, otolith-core chemical

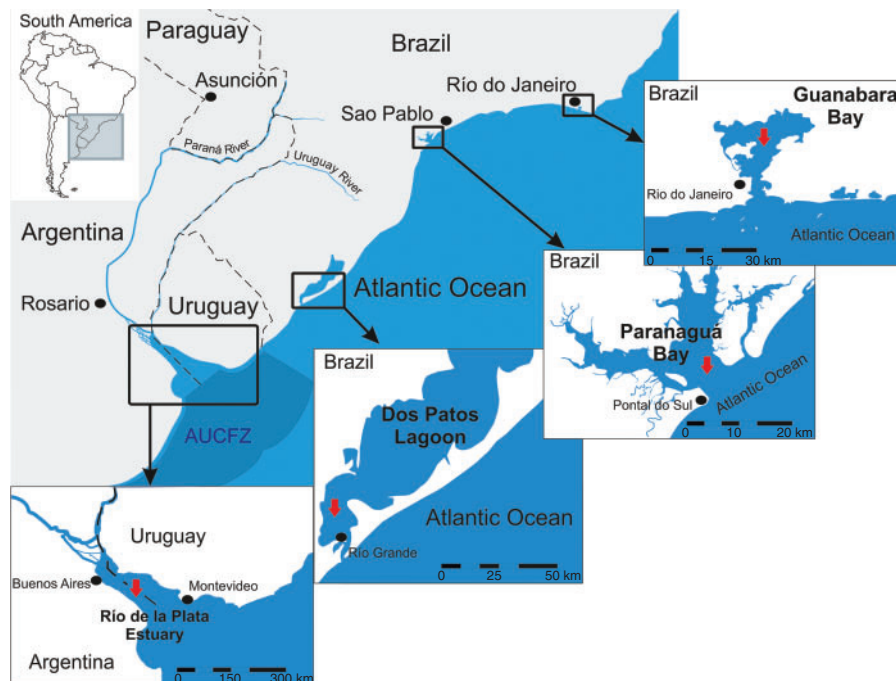


Fig. 2. Sampling sites of the anadromous catfish, *Genidens barbuis*. AUCFZ, Argentine–Uruguayan common fishing zone.

signatures of sexually mature adults (age >8 years; Reis 1986) were compared among different estuaries (De la Plata River in Argentina, and Patos Lagoon, Paranaguá Bay and Guanabara Bay in Brazil). This was undertaken so as to distinguish several scenarios related to migration and connectivity among possible nursery areas (Fig. 1). In this context, the specific objectives were (1) to assess whether the nursery areas can be discriminated by using microchemical signatures (barium (Ba) : calcium (Ca), lithium (Li) : Ca, magnesium (Mg) : Ca, manganese (Mn) : Ca, strontium (Sr) : Ca, and zinc (Zn) : Ca ratios) of the *lapillus* otolith core, (2) to assess the accuracy of classifying fish in relation to natal nursery area (connectivity) and (3) to discuss the possibility of the existence of homing behaviour.

Materials and methods

Study area

The adults were caught in four south-western Atlantic estuaries distributed across three countries (Fig. 2). With respect to the size of the estuaries and the volume of catches, these are the main systems that could function as nursery areas, taking into account virtually all distribution of the species (Velasco and Reis 2004; Avigliano *et al.* 2015a, 2015b). Environmental characteristics of each sampling site are described briefly in Table 1. The Guanabara Bay (GB) estuary, located in the state of Rio de Janeiro, has an area of 338 km² (Fig. 2). The bay measures 28 km from west to east and 30 km from south to north, whereas the narrow entrance to Guanabara Bay is only 1.6 km wide (Kjerfve *et al.* 1997). The Paranaguá Bay (PB) estuary, in the Paraná State, has an area of 601 km² (Fig. 2). This system is connected to the adjacent continental shelf by the channels Galheta and Sudeste (Angulo and Araújo 1996). The estuary

