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Original Article

Reproductive biology of *Donax striatus* (Linnaeus, 1758) (Mollusca: Bivalvia) on Gado Bravo Beach, municipality of Tibau do Norte, state of Rio Grande do Norte, Brazil

Biologia reprodutiva de *Donax striatus* (Linnaeus, 1758) (Mollusca: Bivalvia) na Praia Gado Bravo, município de Tibau do Norte, estado do Rio Grande do Norte, Brasil

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Abstract

Aspects of the reproductive biology of *Donax striatus* were studied from individuals collected from Gado Bravo Beach in the municipality of Tibau do Norte, state of Rio Grande do Norte, Brazil. *Donax striatus* is a dioic species without external (on the shell) or internal (gonads) macroscopic dimorphism. Thus, a microscopic examination of the reproductive cells is necessary. For the characterization of the gonadal development stages and determination of the size at first sexual maturity (L_{50}), 30 specimens were selected monthly between February 2021 and January 2022 and submitted to histological processing. The condition index (CI) of each individual was estimated and monthly variations were statistically assessed. The size at first maturity (L_{50}) was estimated to be 14.2 mm in shell length. To foster conservation of the species, catches of individuals larger than 14.2 mm is recommended. The lowest condition indices were found in the dry season, with a greater occurrence of organisms in the elimination stage and exhibiting gonad tissue reorganization. Higher indices were found in the rainy season, with the presence of mature individuals. The continuous nature of gametogenesis in *Donax stritatus* reflects the influence of rainfall in the region. Males and females have peak gamete elimination with pauses during the year, but with the presence of maturing and eliminating individuals throughout the year. As shellfish gathering targeting *Donax striatus* is excessive on Gado Bravo Beach in the state of Rio Grande do Norte, it is hoped that the results of the present study can contribute to the establishment of management measures for the activity and conservation strategies for the species.

Keywords: sexual maturity, reproduction, histology, bivalves.

Resumo

Aspectos da biologia reprodutiva de *Donax striatus* foram estudados. Os indivíduos foram coletados mensalmente de fevereiro de 2021 a janeiro de 2022 na praia Gado Bravo, no município de Tibau do Norte, estado do Rio Grande do Norte, Brasil. *Donax striatus* é uma espécie dióica sem dimorfismo macroscópico externo (na concha) ou interno (gônadas). Assim, é necessário um exame microscópico das células reprodutivas. Para a caracterização dos estágios de desenvolvimento gonadal e determinação do tamanho na primeira maturidade sexual (L_{50}), 30 espécimes foram selecionados mensalmente entre fevereiro de 2021 e janeiro de 2022 e submetidos ao processamento histológico. O índice de Condição (IC) de cada indivíduo foi estimado e as variações mensais foram estatisticamente avaliadas. O tamanho de primeira maturação (L_{50}) foi estimado em 14,2 mm de comprimento de concha. Para promover a conservação da espécie, recomenda-se a captura de indivíduos maiores que 14,2 mm. Os menores índices de condição foram encontrados na estação seca, com maior ocorrência de organismos em fase de eliminação e exibindo reorganização do tecido gonadal. Índices maiores foram encontrados no período chuvoso, com presença de indivíduos maduros. A natureza contínua da gametogênese em *Donax stritatus* reflete a influência das chuvas na região. Machos e fêmeas apresentam pico de eliminação de gametas com pausas durante o ano, mas com presença de indivíduos em maturação e eliminação ao longo do ano. Como a mariscagem visando *Donax striatus* é excessiva na Praia de Gado Bravo, no estado do Rio Grande do Norte, espera-se que os resultados do presente estudo possam contribuir para o estabelecimento de medidas de manejo da atividade e estratégias de conservação da espécie.

Palavras-chave: maturidade sexual, reprodução, histologia, bivalves.

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1. Introduction

Studies on the reproduction of mollusks enable understanding aspects of the biology and ecology of these organisms and can therefore assist in the management of natural populations and the conservation of species. Information on the condition index combined with histological determinations of gonadal development stages is the most widely used, efficient method for the determination of the reproductive cycle of bivalves (Rocha-Barreira and Araújo, 2005; Araújo and Nunes, 2006). Such information is also considered essential to the determination of the size at which individuals reach sexual maturity, which is especially important when populations are commercially exploited (Oliveira, 2019). Among bivalves, species of the genus *Donax* belong to a globally dominant group in invertebrate communities on sandy beaches. However, few studies have been conducted on the reproductive biology of these species in tropical regions (Gadelha et al., 2019).

The mollusk bivalve *Donax striatus* (Linnaeus, 1758) has broad distribution in coastal zones of the western Atlantic from the Caribbean Sea to Venezuela, Suriname, and Brazil, where the species is found in the states of Rio Grande do Norte, Ceará, Piauí, Maranhão, and Pará (Sousa et al., 2014; Barroso et al., 2013; Gadelha et al., 2019). These bivalves live buried in the surface sand on tidal flats of sandy beaches, performing tidal migrations accompanying the sweep of the waves and variations in the rise and fall of the tide (Ansell, 1983; Sousa et al., 2014).

