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Research Article

Use of otolith microchemistry as habitat indicator of *Anchoa tricolor* (Spix & Agassiz, 1829) in a subtropical estuary

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ABSTRACT. The objective of the present study was to evaluate the use of sagitta otolith microchemistry (Sr:Ca, Ba:Ca ratios) as habitat indicator of *Anchoa tricolor* between the estuary and the adjacent continental shelf. We analyzed 162 specimens of *A. tricolor* in the Paranaguá Estuarine Complex (Brazil), and salinity and rainfall data. The biological and environmental data were grouped into sectors (internal, intermediate, and external) and periods (early and late wet, and dry periods). The concentrations of Ca, Ba and Sr were determined using an ICP-OES. The Sr:Ca ratio indicated the existence of two groups, probably from different origins, that use the estuary at the same time, but occupying different sectors of the estuary. The Ba:Ca ratio did not differentiate the groups as to the use of estuary, but rather revealed low values, suggesting that this species does not use freshwater environments. However, this ratio showed a high variation in the wet period, indicating a greater availability of barium in the estuarine regions affected by the river discharge. Considering our results, it is possible to classify *A. tricolor* as an estuarine migrant species. Also, the Sr:Ca and Ba:Ca ratios can be indicative of the habitat use in a subtropical estuary.

Keywords: *Anchoa tricolor*, Engraulidae, life cycle, habitat, otolith microchemistry, subtropical Brazil.

INTRODUCTION

Life history, migration processes and the population structure of various species of fishes have been described through otolith microchemistry in recent decades (Bradbury *et al*., 2008; Geffen *et al*., 2011; Reis-Santos *et al*., 2012). Concentrations of strontium (Sr) and barium (Ba) are the most studied for the chemical characterization of this structure (Campana, 2005; Elsdon *et al*., 2008; Avigliano & Volpedo, 2013; Avigliano *et al*., 2014). The Sr:Ca and Ba:Ca otolith ratios are susceptible to changes in the physical and chemical characteristics of water, particularly salinity (Reis Santos *et al*., 2013; Avigliano *et al*., 2014). These ratios reflect the availability of these elements in the environment, revealing the permanence of organisms in different freshwater or marine environments (Secor *et al*., 1995; Campana & Tzeng, 2000; Avigliano & Volpedo, 2013). In this way, it is possible to analyze the displacements of species in their ontogenetic stages and study the connectivity between ecosystems (Gillanders, 2002; Thorrold & Stephen, 2009; Reis-Santos *et al*., 2012). The otolith microchemistry may also reflect the environmental fidelity or plasticity (Gillanders *et al*., 2012; Albuquerque *et al*., 2012; Mai *et al*., 2014).

Anchovy, *Anchoa tricolor* (Spix & Agassiz, 1829) is common in different environments of the east coast of the South America, with ecological key role in transferring energy from plankton to higher trophic levels (Hackradt *et al*., 2011). It is a small pelagic fish with distribution range between 03°40'S and 37°58'S

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(Whitehead *et al*., 1988) that inhabit shallow coastal waters and transitional environments such as estuaries, mangroves and bays (Froese & Pauly, 2015). In the southwest Atlantic, one of the most important estuarine ecosystems is the Paranaguá Estuarine Complex (PEC) (22º54'-23º04'S, 43º34'-44º10'W), which has been traditionally used as a model of integrated studies in marine science (Lana *et al*., 2001; Noernberg *et al*., 2004). Particularly the PEC, as well as other coastal ecosystems, is used by early stages of *A. tricolor* for feeding, growth and shelter, while the adults use the continental shelf (Araújo *et al*., 2008a). The anchovy has a high growth rate in the PEC, reaching 75 mm of total length in 9 months when compared to other fishes inhabiting this estuary (Carvalho *et al*., submitted). Nevertheless, these shifts between the marine environment and the estuary, throughout its ontogeny, are not clear, and generate different hypotheses about whether it is a marine migrant species or estuarine migrant species (Araújo *et al*., 2008b; Vilar *et al*., 2011). Thus, the knowledge on the movements of this species is of utmost importance given its abundance in this environment (Vilar *et al*., 2011), its ecotrophic importance (Bornatowski *et al*., 2014) and its use as a fishery resource (Cardoso & Nordin, 2006).

