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Identification of potential fish stocks and lifetime movement patterns of *Mugil liza* Valenciennes 1836 in the Southwestern Atlantic Ocean.

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Abstract

The mullet *Mugil liza* is the Mugilidae that lives southernmost in the western Atlantic Ocean. Knowledge about migration, movements and identification of stocks of this important fishery resource is scarce. Thus, we aim to study movement patterns and to identify the presence of different fish stocks in the southwestern region of the Atlantic

Ocean, using cumulative otolith shape morphometric and microchemical analyses of sagittae otoliths. Specimens (n = 99) were obtained in four coastal areas: Paranaguá Bay in Brazil, Samborombón Bay, Mar Chiquita Coastal Lagoon, and San Blas Bay in Argentina. Otolith shape indices (Circularity, rectangularity, aspect ratio, percentage occupied by sulcus, ellipticity and form factor) were used for stock identification analysis; and otolith microchemistry using LA-ICP-MS (Sr/Ca and Ba/Ca ratios chronological variation) was used for both the analysis of movement behaviors and the identification of fish stocks (otolith edge ratios). Morphometrical indices did not revealed a clear separation among areas. San Blas bay individuals presented otoliths tending to be longer than wider, with a more elliptic shape than the otoliths from other studied areas; also, did not share individuals with the most northern one, Paranaguá Bay in Brazil. The analysis of microchemical lifetime profiles revealed three types of behavior pattern: Type I: most frequent use of estuarine environments; Type II: a fluctuating behavior between estuarine and sea/high salinity waters; Type III: most frequent use of sea/high salinity habitats. Otolith edge analysis did not reveal differences among Sr/Ca and Ba/Ca ratios for the different areas. Thus, it cannot be assured that there is more than one stock in the studied region. *Mugil liza* revealed different environmental migratory behaviors in the Southwestern Atlantic Ocean showing a facultative use of estuarine waters; hence, the species appears to be mostly coastal with the use of low estuaries, as seen also by the Sr/Ca otolith cores ratios; differing from the general mugilid behavior previoulsy described.

Keywords

Mugilidae; Displacements; Fish Stocks; *Sagitta* morphometry; Sr/Ca & Ba/Ca ratios

1. Introduction

Members of the family Mugilidae, generally known as mullets, are coastal marine fishes with a worldwide distribution including all temperate, subtropical and tropical seas. The mullet *Mugil liza* Valenciennes, 1836 is the Mugilidae species that lives southernmost in the west Atlantic Ocean, its distribution range goes from the Caribbean Sea to northern Patagonia in Argentina (Garbin et al., 2014). It inhabits offshore and coastal waters, but also spends part or even their whole life cycle in coastal lagoons, lakes and/or rivers (González-Castro and Minos, 2016; Harrison, 2002; Heras et al., 2009; Thomson, 1997). *M. liza* has been previously confused with *Mugil cephalus* and *Mugil platanus* (Heras et al., 2016; Whitfield et al., 2012). *Mugil liza* range was believed to be found as far as South of Rio de Janeiro in Brazil (Heras et al., 2009), and *M. platanus* was assumed to be a different mugilid species with a southern distribution. Nowadays, it is established that *M. platanus* is a synonym of *M. liza* (Menezes et al., 2010). Even though there is a difference among samples of *M. liza* from Rio de Janeiro (23°S) and those from all Southern locations (São Paulo to Argentina) given by significant genetic differentiation (Mai et al., 2014a), the authors only recognized the presence of two different demographic clusters of *M. liza* in the southerwestern Atlantic Ocean. Referring to *M. cephalus*, both species have been proposed to be part of a species complex (Durand et al., 2012; Whitfield et al., 2012); however, since they have allopatric distribution ranges, both can be considered different species (González-Castro and Ghasemzadeh, 2016; Menezes et al., 2015; Whitfield et al., 2012). *Mugil liza* is a commercially important species, in all of its distribution, mostly from artisanal catches supporting the local market and communities (González-Castro et al., 2009a; Mendonça, 2007). Particularly in Brazil, thousands of tons of this mullet are extracted from most of the coastal states of the country (IBAMA, 2007; Miranda et al., 2011). In Argentina, *M. liza* is part of a small-scale fishery, mainly in the northern coast of the Buenos Aires province, where it is used as food resource, or as fishermen sport (González-Castro et al., 2009a).