is partially mixed with stratification of the water column (Kjerfve *et al.* 1997; Table 1). The Patos Lagoon (PL), one of the most important coastal ecosystems in Brazil, is a large coastal lagoon located on the coast of Rio Grande do Sul State, with a total area of 10 360 km². The southern part of the system is occupied by an estuary, the total area of which accounts for 10% of the lagoon area. The system is connected to the Atlantic Ocean by a narrow channel that is 4 km long and 740 m wide (Fig. 2). De La Plata River (PR) estuary is a fluvio-marine environment from the Plata Basin (Argentina and Uruguay), with a total area of 10 360 km² and an average discharge of 23 000 m³ s⁻¹ towards the Atlantic Ocean (Guerrero *et al.* 1997). The outer section is 300 km wide (Guerrero *et al.* 2010; Fig. 2).

Sample collection

In total, 88 adult fish (age range: 7–13 years; total-length range: 42–120 cm) were caught in all study sites (Fig. 2) with longlines, gill-nets and hooks, at depths ranging from 10 to 33 m (Fig. 1). Fish were collected between November 2010 and May 2015.

The total length (mm) was recorded and both *lapillus* otoliths were removed, rinsed with ultrapure water and cleaned of any remaining tissue with a plastic toothbrush.

We preferred using *lapillus* otoliths rather than *sagitta* or *asteriscus* otoliths because they are larger in the Ariidae catfishes, and allow less measurement error (Avigliano *et al.* 2015a).

Age determination and sample preparation

The method applied in the present study for the isolation of the core area by micromilling of the *lapillus* otolith has been described by Avigliano *et al.* (2015a).

Table 1. Characteristics of sampling areas for *Genidens barbatus*

Sampling area	Surface temperature	Salinity	Depth	Ecoregion	Location
Guanabara Bay	Temperature varies from 24.5°C in winter to 31°C in summer (Kjerfve <i>et al.</i> 1997; Fernandes <i>et al.</i> 2002)	The maximum salinity varies from 32.5 in winter to 36 in summer (Fernandes <i>et al.</i> 2002)	Maximum depth is 58 m (Kjerfve <i>et al.</i> 1997; Fernandes <i>et al.</i> 2002)	Mountain tropical rainforest	22°50'58.15"S, 43°10'15.74"W
Paranaguá Bay	Temperature varies from 18–25°C in winter to 23–30°C in summer (Lana <i>et al.</i> 2001)	Salinity varies from 12–29 in winter to 20–34 in summer (Lana <i>et al.</i> 2001)	The main channel has a depth of more than 10 m (max. 33 m) (Lana <i>et al.</i> 2001)	Mountain subtropical rainforest	25°28'53.21"S, 48°24'41.06"W
Patos Lagoon	Temperature range is 12°C in winter to 27.5°C in summer (Muxagata <i>et al.</i> 2012)	Changes in salinity over hourly periods, but the average salinity (0–32) follows a seasonal pattern (dry summer and wet winter), being influenced primarily by rainfall and wind direction (Seeliger <i>et al.</i> 1997)	Maximum depth is 6 m in the estuary and 18 m in the navigation channel (Guerrero <i>et al.</i> 2010)	Plain	31°29'59.31"S, 51°40'46.24"W
De la Plata River	Temperature varies from 8°C to 24°C (Guerrero <i>et al.</i> 1997)	Highly variable water salinity (0–32) and stratification during the year (Marcelo Acha <i>et al.</i> 2008)	Maximum depth ranges between 5 and 35 m	Pampan plain	35°48'6.07"S, 6°14'52.92"W

Table 2. Number of specimens (*N*) of *Genidens barbatus* per group (birth year) and sampling site
RP, De la Plata River; PL, Patos Lagoon; PB, Paranaguá Bay; and GB, Guanabara Bay

Birth year	RP	PL	PB	GB	Total
2002	18	6	–	–	24
2003	14	8	2	–	24
2006	9	–	19	4	32
2007	–	–	4	4	8
Total	41	14	25	8	88