Otolith microchemistry has been successfully applied to the study of small coastal pelagic fish using transitional environments (Kimura *et al*., 2000; Secor & Rooker, 2000; Castro, 2007) and demersal fishes (Alburqueque *et al*., 2010; Avigliano *et al*., 2014). The objective of the present study was to evaluate the use of sagitta otolith microchemistry (Sr:Ca, Ba:Ca ratios) as an indicator of the habitat use of *Anchoa tricolor*.

MATERIALS AND METHODS

Study area

The Paranaguá Estuarine Complex (PEC) (Fig. 1) is 601 km^2 in area, one of the most important coastal ecosystems in the eastern coast of South America. It has humid subtropical climate, with wet period between the late spring and late summer (between October and March) (Lana *et al*., 2001). The PEC exports freshwater to the shallow continental shelf (up to 20 m isobath), with 7×10^6 m^3 day^{-1} in the dry period and 28×10^6 m^3 day^{-1} in the wet period (Marone *et al*., 2005). It has two main axes: the north-south axis, comprising the bays of Guaraqueçaba and Laranjeiras, with 30 km long, and the east-west axis, formed by the bays of Paranaguá and Antonina, with an estimated length of 56 km, where the major tributaries are the Guaraquaçú, Maciel, Cachoeira, Faisqueira and Nhundiaquara rivers (Lamour *et al*., 2004). This system is interconnected to the adjacent continental shelf by the channels Galheta and Sudeste (Angulo & Araújo, 1996).

The estuary hydrodynamics is determined by the flood and low tides (Noernberg *et al*., 2007). The estuary is partially mixed (Marone *et al*., 2007) with stratification of the water column (Mantovanelli *et al*., 2004). It has semidiurnal tides with amplitudes between 0.5 and 2.0 m, which exert influence by the local geomorphology, with an average of 2.2 m, and salinity and water temperature varying in summer (12-29 and 23-30ºC) and winter (20-34 and 18-25ºC) (Lana *et al*., 2001).

Sampling

We collected 162 specimens of *Anchoa tricolor* with a beach seine net during the neap tide in the intertidal areas along the north-south axis of the PEC during periods defined as early wet (EW, October, November and December 2010), late wet (LW, January, February and March 2011) and early dry (ED, April, May and June 2011). The sampling sites were separated into three sectors according to the distance from the estuary mouth, which are internal (INT), intermediate (INTER) and external (EXT) (Fig. 1).

In each area, we performed a profiling with CDT for the measurement of salinity, for the first meter of the water column. Monthly, cumulative rainfall data were obtained from the meteorological stations in the municipalities of Antonina and Guaraqueçaba (Simepar, 2014). The rainfall in Antonina influences the discharge of the rivers and the runoff in the intermediate and external sectors. The Paraná Meteorological System (Simepar, 2014) provided these data for the study period. The monthly average values of cumulative rainfall data, between 2005 and 2009, in the municipality of Antonina were also, used and provided by Simepar.

In laboratory, we measured the total length (TL mm) of fishes and their sexes were identified macroscopically. Sagitta otoliths were extracted, washed, dried and stored for further analysis. Considering the individuals of each period and sector, the otoliths were weighed (WO, g) on an analytical balance (accurate to 0.001 g) and then digested with 50% nitric acid.

Otolith microchemistry

The otolith concentrations of Sr (407.771 nm), Ba (233.527 nm) and Ca (317.933 nm) were determined via inductively coupled plasma-atomic emission spectrometry (ICP-OES), using a Perkin Elmer® Optima 2000 DV (Überlingen, Germany) equipped with cross-flow nebulizer and a quartz ICP torch (EPA 200.7 method) (EPA, 1994). A Perkin-Elmer AS-90 Plus

