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Original article

Discrimination of habitat use between two sympatric species of mullets, *Mugil curema* and *Mugil liza* (Mugiliformes: Mugilidae) in the rio Tramandaí Estuary, determined by otolith chemistry

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Two sympatric species of marine mullets, *Mugil curema* and *M. liza*, use the rio Tramandaí Estuary as nursing grounds. When two closely related species are sympatric, various mechanisms may permit their coexistence, including spatial or temporal segregation that results in the divergent use of the resources for which they compete. To investigate the spatial segregation, we used otolith chemistry inferred through laser ablation-inductively coupled plasma mass spectrometry. Our results indicate that in the rio Tramandaí Estuary, *M. curema* is associated with high salinity waters and can be classified as a Marine Migrant in the Marine Estuarine-opportunist subcategory. *Mugil liza* is associated with lower salinity and can be classified as a Marine Migrant in the Estuarine Dependent subcategory. The intra-specific variation in estuarine habitat use indicates that the migratory behaviors in mullets are far more complex than previously known.

Keywords: Estuarine use, LA-ICPMS, Marine migrant, Migratory behavior, Otolith elemental fingerprint.

Duas espécies de tainha simpátricas, *Mugil curema* e *M. liza,* usam o estuário do Rio Tramandaí como zona de berçário. Quando espécies do mesmo gênero são simpátricas, vários mecanismos podem permitir sua coexistência, incluindo segregação espacial ou temporal que resultam no uso distinto dos recursos pelos quais elas competem. Para investigar a segregação espacial nós usamos a análise de elementos químicos em otólitos inferidos por espectrometria de massas através de plasma indutivamente acoplado com amostras extraídas a laser (LA-ICPMS). *Mugil curema* está associada a águas de alta salinidade podendo ser classificada como Marinho Migrante sub-categoria Marinha estuarina-oportunista. *Mugil liza* está associada a águas menos salinas, devendo ser classificada como Marinha Migrante sub-categoria Estuarina Dependente. A variação intraespecífica no uso do habitat estuarino indica um comportamento migratório mais complexo que o previsto em tainhas.

Palavras-chave: Assinatura química no otólito, Comportamento migratório, LA-ICPMS, Marinho migrante, Uso do estuário.

Introduction

Eight congeneric species of mullets inhabit the coast of South America: *Mugil brevirostris* (Ribeiro, 1915); *M. curema* Valenciennes, 1836; *M. curvidens* Valenciennes, 1836; *M. incilis* Hancock, 1830; *M. margaritae* Menezes, Nirchio, Oliveira & Ramirez, 2015; *M. rubrioculus* Harrison, Nirchio, Oliveira, Ron & Gavíria, 2007; *M. trichodon* Poey, 1875; and *Mugil liza* Valenciennes, 1836 (Menezes *et al.*, 2015). In southern Brazilian estuaries, the juveniles of *M. curema* and *M. liza* occur in large numbers and *M. brevirostris* occurs in lower numbers (Vieira, 1991; Ramos, Vieira, 2001).

Mugil curema and *M. liza* are total spawners that reproduce in oceanic areas on the continental shelf (Ibáñez-Aguirre, Gallardo-Cabello, 2004; Silva, 2007; Fernandez, Dias, 2013; Lemos *et al.*, 2014). After reproduction, the juveniles of both species move toward the surf-zone and recruit into lagoons and estuaries, where they spend most of their life cycle (Blaber, Blaber, 1980; Vieira, 1991; Ibáñez-Aguirre, 1993; Albieri *et al.*, 2010). Therefore, mullets are generally assumed to be catadromous, migrating from estuaries to offshore for spawning (Silva, 1980). Nevertheless, recent studies have demonstrated high intra-specific variability in the migration patterns among several distinct species of mullets (Chang *et al.*, 2004; Chang, Iizuka, 2012; Ibáñez-Aguirre *et al.*, 2012; Wang, 2014; Avigliano *et al.*, 2015).

When two distinctive related species are sympatric, various mechanisms may permit their coexistence, including spatial or temporal segregation that results in the divergent use of the resources for which they compete (Ibáñez-Aguirre, 1993; Albieri *et al.*, 2010). In this study, we used otolith chemistry to discriminate the habitat use between two of the tree species of mullet that occur in the rio Tramandaí

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Estuary: *M. curema* and *M. liza*. The differences in the depositional patterns of strontium (Sr) and barium (Ba) in the otoliths of fishes offer a tool to reveal different habitat uses of diadromous fishes (Gillanders, 2005; Walther, Limburg, 2012).