In some coastal regions, this bivalve is an essential resource for food and the development of the local economy through shellfish gathering and traditional fishing (Wade, 1967; Vasconcelos et al., 2013; Meireles et al., 2007; Sousa et al., 2014). Its capture is performed by hand and often in a disorganized manner without proper monitoring, as the activity does not require sophisticated equipment. Although artisanal, this practice, along with the urbanization of beaches, places considerable pressure on the stocks of this bivalve, causing a reduction in its populations (Rocha-Barreira et al., 2002; Sousa et al., 2014).

Information on the reproductive biology of *Donax striatus* is still scarce in the literature, except for the study carried out by Gadelha et al. (2019), on reproductive cycle and gonadal development on a sandy beach in the northern region of Brazil . Thus, such studies are needed to enable a better understanding of the reproductive biology of *Donax striatus* while taking into consideration its relevance to local communities (Medeiros et al., 2015), contributing to update the information on the reproductive behavior of the species.

The aim of the present study was to characterize the population structure and reproductive biology of *Donax striatus* on Gado Bravo Beach in the municipality of Tibau do Norte in the state of Rio Grande do Norte, Brazil.

2. Materials and Methods

2.1. Sampling area

Sampling was performed on a natural sandy-muddy bank measuring 80 m in width and 565 m in length located on Gado Bravo Beach in the municipality of the Tibau do Norte on the western coast of the of Rio Grande do Norte state, Northeast Brazil (Figure 1). Specimens were collected

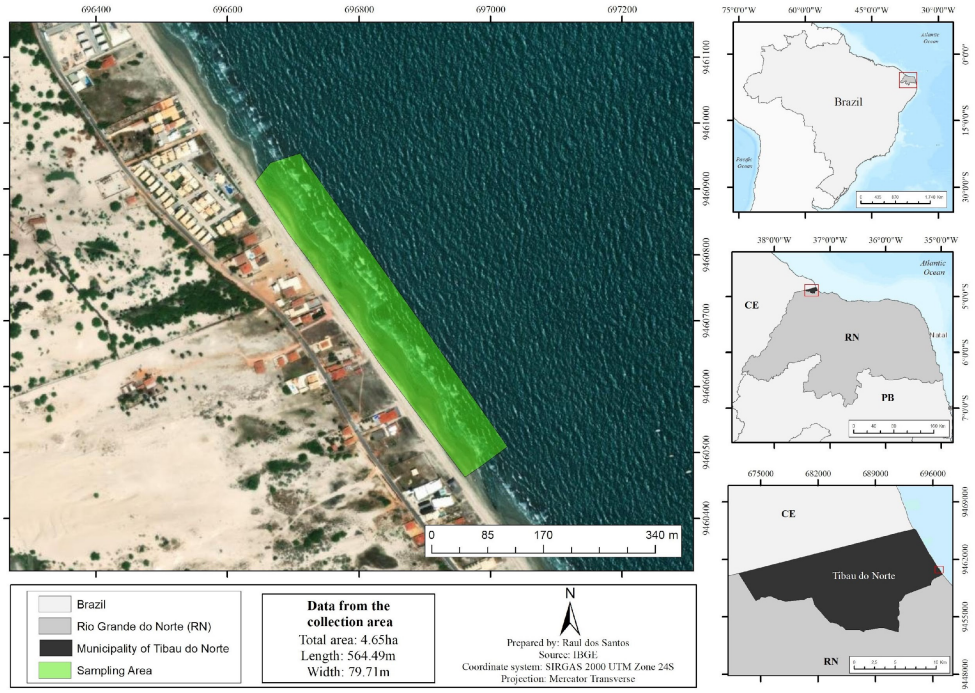

Figure 1. Location of *Donax striatus* sampling area on natural bank on Gado Bravo Beach, Tibau do Norte, RN, Brazil.

in a radial area around a fixed georeferenced mark (04° 52' 34.75" S and 37° 13' 34.54" W). The beach is a deposit of sandy sediment accumulated by wave action, serving as an important element of the coastal conformation. Fishing is the main provision service in the area, along with the regulation service of oceanic and geomorphological processes and cultural aspects through the services of leisure and geotourism offered by the beach (Terto, 2021).

2.2. Collection and processing of samples

Sampling was conducted monthly over a 16-month period during daylight hours at low spring tide. Specimens of *Donax striatus* were collected by two to three collectors for one hour through manual collection with the aid of a sieve (mesh size: approximately 2 mm) to separate sediment from the biological material.

