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ARTICLE

Otolith as a tool to differentiate juveniles of two species Centropomidae

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Abstract. *Centropomus undecimalis* and *Centropomus ensifurus* are a species belonging to the family Centropomidae, which has an important role in estuarine ecosystem. The present study aimed at comparing the otoliths shape of juveniles both species collected in in the lower São Francisco River (10°28'34.02"S, 36°24'27.02"W). In the laboratory, 52 otoliths were extracted, photographed, measured and the contour was analyzed by the wavelet method. The otolith contours varied between species (n = 28 *C. ensiferus* and n = 24 *C. undecimalis*). The Linear Discriminant Analysis correctly reclassified 92.3% of all otoliths among species. MANOVA also evidenced significant differences in contour between species ($F = 3.73$; $p < 0.0001$). The results suggest that *C. ensiferus* is adapted to enviroments with higher turbidity and the *C. undecimalis* tends to colonize environments with lower turbidity and spends more time in the water column.

Keywords. Common snook; Swordspine snook; Estuary; Use habitat; River; Beach seine net.

INTRODUCTION

Otoliths are metabolically inert structures composed by calcium carbonate in a protein matrix, precipitated mainly in the form of aragonite (Campana, 1999; Schulz-Mirbach *et al.,* 2019). They are located in the inner ear of the bony fishes, being an important component of their mechanoreceptor system (Assis *et al.,* 2003). They have interspecific patterns among species and some morphological changes may occur influenced by various physiological or external factors (Popper & Fay, 2011). Growth, sex, reproduction and feeding are examples of physiological factors that influence the morphology of the otolith (Gagliano & McCormick, 2004; Tombari *et al.,* 2005; Carvalho *et al.,* 2020; Medeiros *et al.,* 2021). Environmental factors can also influence the morphology and morphometry of the otoliths as for example: depth is related to alterations in the *sulcus acusticus* area, allowing a greater sound perception (Torres *et al.,* 2000; Cruz & Lombarte, 2004) and salinity influences in the biomineralization of the otoliths (Avigliano *et al.,* 2014). Otoliths are excellent tools for understanding the connectivity and patterns of fishes through analysis of their chemical concentrations (Morissette & Whitledge, 2022). Using morphology and morphometry it is possible to identify patterns of habitat use and patterns of growth (Verocai *et al.,* 2023). For example, studies using otoliths have helped to identify patterns of habitats use and patterns of growth of Centropomidae species (Daros *et al.,* 2016; Medeiros *et al.,* 2021).

The family Centropomidae comprises 12 species, commonly called "snooks", they occur in tropical and subtropical regions of the Pacific and Atlantic Ocean (Froese & Paully, 2023). Five

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of this species are present on the Brazilian coast: *Centropomus undecimalis* (Bloch, 1792), *Centropomus parallelus* (Poey, 1860), *Centropomus ensiferus* (Poey, 1860), *Centropomus pectinatus* (Poey, 1860), *Centropomus poeyi* (Chavez, 1961) and *Centropomus irae* (Carvalho-Filho, Oliveira, Soares & Araripe, 2019) (Carvalho-Filho *et al.,* 2019; Figueiredo-Filho *et al.,* 2021). The species from the genera *Centropomus* are stenothermic and thermophilic and they are not found in temperatures below 15°C (Rivas, 1986). Snooks are predators feeding fishes and/or invertebrates, use estuaries for growth and feeding, and have a complex life cycle, with some species being sequential hermaphroditic (Daros *et al.,* 2016; Lira *et al.,* 2017; Medeiros *et al.,* 2021). *Centropomus* species are an important fishery resource mainly by sport fishers (Freire *et al.,* 2016). Species of this family also are important because of their high commercial value and the potential for aquaculture (Junior *et al.,* 2007; Ostini *et al.,* 2007).

In the Northeast Brazilian coastal environment, the largest number of *Centropomus* species are: *C. parallelus, C. pectinatus, C. undecimalis* and *C. ensiferus* (Figueiredo-Filho *et al.,* 2021). The focus of the present study was *C. undecimalis* and *C. ensiferus,* because both species use shallow estuarine environments for growth and feeding fishes and/or invertebrates (Bot Neto *et al.,* 2023; Froese & Pauly, 2023). *C. undecimalis* presents immature individuals smaller than 10 cm, but for *C. ensiferus* there is no information about the length at first maturation (Medeiros, *et al.,* 2021; Froese & Pauly, 2023) and habitat use by juveniles of these species is unknown. There is a lack of knowledge about the habitat use of *C. undecimalis* and *C. ensiferus* juveniles, due to this the aims are to use the otolith shape of juveniles from both species and correlate with the habitat use of these species in a tropical environment.