In this study, we test the hypothesis that the habitat uses of *M. curema* and *M. liza* differ within the same estuary. To test this hypothesis, we used two different approaches: first the concentration ratios of Sr:Ca and Ba:Ca at the cores and edges of otoliths were compared between species using the Mann-Whitney U test; next, the entire otolith readings of each specimen were visually compared throughout the life stages.

Material and Methods

We analyzed 23 specimens of *M. curema* (300 to 370 mm in TL) and 23 specimens of *M. liza* (320 to 490 mm in TL). The specimens were captured by artisanal fishermen between June 2014 and August 2014 in the rio Tramandaí Estuary, Rio Grande do Sul, Brazil. The rio Tramandaí Estuary drains an area of 2,700 km^2 and consists of 30 lagoons connected by channels flowing to the Atlantic Ocean (29°18’ to 30°26’S, 49°45’ to 50°34’W; Fig. 1). Only two of these lagoons, Tramandaí and Armazém, have daily salinity fluctuations, and the salinity varies from 0 to 11 in the estuary (Kapusta *et al.*, 2006). Voucher specimens of *M. curema* (FURG 2872, four specimens) and *M. liza* (FURG 2817, two specimens) that were collected during the same events were deposited in the Coleção de Peixes da Universidade Federal do Rio Grande.

The left sagittae otoliths were embedded in crystal polyester resin, and transverse sections were made through the core using a low-speed, diamond blade Isomet saw (Wang, 2014; Avigliano *et al.*, 2015). Thin sections with 0.4 mm width were mounted onto glass slides with cyanoacrylic glue. Prior to laser ablation inductively coupled plasma mass spectrometry analysis, otolith surfaces were polished with silicon carbide paper (No. 8000), washed with ultrapure DI water (Milli-Q, Millipore, Bedford, USA), sonicated for 5 minutes, and rinsed three times with ultrapure water. The slides were then dried in a laminar flow cabinet before analysis.

Laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) was used to quantify the concentrations of Ca^{43}, Sr^{86}, and Ba^{137} with a PerkinElmer DRC-e ICPMS coupled to a New Wave Nd-YAG 193-nm laser (Electro Scientific Industries) at the Instituto de Geociências da Universidade de São Paulo. The laser was operated at a pulse frequency of 10 Hz, a scan speed of 30 µm/s, and energy outputs of 10 and 12 J/cm^2 per pulse. Under these conditions, the crater width was approximately 30 µm. The ablated material was conducted through a Teflon-coated tube into the ICP-MS using argon as a carrier gas. After each otolith ablation, the background intensity was measured for 60 s. Strontium and calcium counts per second (cps) were subtracted from the background level, and element:Ca ratios were then calculated for all otoliths. The ICP-MS had been previously optimized for daily performance, with maximum analyte intensities and minimum interferences determined using oxides and double charged ions. The otolith samples were read in random order. A certified glass reference standard National Institute of Standards and Technology - NIST 612 was run at the start and end of each session and after every 10 otoliths to correct for mass bias and machine