The specimens were transported to the *Laboratório de Tecnologia e Oceanografia Pesqueira* (LABTOP [Fishery, Oceanography and Technology Lab]) in closed plastic bags containing seawater. The organisms were fixed in 10% saline formol for 24 hours and preserved in 70% ethyl alcohol until analysis. The samples were fixed with the soft parts still in the shell and the valves partially open.

Digital calipers with a precision of 0.1 mm were used to determine the size of the specimens. Height (maximum dimension between the umbo and edge of the shell), length (maximum dimension between the anterior and posterior regions), and width (maximum dimension between the two valves) were determined in accordance with the anatomical axis.

For the characterization of the gonadal development stages and determination of the size at first sexual maturity (L_{50}), 30 specimens were selected monthly between February 2021 and January 2022 and submitted to histological processing.

Weight was measured using a precision scale with the entire individual and with the soft parts separated from the valves. The visceral mass, where the pair of gonads is located, was extracted from each specimen.

Monthly precipitation was obtained from the *Fundação Cearense de Meteorologia e Recursos Hídricos* (FUNCEME [Ceará Meteorology and Water Resource Foundation]) located near the collection site.

2.3. Histological analysis

For the preparation of histological slides the samples were submitted to dehydration in a set of solutions with increasing concentrations of alcohol, cleared with xylol, and embedded in paraffin, following the protocol used by Santana (2010). Cross-sections were cut on a manual microtome to a thickness of 5 µm, following the method adopted by Gadelha et al. (2019). The specimens were then stained with aqueous Harris hematoxylin and eosin, following the protocols of the Applied Animal Morphophysiology Lab, where the procedures were performed. Reproductive cells were photographed with a digital camera coupled to an optical microscope (magnifications: 400x and 1000x).

Measurements of the reproductive cells were performed on the images with the aid of the Image J program. Four measurements were made of each oocyte for the determination of the mean diameter (adapted from Corte, 2011), adopting the nucleus in the center of each oocyte. Only cells with an apparent nucleus were selected. The classification of the gonadal stages was performed based on Lamine et al. (2021), Gadelha et al. (2019), Corte (2011), Rocha-Barreira and Araújo (2005), and Gil and Thomé (2004a).

2.4. Size at first sexual maturity (L_{50})

Length at first maturity (L_{50}), which indicates the size at which 50% of the population reaches sexual maturity, and the proportion of mature/immature individuals per shell size class were estimated using the SizeMat package (Torrejon-Magallanes, 2020) available in the R software (R Core Team, 2022), as follows (Equation 1):

$$P = \frac{1}{1 + e^{-(\ddot{u} + \cdot)}} \quad (1)$$

which P is the probability of an individual being mature at a particular size, a is the intercept, b is the angular coefficient and L is shell length.

2.5. Determination of condition index

The condition index (CI) of each individual was estimated from the ratio between the total fresh mass of the soft parts and shell mass, based on Santana (2010) (Equation 2):

$$CI = \left(W_{TM}/W_S\right) \times 100 \quad (2)$$

in which W_{TM} is the total fresh mass of the soft parts in grams and W_S is shell weight in grams.

The Shapiro-Wilk test demonstrated that CI values did not have normal distribution ($W = 0.927$; p-value = 2.2×10^{-16}). Therefore, monthly variations in the index were analyzed using the nonparametric Kruskal-Wallis test followed by Dunn's post hoc test for the determinations of which months differed significantly from each other.

3. Results

3.1. Population structure

A total of 2569 specimens of *Donax striatus* were collected between October 2020 and January 2022. The largest individuals measured 27.63 mm in length and the smallest measured 3.95 mm. Mean length and standard deviation among the specimens was 17.97 ± 2.89 mm. The size distribution per length class is displayed in Figure 2.

3.2. Reproductive cycle

Donax striatus is a dioic species without external (shell) or internal (difference in coloration of gonads) morphological characteristics that enable the visual

differentiation of the sexes. Macroscopically, *Donax striatus* has whitish soft parts, with a digestive gland in greenish, yellowish, and/or brownish tones.

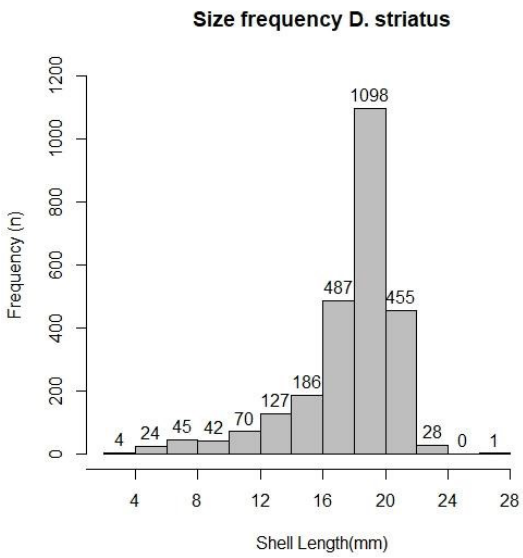

Figure 2. Frequency distribution per shell length class of *D. striatus* on Gado Bravo Beach, RN, Brazil.

