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Reproduction and age of *Opsanus beta* (Goode & Bean, 1880) in a new environment

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ABSTRACT. *Opsanus beta* is endemic to the Gulf of Mexico and has recently been introduced to the Brazilian coast; probably the introduction is via ballast water and/or oil rigs. In this study, the reproduction and age of the species were recorded for the first time in Guaratuba Bay. The two specimens analyzed in this study were caught by sport fishermen. Sex and stage of gonadal maturation were histologically analyzed, and the age was determined by cross-sectioning the sagitta otoliths. The female showed 29 cm total length (TL) and 396 g total weight (TW), and the male showed 21 cm LT, 168 g WT, and 2 years of age. The gonadal histology demonstrated the female and male as capable of spawning. Despite the small sample, reproduction shows that there is an established population of *O. beta* in Guaratuba Bay.

Keywords: Alien species; Batrachoididae; Guaratuba Bay; subtropical region of Brazil.

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Introduction

Intraspecific biological parameters such as age, reproduction, and growth rate help understand habitat use, population growth, and the dispersion of a species (Vaz-dos-Santos, & Rossi-Wongtschowski, 2007; Soeth et al. 2018). The knowledge of individual and population parameters (e.g., age, growth, reproduction) of non-native species helps in understanding the establishment and the impact of the bioinvasion process on the novel environment and has planned control measures (Mizrahi, Chapman, Gough, Humber, & Anderson, 2017; Fogg et al. 2019; Carvalho, Freitas, Lapuch, Volpedo, & Vitule, 2022a). Non-native species are those occurring in an area far from their native distribution limit (Blackburn, Bellard, & Ricciard, 2019). These species are introduced from outside their original geographic distribution range by human activities, such as aquaculture, aquarism, sport fishing, offshore platform transportation, and ballast water (Watkins, Yan, Dunic, & Côté, 2021). Colonization of new environments depends on the species' ability to adapt to new environmental parameters, reproduction, and the dispersion of recruits in the recently colonized environment (Richardson et al. 2000; Wonham, Carlton, Smith, & College, 2000; Olenin, Gollasch, Lehtiniemi, Sapota, & Zaiko, 2017).

This is especially true for the south-southeastern coast of Brazil, where the records of non-native marine and/or estuarine fish species are increasing (Castro, Fileman, & Hall-Spencer, 2016). *Opsanus beta* (Goode & Bean 1880) belongs to the family Batrachoididae and is endemic to the Gulf of Mexico in estuarine habitats; it is a cryptic and sedentary species but can also make short-scale migrations (Collette 2002; Greenfiel, Winterbotton, & Collette, 2008). It is a polyphagic nocturnal predator, feeding mainly on fish, crustaceans, and mollusks (Yáñez-Arancibia, Lara-Domínguez, & Day Jr., 1993). Their spawning was recorded once a year in Florida (Malca, Barimo, Serafy, & Walsh, 2009) and between November and January in Mexico (Franco - López et al. 2017), with a short life cycle reaching up to 6 years of age in its natural distribution area (Malca et al. 2009, Franco - López et al. 2017). The first records of *O. beta* on the Brazilian coast occurred in Santos and Paranaguá Basy in the early 2000s. It is possible to affirm that this species is expanding its geographic distribution with recent records in Guanabara Bay, Sepetiba, Guaratuba, and Laguna (Caires, Pichler, Spach, & Ignácio, 2007; Tomás, Tutui, Fagundes, & Souza, 2012; Carvalho, Ferreira Junior, Fávaro, Artoni, & Vitule, 2020; Cordeiro, Bertoncini, Abrunhosa, Corona, Araújo, & Santos, 2020; Andrade-Tubino, Salgado, Uehara, Utsunomia, Araújo, 2021; Carvalho et al.

2022). *Opsanus beta* was probably introduced in Brazil by ballast water or aquarism; only two populations were recorded in an estuary without a port terminal (Guaratuba Bay, Paraná - Carvalho et al. 2020, and Laguna, Santa Catarina - Carvalho, Freitas, Lapuch, Volpedo, & Vitule, 2022b).