The otoliths were weighed, washed with ultrapure water and dried. The left otolith of each pair was embedded in crystal epoxy resin and sectioned transversely through the core to a thickness of 1000 µm with a Buehler Isomet low-speed saw (Hong Kong, China) equipped with twin diamond edge blades and spacers. The number of rings in the otolith section was counted with the piece immersed in ultrapure water, using a stereomicroscope (Leica EZ4-HD, Singapore) at 8× magnification, by two independent observers. The sections were discarded if observers disagreed about the number of counted rings. Age determination by counting the ring (*annuli*) number in *lapillus* otoliths of *G. barbatus* was validated by Reis (1986). To avoid possible year-class or cohort effect in trace-element composition of the otoliths, the year of birth of the fish was calculated as capture date – otolith ring number (FAO 1974). Then, the year class or cohort represents the year the fish was born (Table 2).

Prior to micromilling, the portion of the otolith corresponding to the first year of life was identified (by observation of the otolith sections immersed in ultrapure water) by using a stereomicroscope (Leica EZ4-HD). Otolith core was exposed in the sections by polishing the rings from the margin to the core with a rotatory saw (Fig. 3) under a stereomicroscope (Leica Zoom 2000 Z30V, Buffalo, NY, USA) in a laminar-flow hood. The polished sections

were washed with Milli-Q water (18.2 MΩ, Millipore, Sao Paulo, Brazil) to remove dust contamination from the micromilling process, and dried. The core material was then removed carefully from the section with forceps, washed in water Milli-Q water and, once dry, cores were transferred to a sterile centrifuge tube and weighed using a Sartorius AG ED2242 (Göttingen, Germany) microbalance to the nearest 0.0001 g. After weighing, cores were decontaminated with 1% HNO₃ (Merck KGaA, Garmstadt, Germany) (Arslan and Secor 2008) and finally rinsed five times with Milli-Q water to remove any contamination from weighing and micromilling, transferred to new sterile centrifuge tubes and dried overnight in a laminar-flow hood. On the basis of a comparison of whole otoliths and otoliths cut into pieces with a diamond saw, Dove *et al.* (1996) and Arslan and Secor (2008) concluded that a similar procedure of rinsing otolith pieces with a combination of Milli-Q water and dilute acid removed any contamination acquired during the sawing and micromilling process.

Otolith microchemistry

The cores were digested with 10% nitric acid over 48 h (Avigliano *et al.* 2015a). The otolith ¹³⁷Ba, ⁴⁴Ca, ⁷Li, ²⁴Mg, ⁵⁵Mn, ⁸⁸Sr and ⁶⁶Zn concentrations were determined by inductively coupled plasma–mass spectrometry (ICP–MS; Agilent 7500, Agilent, Waldbronn, Germany) equipped with a Micro Mist nebuliser (Glass Expansion, Melbourne, Australia) and a Scott double-pass spray chamber (Melbourne). The equipment was cleaned at regular intervals with Milli-Q water (Millipore) and 2% nitric acid matrix to prevent effects the sample memory.

External calibration was performed using the atomic spectroscopy standard QCS 21 (Quality Control Standard, Perkin Elmer Pure, Waltham, MA, USA). In addition, scandium, yttrium, terbium and holmium were used as internal standards. All measurements were performed in triplicate (RSD < 4%).

The detection limits for water samples (LOD) in micrograms per litre based on three times the standard deviation of the blank

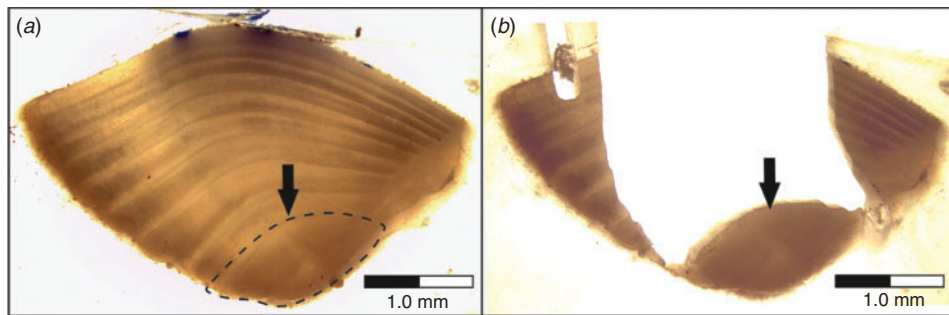


Fig. 3. Isolation of core material in anadromous catfish, *Genidens barbuis*. (a) Medial wing in sectioned lapilli otolith. The dashed line indicates target region to be isolated. (b) Isolated core region following micro-milling procedure.