From the 360 processed gonads, 304 individuals were able for histological analysis. The examination of the reproductive structures led to the identification of 29 immature individuals, 62 males, 104 females and 109 individuals of undetermined sex.

Germ cells were described based on the degree of gonadal development. The following cell types were identified in females: I- ovogonia; II- pre-vitellogenic oocytes; III- vitellogenic oocytes; and IV- mature oocytes. These cells are located within the ovarian follicles.

The ovogonia are cells fixed to the germ follicle wall measuring 1.5 to 11.25 µm in diameter. Pre-vitellogenic oocytes have an oval shape with a diameter between 3.75 and 19.25 µm and are still attached to the follicular wall. The cytoplasm is slightly acidophilic. The nucleus is voluminous and quite basophilic. Vitellogenic oocytes have an oval or peduncular shape and can have a smaller area fixed to the germ cell wall or may be free in the interior of the follicle, measuring 7.5 to 34.5 µm in diameter. Mature oocytes are free mature cells in the center of the follicle with an oval shape, basophilic nucleus, and acidophilic cytoplasm, measuring 7.5 to 28.75 µm in diameter (Figure 3a and 3b).

The following germ cells were found in males: I- spermatogonia; II-spermatocytes; III- spermatids; and IV- spermatozoids. These cells are located in the testicular tubules.

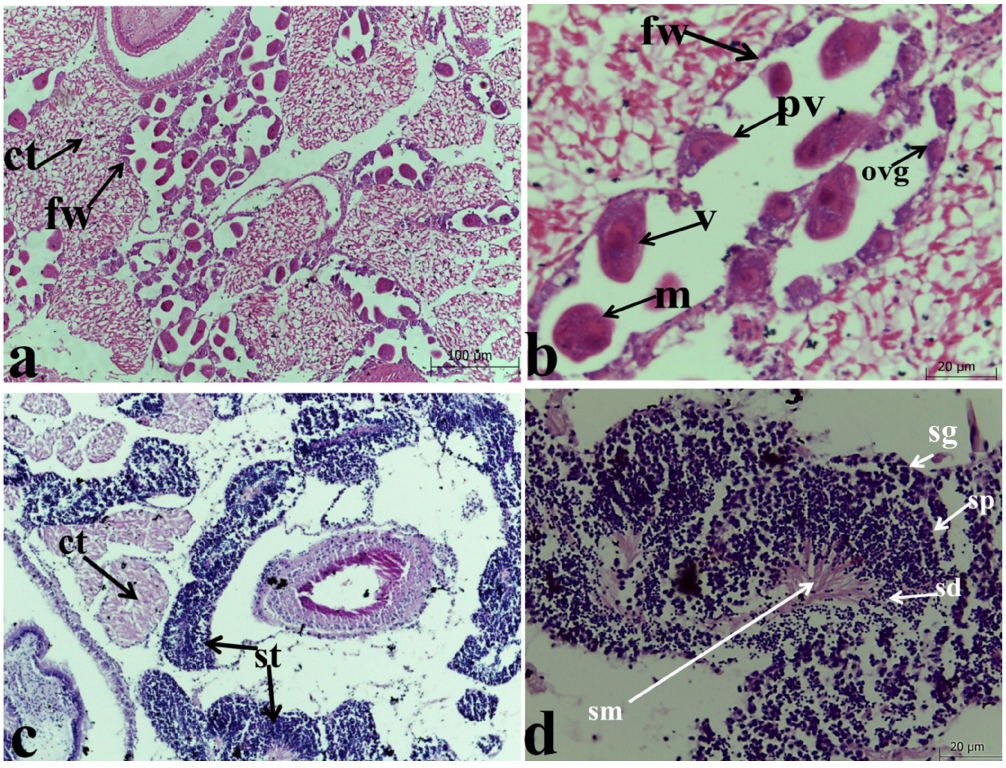

Figure 3. Photomicrograph of female and male germ cells of *Donax striatus*. Ovarian follicle with female germ cells (a and b): fw = follicle wall; ct = connective tissue; ovg = ovogonia; pv = pre-vitellogenic oocyte; v = vitellogenic oocyte; m = mature oocyte; Male gonads germ cells (c and d): st = spermatic tubules (follicles); ct = connective tissue; sg = spermatogonia; sp = spermatocytes; sd = spermatid; sm = spermatozoid.