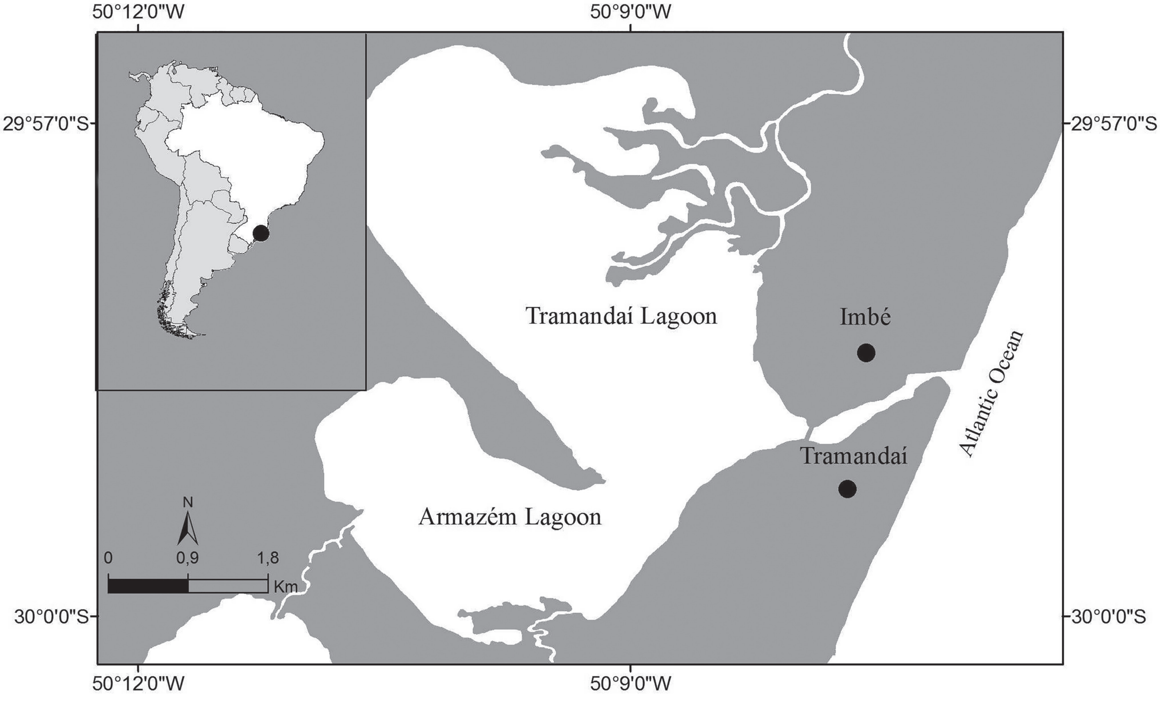

Fig. 1. Maps showing the sampled area, Southwest Atlantic, rio Tramandaí Estuary.

drift. Estimates of external precision (% relative standard deviation) were assessed by measurements of a calcium carbonate certified reference material MACS-3 (United States Geological Survey) and were as follows: 5.7% (^{86}Sr), and 3.2% (^{137}Ba). Data reduction, including background corrections, conversion of mass count data to concentrations (ppm) and limits of detection were all performed for each sample via Glitter software GEMOC, Macquarie University.

The transects of the otoliths from the nucleus to edge were used to analyze the elemental concentrations through the entire life history of each individual. The chemical signatures of the first 20 reads of the otolith core were used to represent the physical-chemical properties of the birthplace (core), and the last 20 reads of the edge were used to represent the most recently deposited material. For these comparisons, we applied the Mann-Whitney U test (Avigliano *et al.*, 2015).

The habitat use profiles were visually interpreted and based on two assumptions: first, Ba:Ca ratios in the otolith below the 0.5 μmol.mol^{-1} threshold indicate that the fish was in a marine habitat. This assumption considered the upper concentration limit of the Ba:Ca ratio found in the cores of otoliths of both species, and the fact that they both spawn at sea (Ibáñez-Aguirre, Gallardo-Cabello, 2004; Lemos *et al.*, 2014); second, Ba:Ca ratios above the 1.8 μmol.mol^{-1} threshold suggest that the fish spent time in freshwater habitat. This assumption was validated using ten specimens of *M. liza* sampled from the limnetic zone of the Laguna dos Patos, which is located 100 km from the study area.

Results

The Sr:Ca ratios in the otolith cores were similar in both species: *Mugil curema* 1.91±1.55 mmol.mol^{-1}, *Mugil liza* 2.06±1.54 mmol.mol^{-1}; Mann-Whitney U-test, $p = 0.064$ (Fig. 2). These high values of Sr in the core provide evidence that both species spawn in marine waters. Moreover, the Sr:Ca ratio at the edge of otoliths was significantly higher in *M. curema* than *M. liza* (2.6±2.9 and 1.7±1.1 mmol.mol^{-1}, respectively) (Mann-Whitney U-test, $p < 0.05$). The Ba:Ca ratios in the otolith cores and edges were significantly lower in *M. curema* (core 0.21±0.20 μmol.mol^{-1}, edge 0.24±0.25 μmol.mol^{-1}) (Mann-Whitney U-test, $p < 0.05$ for both) than in *M. liza* (core 0.53±0.48 μmol.mol^{-1}, edge 1.58±1.23 μmol.mol^{-1}).

The cross-otolith lifetime profiles derived from the Sr:Ca and Ba:Ca ratios showed differences in habitat use within *M. curema* and *M. liza* individually (Figs. 3-4). Nevertheless, the Sr:Ca ratios did not show a pattern that would aid in our interpretation of estuarine habitat use for either species (Figs. 3-4).

