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Research Article

Age, growth, and ontogenetic variation in the *sagitta* otolith of *Opsanus beta* (Goode & Bean, 1880), a non-native species in a wetland of international importance

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ABSTRACT. Invasive species are among the most important problems for biodiversity conservation worldwide, particularly in megadiverse countries such as Brazil. However, there is no biological information to develop policies for managing invasive species populations in many cases. The life history parameters and otolith variations are essential to understanding the adaptations of the species introduced in marine environments. This study aimed to identify the age structure and ontogenetic variation in the *sagitta* otolith of *Opsanus beta*. Fish samples were obtained monthly in the Paranaguá Estuarine Complex (PEC), south of Brazil. The shape indices verified ontogenetic variations in the otoliths (PERMANOVA; $F = 110$; $P < 0.0001$), but no sexual variations were observed in the shape of the otoliths (PERMANOVA; $F = 3.65$; $P > 0.05$). Specimen aged between 1 to 9 years were observed in the PEC, with the highest occurrence of individuals between 3 and 6 years (78%). No sexual differences were observed between the ages by the Kimura test. Our results confirm that the *O. beta* population is well established with age groups similar to that observed in the region where the species is native.

Keywords: *Opsanus beta*; Batrachoididae; alien species; life history; Paranaguá Estuarine Complex; Brazil

INTRODUCTION

The introduction of species into marine ecosystems is facilitated by various human activities, such as aquaculture, sport fishing, transport of platforms, and ballast water (Ojaveer et al. 2018, Vitule et al. 2019). The species is considered non-native when introduced outside its original geographic range (Bax et al. 2001). Invasive species significantly impact areas when they establish as a viable population (Jägerbrand et al. 2019) and adapt their bioecology to the new environment (Wonham et al. 2000, Olenin et al. 2017). The knowledge of individual and population parameters (e.g. age, growth, reproduction) of the non-native species help in understanding the establishment and the impact phases of the bioinvasion process in the novel ecosystem and has planned control measures (Curtis et al. 2017, Mizrahi et al. 2017, Fogg et al. 2019).

In Brazil, the number of marine bioinvasions of several taxa has increased (Ferreira et al. 2009, 2015, Bumbeer & Rocha 2016, Miranda et al. 2018), and among these, invasive non-native marine fish increased

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in the last two decades (Gerhardinger et al. 2006, Lasso-Alcalá et al. 2011, Tomás et al. 2012, Contente et al. 2015, Ferreira et al. 2015, Côa et al. 2017, Soeth et al. 2018, Adelir-Alves et al. 2019). For example, *Opsanus beta*, an endemic species of the Gulf of Mexico, was first observed on the southwestern Atlantic coast in the 2000s (Caires et al. 2007). This species resides in estuarine habitats, has cryptic and sedentary behavior, although it can also make short-scale migrations (Collette 2002, Greenfield et al. 2008). In the Gulf of Mexico (GM), this species is an opportunistic generalist that feeds on mollusks, crustaceans, and fish (López et al. 2017). *O. beta* is classified as a batch spawner. Their spawning was recorded once a year (March) in Florida (Malca et al. 2009) and between November and January in Mexico (López et al. 2017), presenting a short life cycle reaching up to 6 years of age in its natural distribution area (Malca et al. 2009, López et al. 2017).

In the Southwestern Atlantic Ocean, the first records of *O. beta* were evaluated in port regions, around Guanabara Bay (GB, 22°49'S, 43°10'W), Santos Bay (SB, 23°30'S, 46°30'W) and in the Paranaguá Estuarine Complex (PEC, 25°30'S, 48°25'W) (Caires et al. 2007, Tomás et al. 2012, Cordeiro et al. 2020). Recently, new records of the *O. beta* occurred in the Guaratuba Bay (25°51.8'S, 48°38.2'W) site that does not have large-scale ports and oil terminals (Carvalho et al. 2020). Despite the potential impact of the species on native populations, few studies have been carried out to evaluate the populations of *O. beta* on the Brazilian coast (Caires et al. 2007, Tomás et al. 2012). In this sense, this study aims to analyze the (age and growth) pattern in the PEC and compare the results with the GM native population. Complementarily, we will describe the ontogenetic variation of the *O. beta* otoliths for future trophic ecology studies.

MATERIALS AND METHODS

Ethical statement

The capture was fulfilled 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 69400.

Study sites

Samples for *Opsanus beta* were obtained monthly between September 2011 and March 2012 in the PEC, south of Brazil (25°28'39.68"S, 48°32'41.15"W; Fig. 1). This estuary is an important 601 km^2 ecosystem in the South Atlantic Ocean, with a humid subtropical climate. The PEC is divided into north-south and east-west axes (Lana et al. 2001). With the largest rivers, Cachoeira and Nhundiaquara, on the east-west ax. In the more rainy periods, the estuary flow is 28×10^6 m^3 d^{-1} (Marone et al. 2005).