Spermatogonia are pioneering cells that undergo mitosis and meiosis, giving rise to various cells. As these cells undergo morphological changes, they migrate toward the center of the tubule (lumen) and become mature.Spermatocytes are smaller than spermatogonia and are located further from the wall of the tubules. Spermatids are smaller than spermatocytes and arise from the meiotic divisions of spermatocytes. Spermatozoids are mature cells with characteristics that develop from spermatids. These cells are found at the lumen of the testicular tubules and are morphologically different (elongated) in comparison to the previous cells (Figure 3c and 3d).

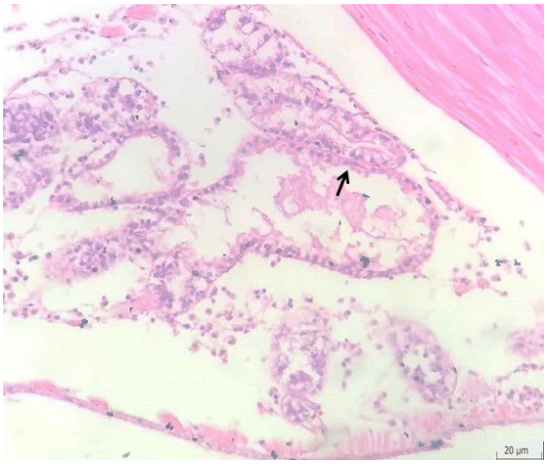

Figure 4. Photomicrograph of indetermined germ cells of *Donax striatus* in interior of follicles. Arrow indicates mother-cells adhered to inner wall of the follicular basal membrane.

Five gonadal stages were described: indetermined, maturing, mature, eliminating, and eliminated.

Indetermined individuals (Figure 4) exhibited the onset of the formation of the follicles, with clusters of mother-cells adhered to the inner wall of the follicular basal membrane with an empty space from which the first germ cells (spermatogonia and oogonia) will arise; it is not possible to differentiate the sex in this stage.

The following characteristics were identified for the stages in females:

- Maturing (Figure 5a) – different phases of germ cells, with a predominance of oogonia, pre-vitellogenic oocytes and vitellogenic oocytes; small, well defined ovarian follicles with thick walls; considerable quantity of extrafollicular connective tissue;

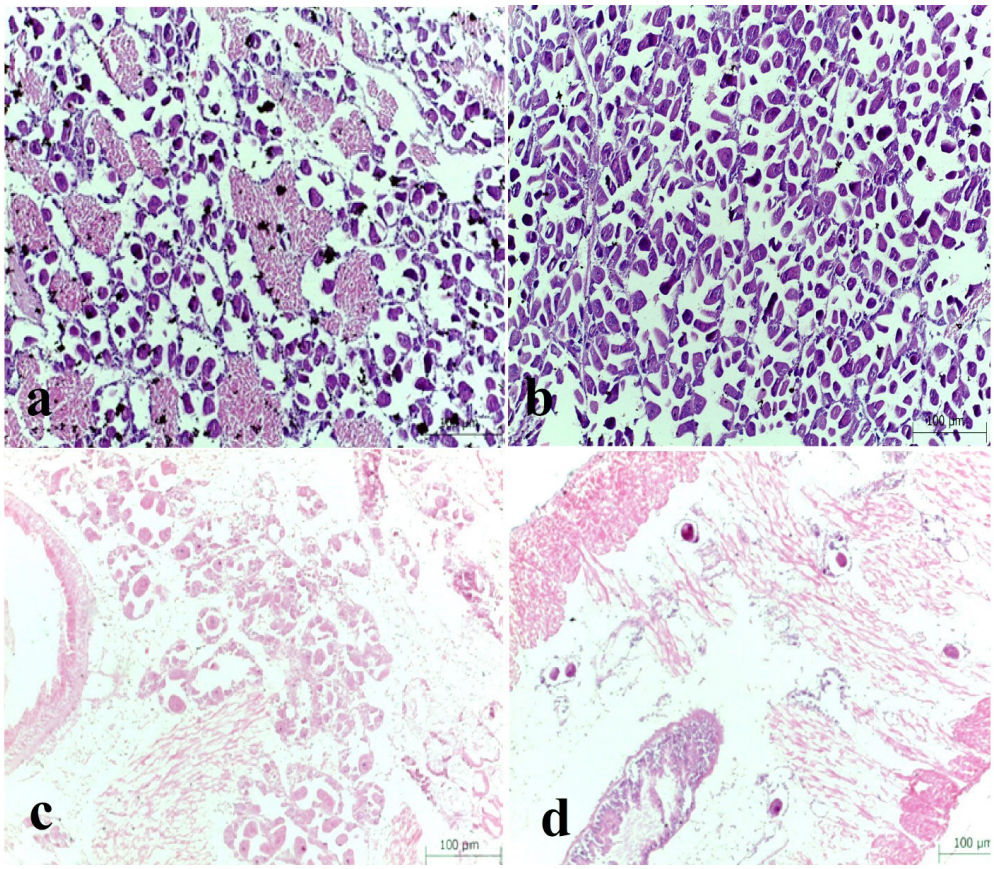

Figure 5. Photomicrograph of female gonads of *Donax striatus* in different stages of development. (a) maturing, (b) mature, (c) eliminating, (d) eliminated.