Mugil curema has four patterns of habitat use. In 27% of the specimens, low Ba:Ca values throughout the entire section indicate that the specimens probably spent their entire lives in high-salinity water (Fig. 5a). In 30% of the specimens, the high Ba:Ca values over short periods indicate sporadic entries into brackish waters (Fig. 5b). In the third pattern, 13% of the specimens have episodes of very high Ba:Ca ratios, suggesting the use fresh water for a short period of time (Fig. 5c). Finally, 30% of the specimens have high Ba:Ca values over long periods, which indicates the use of brackish waters for longer periods of time with incursions into fresh water for short time periods (Fig. 5d). As *M. curema* exhibits partial migration, the contingents show different patterns of habitat use.

Mugil liza showed two patterns of habitat use. The first group included individuals that had Ba:Ca ratios within the brackish water range, which indicates the use of estuarine waters during most of their life cycle; the second group includes 56.5% of the individuals that had episodes of very high Ba:Ca ratios, which indicates the use of both brackish and freshwater habitats throughout their life (Figs. 6a-b, respectively). These multiple contingents of *Mugil liza* showed different uses of the estuary, but the species appeared to be more dependent on the estuary.

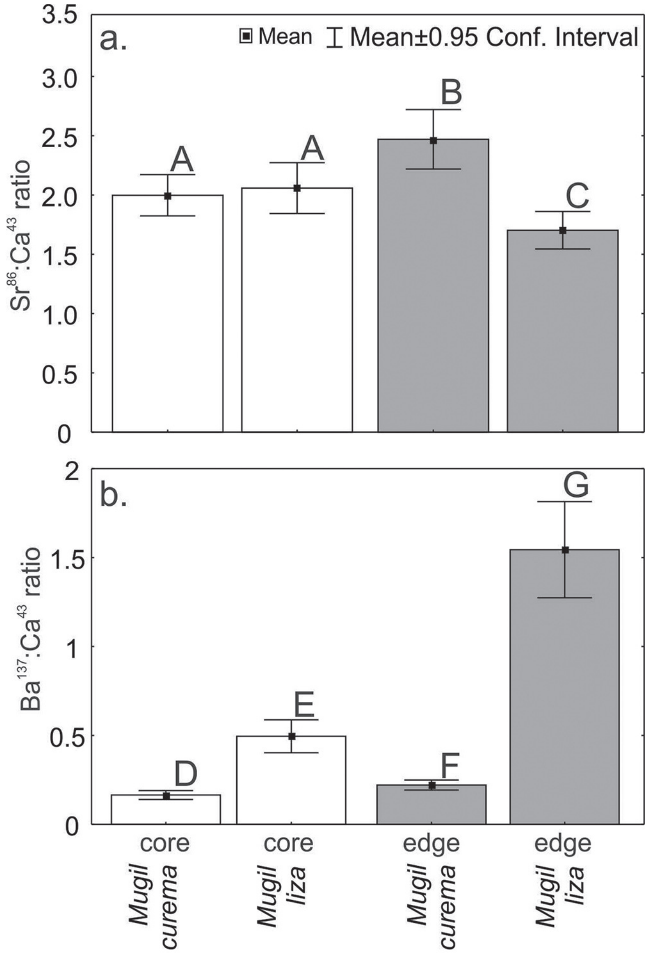

Fig. 2. Means and standard deviations (SD) of (a) Sr^{86}:Ca^{43} (mmol.mol^{-1}); and (b) Ba^{137}:Ca^{43} ratio (μmol.mol^{-1}) in otoliths of the inner 20 measurements (core) and the outer 20 measurements (edge) of *Mugil curema* and *M. liza* caught in the Tramandaí River Estuary, Brazil. Different letters within a spruce stand denote significant differences between species (Mann-Whitney U test, $p<0.05$).