signal ($n = 10$) were $0.2 \mu\text{g L}^{-1}$ for Ba, Mg, Mn and Zn and $15 \mu\text{g L}^{-1}$ for Sr and Ca. The digestion and analytical procedures were checked by the analysis of otolith-certified reference material for trace elements (FEBS-1, National Research Council, Ottawa, ON, Canada). Replicate analysis of these reference materials showed good accuracy, with the following metal-recovery rates: 99% for Ba, 114% for Ca, 105% for Mg, 104% for Mn, 93% for Sr and 114% for Zn.

Concentrations of trace elements were expressed as molar ratios (element : Ca = mmol mol^{-1}) to account for fluctuations in the amount of material analysed and the loss of material during the preparation process (Sinclair *et al.* 1998; Bailey *et al.* 2015).

Data analysis

Since interannual differences in element : Ca ratios were found for all sites (exact Wilcoxon test, $0.0001 < P < 0.04$), each born year was treated separately (Table 2).

The general statistical treatment applied has been described by Kerr and Campana (2013). The element : Ca ratios did not fit the normal distribution and homogeneity of variance (Shapiro–Wilk, $P < 0.05$; Levene, $P < 0.05$), even after transformation $\log(x + 1)$. Therefore, non-parametric statistics were used to compare variables among study sites.

To ensure that the differences in otolith weight (\approx fish size) among samples did not confound any spatial patterns in elemental composition, the effects of core and total otolith weights on element ratios were examined by using analysis of covariance (ANCOVA, otolith weight as covariate; Longmore *et al.* 2010; Kerr and Campana 2013). ANCOVA is robust to violations of the assumption of homogeneity of variance (Olejnik and Algina 1984). The element : Ca ratios were not significantly ($P > 0.05$) correlated with core and total otolith weights and it was not necessary to correct any variable.

Each element : Ca ratio was compared among sampling sites through the use of Kruskal–Wallis or Mann–Whitney U tests to determine whether differences occurred among regions. Bar plots were used to graphically represent the element : Ca ratios.

Mardia's skewness and kurtosis tests showed multi-dimensional non-normality; hence, permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was used to evaluate the otolith-core multi-elemental fingerprints and to detect differences in core area from different sampling

sites. The analysis was based on Gower distances with 9999 permutations (Gower 1966). Modified Gower dissimilarity matrix is more appropriate for dealing with multivariate heterogeneity of variance than is the Bray–Curtis measure (Anderson *et al.* 2006).

Finally, discriminant function analyses were used to assess the ability of otolith elemental ratios to classify adults to their region of origin (Kerr and Campana 2013; Bouchard *et al.* 2015). Because the assumption of homogeneity of variance–covariance matrices was not met (verified with Bartlett test), quadratic discriminant function analysis (QDA) was used. Multicollinearity between variables was analysed, thus preventing a false outcome in the QDA analysis and the use of redundant variables in the study (Graham 2003). All statistical tests were performed by using the software InfoStat (University of Córdoba, Córdoba, Argentina, see <http://www.infostat.com.ar/>), Past ver. 3.01 (Hammer *et al.* 2001; Natural History Museum, University of Oslo, Norway, see <http://folk.uio.no/ohammer/past/>) and SPSS ver. 19.0 (IBMSPSS Statistics, IBM, USA, see <http://www.spss.com>).