- Mature (Figure 5b) – predominance of free mature oocytes in the lumen, with other types of germ cells in lower quantity; follicles with considerable quantity of oocytes and distended follicular wall; reduction in interfollicular tissue;
- Eliminating (Figure 5c) – reduction in germ cells in pre-vitellogenic; vitellogenic and mature phases, leaving space within ovarian follicles;
- Eliminated (Figure 5d) – thin follicular wall with ovarian follicles either empty or with residual cells in cytolysis.

The following characteristics were identified for the stages in males:

- Maturing (Figure 6a) – germ cells in spermatogonium, spermatocyte, and spermatid phases. Spermatozoids observed in a more advanced phase disperse in testicular ducts, which become gradually wider with reduced intrafollicular space;
- Mature (Figure 6b) – thin follicular wall and large quantity of spermatozoids in lumen;
- Eliminating (Figure 6c) – signs of emptying due to release of spermatozoids, leaving intrafollicular and interfollicular spaces;
- Eliminated (Figure 6d) – emptying of testicular ducts, which become narrow; large quantity of connective tissue between ducts.

Individuals in the indetermined stage of development were found throughout the study period, with the least occurrence in May 2021 and the greatest in September 2021. Males and females displayed the peak elimination of gametes with pauses during the year, but with maturing and eliminating individuals found throughout the year, indicating continuous reproduction.

The precipitation index in the region revealed that the wettest period was from March (109 mm) to May 2021 (60 mm), when a predominance of maturing and mature individuals was found. Two gonadal cycles were found in the dry period – one from June to September 2021, with a predominance of individuals in the eliminating and indetermined stages, and another from

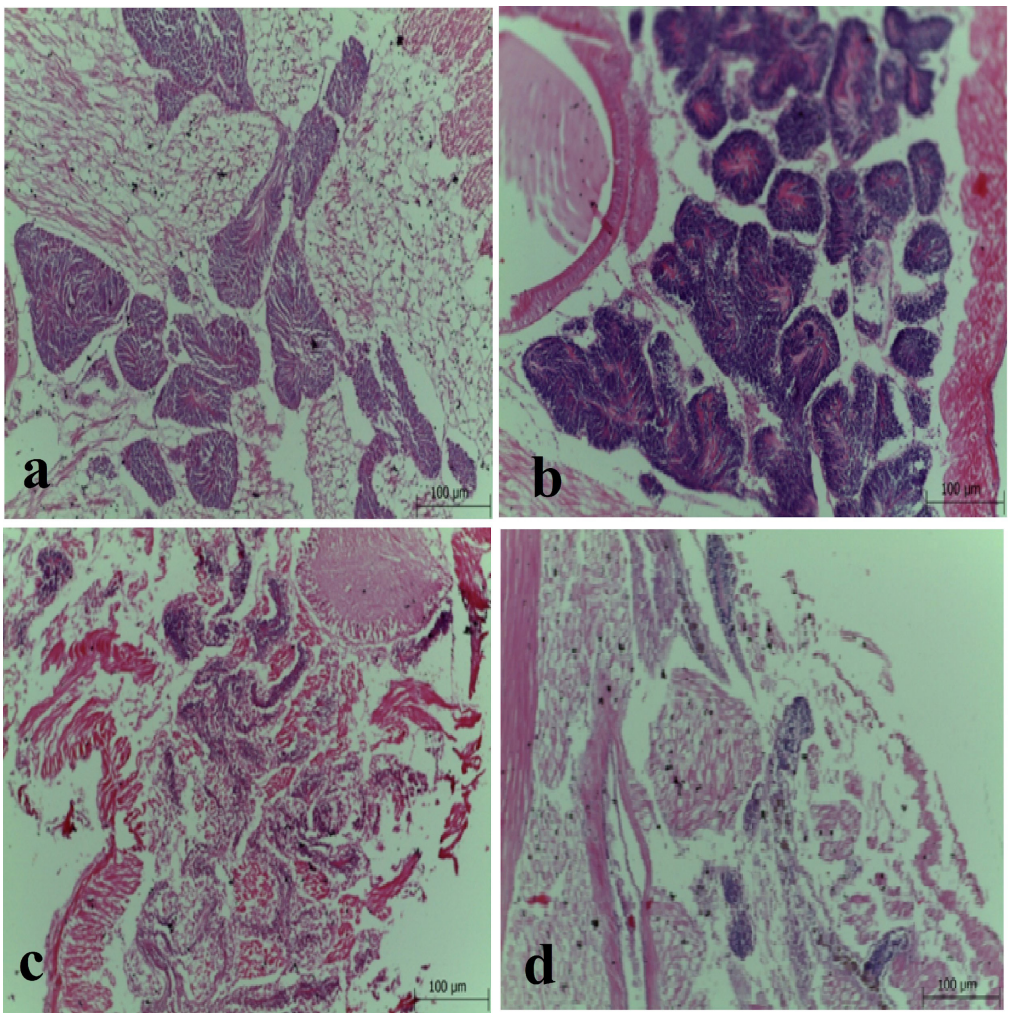

Figure 6. Photomicrograph of male gonads of *Donax striatus* in different stages of development. (a) maturing, (b) mature, (c) eliminating, (d) eliminated.