The several studies that have been carried out focused mostly in *M. liza* growth (Garbin et al., 2014; González-Castro et al., 2009a; Okamoto et al., 2006), reproduction (Albieri and Araújo, 2010; Albieri et al., 2010; Esper et al., 2001; González-Castro et al., 2011), abundance (De Araújo Silva and De Araújo, 2000), parasites (Alarcos and Etchegoin, 2010; Knoff et al., 1997, 1994); and importance as bioindicators of contamination (Hauser-Davis et al., 2012; Marcovecchio, 2004). However, there is a gap in the knowledge about migration, movements and identification of stocks of this important fishery resource.

Different methods have been used to study displacements and identify fish stocks such as mark-recapture, parasites, genetics and the analysis of calcified structures like scales and otoliths (Avigliano et al., 2014; Clément et al., 2014; Kerr and Campana, 2014; Sturrock et al., 2012). The research on otolith (complex calcium carbonate structures located in the inner ear (Campana, 1999)) has widen the knowledge of fish movements and migrations and stock identification of important commercial species (Avigliano et al., 2014; Avigliano et al., 2015a; Gillanders, 2005; Tabouret et al., 2010; Tracey et al., 2012). Morphometrical analysis of features like shape and contour (Lestrel, 1997) allows stock identification of species (Avigliano et al., 2015c; Sadighzadeh et al., 2014; Tuset et al., 2003b). Also, the study of the otolith chemical composition has been increasingly used to study fish displacements and identify fish stocks (Kraus and Secor, 2004; Schuchert et al., 2010; Tabouret et al., 2010). The analysis of elemental signatures throughout otolith growth serves as a natural maker and can be used to reconstruct their lifetime movement patterns

(Campana et al., 2000; Wang et al., 2010), as the chemicals deposited in the otolith represent a permanent record of the environmental conditions experienced by the fish at a particular time (Campana et al., 2000; Ruttenberg et al., 2005). Nowadays, Sr/Ca and Ba/Ca otolith ratios have been simultaneously used by some authors for stock and migration studies (Avigliano et al. 2015b; Schuchert et al. 2010; Tabouret et al. 2010). These elements vary between freshwater and seawater and are useful to understand diadromous fish migration behavior (Campana et al., 2009; Milton et al., 2008; Wang et al., 2010). Sr is correlated positively to salinity of water while Ba correlates negatively, hence the former element is strongly associated to marine waters while the second is associated to freshwater environments (Kraus and Secor, 2004; Elsdon and Gillanders, 2005; Miller, 2011; Tabouret et al., 2010).

The aim of this study was to identify the presence of more than one potential fish stock of *Mugil liza*, and to provide information on its movement patterns in the southwestern region of the Atlantic Ocean. For this, cumulative analyses of morphometry and microchemistry of *sagittae* otoliths were performed.

2. Materials and Methods

2.1 Study area and fish sample collection

Four coastal areas were selected in the southwestern Atlantic: Paranaguá Bay in Brazil, Samborombón Bay, Mar Chiquita Coastal Lagoon and San Blas Bay (sea coast) in Argentina (Figure 1). Adult individuals of *Mugil liza*, all beyond the length at first maturity (L_{50}) according to González-Castro et al. (2011) were obtained from artisanal catches with gill nets or rods in the years 2012-2013 (Table 1). In all sampled areas, in spite of their different geomorphological features, sampling sites were located inside each estuary approximately at the same distance from the coastline (3 km). Also, no river outfall was located near sampling sites, so there were not local or temporal changes in water masses where fish were obtained. A total of 99 individuals were collected and taken to the laboratory. Standard length (SL in mm) was recorded and their *sagittae* otoliths were removed. After extraction, otoliths were dried and stored for further use.

2.2 Otolith morphometry

Otolith morphometry was used to test differences among fishes from sampling areas as an attempt to identify potential fish stocks. Right side otoliths were selected and the respective medial face photographed with a digital camera attached to a stereomicroscope (Leica® EZ4 HD). Images were analyzed and morphometric variables were measured using image processing systems (Image-Pro Plus 4.5®). The variables registered were: otolith length (OL), otolith height (OH), otolith perimeter (OP) and sulcus perimeter (SP) in mm (Figure 2); and otolith area (OA) and sulcus area (SA) in mm^2. Afterwards, shape indices were calculated using these variables: circularity (OP^2/OA) (Tuset et al. 2003a, 2003b, 2008), rectangularity (OA / [OL × OH]) (Tuset et al. 2003a, 2003b, 2008), aspect ratio (OH/OL) (Tuset et al., 2008), percentage of the otolith area occupied by the sulcus (SA/OA) (Avigliano et al. 2014), ellipticity ([OL–OH] / [OL+OH]) (Tuset et al., 2003a, 2003b), and form factor ($[4\pi \times OA/OP^2]$) (Tuset et al., 2003a, 2003b).