In the citizen science study carried out by Carvalho et al. (2022), six specimens of *O. beta* were caught in Guaratuba Bay; compared to the other Brazilian estuaries, it is a reduced number of individuals captured of this species (Tomás et al. 2012; Andrade-Tubino et al. 2021; Carvalho et al. 2022b). This fact raises hypotheses, does *O. beta* colonize Guaratuba Bay? Or is Guaratuba Bay under pressure from *O. beta* propagules originating from other nearby estuaries? One way to measure the colonization success of a species is to describe the reproductive parameters, longevity, and age of the specimens in the recently colonized environment (Carlton, 1996). In this context, this study aimed to describe the sex, maturation stage, and age of the largest specimens of *O. beta* caught in Guaratuba Bay

Material and Methods

Study area and sampling

Guaratuba Bay (25°51.8'S and 48°38.2'W) is located in the South-western Atlantic Ocean (Figure 1). It is a small estuary with a 50.19 km^2 area surrounded by mangroves associated with the cities of Matinhos and Guaratuba. This estuarine system has an average depth of 3 meters, with greater depths (27 m) at the mouth, temperature between 20 and 26°C, and salinity < 26 (Marone et al., 2006; Mizerkowski, Machado, Brandini, Nazario, & Bonfim, 2012).

Samples were caught by pole and line fishing in the two sites of Guaratuba Bay. Each sample was measured for total length (TL, cm) and total weight (TW, g). The gonads were removed and weighed (WG, g) and macroscopically examined to determine the sex and reproductive phase. Sagittal otoliths were removed, rinsed, and stored dry for later age determination.

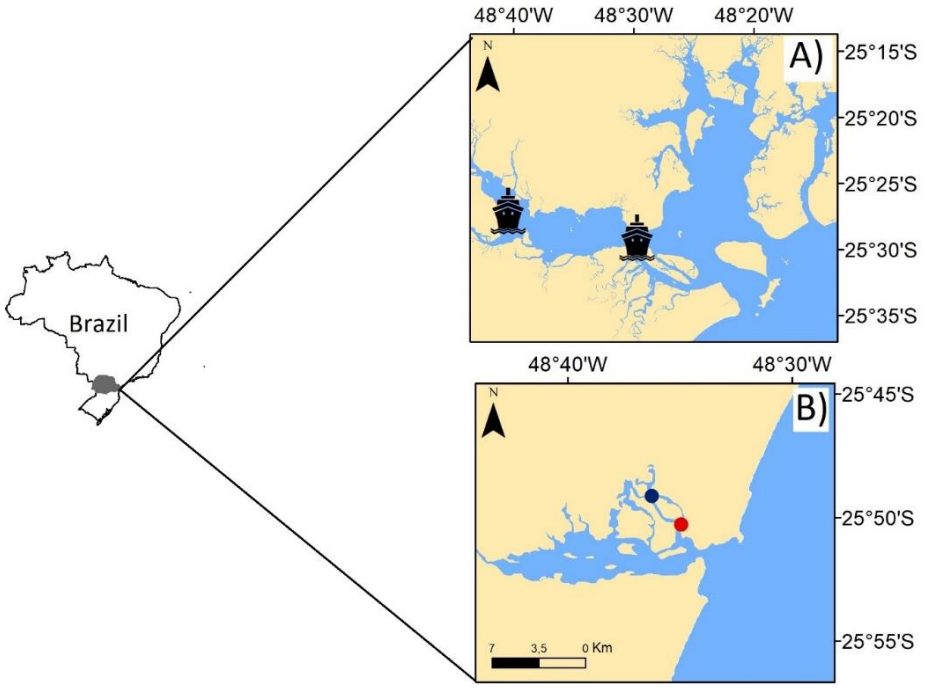

Figure 1. A) Paranaguá Estuarine Complex in the Subtropical Southwestern Atlantic Ocean and the ships indicate the Antonina (1) and Paranaguá Port (2). B) Sampling sites where *Opsanus beta* was caught in the Guaratuba Bay, male (blue circle) and female (red circle).