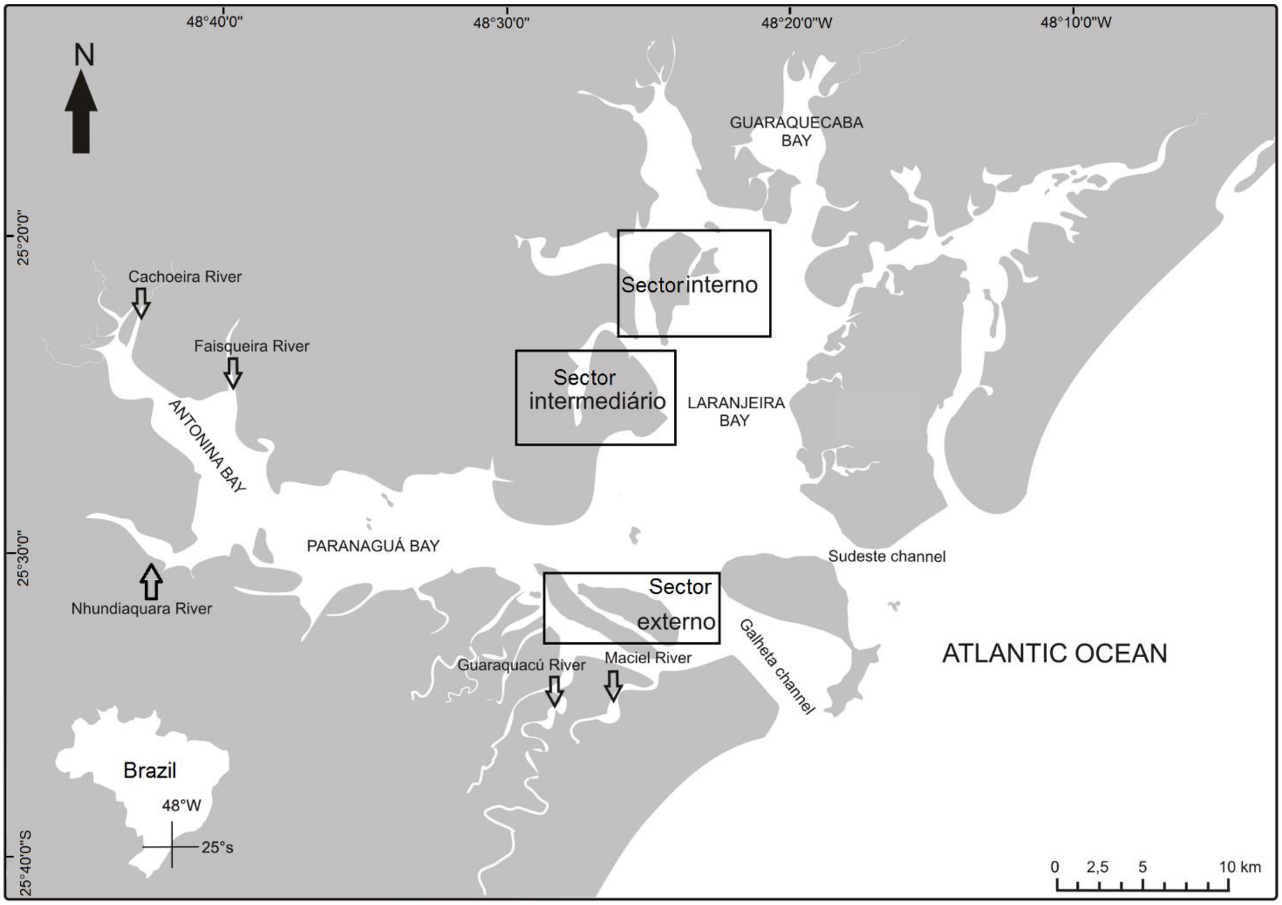

Figure 1. Map of the Paranaguá Estuarine Complex illustrating the location of the sampling sectors (squares) of *Anchoa tricolor*.

autosampler was used for the automated sample handling. The equipment was cleaned at regular intervals with MilliQ water (Millipore, São Paulo, Brazil) and 10% nitric acid matrix in order to prevent the sample memory effects. The detection limits of ICP-OES were 8, 10 and 10 µg L^{-1} for Ba, Sr and Ca, respectively. The external calibration was carried out using the atomic spectroscopy standard QCS 21 (Quality Control Standard, Perkin Elmer® Pure, USA). All the measurements were performed in triplicate (RSD <4%). The digestion and analytical procedures were checked by the Otolith Certified Reference Material analysis for trace elements (FEBS-1, National Research Council, and Canada). The analyses in replicate of these reference materials showed good accuracy, with the following metal recovery rates: 98% for Sr, 94% for Ba, and 101% for Ca.