All variables were tested for normality with Shapiro-Wilks test and homogeneity of variances with Levene’s test. An ANCOVA analysis ($p < 0.01$) was used to correct the effect of fish size on the morphometric indices using the common with-in slope (b) (Burke et al., 2008; Campana et al., 2000; Galley et al., 2006). The constants used for the correction were: circularity: $b = 0.01$; rectangularity: $b = -3.4 \times 10^{-5}$; aspect ratio: $b = 2.5 \times$

10^{-4}; percentage area occupied by the sulcus: b = -1.2×10^{-4}; ellipticity: b = -2.3×10^{-4}; and form factor: b = -2.3×10^{-4}. A Canonical Discriminant Analysis (CDA) using the morphometrical variables was applied to obtain the cross-classification matrix and determine the capacity of these indexes to locate the site where the fish were captured, thus, to assess the possibility of fish stock discrimination. Furthermore, a Principal Component Analysis (PCA) was performed to identify the morphometric variables that could best explain variability among sampling sites. For interpretation, the selection of axes for the analysis was made using a screen plot (Avigliano et al., 2015b; Hubert et al., 2009). All analyses were performed using InfoStat® software.

2.3 Otolith microchemistry

Otolith microchemistry was used for the analysis of movement behaviors and the identification of potential fish stocks, and spawning areas. For this, a random sample of otoliths of individuals captured in the four studied areas (Table 1) were selected. The differences among microchemical samples of the sites were due to the variation in total sample size of each sample area. All otoliths were weighted to the nearest 0.0001 mg in an analytical balance and then embedded in crystalline epoxy resin (EC 141). Transversal sections just above the core were made using a Buehler Isomet low speed saw. The core was then exposed using sanding paper (4000 down to 500-grit) and its surface smoothed using a 1 μm polishing cloth (©Buehler). Sections were sonicated in Milli-Q water for 5 min and dried under a laminar flow for 24 h prior to the laser ablation ICP-MS analysis. An Agilent 7500ce (Tokyo, Japan) inductively coupled plasma mass spectrometer (Q-ICP-MS), coupled to a 213 nm Nd:YAG laser unit (LA) LSX-213 (Cetac Technologies, Omaha, USA) was used to analyze the chronological variation of ^{88}Sr, ^{138}Ba and ^{43}Ca in the otoliths. Sectioned samples were placed in the ablation chamber and a transect was ablated from core to edge (crater width: 50 μm) along the longest radius of the otolith. The laser operated at a pulse rate of 20 Hz, a scan speed of 10 $\mu m \cdot s^{-1}$ and an energy output of 100% (5.6 mJ max.). LA-ICP-MS coupling was daily optimized using a SRM NIST 612 silicate glass standard (National Institute for Standards and Technology-NIST, Gaithersburg, MD, USA) for high sensitivity and low background intensity. Otolith element composition was obtained and element/Ca ratios were calculated for statistical analysis of lifetime variation of elements for each fish.

To identify behavior patterns, individuals were analyzed and the obtained chronological Sr/Ca profiles were classified according to their environmental components. Chemical thresholds for different environments were assumed as described by other researchers for *Mugil cephalus*, the most studied species of Mugilidae, as information for *M. liza* is lacking and both species are closely related (Durand et al., 2012; González-Castro and Ghasemzadeh, 2016). Freshwater behavior was assumed when Sr/Ca ratios were below 3.5×10^{-3} mmol/mol; ratios from 3.5 to 7.0×10^{-3} mmol/mol represented estuarine-brackish waters; and values $> 7.0 \times 10^{-3}$ mmol/mol were considered as representing marine habitats (Chang et al., 2004; Górski et al., 2015; Wang et al., 2010). A Canonical Discriminant Analysis (CDA), using InfoStat® software, was performed to study the correct identification of individuals to the recognized habitat use patterns. This multivariate test was executed using the average Sr/Ca and Ba/Ca ratios of each profile and the respective standard deviations (Sd) as dependent variables. Prior to that, a fish size effect was detected for the microchemical variables (ANCOVA analysis: $p < 0.01$). Thus, they were corrected using the common within-group slope (Burke et al., 2008; Campana et al., 2000; Galley et al., 2006) for further analysis. Constants used were: Sr/Ca, b = 0.01; SdSr/Ca, b = 1.6×10^{-3}; Ba/Ca, b = 2.6×10^{-4}, and SdBa/Ca: 5.5×10^{-4}; successfully removing the significant correlation with fish length.