MATERIAL AND METHODS

Ethical Statement

The capture complied with animal welfare laws, guidelines and policies, approved by the national licensing authority the "Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, IBAMA" with license number 56379.

Study area

The Brazilian Northeast Coast is characterized by water temperatures ranging between 25.5°C and 29.6°C and is also considered an oligotrophic environment (Heileman, 2009). This coast is subjected to a marked period of high precipitation in the austral winter (rainy season, June/July to August/September) (Oliveira *et al.,* 2018). The São Francisco River Basin is located between geographical coordinates of 7.0°-21.0°S and 35.0°-47.7°W, has an area of 638,576 km^2 and an extension of 2,860 km. This river has its origins in the Canastra National Park and

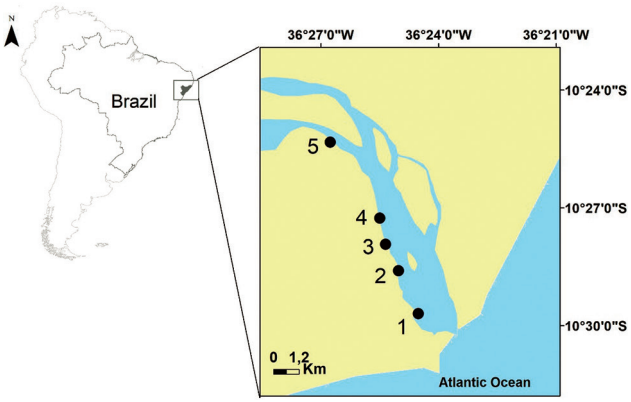

Figure 1. Sampling sites in the lower São Francisco River, State of Sergipe, Brazil.

the outfall is on the Atlantic Ocean. The flow average annual of 2,850 m^3.s^{-1}, ranging from 1,077 to 5,290 m^3.s^{-1} (Bezerra *et al.,* 2019).

Sample processing

The specimens of *Centropomus undecimalis* and *C. ensiferus* was sampled monthly, between May 2017 and April 2018, at five sampling sites distributed between the mouth of the São Francisco River and the municipality of Brejo Grande (Fig. 1), in the region of lower São Francisco River (10°28′34.02″S, 36°24′27.02″W). For collection, a beach seine (30 m long, 2.8 m high, and 5 mm mesh between opposite knots) was used. Subsequently, the caught fish individuals were refrigerated, identified to the species taxonomic level using specialized literature (Figueiredo & Menezes, 1980), measured (total length TL; 0.01 cm and standard-length SL; 0.01 cm), weighed (total weight TW; 0.1 g). The sagitta otoliths were extracted, packed in identified plastic bags and subsequently photographed.

Otolith contour analysis

The right otolith was photographed and the otolith length (OL, in mm), height (OH, in millimeters) and area of the sulcus acusticus (AS, in mm^2) were measured through these images (Fig. 2A). The classification of the

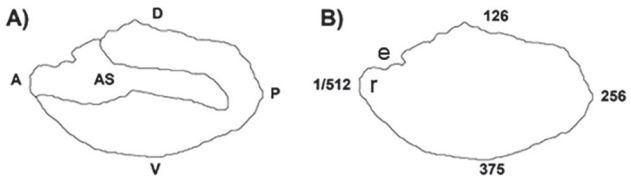

Figure 2. (A) Schematic drawing of the sagitta otolith of *Centropomus undecimalis* and *Centropomus ensiferus* caught in the lower São Francisco River, Sergipe, Brazil; (B) Contour of the otolith using 512 equidistant coordinates in the *sagitta* otolith; Abbreviations: A = anterior region, D = dorsal region, V = ventral region, P = posterior part of the otolith, AS = *sulcus acusticus,* r = *rostrum* and e = *excisura.*

Table 1. Mean and standard deviation of fish total length (TL), standard-length (SL) and otolith length (OL), height (OH) and areas of the *sulcus acusticus* (AS) of *Centropomus ensiferus* and *Centropomus undecimalis* by location and "n" number of specimens analyzed in Sergipe (SE), northeastern Brazil.