The estuary hydrodynamics is determined by the flow of the rivers and the tidal waves (Noernberg et al. 2007). The tidal is semidiurnal with amplitudes between 0.5 and 2 m; the salinity and water temperature vary seasonally. In the summer occurred the lowest salinities and the highest temperatures (12-29 and 23-30°C), and in the winter, the highest salinity and the lowest temperatures (20-34 and 18-25°C) (Lana et al. 2001). The PEC is one of the largest in the Americas and bordered by the Atlantic rainforest ridge, and at current is considered is 'biosphere reserve' by UNESCO in 1995. About 16 conservation units are registered in this region. This site has been included in the Ramsar's list of wetlands of international importance since the year 2000. In contrast to its ecological importance, the biggest port for grain export in South America (Marone et al. 2005), the ports of Paranaguá and Antonina operate in this region.

Sample collection

The sample of this study occurred in the axes west-east of the PEC. Collections were carried out monthly using five longlines near rocky shores, and each longline contained 100 number 10 hooks with a total length of 100 m; the longlines remained in the water for 24 h.

After the capture, they were packaged on ice. In the laboratory, individuals were measured for total length (TL, in cm) and weighed (TW, in g), and the sex was characterized macroscopically based on Vazzoler (1996). *Sagittae* otoliths were extracted, cleaned, and stored in the Eppendorf. After, the samples were separated into six interval classes of the individuals: 1) 05-10; 2) 10.1-15; 3) 15.1-20; 4) 20.1-25; 5) 25.1-30; and 6) 30.1-35 cm.

Length-weight relationships

The length-weight relationships (LWR) were adjusted through the equation $TW = a\ TL^b$ (Huxley 1929), where a is the proportionality coefficient, and b is the allometric coefficient (Carvalho et al. 2017, Possamai et al. 2020).

Morphometry of otolith

All right otolith was photographed, and the internal face was classified according to Tuset et al. (2008). In this classification, the otolith shape is characterized by *sulcus acusticus* types, absence or presence of *rostrum* and excisura, and anterior and posterior otolith type re-

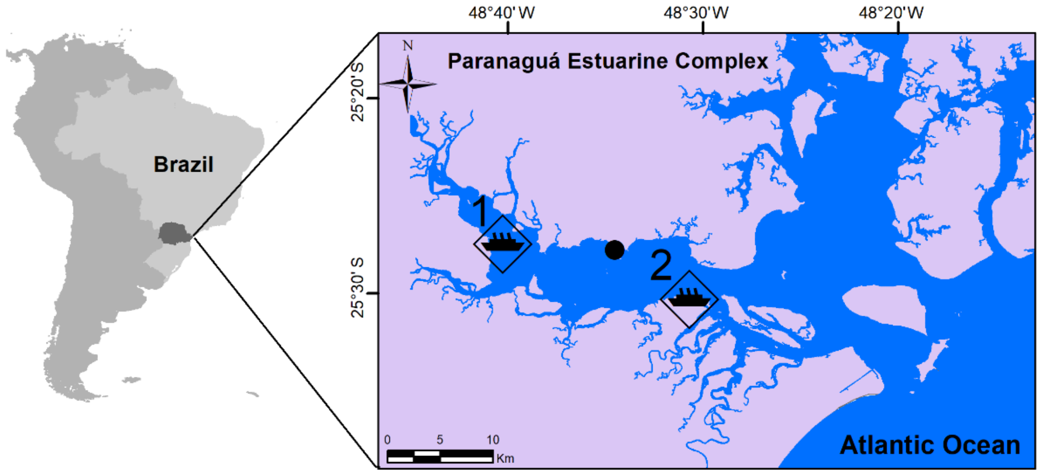

Figure 1. Sampling sites of *Opsanus beta* in the Paranaguá Estuarine Complex in the Subtropical Southwestern Atlantic Ocean. The black circle is the site of the sample, and the ship is the position of the Antonina (1) and Paranaguá Port (2).

gions. Measurements of the right otoliths were taken using the ImageJ program, namely: length (OL, greater longitudinal distance in mm) and height (OH, greater perpendicular distance in mm) and area (AO) of the otolith, area (mm^2) of the *sulcus acusticus* (AS) and otolith perimeter (PeS, in mm) (Fig. 2).