To study the presence of potential fish stocks, microchemical signals in otoliths edges of each fish were analyzed for all study sites. Variables were corrected, as mentioned before, to remove the fish size effect (constants: EdgeSr/Ca, b = -0.004; EdgeBa/Ca, b = -0.000059). Then, after verifying normality and homoscedasticity, element/Ca ratios were compared using one-way analysis of variance (ANOVA) and Tukey test was applied for *post-hoc* multiple-comparison among locations. Finally, Sr/Ca ratios in otolith cores were compared to the previously assumed chemical thresholds for different environments to identify spawning habitats for the species.

3. Results

3.1 Otolith morphometry

The mean of the morphometrical indices calculated for all studied sites are shown in Table 2. San Blas Bay presented higher values for rectangularity, percentage occupied by sulcus and ellipticiy indexes, and lower for aspect ratio and form factor indexes than the other studied areas (Table 2). The Canonical Discriminant Analysis did not reveal a clear separation among areas (Table 3). However, the most southern location, San Blas Bay in Argentina, did not share individuals with the most northern one, Paranaguá Bay in Brazil, and had the highest percentage of correct area identification of fish (77%) (Table 3). Also, San Blas Bay only shared individuals with Samborombon Bay, not the closest sampled area, but the area with the same feature of coastal lagoon. The other studied sites showed a lower percentage of separation (36% Paranaguá Bay, 48% Mar Chiquita Lagoon and 50%

Samborombón Bay) with specimens allocated to all sampled sites; nevertheless, their lowest percentages were obtained for fish assigned to San Blas Bay (Table 3).

When analyzing the PCA, four principal components that accounted for the total variance (100%) of the six morphological variables were extracted. The first axis (PC1) explained 42.4% of the total variability (Figure 3A). In this axis, circularity and form factor indices were negatively correlated (correlation coefficient = -0.90), as well as ellipticity and aspect ratio ones (correlation coefficient = -1.00); these were also the morphometric variables that contributed most to the observed spatial gradient of the PC1 scores (eigenvector = -0.51 and 0.49 respectively) (Figure 3A). In the first PC, San Blas bay individuals presented otoliths tending to be longer than wider, therefore with a more elliptic shape than the otoliths from other studied areas. The second axis (PC2) explained 28.6% of the total variability. The most important variables for this axis were rectangularity (eigenvector = 0.49), and aspect ratio (eigenvector = -0.45) that also correlated with ellipticity (Figure 3A). In this PC the otoliths from specimens captured in San Blas bay also showed otoliths that may be characterized by being relatively longer (in relation to height) and closer to a rectangle than otoliths from other regions. The PC3 explained 18.9% and PC4 9.9% of the total variability (Figure 3B). For the third axis (PC3) the most important index was the percentage occupied by the sulcus (eigenvector: 0.85); and for the fourth one (PC4), rectangularity was the most important index (eigenvectors: 0.72). There was not a clear association between areas and morphometric indices for PC3 and PC4 (Figure 3B).

3.2 Otolith microchemistry

The analysis of lifetime profiles revealed three behavior patterns (Figure 4). Pattern Type I corresponds to a most frequent use of estuarine environments (estuarine resident) (Figure

4a); Type II corresponds to a fluctuating behavior among estuarine and sea/high salinity waters throughout their lifetime (mixed) (Figure 4b); and Type III corresponds to a sea/high salinity water habitat use during most of their life (seawater resident) (Figure 4c).

The CDA correctly assigned individuals to the detected patterns with high accuracy (Type I: 95%; Type II: 75%; and Type III: 100%) (Table 4). The first canonical axis explained 94.4% of the variance among patterns. The plot shows a clear separation of types I and III towards opposites margins of the first canonical axis (Figure 5). Type II individuals are distributed in between of the other two groups (Figure 5).

All movement patterns were identified in the studied areas. Type I and Type II were the most common patterns among individuals. Paranaguá Bay showed a higher percentage of type I (50%); Mar Chiquita Lagoon and San Blas Bay had more individuals with type II pattern (53% and 58% respectively); and Samborombón Bay specimens showed equally type I and II patterns (36%) (Table 5).