Results

Univariate analysis

Lithium concentration was below the detection limit for the 46% of individuals from PR, 36% from PL and 23% from PB. For this reason, the Li : Ca ratio was not statistically compared among these sites and is not included in Fig. 4 (except for 2007). In 2007, the otolith Li : Ca ratio was similar ($P > 0.05$) between fish from PB and GB (0.009 ± 0.005 and 0.010 ± 0.002 respectively).

The average values and deviations from element : Ca ratio are shown in Fig. 4. For the 2002 cohort, significant differences were observed only for the Zn : Ca ratio (exact Wilcoxon, $P = 0.02$) between the PR and PL samples. For 2003 cohort, the Ba : Ca ratio was significantly higher for PL than for PR and PB (Kruskal–Wallis, $P < 0.05$). Tendencies among sites were similar for both years for Ba : Ca, Mg : Ca and Zn : Ca ratios. In 2006, the Ba : Ca ratio was significantly higher for the PR and lower for GB, being intermediate for PB (Kruskal–Wallis, $P < 0.001$). The Mn : Ca ratio was higher for PR than for PB and GB (exact Wilcoxon, $P = 0.003$). The Mg : Ca ratio followed the same not-significant tendency. The Sr : Ca and Zn : Ca ratios were lower for GB than for the other sites