Age

The right sagittal otolith of each fish was embedded in epoxy resin, cross-sectioned using a low-speed metallographic saw, sanded, and polished with alumina. Subsequently, the sections were photographed under a polarized-light stereo microscope for reading the age rings of increments (each increment = one translucent plus one opaque zone; Figure 2). The otolith increment readings were taken for each otolith sample at different times by the same reader (Malca et al. 2009).

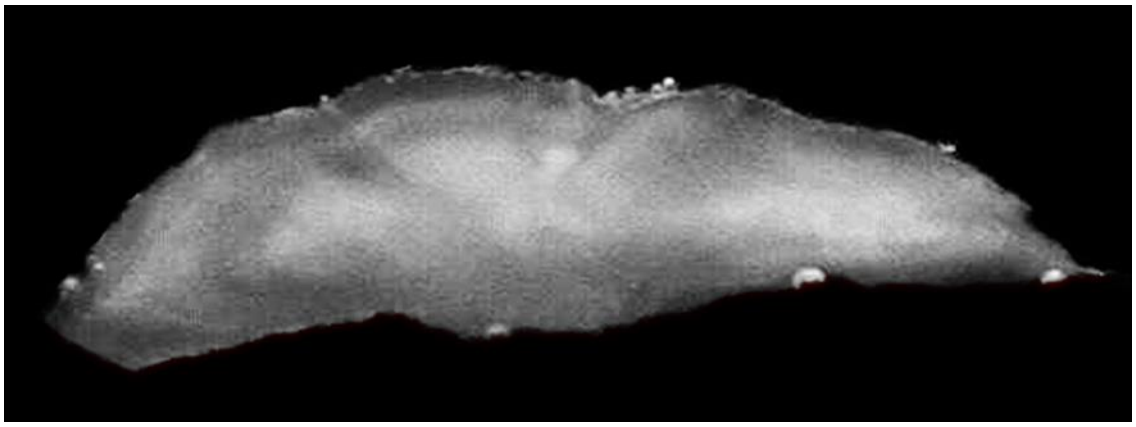

Figure 2. Cross-section of the otolith (*sagitta*) of *Opsanus beta* male specimen with TL = 21 cm collected in a subtropical environment of the South Atlantic.

Reproduction

Gonads remained in the preservative for 18 to 24 hours. Tissues were processed using a standard methodology for histological paraffin embedding and hematoxylin and eosin-Y staining. The maturity and reproductive phases of the histological samples were assessed according to a modified classification based on Vazzoler (1996) and Brown-Peterson, Wyanski, Saborido-Rey, Macewicz, and Lowerre-Barbieri, (2011). The gonadosomatic index (GSI), expressed by the formula GSI = (WG/TW)*100, was determined for each individual.

Results

Two specimens of *O. beta* were analyzed, one female and one male. The female showed LT = 29 cm, WT = 396 g, and GSI = 8.84, and the male showed LT = 21 cm, TW = 168 g, and GSI = 0.27. The age of the female was not determined because the otolith broke during sample processing. The age estimated by counting the rings of the male *O. beta* was two years of age.

The female and male were classified macroscopically and microscopically as spawning capable. The gonadal histology demonstrated the female with hyalinized ovarian follicles (Figure 3A) and the male with seminiferous tubules filled with sperm (Figure 3B).