September to December 2021, with a predominance of indetermined individuals and a smaller proportion of maturing individuals (Figure 7).

3.3. Size at first maturity (L_{50}) and condition index

The adjustment of the logistic model indicated that 50% of the individuals (L_{50}) reach sexual maturity at a length of 14.2 mm (Figure 8). Significant differences were found in the Condition Index of *Donax striatus* among the months of the study [Kruskal-Wallis (H = 604.96; p-value = 2.2×10^{-16})]. The highest means were found in January to April 2021 and the lowest were found between May and December 2021 (Figure 9).

4. Discussion

The size of organisms is important to establishing the age range correlated to the respective stages of maturity – whether these stages are identified microscopically with the examination of germ follicles or macroscopically by the tonalities of shell colors. The range of sizes found in the present study is similar to patterns reported by Medeiros et al. (2015), who studied the population density of *D. striatus* in an area near that analyzed in the present investigation and found juveniles and adults in all sampling periods, lending strength to the hypothesis of continuous spawning throughout the year.

Shell length at first maturity (14.2 mm) found in this study is in agreement with lengths previously reported for the genus by different authors. In a previous study conducted with *Donax hanleyanus* in South Brazil, Gil and Thomé (2004b) established size at first maturity (L_{50}) using a combination of two methods: the calculation of the average length of the smallest individual in the state of emission/spawning and the length at first sexual maturity distribution method. First maturity for *D. hanleyanus* was estimated as a shell length of 12.0 mm and the greatest frequency of individuals in sexual activity was found between the length classes from 13.0 to 15.0 mm (Gil and Thomé, 2004b). For *Donax punctatostriatus* in Sinaloa, México, size at first maturity was estimated at 12.14, 12.63, and 12,03 mm for females, males, and both sexes together, respectively, using the logistic model (Esqueda-González et al., 2018). For *Donax trunculus* in its first year of life on the coast of Argentina, L_{50} was estimated at 16 mm and the size of juveniles ranged from 8 to 16 mm (Moueza and Frenkiel-Renawt, 1973). Size at first maturity of *Donax trunculus* on the southern coast of the Adriatic Sea in the second year of life was estimated at 18 mm (Zeichen et al., 2002), 19.1 mm in the Sea of Marmara, Turkey (Deval, 2009), and 19.04 mm on the Iberian Coast between the waters of the Atlantic and Mediterranean (Patiño et al., 2021). Sexual maturity is a function of age rather than size, such that this species initiate reproductive maturity in the first year of life (13 to 21 mm) (Gaspar et al., 1999). The result of the present investigation is quite similar to that reported by Gadelha et al. (2019), who stated that species of the genus *Donax* initiate maturity irrespective of the climate to which they are submitted, with L_{50} estimated at 12.5 mm, for pooled sexes.

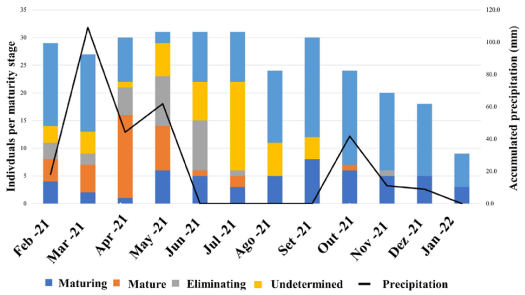

Figure 7. Monthly accumulated precipitation (mm) and frequency of individuals of *Donax striatus* in different gonadal development stages *Donax striatus* on Gado Bravo Beach, RN, Brazil, between February 2021 and January 2022.

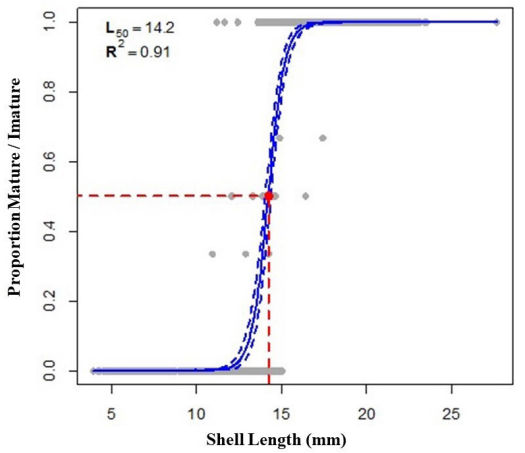

Figure 8. Probability of maturity of *Donax striatus* considering shell length class. Dotted line in red indicates length at which 50% of the individuals reach morphological sexual maturity (L_{50}).