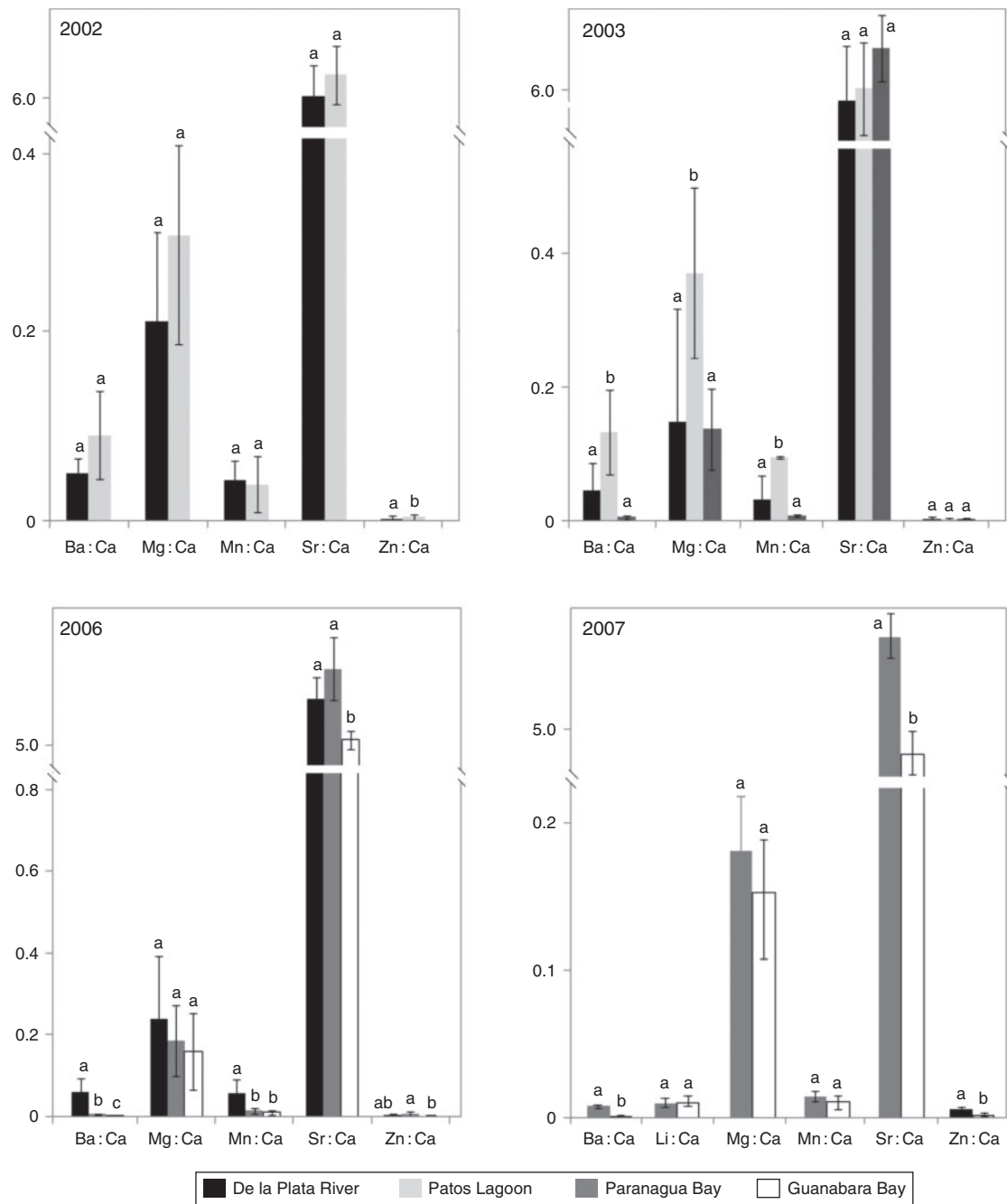


Fig. 4. Mean \pm s.e. elemental ratio (mmol mol^{-1}) in otolith cores from different sampling locations for year-classes. For each region and each elemental ratio, values with different letters are significantly different.

(Kruskal–Wallis, $P < 0.001$ and 0.007 respectively). For the cohort of 2007, the Ba : Ca, Sr : Ca and Zn : Ca ratios were higher for PB (exact Wilcoxon, $P < 0.03$). Tendencies were similar for all the elements between 2006 and 2007.

Multivariate analysis

Results from PERMANOVA analysis showed significant ($0.0001 < P < 0.04$) differences in the multi-element signatures of the otolith cores among sampling sites for all year classes.

The P -value was 0.04 for the comparison between PR and PL (Year class: 2002), $0.0001 < P < 0.04$ for PR, PL and PB (2003), $0.0001 < P < 0.009$ for PR, PB and GB (2009) and $P < 0.03$ for PB and GB (2007).

The QDA was shown to have greater accuracy in classifying (80–100%) among sampling sites for all groups (Table 3). In 2002, overall jackknife re-classification success was 96 and 80% for PR and PL respectively. In 2003, 2006 and 2007, the percentage of correctly classified individuals was 100% (Table 3).

Table 3. Percentage of adult fish classified to each region by quadratic discriminant function analyses on the basis of multi-elemental composition (barium (Ba): calcium (Ca), lithium (Li): Ca, magnesium (Mg): Ca, manganese (Mn): Ca, strontium (Sr): Ca, and zinc (Zn): Ca ratios) of otolith core

Bold numbers indicate the percentage of correctly classified individuals. RP, De la Plata River; PL, Patos Lagoon; PB, Paranaguá Bay; and GB, Guanabara Bay

Birth year	Actual region	Predicted region			
		RP	PL	PB	GB
2002	RP	96	4	–	–
	PL	20	80	–	–
2003	RP	100	0	0	–
	PL	0	100	0	–
2006	PB	0	0	100	–
	RP	100	–	0	–
	PB	0	–	100	0
2007	GB	0	–	0	100
	PB	–	–	100	0
	GB	–	–	0	100

Discussion

Water concentrations of Li, Mg and Sr are, in general, positively correlated with salinity (Campana 1999; Avigliano and Volpedo 2013; Avigliano *et al.* 2014; Bouchard *et al.* 2015). Conversely, environmental concentrations of Mn and Ba are generally higher in freshwater than in saltwater (Campana 1999; Tabouret *et al.* 2010; Bouchard *et al.* 2015). However, incorporation of these elements in the otoliths is not always related to the salinity (Brown and Severin 2009; Sturrock *et al.* 2012; Bouchard *et al.* 2015). For example, Bouchard *et al.* (2015) found a positive ratio between Ba : Ca and salinity in juvenile *Boreogadus saida*. In contrast, incorporation of Zn into the otoliths is mainly influenced by fish diet and may be independent of water concentration (Ranaldi and Gagnon 2008). Temperature has been shown to influence incorporation of elements into otoliths of larval and juvenile marine fish (Bath *et al.* 2000; Martin *et al.* 2004; Martin and Thorrold 2005; Bouchard *et al.* 2015). All of these factors can make it difficult to know exactly how the environmental characteristics affect otolith microchemistry.