Species	n	TL (cm)	SL (cm)	OL (mm)	OH (mm)	AS (mm^2)	OH/OL*100
C. ensiferus	28	7.6 ± 1.69	6.0 ± 1.28	3.5 ± 0.71	2.2 ± 0.40	1.7 ± 0.62	63.1 ± 3.16
C. undecimalis	24	6.9 ± 1.85	5.8 ± 1.94	3.0 ± 0.61	2.0 ± 0.77	1.2 ± 0.40	62.1 ± 5.38

Table 2. Reclassification of *sagittae* otolith of *Centropomus ensiferus* and *Centropomus undecimalis* between those collected in the northeastern (Sergipe, SE) by the linear discriminant analysis (LDA).

Species	*Centropomus ensiferus*	*Centropomus undecimalis*	Total
Centropomus ensiferus	27 (92.3%)	1(7.7%)	28
Centropomus undecimalis	3 (12.5%)	21 (87.5%)	24
Total	30	22	52

otolith shape was performed according to Tuset *et al.* (2008) and Brenha-Nunes *et al.* (2016).

The wavelet function was used to define the otolith contour (Parisi-Baradad *et al.,* 2010; Sadighzadeh *et al.,* 2014) (Fig. 2B). The wavelet is the result of the expansion of a signal in a family of functions representing expansions and translations of a mother function, *i.e.:* $\Psi s(x) = 1/s\Psi(\varphi/s)$, where Ψ is a function with local support in a limited amplitude on the abscissa axis; φ lower the step filter; s is a scale parameter (Mallat, 1991). A total of 512 points, with equidistant coordinates from each otolith, were extracted with the rostrum as the contour origin (Parisi-Baradad *et al.,* 2010). The fourth and fifth wavelet are more appropriate for identifying stocks or populations, as they describe the contour of the otoliths more sensitively (Sadighzadeh *et al.,* 2014). The image processing was performed using AFORO (http://aforo.cmima.csic.es) (Parisi-Baradad *et al.,* 2010).

A Principal Component Analysis (PCA), based on the variance-covariance matrix, was applied to reduce wavelet functions without losing information (Tuset *et al.,* 2015). Principal components (PCs) explaining data variability were selected by the Broken-stick method (Gauldie & Crampton, 2002). Subsequently, the effect of the allometry of fish size was removed using the residuals of the linear regression between the significant principal components and the otolith length. From these, a new PCA was run (Stransky & MacLellan, 2005) to check for variations in the otolith contour for each species. The allometry was testes and no significance difference was found. A Linear Discriminant Analysis (LDA) was applied between sites to verify the correct percentage of otolith reclassification. A multivariate analysis of variance (MANOVA) was performed with the length and height of otoliths and the PC without the effect of allometry, to check for differences in the shape of otoliths collected in those three states. All statistical analyses were performed using the Past software version 4.03 (Hammer *et al.,* 2001).

RESULTS

A total of fifty-two otoliths of *C. ensiferus* (n = 28) and *C. undecimalis* (n = 24) from Sergipe were analyzed (Ta-