The element:Ca ratios were expressed in mmol mol^{-1} in order to standardize the concentrations of trace elements in relation to Ca.

Data analysis

The data of salinity, fish total length and otolith weight were described in the form of mean and standard deviation. The influence of salinity on the Sr:Ca ratios and the rainfall on the Ba:Ca concentrations was verified through Spearman correlation (Zar, 2010).

As for the concentration values, we calculated the initial values of the Sr:Ca and Ba:Ca ratios. In order to minimize the otolith weight effect on the elemental ratios, an ANCOVA was run between the values of Sr:Ca and Ba:Ca and otolith weight (WO). The coefficients $b_{Sr:Ca} = -504.38$ and $b_{Ba:Ca} = -3.46$ estimated by ANCOVA were used to fit these ratios according to the equation: $[Ratio_i] - b(OW_{average} - OW_i)$, where *i* represents the value of variables for each individual (Campana *et al*., 2000; Avigliano *et al*., 2014). With the values of ratios corrected, after checking the normality and homogeneity, a Kruskal-Wallis test followed by a Student Newman Keuls test (SNK) were performed in order to compare the different periods and sectors (Zar, 2010).

RESULTS

The salinity ranged from 13 to 27 in the PEC. In the internal sector of the estuary (INT), the salinity ranged from around 21 ± 6, in the intermediate sector (INTER) the salinity was 26.31 and in the external sector (EXT) the salinity ranged from around 20.91 ± 7.15. The rainfall in Antonina showed a large variation (76.2-652.4 mm) between October 2010 and June 2011. In Guaraqueçaba, the rainfall presented low variation (from 5.7 to 23.8 mm) between February and June 2011 (Fig. 2). The rainfall in Guaraqueçaba was lower than in Antonina. Considering Antonina, during the study period there was an increase in the rainfall in relation to the average in previous years, increasing consequently the river inflow for the PEC, in the east-west axis, and thus influencing the external sector (Fig. 2).

The total length of *A. tricolor* varied between 25 and 75 mm TL, with record of juvenile and adult individuals (Table 1). The largest specimens were found in the early wet period. During the late wet period, smaller specimens were caught in the internal and external sectors, respectively. In the late wet period, anchovies with intermediate sizes were registered. The weight of otoliths varied between 0.05 and 1.95 mg (Table 1). The Sr:Ca ratio was positively correlated ($P < 0.004$, $R^2 = 0.2053$) with salinity and the Ba:Ca with rainfall ($P < 0.0001$, $R^2 = 0.856$).

The Sr:Ca ratio varied significantly between sampling sites (H = 44.966, $P < 0.001$) due to ratio of the intermediate sector (SNK $P < 0.05$) (Fig. 3a). The Ba:Ca ratio also varied significantly between sampling sites (H = 121.725, $P < 0.001$), due to the highest ratios of the external sector (SNK < 0.05). In the external sector, the otoliths were obtained only in the late wet period (5.78 ± 1.27 mmol mol^{-1}), with a higher mean value than the intermediate sector but within the range of variation of the internal sector. The Ba:Ca ratio in the external sector is very variable (Fig. 3b).

The Sr:Ca ratio did not present differences regarding the rainfall ($P > 0.05$) (Fig. 3c). However, the Ba:Ca ratio showed significant differences (H = 62.115, $P < 0.001$) in the different periods, due to the highest values in the late wet period (Fig. 3d).

In the early wet period, we observed the highest mean value (± standard deviation) of the Sr:Ca ratio (8.61 ± 0.45 mmol mol^{-1}), followed by a sharp decline in the late wet period (4.88 ± 1.57 mmol mol^{-1}) (Fig. 3c). In the early dry period, the mean value was intermediate (6.80 ± 1.39 mmol mol^{-1}). In the intermediate sector, we analyzed only otoliths in the early wet period, with the record of the lowest mean value for this ratio (4.28 ± 0.37 mmol mol^{-1}).