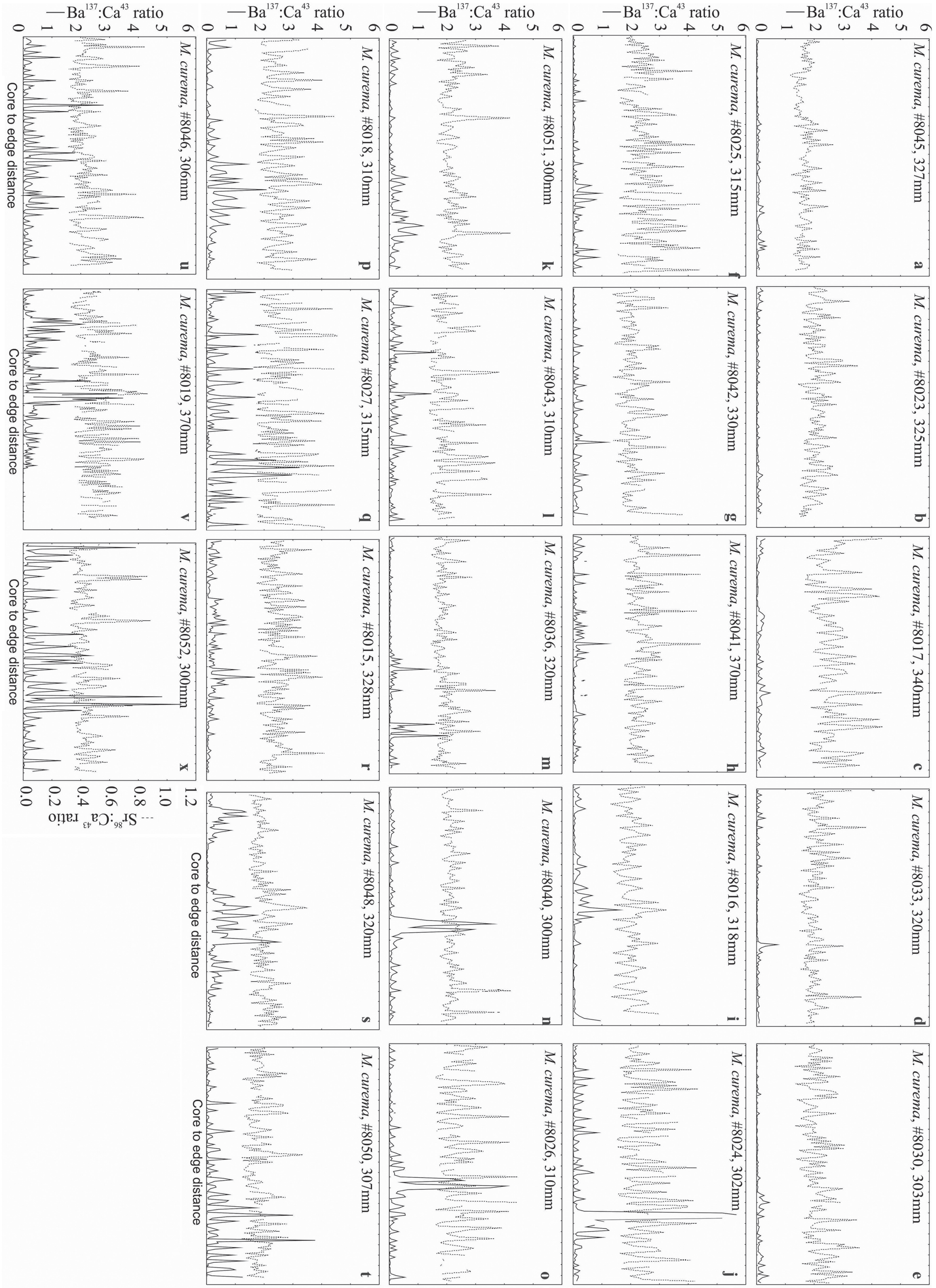

Fig. 3. Otolith transects of *Mugil curema* measured by LA-ICP-MS from the core to the edge. Ba^{137}:Ca^{43} (line) and Sr^{86}:Ca^{43} (dashed line). The identification code and total length (mm) of each fish are indicated on the graph.