In our study area, there was a decreasing temperature gradient from north–south direction. Furthermore, estuaries had different climatic and topographic features, depths, salinity ranges and hydrographic dynamics (Table 1). All these factors undoubtedly affect the characteristics of the water and could have printed unique and distinctive signatures in the otoliths, which explains the multi-elemental differences found in the present work.

Avigliano *et al.* (2015a) compared the ratios Sr : Ca, Ba : Ca and Mg : Ca from the first four otolith rings between RP and PL and found significant differences that suggested the existence of two nursery areas. However, a later study (Avigliano *et al.* 2015b) showed that catfish tend to move between the estuary and the ocean in the early years of life. This could have consequences for the interpretation of the results when groups of rings are analysed to evaluate the life history. Therefore, in

the present work, only the otolith core was used and trace-element analyses were incorporated. Even taking into account all these precautions, the multi-elemental differences found here matched those reported by Avigliano *et al.* (2015a).

In the discriminant analysis, percentages of classification a result of chance differ when comparing two (50%) or three groups (33.3%). In the present work, values obtained widely exceeded chance percentages (between 80–96% for two-group comparisons and 100% for three groups). There have been other studies where univariate marked differences were not observed, but high percentages of classification were obtained with discriminant analysis. For example, Rooker *et al.* (2003) analysed Li, Mg, Ca, Mn, Sr and Ba in bluefin tuna otolith cores and found significant differences only in Li concentration, obtaining a classification percentage of 71%. In contrast, several authors have found significant multi-elemental differences, even at a lower geographical scale (~12 km). Thorrold *et al.* (1998) identified small spatial-scale differences within and among river systems when studying American shad (*Alosa sapidissima*) and Dorval *et al.* (2005) observed small spatial-scale differences among estuaries when studying weakfish (*Cynoscion regalis*). Bailey *et al.* (2015) found differences in the chemical signature in juvenile flounder (*Pseudopleuronectes americanus*).

Unlike the analysis of microchemistry in the otolith cores of juvenile fish, the same analysis performed in adult fish suggested ontogenetic movements and homing behaviour. For example, the absence of significant differences in the chemical signatures of otolith cores (adult fish) in different areas would indicate high connectivity, as long as these areas show differences in the chemical composition of the water (Kerr and Campana 2013). A representation of this situation is observed in Scenarios 2 and 3 in Fig. 1. Moreover, the existence of significant differences in the chemical signatures of otolith cores in adult fish suggests low connectivity (Scenario 1, Fig. 1).

In the present work, the differences observed in the microchemical signature indicated that otolith microchemistry represents a good habitat marker for studying and monitoring of nursery areas of *G. barbuis*. The multi-elemental differences among sampling areas usually remained constant in the studied cohorts (Table 3). This suggests that the studied estuaries could be used as nursery areas year after year (at least during the studied period). On the basis of these results, the most parsimonious scenario is Scenario 1 in Fig. 1, where every estuary would represent a nursery area and adults tend to return to the same estuaries for reproductive purposes.

In addition, taking into account that all the great estuaries where the species reproduces were considered and that classification percentages of 100% were obtained (except for 2002 cohort), the results might suggest that the catfish individuals use their birth areas as breeding sites and tend not to mix among large systems, supporting the homing hypothesis proposed by Avigliano *et al.* (2015a) (Scenario 1, Fig. 1).