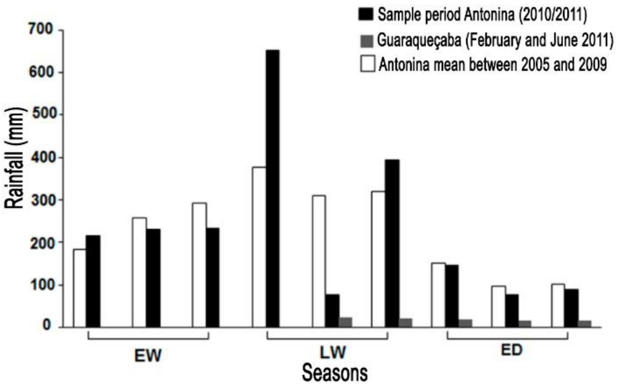

Figure 2. Monthly rainfall data in the study period in the municipalities of Antonina and Guaraqueçaba: early wet rainy (EW, October, November and December 2010), late wet rainy (LW, January, February and March 2011) and early dry periods (ED, April, May and June 2011) in the Paranaguá Estuarine Complex, Brazil.

In the samplings during the early wet period, we caught the largest specimens of *A. tricolor*, with different Sr:Ca ratios (Fig. 4). The group with higher Sr:Ca ratios (from 7.11 to 11.03) was related to the internal sector of the estuary, however the group with lower Sr:Ca ratios (from 3.19 to 4.19) was related to the intermediate sector (Fig. 4a). The Ba:Ca ratio also indicates the presence of two groups for both juveniles and adults in the late rainy period (Fig. 4b).

DISCUSSION

The microchemistry of sagitta otoliths showed variation in the habitat use by *A. tricolor* in the Paranaguá Estuarine Complex. The complexity in habitat use by this small pelagic fish was also observed for other species (Kimura *et al.*, 2000; Secor & Rooker, 2000; Yang *et al.*, 2006; Labonne, *et al.*, 2009; Morais *et al.*, 2010; Geffen *et al.*, 2011; Correia *et al.*, 2014; Mai *et al.*, 2014). In the literature, *A. tricolor* is mentioned as a marine migrant (Araújo *et al.*, 2008b) or estuarine migrant fish (Vilar *et al.*, 2011). According to Elliot *et al.* (2007), estuarine migrant species has part of its life cycle developed outside the estuaries, unlike the marine migrants that spawn in truly marine environments and enter the estuaries in large numbers for feeding and growth. The results suggest that the juveniles of *Anchoa tricolor* that enter in the estuary coming from spawnings occuring in the continental shelf have already a high value of the Sr:Ca ratio until reaching the most internal sector of the estuary. They remain growing in the estuary for 9 months (Carvalho *et al.*, submitted). This behavior would be related to the characteristics defined by Elliot *et al.* (2007), as a estuarine migrant species.

Table 1. Mean values, standard deviation, length range (TL), otolith weight (OW), and sex determination of *A. tricolor* in the Paranaguá Estuarine Complex, Brazil, where *n* is the number of samples, F female, M male and In indeterminate sex. EW (October to December, LW (January to March), ED (April to June).

		n	TL (mm)	Range TL (mm)	Sex	WO (mg)
Sampling sites	Internal	83	51.24 ±14.36	25 - 51	F = 15, M = 13, In = 55	0.0007 ± 0.0005
	Intermediate	20	72.5 ± 1.98	70 - 72	F = 8, M = 8, In = 4	0.0017 ± 0.0001
	External	59	54.00 ± 16.00	25 - 54	F = 0, M = 1, In = 58	0.0007 ± 0.0004
Seasons	EW	24	67.26 ± 1.96	65 - 71	F = 10, M = 10, In = 4	0.0013 ± 0.0002
	LW	119	49.38 ± 14.64	25 - 75	F = 5, M = 4, In = 110	0.0006 ± 0.0004
	ED	19	72.33 ± 1.9	70 - 75	F = 8, M = 8, In = 3	0.0017 ± 0.0001