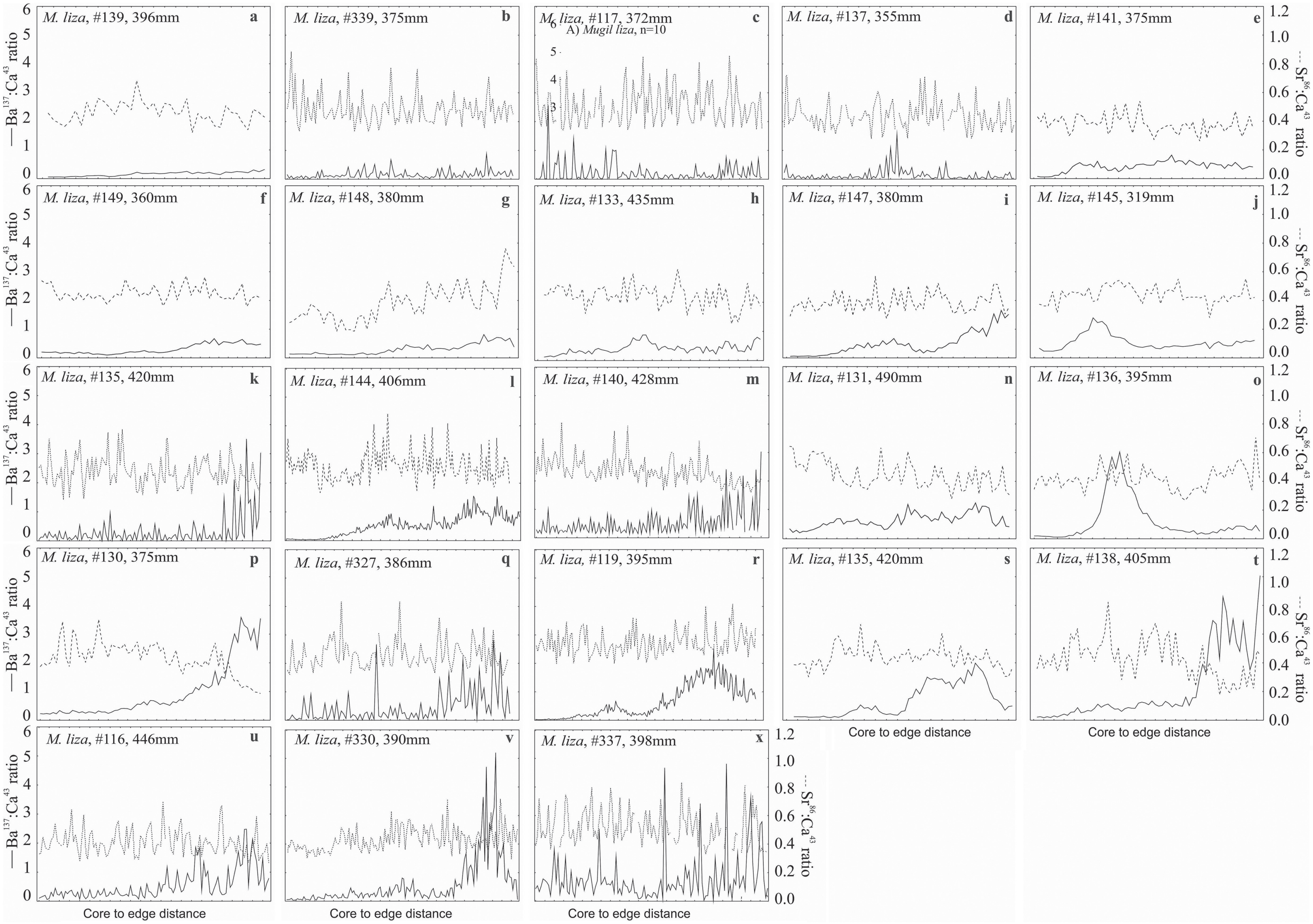

Fig. 4. Otolith transects of *Mugil liza* measured by LA-ICP-MS from the core to the edge. Ba^{137}:Ca^{43} (line) and Sr^{86}:Ca^{43} (dashed line). The identification code and total length (mm) of each fish are indicated on the graph.