To describe the sexual and ontogenetic variation the following shape indices were used: OL/TL and OH/OL% aspect ratios (Volpedo & Echeverría 2003), form factor [FF = (4π × A) PeS-1] (Tuset et al. 2003), rectangularity [Rc = (AO / (OL × OH)] (Carvalho et al. 2020), ellipticity [E = (OL – OH / OL + OH)] (Tuset et al. 2003, Volpedo & Vas-dos Santos 2015) and relative surface of the *sulcus acusticus* [Rss = AS / AO] (Lombarte & Lleonart 1993). The size effect of all individual data was removed by normalization, according to Lombarte & Lleonart (1993). For this, the following equation was applied $y' = y\ (x_0 / x)^b$, where: y' is the normalized variable, y is the raw value of the data, x_0 is the reference value ($x_0 = 6.6$ cm; b = 3.21). To avoid the influence of the physiological processes of the first maturation, in the growth differences between sexes and ontogenetic variation in morphometric indices, only adults were used, grouped in six size classes 15.1-20; 20.1-25; 25.1-30, and 30.2-35 cm.

Age and growth

The otoliths were extracted from 12.0 to 31.2 cm LT field-collected individuals. Sagittal otoliths were processed following standard protocols (Secor et al. 1991). Sagittal otoliths were embedded in epoxy resin, and then they were cross-sectioned with a low-speed metallographic saw, sanded, polished with alumina, and then sections were photographed. Annuli were counted as the opaque rings when viewed under transmitted light. Growth increments were recorded independently twice for each otolith sample (n = 110), with no reference to the previous reading and without information of fish total length. The otolith was removed from the sample if reads did not agree (Fig. 3).

The accuracy of reading and precision was verified by calculating the average percentage error (APE) and using the coefficient of variance (CV), expressed by the formula $CV = \frac{100}{n}\left[\sum_{i=1}^{n}\left(\frac{sdi}{\bar{a}i}\right)\right]$, where: *sdi* is the standard deviation of ages attributed to the individual I and Ii is the mean of readings (Beamish & Fournier 1981, Campana 2001). The Von Bertalanffy growth model (1938) was fitted to age-length relationship estimated for each and grouped sexes obtained by adjusting the mathematical expression: $TL = L_{\infty}\left[1 - e^{-k(t-t_0)}\right]$ where for both expressions: e is the Napierian logarithm; TL = total length (cm) at age t, L_{∞}= asymptotic length, k = instantaneous growth coefficient and t_0 = age at which fish have a theoretical length equal to zero (Ogle 2015). Application of the Kimura (1980) method indicated that von Bertalanffy's growth is sex-specific. Kimura's likelihood ratio test was used to test for significant differences between growth parameters (Haddon 2001).

Statistical analysis

The normality and homogeneity of the data were tested in the shape indices (Shapiro-Wilk: $P < 0.05$; Bartlett's test: $P < 0.05$). Only adults were used to verify sexual and ontogenetic variation (interval class: 15.1-20; 20.1-25; 25.1-30, and 30.2-35 cm) to avoid the influence of the first maturation physiological processes.

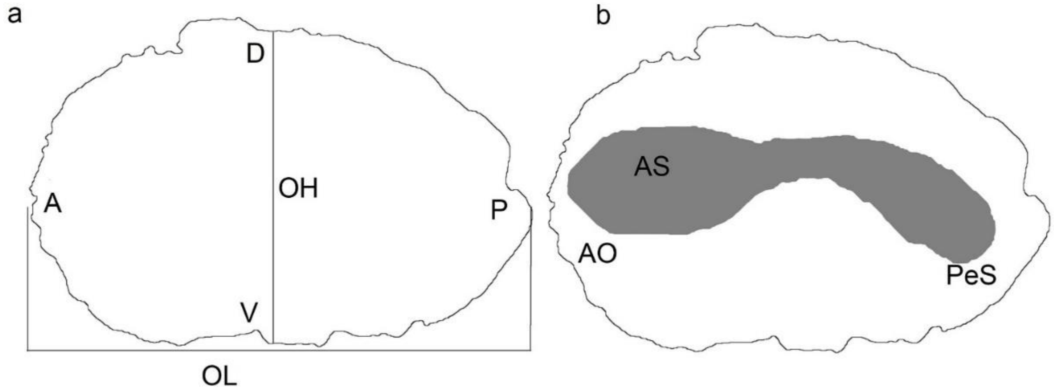

Figure 2. Scheme of linear morphometry applied to the right otolith of *Opsanus beta* collected in the Subtropical Southwestern Atlantic Ocean. a) A: anterior region, D: dorsal region, V: ventral region, P: posterior region, OL: maximum longitudinal length, OH: maximum perpendicular height. b) AO: area of the otolith and AS: area of the *sulcus acusticus* and PeS: perimeter of the *sulcus acusticus*.