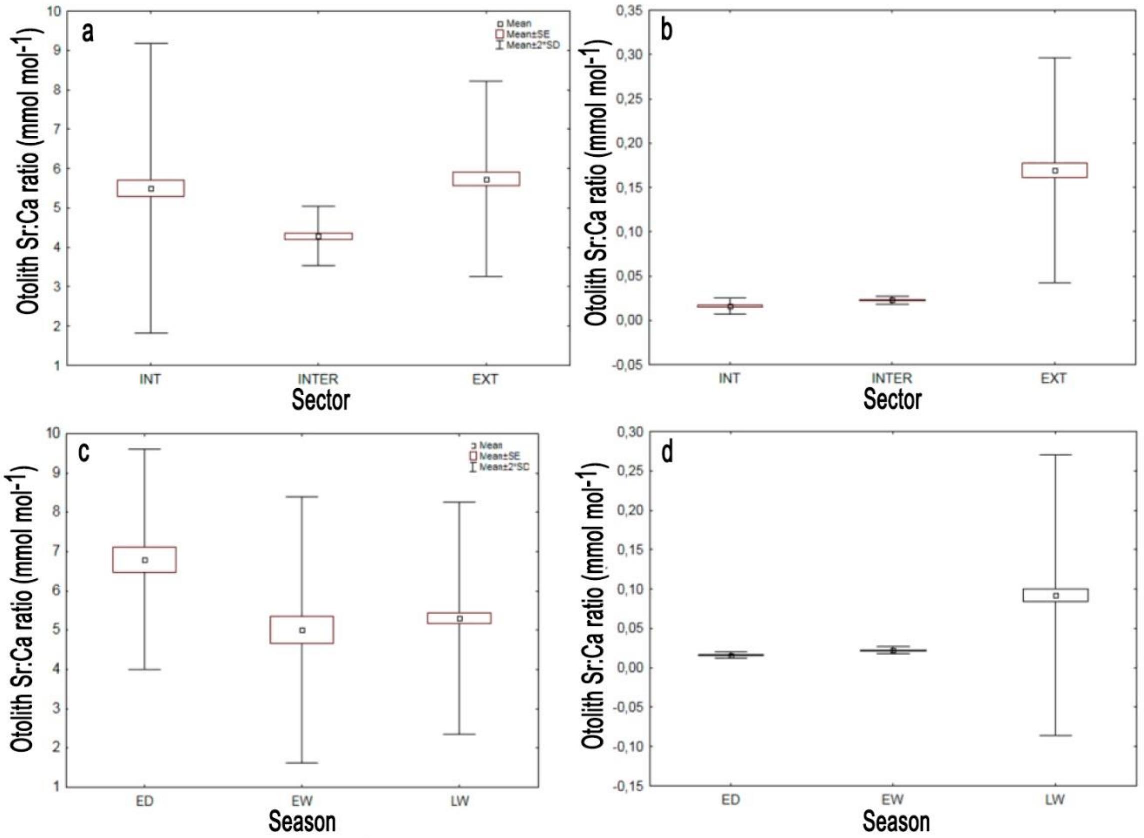

Figure 3. Box plot for ratios a) Sr:Ca ratio for otolith by sector, b) Ba: Ca ratio for otolith of *A. tricolor* by sector, c) Sr:Ca ratio for otolith by seasons, and d) Ba:Ca ratio for otolith by seasons. Those seasons are: early dry (ED), early wet rainy (EWER), late rainy (LR). As for the sectors, they are: internal (INT), intermediate (INTER) and external (EXT).

The specimens with a larger length in the EW period, when they present differences in the Sr:Ca ratio, are associated with the internal and intermediate sectors and could have different origins, coexisting in the estuary during this period. On the other hand, the Ba:Ca ratio from fish groups of the internal and intermediate sectors are similar, suggesting that these two groups, probably from different origins, use the estuary at the same time but occupy different sectors of the estuary. In the external sector, the Sr:Ca and Ba:Ca ratios are variable, which could be related to the input of river water and rainfall in this zone of the estuary.