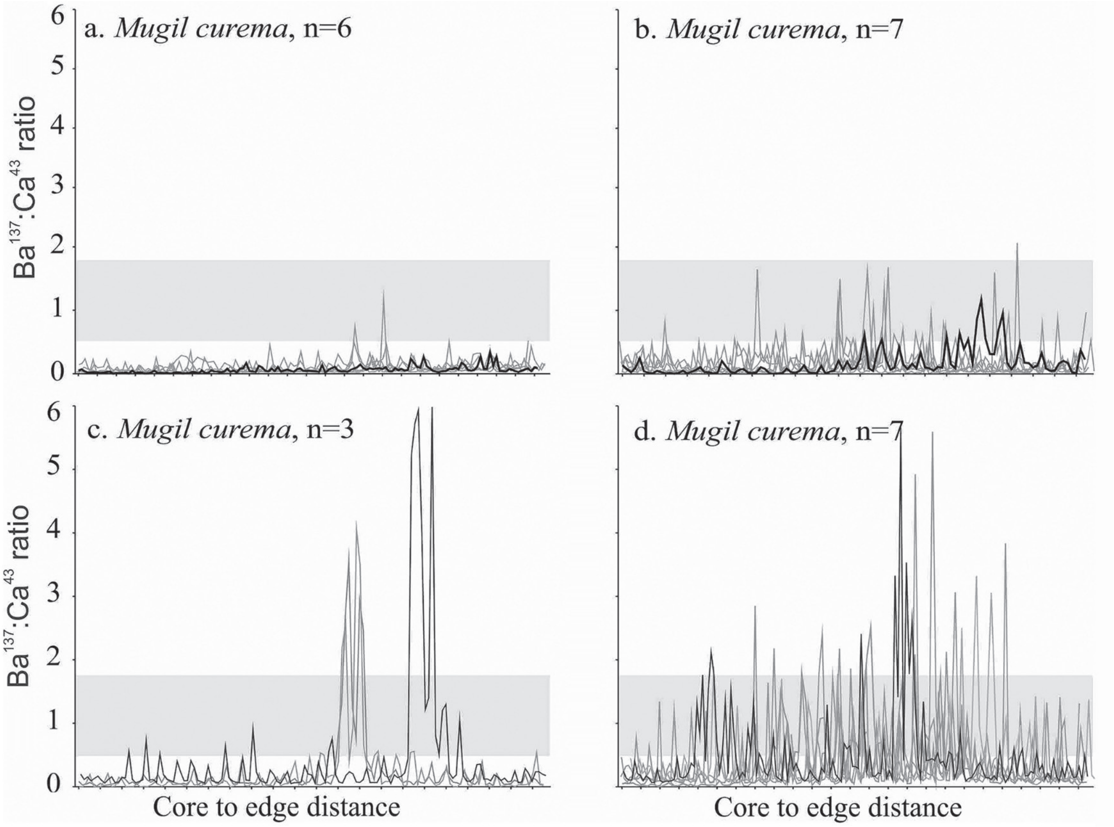

Fig. 5. *Mugil curema*. Ba^{137}:Ca^{43} variations (µmol.mol^{-1}) along transects from the core to the edge of the otolith. Individuals were from the rio Tramandaí Estuary, Brazil. The horizontal gray bands between the Ba^{137}:Ca^{43} ratios of 0.05 and 0.18 indicate migration in brackish waters (see M&M).

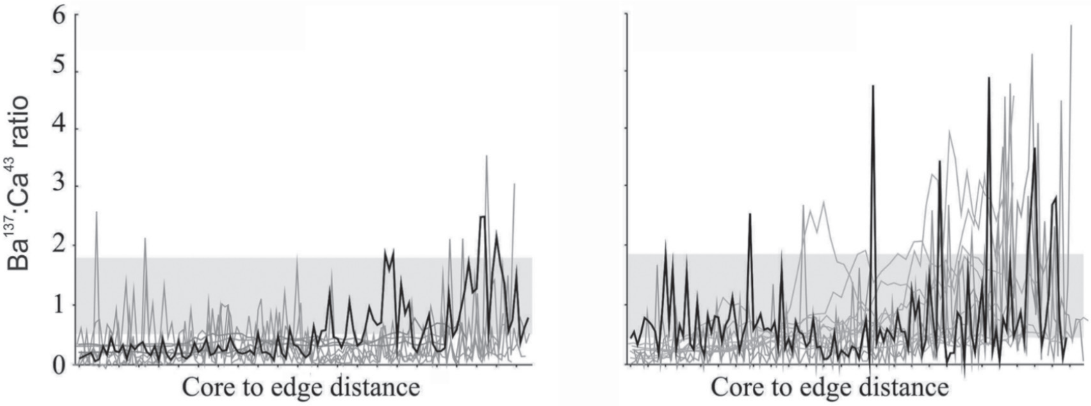

Fig. 6. *Mugil liza*. Ba^{137}:Ca^{43} variations (µmol.mol^{-1}) along transects from the core to the edge of the otolith. Individuals were from the rio Tramandaí Estuary, Brazil. The horizontal gray bands between the Ba^{137}:Ca^{43} ratios of 0.05 and 0.18 indicate migration in brackish waters (see M&M).