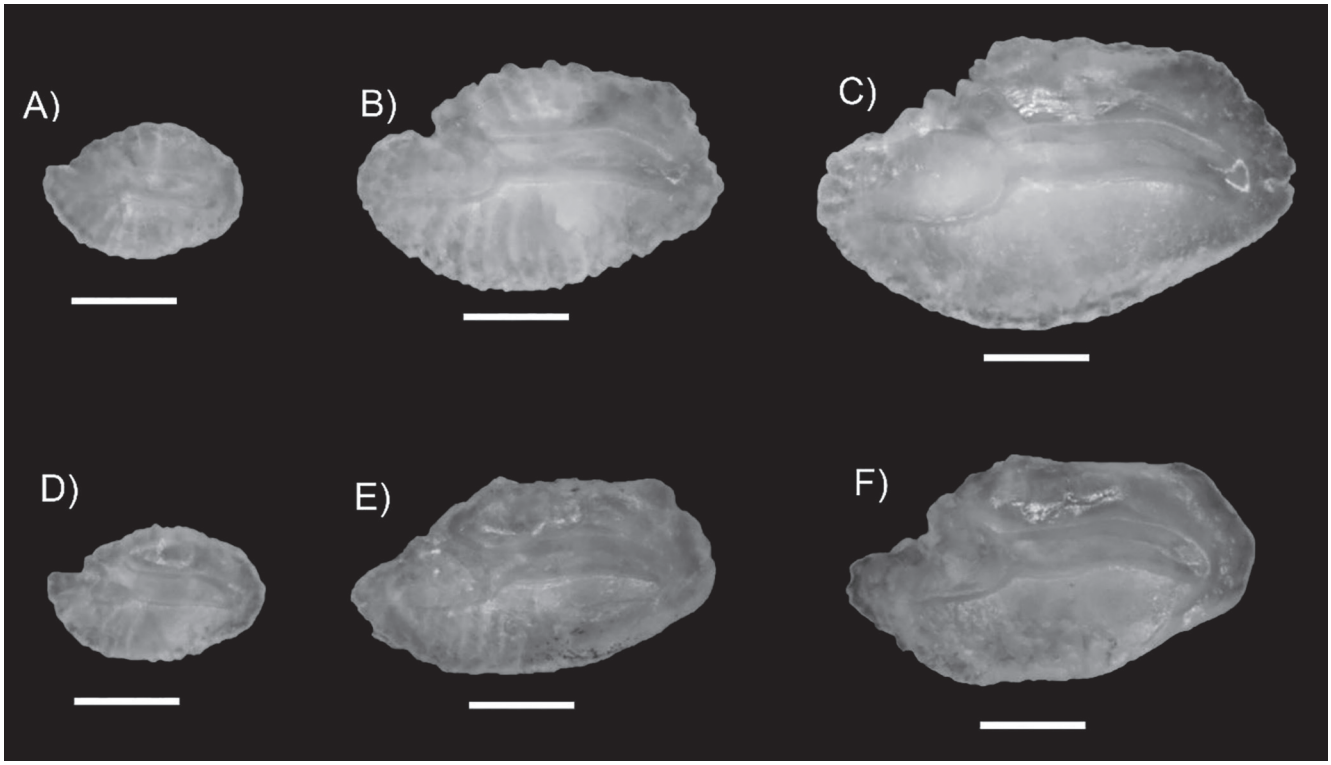

Figure 3. Rigth sagitta otoliths *Centropomus ensiferus* (A) 3.9 cm, (B) 7.6 cm, (C) 9.8 cm and *Centropomus undecimalus* (D) 3.9 cm, (E) 7.5 cm, (F) 9.9 cm caught in the lower São Francisco River, Sergipe, Brazil. Scale bars = 1 mm.

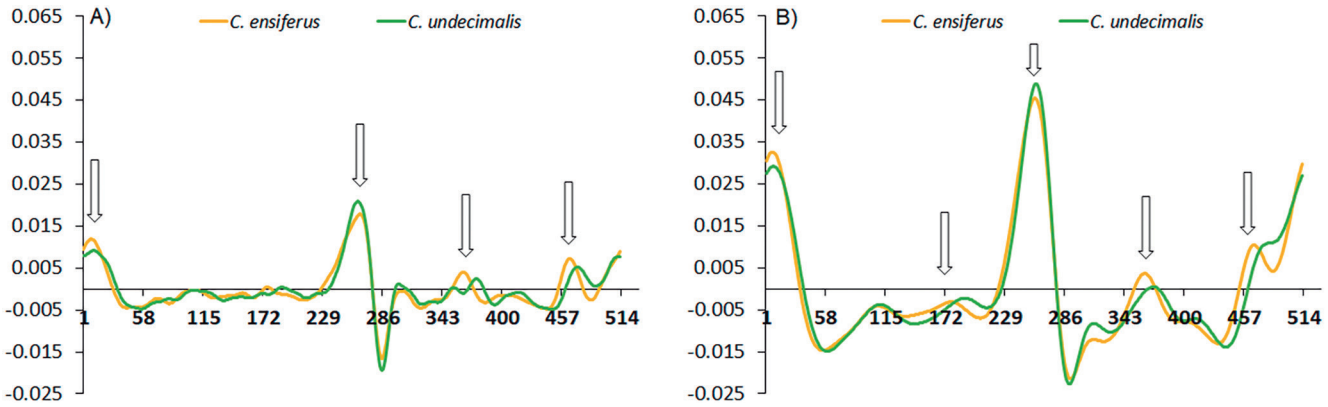

Figure 4. Contour decomposition of the *sagittae* otolith of *Centropomus ensiferus* and *Centropomus undecimalis* collected in Sergipe (SE), northeastern Brazil: (A) Wavelet 4 and (B) Wavelet 5.

ble 1). OL (U = 191,5; p < 0.05), OH (U = 183; p < 0.05) and AS (U = 174; p < 0.05) showed significant variations between species. TL (U = 250; p > 0.05), SL (U = 353; p > 0.05) and OH/OL*100 (U = 309; p > 0,05) did not show significant variations between species.