Araújo *et al.* (2008a) studied the different maturity stages of *A. tricolor* in the Sepetiba Bay, identifying the different uses of this estuary by the species, corroborating our results. According to these authors, juveniles are more concentrated in the innermost and protected regions than the adults, which is consistent with the occurrence of anchovy in the early wet period in the internal sector. Fish with the greater values of

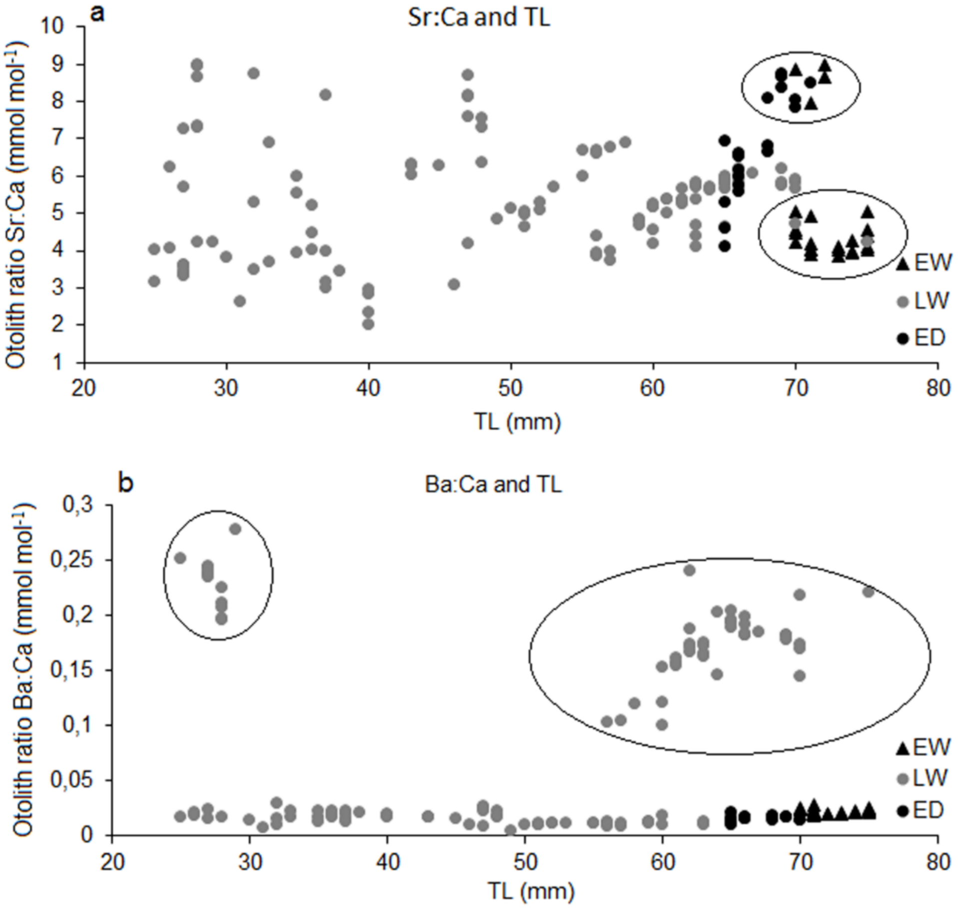

Figure 4. Scatterplot for ratios of elements according to length a) Sr:Ca ratio, and b) Ba:Ca ratio of otoliths of *Anchoa tricolor*. The periods are: early dry (ED), early wet (EW), late wet (LW).

total length and Sr:Ca and Ba:Ca ratios remained longer in waters with lower variations in salinity (possibly in the continental shelf) and for short periods in waters with higher variations in salinity as, for instance, the estuary. This suggests the connectivity between environments. On the other hand, in this same period, fish of the intermediate sector remained in less saline waters, in which, given their central location in the PEC, prevent the detection of their origins. This pattern shown by the Sr:Ca ratio of different groups using the same area was also described for *Clupea harengus* in the Irish Sea (Geffen *et al.*, 2011).

In the late wet period, there was an increase in the Ba:Ca ratio, indicating higher availability of Ba due to the water inflow from rivers of the east-west axis of the PEC (Nhudiaquara, Cachoeira, Maciel and Faisqueira) and the storm water, which is more intense in this period when compared to the previous years (Fig. 2). Therefore, the Ba:Ca ratio of *A. tricolor* otoliths can be a good indicative of events resulting in large supplies of freshwater. The results of the Ba:Ca ratio in anchovies, during the same period for the internal sector corroborate this functionality (Fig. 2). The Sr:Ca ratio for anchovies in the internal sector in the wet period demonstrated the tidal influence, since in this sector occurs lower oceanic influence, as also described for *Anchoa mitchilli* (Kimura *et al.*, 2000) and *Engraulis encrasicolus* (Morais *et al.*, 2010). The three species (*A. tricolor, A. miichilli* and *E. encrasicolus*) showed an increasing pattern for the otolith Sr:Ca ratio in response to the increased salinity in the estuaries, indicating the magnitude of the tidal influence on this ratio.