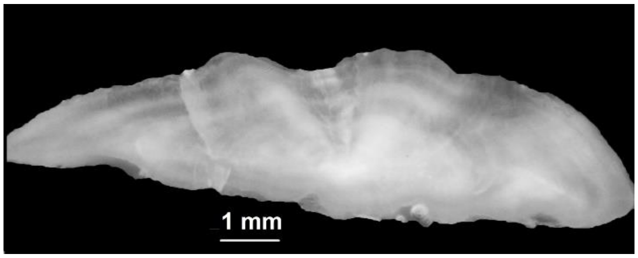

Figure 3. Cross-section of an *Opsanus beta* otolith (*sagitta*) female specimen TL = 25 cm, collected in the Paranaguá Estuarine Complex in the Subtropical South-western Atlantic Ocean.

We use the permutational analysis of variance (PERMANOVA) between the interval class and shape indices, and the Bonferroni test was used to identify between which intervals the interactions were significant ($P < 0.005$). The principal component analysis (PCA) was used to verify the shape variation between sexual and interval classes. Posteriorly, with the main components (PCs), a canonical variable analysis (CVA) was performed to verify the percentage of correct reclassification of otolith between interval class and sex (Linde et al. 2004). All statistical analyses were performed using the Past 3.25 (Hammer et al. 2013). The parameters of the von Bertalanffy curves for both sexes were compared using Kimura's likelihood ratio test (Haddon 2001).

RESULTS

Length-weight relationships

A plot of all *Opsanus beta* TL and TW data collected (n = 120; $TW = 0.0116\ TL^{3.1272}$; $R^2 = 0.987$) is shown (Fig. 4). While male specimens attained larger sizes than females, the difference between sexes in their TL and TW relationships was not statistically significant (ANCOVA, n = 121, $P > 0.05$). LWR showed that the species has positive allometric growth. Immature specimens in the interval class 5-10 and 10.1-15.0 cm prevailed while females predominated the smaller classes (<20 cm), males were more representative in the larger sizes (>20 cm) (Fig. 4b).

Ontogeny and sexual variation

In total, 121 otoliths of the *O. beta* (TL mean: 21.86 ± 5.62 cm; TW mean: 217.60 ± 135.25 g) (Fig. 4a) were analyzed. The morphological classification of the otolith showed that along the ontogeny constant characteristics were the shape of otolith elliptic and heterosulcoid *sulcus acusticus* (Fig. 5). The anterior and posterior type otolith regions demonstrated variations (Fig. 6). The interval class 20.1-25 and 25.1-30 cm presented four anterior type regions and three posterior regions (Figs. 6a-b). Table 1 shows the means and standard deviation of the indices per interval.

The data of the shape indices did not meet the assumptions of parametric analysis (Shapiro-Wilk $P < 0.05$ and Bartlett $P < 0.05$). PERMANOVA showed significant differences between shape indices and interval class (F = 110; $P < 0.0001$). The Bonferroni test showed significant differences between all interval class and shape indices otolith, except in the RSS where no significant differences occurred between 25.1-30 and 30.1-35 cm interval class ($P = 0.47$).

An elevated variation in the shape of the otoliths along this species' ontogeny was observed (Fig. 7). The PC1 explained the high variation of the otolith shape 96.93%; in the positive PC1 values, more elongated

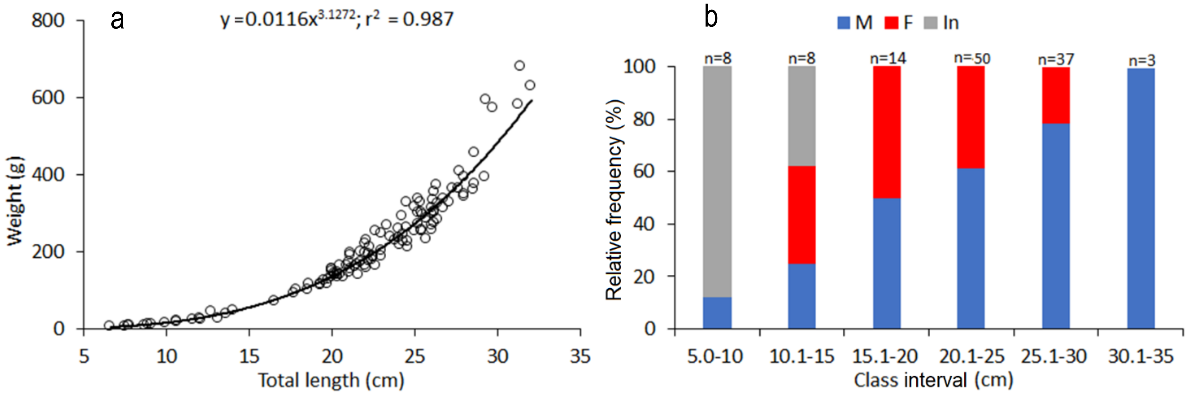

Figure 4. a) Length-weight relationships, b) distribution of sexual frequency per class interval of *Opsanus beta* in the Subtropical Southwestern Atlantic. M: males, F: females, Im: immatures.