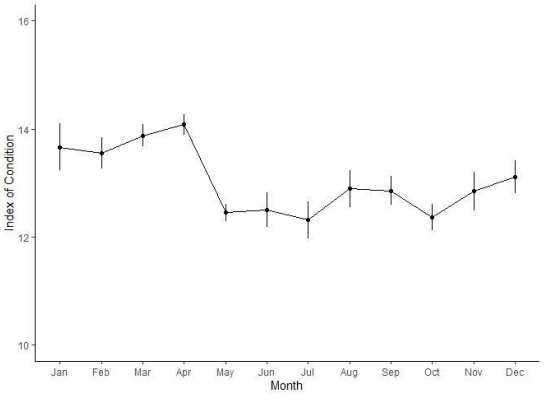

Figure 9. Monthly variation in condition index of *Donax striatus* on Gado Bravo Beach, Rio Grande do Norte, Brazil.

We can infer that this variation may be influenced by regional issues and other particularities that these individuals face, whether anthropogenic, physical, chemical, and/or biological. Geographical differences in life history traits as a function of the environment is a general phenomenon among marine taxa (Patiño et al., 2021). Environmental factors such as temperature, salinity, and the availability of food sources are taken into consideration because may exert an influence on the reproduction cycle in bivalves (Lunetta, 1969). The difference in length at first maturity is normally affected by the growth pattern and particle size of the substrate (Zakaria et al., 2019).

The population of *Donax striatus* on the sandy beach of Ajuruteua in the Amazon region had a greater abundance of females and continuous reproduction cycle (Gadelha et al., 2019). The same pattern was found in the present study for the population of *D. striatus* on Gado Bravo Beach on the western coast of the state of Rio Grande do Norte. The same patterns have also been reported for other species of the genus distributed throughout the world, such as *Donax purpurascens* in Indonesia (Zakaria et al., 2019), *Donax Denticulatus* in Venezuela (Rojas, 1985), *Donax truculus* on the Iberian coast between the waters of the Atlantic and Mediterranean (Patiño et al., 2021), and *Donax punctatostriatus* in Mexico (Esqueda-González et al., 2018).

The reproductive characteristics of *Donax stritatus* observed in this study reflect continuous gametogenesis, determining a continuous reproductive cycle influenced by precipitation in the region. Greater occurrences of individuals eliminating gametes on the Amazon beach were recorded in the rainy season and the highest incidence of mature individuals occurred in the dry season (Gadelha et al., 2019). These findings differ from the results of the present study, in which the predominance of individuals with gonadal maturity occurred mainly in the rainy season, with peak elimination occurring at the onset of the dry season. Boussoufa et al. (2015) found that the main peak of gamete elimination of the species *Donax trunculus* in Tunis Bay in the north of Tunisia occurred from June to October due to the higher temperatures, as also found for the species on Gado Bravo Beach, RN, Brazil.

The condition index of the organisms indicated changes in gonadal mass throughout the year, with higher values when the gonads are ripe (Herrmann et al., 2009; Gaspar et al., 1999). In the present study, the lowest condition indices were found in the dry period, with a greater occurrence of eliminating individuals and the reorganization of gonadal tissue, whereas the highest indices were found in the rainy period, with a greater presence of mature individuals.

Further studies are needed on the population aspects of this species, especially its reproductive biology, throughout its geographic distribution. Many beaches inhabited by these bivalves have been undergoing changes due to coastal erosion, the loss of habitat as a result of urban growth and pollution, which has been diminishing the size of the populations. Moreover, this bivalve is used as a resource in many coastal communities to ensure subsistence and complement the family income. Thus, scientific information can contribute to the establishment of appropriate management and conservations measures.

Shellfish gathering targeting *Donax striatus* is significant on Gado Bravo Beach in the state of Rio Grande do Norte and the establishment of regulations for the activity is necessary. Considering the results of the present study, the capture of individuals with a length greater than 14.2 mm is recommended to prevent the capture of immature individuals. More prolonged studies are needed in this region, with the collection of robust data on biotic, abiotic, and anthropogenic factors that can complement the results of the present investigation. The combination of maturity and morphological studies could assist in the understanding of the reproductive and dynamic behavior of the population of this species.

A complete understanding of the reproductive biology of the species could assist in the formulation of management and conservation strategies directed at this resource on the part of governmental agencies and, thus, ensure the sustainable exploitation of the *Donax striatus*, strengthening the local economy while ensuring the survival of the species, as well the comunities that depend on it for sustenance.

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