Homing behaviour is especially interesting knowing that these fish are long-lived (up to 36 years) and remain on the maritime platform for at least 7 years before returning to the nursery area (Velasco and Reis 2004; Velasco *et al.* 2007; Avigliano *et al.* 2015a, 2015b). However, it is clear that our data did not directly demonstrate the presence of homing, but rather suggested that such behaviour could exist in each of the

studied estuaries. Other methods are necessary to confirm the presence of homing. For example, Thresher *et al.* (1994) assessed the composition of the otolith core of adult jackass morwong, *Nemadactylus macropterus*. The data from adults were then used to classify each juvenile and to calculate the probability that it had originated in the breeding populations. Rooker *et al.* (2008) studied the connectivity of bluefin tuna (*Thunnus thynnus*) and demonstrated the presence of natal homing behaviour used juveniles (Age 1).

The homing behaviour has been previously reported for other large silurids from Plata Basin, such as *Pseudoplatystoma corruscans* (Pereira *et al.* 2009). Furthermore, homing and anadromy are two behavioural life-history traits that are well attested in various groups of fish, especially in the family Salmonidae (e.g. *Salmo*, *Salvelinus*, *Oncorhynchus*), and also in other groups such as Clupeidae (American shad, *Alosa spadissima*; Marschall *et al.* 1998; Quinn *et al.* 1999; McDowall 2001). Marschall *et al.* (1998) and Quinn *et al.* (1999) reported that the Atlantic salmon (*Salmo salar*) and sockeye salmon (*Oncorhynchus nerka*) show a strong tendency to return to their birth site. The anadromous behaviour provides fish the opportunity for more rapid growth and higher fecundity through the exploitation of rich food resources, whereas homing fosters adaptation of stocks to favourable local spawning conditions (McDowall 2001).

The reclassification success from the discriminating model suggests that a high level of spatial segregation exists in the life of adult catfish. Nevertheless, significant regional variability was found in the univariate elemental composition of adult catfish otolith cores (especially for PR and PL, 2002–2003 year classes). In addition, the classification percentages were between 80 and 96% for the Age group 2002. This could indirectly represent some connectivity, despite being low, between PR and PL, tending towards Scenarios 2 or 3. Supporting these results, it has been suggested that there is a certain flow of individuals between PR and PL (Avigliano *et al.* 2015a) and this could also exist between neighbouring sites such as GB and PB. The fact of obtaining classification percentages of 100% among the rest of the sites and age classes should not be taken as evidence of a total absence of connectivity, because there is the possibility of not having captured specimens that migrate between estuaries, especially taking into account the size of sampling obtained for GB.

In the case that homing in *G. barbuis* can be confirmed, the diversification of this behaviour would not be a novelty, because it has already been found previously, especially in salmonids (Cury 1994; McDowall 2001). According to McDowall (2001), a low percentage of straying in species that he observed may have long-term evolutionary advantages. In this way, the fish could move into new environments that are, perhaps, more beneficial in terms of food and reproduction, especially if the natal site has changed. Furthermore, a certain migration rate between the spawning sites would also increase the genetic variability of populations (Cury 1994). Some studies on Atlantic salmon have estimated that to maintain a genetic homogeneity within subpopulations of 2500–10 000 individuals, the migration rate between the populations has to be less than only one individual per year (Cury 1994).

In conclusion, otolith-core microchemistry of adult catfish showed promise for identifying the nursery areas and for studying the origin of adult marine catfish. These elemental analyses need

to be linked to specific management to be useful to fisheries managers. Management issues include monitoring the evolution of the fishery resource, entrapment of newly hatched fish (quite large for *G. barbuis*, see Reis 1986; Velasco *et al.* 2007) with trawls and the time frame when bottom dredging is permissible to minimise impacts to benthic fish larvae, eggs or juveniles. On the basis of our results, it is advisable to generate specific management policies for each nursery area (different estuaries) because the nurseries tend to be independent and sustain fairly independent subpopulations. Being able to identify which areas contribute the most recruits (or conversely, the fewest) would provide verification for protecting or permitting changes to specific estuaries. Coupled with these otolith-microchemistry studies, there is a need to understand the abundance of *G. barbuis* young of year or juveniles within these estuaries, so that the relative contribution of each estuary to the adult population can be calculated accurately. Finally, the performance of specific studies to confirm the existence of homing behaviour would be helpful for managing the species.

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