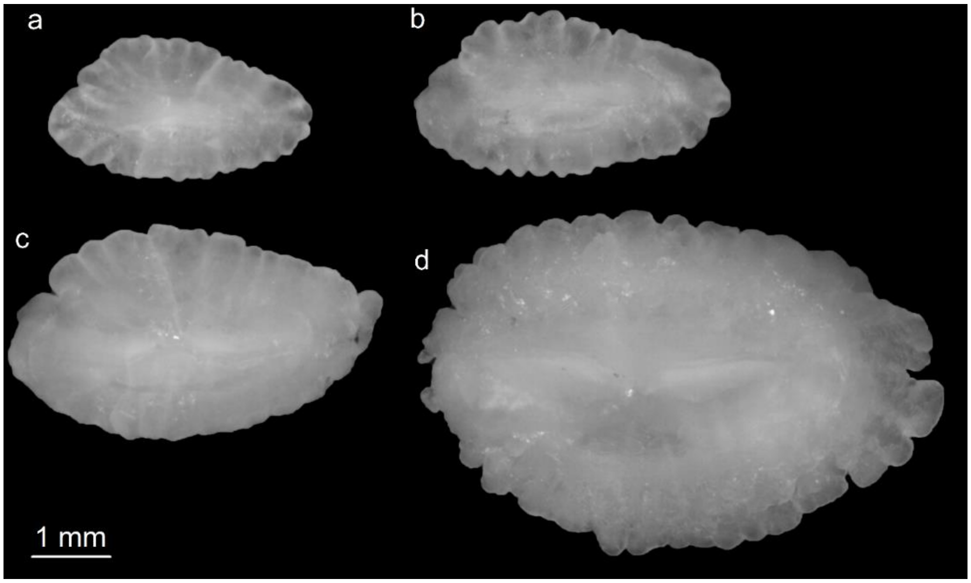

Figure 5. Photograph of the inner face of the right *sagitta* otolith of *Opsanus beta* throughout its ontogenetic development: a) 8.7 cm interval 5-10 cm, b) 10.5 cm interval 10.1-15 cm, c) 17.8 cm interval 15.1-20 cm, and d) 23.7 cm interval 20.1-25.

otoliths were distributed, with crenulate margins and associated peak posterior region 15.1-20 and 20.1-25 cm interval class. In the negative PC1, otoliths with entire margins and peak posterior region prevailed. The positive and negative PC2 identified otoliths with irregular margins, posterior region posterior type flatted and anterior region type peak, round, flatted, and double-round. The PERMANOVA marginally showed a significant difference between shape indices and sex (F = 3.65; *P* = 0.0615).

Age and growth

Increments deposited in the otolith varied in width and contrast, making it difficult to distinguish each annulus in all processed samples; thus, 11 otoliths were discarded when the readings did not agree. The specimens ranged in age from <1 to 9 years (n = 114; Table 2), with the highest occurrence of individuals between 3 and 6 years (78%).

The APE between readings was 6.06%, and CV was 8.57%. By adjusting the growth curve, different values of the growth parameters were observed for all specimens (including three young specimens and all adults) (n = 114; *VB* L_∞ = 24.52 cm; k = 0.38 and t_0 = -2.19) (Fig. 8a), females (n = 51; *VB* L_∞ =23.10 cm; k = 1.55 and t_0 = 0.25) (Fig. 8b) and males (n = 60; *VB* L_∞ = 26.11 cm; k = 0.42 and t_0 = -1.57) (Fig.8b). The growth trajectories for male and female *O. beta* appear