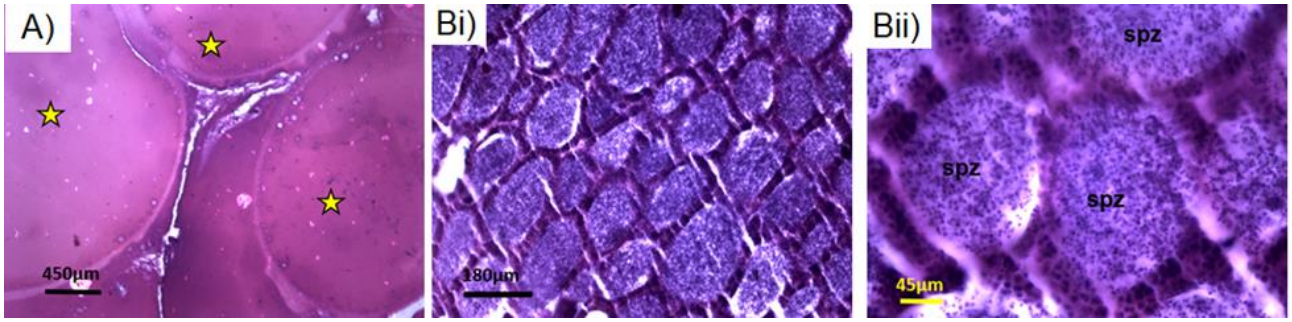

Figure 3. Microscopic analysis of ovaries and testes of *Opsanus beta* in a subtropical environment of the South Atlantic. A) spawning capable ovary - hyalinized ovarian follicles (yellow star); Bi) spawning capable testes; and Bii) seminiferous tubules practically replete with spermatozoa (spz).

Discussion

Our results showed that the analyzed *Opsanus beta* specimens were in reproductive activity with gonads at an advanced stage of development (able to reproduce). Also, through histological analysis of the ovary, hyalinized oocytes were evidenced, which occur close to spawning, indicating that the area where the specimen was caught is used as spawning grounds (Oliveira & Fávaro 2010). Our results corroborate the presence of an established population in Guaratuba Bay despite the low record of catches by sport fishermen compared to other estuaries on the Brazilian coast.

The low density and distribution of the *O. beta* population in Guaratuba Bay compared to other regions of the Brazilian coast may indicate a recent establishment of the population in the referred estuary (Tomás et al.

2012; Cordeiro et al. 2020; Andrade-Tubino et al. 2021; Carvalho et al. 2022a; Carvalho et al. 2022b). Differently from other Brazilian estuaries with the record of this species, Guaratuba Bay does not have port terminals, which indicates that the routes of introduction of this species are not directly related to ballast water or fouling on ships and oil platforms. The lower density of specimens caught by sport fishermen may indicate that the pressure of propagules in this environment is reduced concerning estuaries with port terminals that constantly receive new individuals and rapidly increase their populations.

Opsanus beta is a territorial species; males stay in the nests after fertilization of the adherent eggs until larvae hatch out and can keep the offspring in their mouths for weeks (Robins, & Ray, 1986). However, it is not yet known whether the female remains close to the nest or migrates to deeper regions after spawning. In places with higher population density, it is possible to observe no differentiation in habitat use between males and females (Tomás et al. 2012; Almeida-Tubino et al. 2021; Carvalho et al. 2022a). In Guaratuba Bay, the male and female were caught in different regions, which does not allow us to conclude if there is a separation of habitats after reproduction or if this distribution occurs due to the possible lower population density of *O. beta* in this environment.

In a natural distribution area, *O. beta* reaches up to 10 years of age (Malca et al. 2009), while in Guaratuba Bay, the age of 2 years was determined for the male specimen of 21 cm. However, the relationship between age and length was similar between the natural distribution site and new environments used by the species. such as the Guaratuba Bay (present study) and the Paranaguá estuarine complex (Carvalho et al. 2022a). Thus, the growth rates of *O. beta* in natural areas and colonized environments are suggested to be similar, and the change of habitats and, consequently, different environmental forces do not influence the growth pattern of this species.

Conclusion

From this study, it is possible to affirm the occurrence of a population of *O. beta* in Guaratuba Bay. Due to the lack of studies, it is not yet possible to state the population density of the species in this environment, and the authors suggest that environmental agencies a future management plan for this population, taking advantage of the fact that it is apparently in a low population density in Guaratuba Bay. The government's environmental authorities must take rapid action to prevent the *O. beta* population from causing impacts in Guaratuba Bay.

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