Discussion

The Sr:Ca and Ba:Ca ratios at the core otolith regions in both *M. curema* and *M. liza* indicate that these two species spawn in marine waters. However, the reproductive periods of *M. curema* and *M. liza* are different, suggesting that spawning occurs in distinct environmental conditions. In southeastern Brazil, *Mugil curema* spawns between October and April with two annual peaks in April and November (Fernandez, Dias, 2013). *Mugil liza* spawns between April and July with a single peak in June (Lemos *et al.*, 2014). Indeed, the abundances of juveniles of both species exhibit seasonal differences in the estuaries of southern Brazil. While the juveniles of *M. liza* are more abundant in the winter, those of *M. curema* are more abundant in the summer (Vieira, 1991; Ramos, Vieira, 2001). The different spawning periods associated with warmer waters for *M. curema* and cooler waters for *M. liza* act as a mechanism to reduce the competition among juveniles of these two species after recruitment in the estuary (Vieira, 1991; Albieri, Araújo, 2010). The winter in southern Brazil is characterized by an increase in precipitation, which consequently increases the river and estuarine runoff and reduces the salinities in the estuaries and adjacent coastal areas. On the other hand, the reduced precipitation and the predominance of tropical waters in the coastal region during the summer increase the salinities in the estuaries (Ciotti *et al.*, 1995; Soares, Möller, 2001; Möller *et al.*, 2008).

Only the Ba:Ca ratio was used to reconstruct the habitat use profiles of the individuals because the Sr:Ca ratio fluctuated among individuals of the same species; therefore, a directional trend throughout the otolith transect was not clear. In Marine Migrant species that experience intermediate salinity of 20-35 ppm, the analysis of the Sr:Ca ratio has limited ability to distinguish long periods of residence in fully marine or mid-estuarine habitats (Kraus, Secor, 2004; Chang *et al.,* 2004; Walther, Limburg, 2012). The use of the Ba:Ca ratio in the otoliths may provide a more accurate classification of estuarine habitat use than the Sr:Ca ratio (Elsdon, Gillanders, 2005; Tanner *et al.*, 2013; Gillanders *et al.*, 2015).

The significantly higher Ba:Ca ratios at the edges of the otoliths of larger specimens of *M. liza* (1.58±1.23 μmol.mol^{-1}) suggest a preference for areas with lower salinity. On the other hand, the high Sr:Ca ratios (2.6±2.9 mmol.mol^{-1}) and the low Ba:Ca ratios at the edges of the otoliths (0.24±0.25 μmol.mol^{-1}) of *M. curema* suggest a preference for areas with higher salinities. These findings, in association with the reproductive and recruitment patterns of both species, support our hypothesis of different habitat utilization of *M. curema* and *M. liza* in the rio Tramandaí Estuary.

These characteristics reveal that *M. curema* is a marine species that regularly enters estuaries in substantial numbers, particularly as juveniles, but use nearshore marine waters as alternative habitats to varying degrees. In Mexican coastal waters, báñez-Aguirre *et al*. (2012) found that *M. curema* has a more diverse euryhaline preference than the simple catadromous behavior. In Venezuela lagoons, *M. curema* has four different cohorts, reflecting different ages and different life stages coexisting in the same habitat (Marin *et al.*, 2003). In Pernambuco, Brazil, two habitat use behaviors for *M. curema* were described, as some juveniles appear to use waters of high salinity and others seem to inhabit low-salinity waters (Silva, 2007).

Fortunato *et al.* (2017) suggested three patterns of estuarine use by *M. liza* during most of their lifetime based on individuals sampled at several different locations in Paranaguá Bay, Brazil; Samborombón Bay, Mar Chiquita Coastal Lagoon; and San Blas, Argentina. Type I corresponds to the regular use of estuarine habitat; Type II corresponds to a fluctuating pattern of estuarine and sea (high salinity waters) habitat use; and Type III corresponds to a high preference for saline water. Our results agree with Fortunato *et al.* (2017), as *M. liza* also showed a high variability of migratory patterns in the rio Tramandaí Estuary. These characteristics reveal a marine species that require sheltered estuarine habitats as juveniles but live along coastal areas where there are no such habitats; thus, these species are dependent on the habitats of that type that are present in estuaries.

In conclusion, based on otolith microelement analysis, both *M. curema* and *M. liza* can be classified in as Marine Migrants *sensu* Elliott *et al.* (2007). However, considering the habitat use by juveniles, both species may be classified into two different subcategories: *M. curema* as a Marine Estuarine-opportunist and *M. liza* as an Estuarine Dependent in the rio Tramandaí River Estuary. This important distinction in the life cycles of these two species is fundamental for developing conservation strategies for these fishery resources. Considering that estuary habitats suffer from greater anthropic impacts than surf-zone coastal areas, it seems that *M. liza* is much more vulnerable than *M. curema* in terms of estuarine habitat conservation (Barletta *et al.*, 2010).

Acknowledgments

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