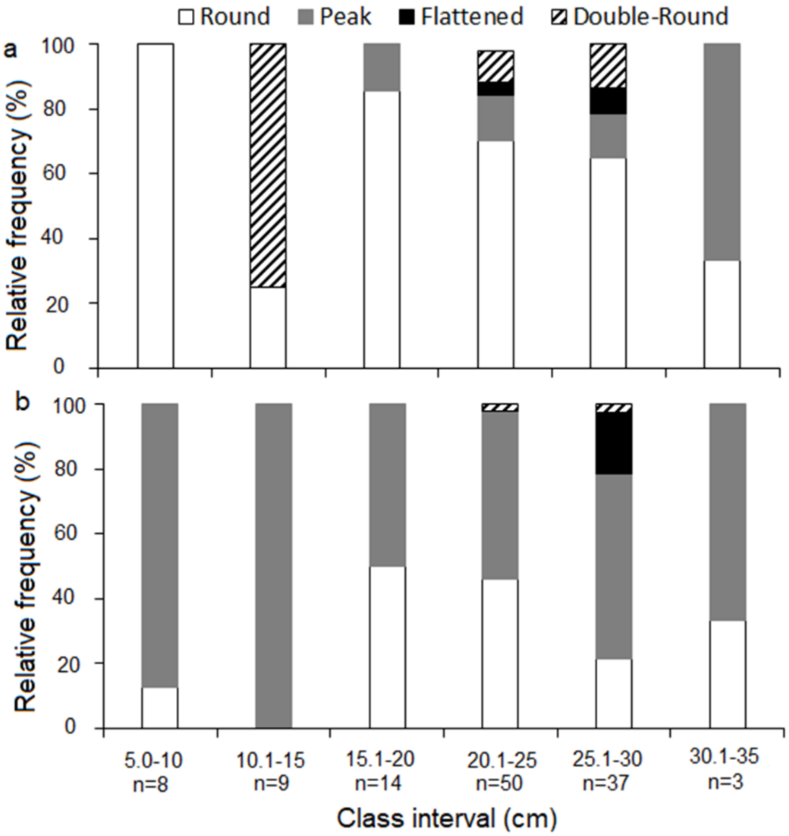

Figure 6. a) Frequency distribution of types anterior otolith regions, b) frequency distribution of types posterior otolith regions by interval class *Opsanus beta* Subtropical Southwestern Atlantic Ocean.

Table 1. Shape indices (mean ± standard deviation) by interval class of *Opsanus beta*. OL/TL: otolith length/total length aspect ratios, OH/OL%: otolith height/otolith length aspect ratios, FF: form factor, RC: rectangularity, E: ellipticity, and RSS: relative surface of the *sulcus acusticus*.

Class interval (cm)	OL/TL	OH/OL%	FF	Rc	E	RSS
15.1-20	0.012 ± 0.003	0.018 ± 0.004	0.028 ± 0.006	0.026 ± 0.005	0.28 ± 0.036	0.008 ± 0.002
20.1-25	0.007 ± 0.002	0.011 ± 0.002	0.017 ± 0.003	0.016 ± 0.003	0.205 ± 0.03	0.005 ± 0.001
25.1-30	0.004 ± 0.001	0.007 ± 0.001	0.009 ± 0.001	0.009 ± 0.001	0.138 ± 0.01	0.003 ± 0.0005
30.1-35	0.002 ±0.0002	0.004 ± 0.0005	0.006 ± 0.0007	0.005 ± 0.0006	0.096 ± 0.005	0.002 ± 0.0001

similar for 0-2 years; however, growth curves diverged past 2 years (Fig. 8a).

The Kimura test did not show significant differences in any of the hypotheses tested, demonstrating significant differences between males and females in the von Bertalanffy curve parameters (Table 3).

DISCUSSION

Opsanus beta showed similar positive allometric growth, in the present study and others, both in its area of natural distribution (GM; Malca et al. 2009, Abarca-Arenas et al. 2012) and in invaded areas (SB; Tomas et al. 2012) suggesting that the change in habitat and consequently the different environmental forces do not influence the growth pattern of this species.

Another similar characteristic observed between the PEC and Biscayne Bay populations (Florida, USA) was the male dominance in the largest size classes (>25 cm). Because the species produces large oocytes (>4 mm; Malca et al. 2009), causing females to invest more energy in the gonadosomatic growth, and consequently they grow less than males, a pattern observed in all locations where the species has been studied. Another explanation may be the territorial behavior in selecting