Several studies have demonstrated the displacements of small pelagic fish. In the genus *Anchoa*, Secor & Rooker (2000) used the Sr:Ca ratio in order to elucidate the life cycle of *Anchoa mitchilli* by capturing young individuals (45 and 50 mm TL) in the estuary of Chesapeake, whose variations in the ratio was between 2.5 and 4×10^{-3} (determined by an electron microprobe JEOL JXA-840A). In this study, the authors reported that the ratio would be compatible with an estuarine species, confirming the estuarine dependence of *A. mitchilli* during the early stages.

Additionally, *Coilia ectenes* is a small pelagic fish, which preferably inhabits the coastal region of China and migrates to freshwater systems to spawn. The fish caught had a length between 120 and 250 mm. This species presented a Sr:Ca ratio of around 4 and 5×10^{-3}, being used X-ray electron microprobe (Yang *et al*., 2006). With regard to the estuarine species *Ethmalosa fimbriata*, which inhabits the Casamance estuary in Africa (seasonally hypersaline), Labonne *et al*. (2009) found an increase in the Sr:Ca ratio between the otolith core and edge, with values not exceeding 4.5×10^{-3} (obtained from electron microprobe Cameca - SX50). Mai *et al*. (2014) demonstrated a high plasticity in the habitat use by *Lycengraulis grossidens* that is adapted to freshwater, estuarine and marine environments, showing values of Sr:Ca ratio lower than those recorded for *A. tricolor*. The species *L. grossidensis* was considered a facultative amphidromous species that inhabits rivers and lakes (Mai *et al*., 2014). These differences in the Sr:Ca ratio between *A. tricolor* and others clupeiform fish indicate differences in salinity and temperature between environments.

The studies mentioned above demonstrate how the Sr:Ca ratio may vary in clupeiform fish, considering that all species (*A. tricolor, A. mitchilli, C. ectenes, E. fimbriata* and *L. grossidens*) had been at some ontogenetic stage in transitional environments, such as estuaries.

The Ba:Ca ratio for *A. tricolor* showed an influence of the freshwater contribution (river discharge, runoff and rain water) on the PEC. As for *L. grossidens*, the Ba:Ca ratio presented the same order of magnitude as the ratio herein, despite the different methods used [LA ICP-MS, Mai *et al*. (2014), and solution-based ICP-OES in this study]. This demonstrates that both species were in the transitional environments and the habitat use is different between them, both in time and space. The same occurs with others migrant fish species, as *Odontesthes bonariensis* (Avigliano *et al*., 2014). The high initial growth rates may also cause variability in the Ba:Ca ratio (Campana *et al*., 2000). Despite the high growth rates of *A. tricolor* (Froese & Pauly, 2015), the Ba:Ca ratios did not vary greatly between different lengths sampled, but rather under the seasonal influence of rainfall. Unlike the observed for *Sardina pilchardus* by Correa *et al*. (2014), who verified spatial variations in the Sr:Ca and Ba:Ca ratios for adults and juveniles.

The different amplitudes of the Sr:Ca and Ba:Ca ratios determined for different clupeiform species (Secor & Rooker, 2000; Yang *et al*., 2006; Labonne *et al*., 2009; Mai *et al*., 2014), regardless of the methodological differences, reflect differences in the life histories of the different species and the environmental influences imposed throughout the ontogenetic development. Thus demonstrating high environmental plasticity of Clupeiformes that can inhabit environments with different salinities.

The present study, using otolith microchemistry, is an indicator of the habitat use of *A. tricolor*, which can be defined as an estuarine migrant species. The Sr:Ca ratio could be a tool showing the shifts of this species in the estuary, since the Ba:Ca ratio could be associated with temporal variations related to rainy periods that characterize subtropical estuaries.

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