When analyzing otolith edges with ANOVA, no differences were found among Sr/Ca and Ba/Ca ratios for the different areas ($F_{3,51} = 2.39$, $p = 0.08$; and $F_{3,51} = 1.84$, $p = 0.15$ respectively). Sr/Ca edge means were: Paranaguá Bay 6.54±1.07 mmol/mol, Samborombón Bay: 5.95±0.82 mmol/mol, Mar Chiquita Coastal Lagoon: 6.29±0.75 mmol/mol, San Blas Bay: 5.67±0.74 mmol/mol; and Ba/Ca edge means were: Paranaguá Bay 0.14±0.09 mmol/mol, Samborombón Bay: 0.11±0.03 mmol/mol, Mar Chiquita Lagoon: 0.09±0.04 mmol/mol, San Blas Bay: 0.11±0.07 mmol/mol. A dispersion analysis of the otolith edge Sr/Ca and Ba/Ca ratios showed overlapped values for all studied coastal regions (Figure 6). However, Paranaguá Bay and San Blas Bay values showed higher dispersion in the Ba/Ca variable towards the positive side of the axis (Figure 6).

When analyzing Sr/Ca ratios in otolith cores to analyze spawning habitats, 63.5 % of the individuals presented estuarine/brackish values (min. 3.79 mmol/mol; max. 6.92 mmol/mol), while the other 36.5 % had marine water values (min. 7,19 mmol/mol; max. 12.61 mmol/mol) (Figure 7), corresponding to the behavioral patterns found for the species.

4. Discussion

Mugil liza is a poorly studied species regarding to movements and connectivity. There is also, no in depth research on stock identification of this important resource.

When analyzing otolith morphometry, our study on the potential number of fish stocks showed differences among sampled areas, distinguishing San Blas Bay from the other northern areas. Otoliths obtained in the southern Argentinian location tended to be longer than wider, resulting in a more elliptical shape. However, the analysis of otolith microchemistry did not revealed a separation among areas, being these variables good indicators of water masses concentrations for diadromous fish (Brown and Severin, 2009). Thus, even if some morphometrical variations were found between the most southern location and the northern ones, it cannot be assured that there is more than one *Mugil liza* stock in the southern region of the southwestern Atlantic Ocean. Different authors have studied genetic populations of the species in its distribution range. Mai et al., (2014a) reported, by the use of microsatellite markers, only one cluster-population for the coastal area of the Atlantic below Rio de Janeiro, Brazil. Siccha-Ramirez et al., (2014), through the use of mitochondrial genes, also suggested that *Mugil liza* is represented by a single population for its entire distribution range from the Atlantic coast of the South Caribbean and South American area. Furthermore, Heras et al., (2016), by the analysis of the mitochondrial control region, found two highly divergent *M. liza* stocks throughout its distribution range, one north of Rio de Janeiro and the other south of the mentioned region. Our findings concur with the previously mentioned researches, given that the sampled areas in the present study are located south of Rio de Janeiro, all included in the southern populational cluster of *M. liza* (Mai et al., 2014a) and coinciding with the most recent genetic research on *M. liza* stock identification (Heras et al., 2016). This has special relevance when considering the use of the resource, provided that having only one stock in the southern region of the Southwestern Atlantic Ocean means that the exploitation of this species is shared by three countries. Thus, joint strategies should be applied for this commercially important resource to generate proper management in all its distribution. When analyzing movement patterns, as it has been reported for other mugilid species (Chang and Iizuka, 2012), different behaviors can be observed in the population of *M. liza*. The study of the elemental composition of the otoliths can reveal population movements and preferred habitats, and their connectivity (Morales-Nin et al., 2014). Elements like Sr and Ba reflect the environment used by fish, correlating with salinity of surrounding water (Kraus and Secor, 2004; Miller, 2011; Tabouret et al., 2010; Kerr and Campana, 2014). By the use of otolith Sr/Ca ratios, Chang et al., (2004) suggested that *Mugil cephalus* movement patterns could be divided into two groups, one that presented a freshwater component in their life cycle and other that did not. When analyzing otolith microchemistry for *M. liza*, in our study, the CDA showed high accuracy for separating chonological profiles of the fish (values obtained by the analysis widely exceed percentages due to chance (25%)). Three types of patterns were found, but none of them involved a preferential freshwater use; they were differenciated by the use of estuarine or sea areas throughout individuals' lifetime, showing that the species appears to be mostly coastal with some use of lower estuaries. Moreover, even if the sampled areas could present a freshwater component, as it happens in Mar Chiquita Coastal Lagoon where salinity fluctuates from 0 to 36 (González-Castro et al., 2009b), extension of the areas is small given the migratory ability of the species. In this research otoliths did not present any chemical markers for freshwaters, this could be a result of the species resting a short time in those environments or having periodic movements between the freshwater-marine water zones of the lagoon, not long enough for the otolith to incorporate elements that could be detected.