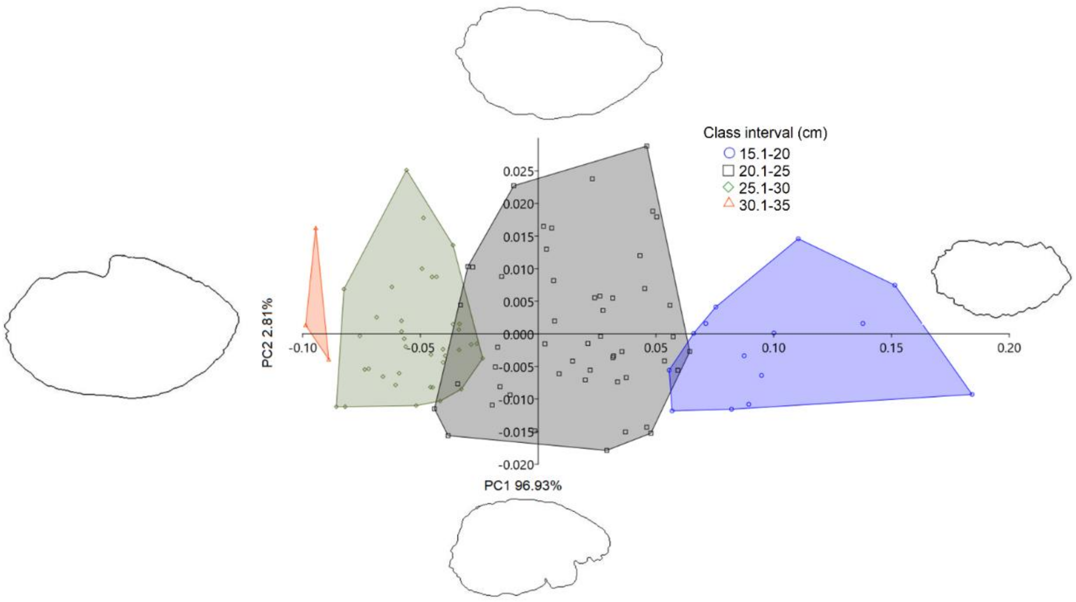

Figure 7. Scatterplot of the principal component analysis of the shape indices of the *sagitta* otolith of *Opsanus beta* along its ontogeny Subtropical Southwestern Atlantic.

Table 2. Age by size class in *Opsanus beta* collected monthly in a Subtropical Southwestern Atlantic Ocean.

Class interval (cm) / age (yr)	<1	1	2	3	4	5	6	7	8	9	Total
5-10	1										1
10.1-15		3	1								4
15.1-20		1		7	2	1					11
20.1-25		3	1	11	20	14	8	4	2	1	64
25.1-30					5	8	12	3	4	1	33
30.1-35					1						1
Total	1	7	2	18	28	23	20	7	6	2	114

larger and "stronger" males through boat whistle announcement calling behaviors. (Thorson & Fine 2002). Several studies demonstrate a correlation between ontogenetic variations in otolith shape and water column use patterns (Assis et al. 2020, Carvalho et al. 2020, Taylor et al. 2020). Otoliths of *O. beta* in the smallest specimens analyzed (size class interval 15.1-20 cm) had an elongated shape suggesting a longer stay in the water column. The largest specimens, rounded otoliths, signify lower mobility and swimming ability (size class interval 30.1-35 cm). These differences in the shape of *O. beta* otoliths may indicate different behavior patterns within the species. Young individuals have a greater swimming capacity until settlement, making adults sedentary and territorial (Thorson & Fine 2002) in agreement with what was proposed by Volpedo & Echeverría (2003), Carvalho et al. (2015), and Assis et al. (2020) for short-moving fish whose otoliths tend to be rounded.

Reducing intraspecific competition through the dispersal of young individuals by tidal currents or by coastal drift currents between estuaries may be one factor that has helped this species colonize the Brazilian coast, which is associated with the elongated form of juvenile fish otoliths. Adults have an elongated form typical of fish prowling in the bottom (Volpedo & Echeverría 2003, Jaramillo et al. 2014). Some marine species show sexual variation in the otolith shape (Leguá et al. 2013, Bose et al. 2016, Carvalho et al. 2020); however, this was not observed for *O. beta* in the PEC.

In this study, the *O. beta* population showed an age structure between <1 and 9 years in a recently colonized environment (around 20 years), around three years more than in previous studies in the GM (distribution of frequency; Serafy et al. 1997, otoliths; Malca et al. 2009). It can be explained due to the colonization and settlement process, favoring greater longevity in the