Otoliths of *C. ensiferus* and *C. undecimalis* presented some morphological variations. *Centropomus ensiferus* have a trapezoidal shape, rostrum and excisura (Fig. 3A and B), specimens above TL 7.5 cm present closed excisura (Fig. 3C). *Centropomus undecimalis* have an elliptic shape, rostrum and excisura presented in all specimens with length less than 7 cm (Fig. 3D).

The reconstruction of the otolith contour using wavelets 4 and 5 showed variability (Fig. 4). The wavelet 4 showed greater variation between the species in the anterior *rostrum* (1-10), posterior (229-286), ventroposterior (290-400), ventroanterior (457-512) (Fig. 4 A). Wavelet 5 presented variation between the species in the anterior *rostrum* (1-10), dorsal (115-220 posterior (229-286), ventroposterior (290-400), ventroanterior (457-512) (Fig. 4B).

The PCA showed high variability in the otolith shape (Fig. 5). PC1 explained 52% and PC2 explained 44% of the otolith shape variability. Along PC1 are distributed more rounded otoliths mainly specimens *C. ensiferus* and on PC2 are distributed more elongated otoliths, with rostrum and excisura mainly specimens *C. undecimalis* (Fig. 5).

The LDA showed correct reclassification of 92.3% of all otoliths among species. *Centropomus ensiferus* otoliths showed the highest reclassification percentage (96.42%) and *C. undecimalis* otoliths showed a smaller percentage of reclassification (87.5%) (Table 2). MANOVA evidenced a significant difference in otolith shape among species (F = 3.73; p < 0.0001).