There is a known general mugilid behavior of spawning offshore and their larvae migrating from the sea to estuarine or freshwater areas (González-Castro and Minos, 2016; González-Castro et al., 2011; Whitfield et al., 2012). In this research, the observed environmental behavior of the studied mullet did not entirely coincide with this well described pattern, showing migration between estuaries and sea for some individuals, or residency in one of the two mentioned environments for others. Recently, Nordlie (2015) has mentioned that some species of the Mugilidae family could enter only lower reaches of estuaries and not spwan in fully marine waters, being our results more consistent with these findgings. Moreover, some specimens showed in their otolith core, Sr/Ca ratios corresponding to estuarine waters. This could be showing that the use of estuarine waters by this species is facultative and that adults are able to spawn in low estuarine areas and larvae, instead of migrating to those enviroments, are also able to use onshore areas for development.

Even though different movement behaviors were observed for the studied mullet species, none of the patterns were specific of a studied area. This could be associated to the migratory capability of the species, but there is a need to revise more in depth the reproductive cycle of *M. liza* linked to the studied wetlands as it has been done for other areas preferred by this species like Lagoa dos Patos or Santa Catarina Region in Brazil

(Herbst and Hanazaki, 2014; Viera, 1991). It is also important to consider that the low number of fish studied by area for life history patterns could be masking other patterns that could have been found with a more abundant otolith sample.

In conclusion, *Mugil liza* revealed different migratory behaviors in the Southwestern Atlantic Ocean as observed by the chronological analysis of otolith microchemistry, with the use of only estuarine and marine waters. Even if there is only one identified stock in the analyzed region, environmental conditions and food availability in the diverse areas frequented by the species could be the influencing factors of the observed displacements (Albuquerque et al., 2012). This species differs from other studied mullets on the particularity that it does not use freshwater environments for long periods in the studied region that could be reflected in otolith chemistry. Some specimens may be exclusively estuarine or marine dependent while others may have different degree of dependence of these areas.

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Figure Captions:

Figure 1. Location of *Mugil liza* sampling sites in the southwestern Atlantic Ocean: 1: Paranaguá Bay; 2: Samborombón Bay; 3: Mar Chiquita Coastal Lagoon; 4: San Blas Bay.

Figure 2. Right *sagitta* otolith of *Mugil liza*. Features and measured variables: OL: otolith length; OH: otolith height; OP: otolith perimeter (dash white line); SP: sulcus perimeter (continuous black line).

Figure 3. Principal Component Analysis based on otolith morphometry data. A. Individuals projected along the first and second principal component axes; B. Individuals projected along the third and fourth principal component axes. Otolith indices: CI: circularity; RE: rectangularity; AR: aspect ratio; SS: percentage of the otolith area occupied by the sulcus; EL: ellipticity; and FF: form factor.

Figure 4. Otolith life history profiles of Sr/Ca ratios for the 3 identified behavior patterns of *Mugil liza* in the four sampled areas in the Southwestern Atlantic measured by LA-ICP-MS from core to edge. Letters refer to identified groups: (a) Type I: estuarine resident (specimen from Mar Chiquita Coastal Lagoon, LS = 394 mm, female); (b) Type II: mixed (specimen from San Blas Bay, LS = 370 mm, female); and (c) Type III: seawater resident (specimen from Paranaguá Bay, LS = 370 mm, sex unknown).

Figure 5. Canonical Discriminant Analysis of otolith microchemical variables for the 3 identified behavior patterns of *Mugil liza* (Type I. estuarine resident; Type II. mixed; Type III: seawater resident).

Figure 6. Relationship between the otolith edge Sr/Ca and Ba/Ca ratios (mmol/mol) of *Mugil liza* for the sampling sites in the Southwestern Atlantic Ocean.

Figure 7. Spawning grounds of *Mugil liza* sampled individuals related to Sr/Ca core ratio values. Microcheminal thresholds considered were: estuarine-brackish waters, Sr/Ca: 3.5–7.0 × 10^{-3} mmol/mol; and marine waters, Sr/Ca: > 7.0 × 10^{-3} mmol/mol (Chang et al., 2004; Górski et al., 2015 and Wang et al., 2010).