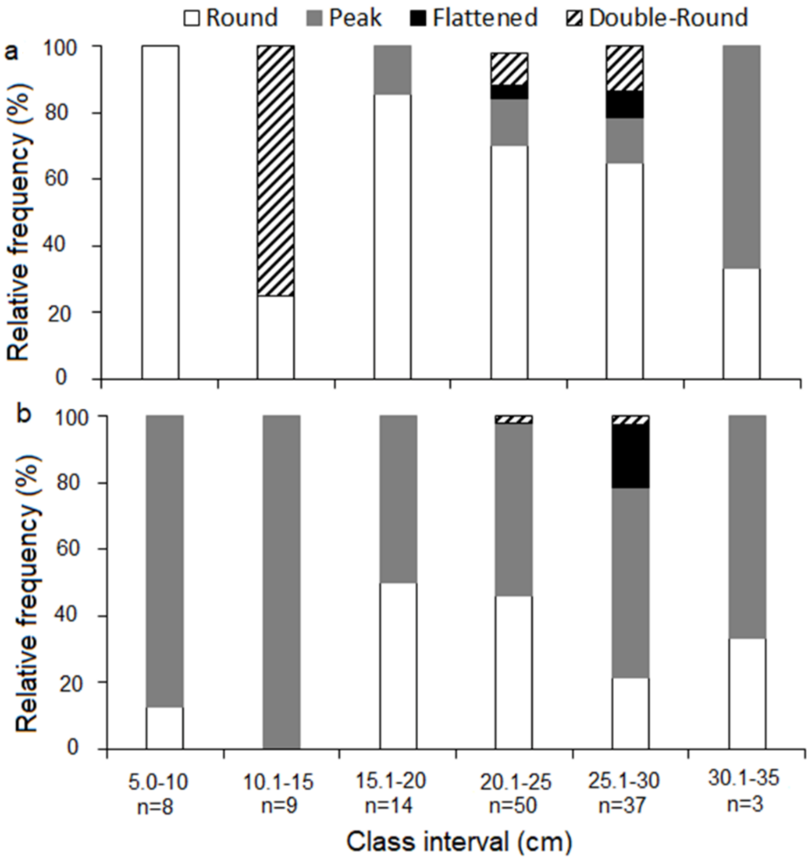

Figure 8. a) Frequency distribution of types anterior otolith regions, b) frequency distribution of types posterior otolith regions by interval class *Opsanus beta* Subtropical Southwestern Atlantic Ocean.

Table 3. Kimura test by sex otolith's readings of *Opsanus beta* from the Subtropical Southwestern Atlantic. χ^2: Chi-square test; $P < 0.05$, df: degrees of freedom, *P: P*-value, L_∞: asymptotic length, *k*: instantaneous growth rate, and t_0: age at which fish have a theoretical length equal to zero. H1: tests if L_∞ between male ($L_\infty 1$) and female ($L_\infty 2$) show significant differences ($P < 0.05$). H_2: tests if *k* between male (*k* 1) and female (*k* 2) show significant differences ($P < 0.05$). H_3: tests if $t_0$between male ($t_0 1$) and female ($t_0 2$) show significant differences ($P < 0.05$). H_4: tests all parameters between male ($L_\infty 1$, *k* 1 and $t_0 1$) and female ($L_\infty 2$, *k* 2 and $t_0 2$) show significant differences ($P < 0.05$).

Males and females				
Tests	Hypothesis	χ^2	df	*P*
H_1	$L_\infty 1 = L_\infty 2$	0.67	1	0.41
H_2	$k\,1 = k\,2$	0.00	1	1.00
H_3	$t_0 1 = t_0 2$	0.03	1	0.86
H_4	$L_\infty 1 = L_\infty 2$, $k\,1 = k\,2$, $t_0 1 = t_0 2$	7.10	3	0.07

invaded environment, initially favored by the great food supply and the absence of natural predators. Comparing the L_∞ values between native and non-native populations, we observed that in the GM (Malca et al. 2009), the species has higher values at younger ages (L_∞ = 39.38 cm; age = 7 year) compared to the PEC (L_∞ = 26.10 cm and age = 9 year). The different fishing efforts and pressure could explain it; however, the species is not seen as a fishing resource, regardless of its occurrence. Sexual variation in the von Bertalanffy model parameters was recorded in the GM by Malca et al. (2009); however, it was not observed in the present study. Malca et al. (2009) explained that differences in male and female growth patterns are probably linked to reproduction and the bioenergetic cost of oogenesis. Based on the assumption that colonization of *O. beta* in the PEC is recent, we believe that females have not reached their full reproductive potential in the PEC, which could be observed in the VB parameters.

Despite the growing record of non-native fish species in Brazilian estuarine and marine ecosystems, the parameters of the life history of these species are still neglected, hindering more effective control actions and strategies. This difficulty is important considering that Brazil is one of the megadiverse countries on the planet. We underscore that much attention has been paid to species just arriving, such as the lionfish, and that well-established and highly important species have been ignored. This fact is somewhat general for ecosystems, as shown by several recent studies (e.g. Watkins et al. 2021). Thus, we believe that the information available in this study can help to support the development of invasive species control plans in this important and vulnerable ecosystem. We encourage that campaigns to promote consumption, create a market and a new source of income for artisanal fishers, contribute to the control of *O. beta* populations, minimize the homogenization of biota, and compete with native species, along with other unknown impacts on the ecosystem.

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