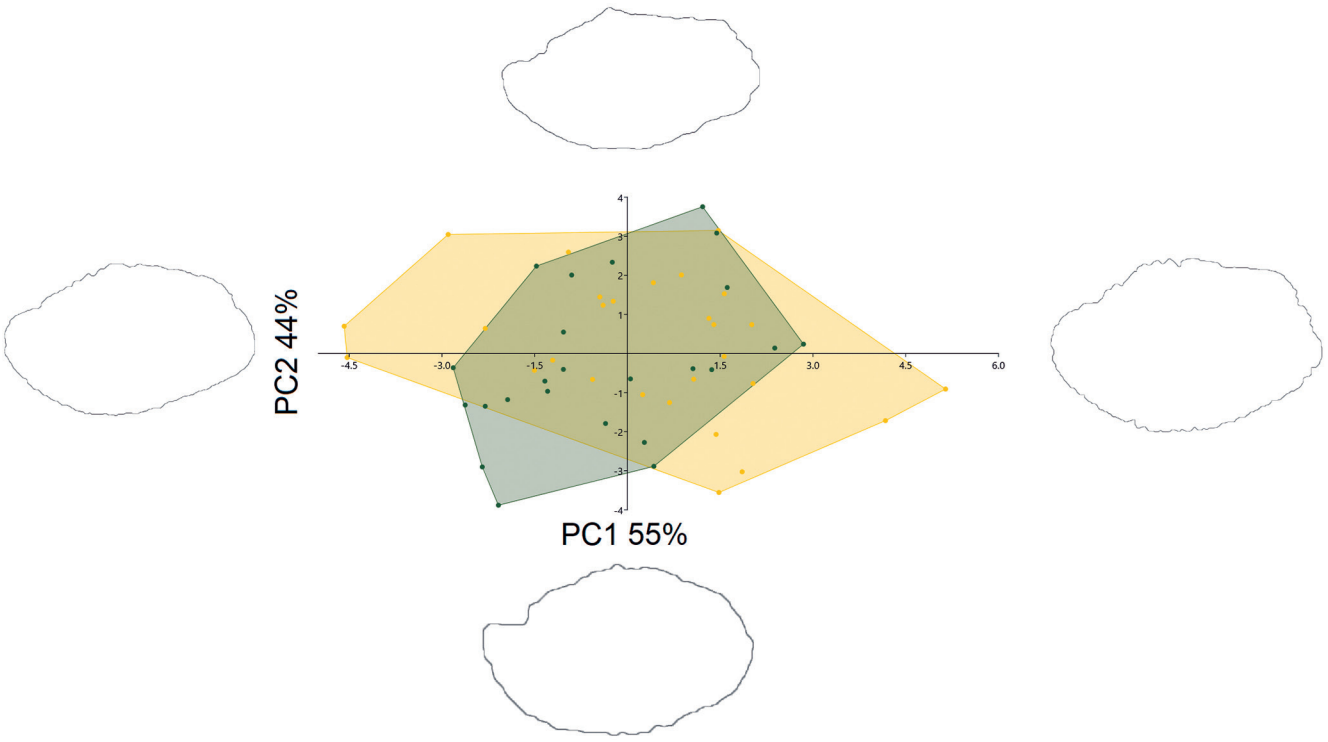

Figure 5. Principal Component Analysis (PCA) scatterplot for the *sagittae* otolith contour of *Centropomus ensiferus* (dots yellow) and *Centropomus undecimalis* (dots green) collected in Sergipe (SE), northeastern Brazil.

DISCUSSION

In this study, we examined the morphometric variation and contour of the *sagittae* otolith from juveniles specimens of two species from the genus *Centropomus* (*C. undecimalis* and *C. ensiferus*). The species showed otolith shape that varied from elliptical to trapezoidal, rostrum and excisura present as a characteristic diagnostic trait for the genus *Centropomus* (Brenha-Nunes *et al.,* 2016; Bot Neto *et al.,* 2020; Granados-Amores *et al.,* 2020). The areas of the *sulcus acusticus* showed significant variations between species, juveniles *C. ensiferus* showed a larger area of the *sulcus acusticus* indicating greater auditory sensitivity compared to *C. undecimalis* juveniles. This difference suggests that in the early-stage *C. ensiferus* can adapt to environments with greater turbidity due to its better hearing ability, whereas *C. undecimalis* would use environments with less turbidity, avoiding competition between species in the initial stages. Through otolith morphometry, it was possible to identify patterns of habitat use in relation to turbidity as saw before for *Menticirrhus americanus* (Carvalho *et al.,* 2020). In future studies of trophic ecology, this difference in the area of sulcus acusticus could allow differentiation between *C. ensiferus* and *C. undecimalis* when they were the prey, as evidenced in other species (Carvalho *et al.,* 2019; Assis *et al.,* 2020). Species adapted to environments with elevated turbidity present a greater heating ability compared to species in regions with lower turbidity, major otoliths are usually correlated with species greater hearing ability for examples species of Sciaenidae family (Verocai, *et al.,* 2023).

According to Volpedo & Echeverria (2003), values in the aspect ratio (OH/OL*100) between 41 and 67 indicate species associated with unconsolidated substrates (composed of silt, clay and sand) and between 35-50 indicate pelagic species. The present study demonstrates that the aspect ratio of juveniles of *C. ensiferus* and *C. undecimalis* fits the definitions of Volpedo & Echeverría (2003). Analyzing larger specimens of *C. undecimalis* (LT between 14.0 and 38.0 cm), the aspect ratio presents lower values than the juveniles analyzed in the present study (OH/OL*100 between 51.66-57.46; Brenha-Nunes *et al.,* 2016). This value of aspect ratio indicates that there is no variation in habitat use between juveniles and adults the *C. undecimalis,* in both life stages *C. undecimalis* uses habitats with unconsolidated sediment and frequents the water column due to the presence of rostrum in juveniles and adults (Bot Neto *et al.,* 2020). For *C. ensiferus,* it is not possible to make correlations of habitat use patterns between juveniles and adults due to the lack of studies otoliths of adults individuals.

Wavelet analysis is more sensitive to small contour variations, than otolith morphometry and aspect ratio. In this study, it was possible to verify greater variation in the contour of the rostrum between the two species analyzed by the Wavelet method. The rostrum is more developed in pelagic species, it also presents a great ontogenetic variation and is correlated with the migratory capacity of several species (Volpedo & Echeverría, 2003; Volpedo *et al.,* 2008; Jaramillo *et al.,* 2014). *Centropomus undecimalis* presents more developed rostrum in the otolith analyzed by wavelet in the relation *C. ensiferus* (Fig. 5), this characteristic suggests that juveniles of *C. undecimalis* in the Brazilian northeast coast remains longer in the water column and probably in less turbid water. The analysis of the contour and morphometry of otolith shape is an important tool which helped to suggests the pattern use habitat within the species of the family Centropomidae along the Brazilian coast. We further recommend studies involving the otolith chemistry and genetics to assess possible migration between the populations that inhabit rivers and/or estuaries.

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