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Reproductive biology of the shorthead drum *Larimus breviceps* (Acanthuriformes: Sciaenidae) in northeastern Brazil

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Abstract :

The shorthead drum Larimus breviceps is one of the main bycatch species of the shrimp fisheries in Brazil. However, studies addressing the biology and fisheries impacts on this species are still scarce. Here we describe the main aspects of the reproductive biology and the female gonadal development of L. breviceps on Paraíba, northeast Brazil. The reproductive tract was described by macroscopic and microscopic analysis. The ovaries were composed by oogonia, pre-vitellogenic, vitellogenic, mature, and atretic oocytes. A total of 970 individuals (549 females and 421 males) were caught between December 2016 and November 2017 through beach seining. The total length (TL) varied from 4.2 to 23.0 cm. Females dominated over males (1 female: 0.77 male). The length-weight relationship did not differ between the sexes, presenting a positive allometric growth. The following stages were thus defined for females: immature, initial development, advanced development, mature, and regressing. The period of highest reproductive activity occurs between November and March and immature individuals occur throughout the year. Mean length at first maturity () was estimated as 11.1 cm TL. The information provided here contributes to the overall knowledge of this species and may be helpful for further development of management practices that ensure the sustainability of marine species exploitation.

Keywords : bycatch, croaker, microscopic description, oogenesis

1. Introduction

Fisheries in tropical coastal ecosystems are commonly known for the great discard of species caught as bycatch, especially in shrimp trawling (Pauly, 2005). Among gears used for shrimp harvesting, the motorized bottom trawling is most common and destructive in Brazil and elsewhere (Pina and Chaves, 2009). This activity causes high incidental mortality and increases the extinction risk of several species (Thomas et al., 2017) by declining populations, catching juveniles, and leading to several alterations in the ecosystem (e.g., great physical disturbance; Diamond et al., 2000; Arendse et al. 2007; Thomas et al., 2017).

In the Northeastern Brazil, shrimp fisheries are predominantly artisanal, carried out mainly by motorized artisanal trawling boats (Dias-Neto, 2011). In the state of Paraíba, northeastern Brazil, the ordinance IBAMA nº 833/1990 prohibited this activity in the coastal areas (3 nautical miles; Moura et al., 2003). Currently, fisheries are carried out exclusively through beach seining targeting mainly the white (*Penaeus schmitti*), pink (*Penaeus subtilis*), and seabob (*Xiphopenaeus kroyeri*) shrimps (Santos, 2010). Despite being limited to areas nearshore, the beach seine can harvest greater diversity of bycatch species than the motorized bottom trawling (Passarone, 2020). Moreover, this activity may cause several impacts (e.g., incidental mortality of juveniles and endangered species) that, given the current state of knowledge, may go mostly unnoticed by scientists, marine resource managers, and conservation biologists.

The shorthead drum, *Larimus breviceps* Cuvier, 1830, distributed in the Central and Southwest Atlantic (from Costa Rica to Santa Catarina in Brazil) (Vianna and Almeida, 2005; Cattani et al., 2011), may reach up to 10% of the bycatch caught by beach seine (Nunes et al., 1998; Passarone et al., 2020). This species has an important role in the food chain, either feeding on components of the ecosystem (e.g., small fishes, shrimps) or integrating the diet of mammals and other fishes (Beneditto, 2017). Furthermore, *L. breviceps* has high socioeconomic importance by serving as a food and income source for the local population, especially in northeastern Brazil (Nascimento, 2019). Although some aspects of its population dynamic and fisheries have been studied in Brazil (Souza and Chaves, 2007; Silva-Júnior et al., 2013; Silva-Júnior et al., 2015; Chaves et al., 2018; Bomfim et al., 2019), key information on the biology and ecology of this species are still scarce.

One key basic knowledge concerns reproductive biology, which provides important information for fish stocks estimations (e.g., mean length at first maturity), anthropogenic impacts evaluation, and implementation of management actions (Sadovy, 1996; Begg, 1988). As an example, accurate estimation of mean length at first maturity may subsidize the optimization of more sustainable fishing gear and size restriction measures; while the description of the spawning season and grounds may provide managers the appropriate closed season and adequate no-taken zones (Silva-Júnior et al., 2015; Eduardo et al., 2018). However, information on reproductive biology lacks for several species, especially those caught as bycatch (Silva-Júnior et al., 2015).

This study aims to characterize the reproductive biology of *L. breviceps*. For that, we i) define the first histological classification of the oocyte and maturity stages of *L. breviceps* ovaries, ii) investigate its spawning activity based upon seasonal variation in gonadal development stages, and iii) establish a mean size at first maturity of the shorthead drum in northeastern Brazil. The new information provided here contributes to the overall knowledge to ensure the sustainability and conservation of the species.

2. Material and Methods

Specimens of *L. breviceps* were collected monthly from the bycatch of an artisanal shrimp fishery in north coast of Paraíba, northeastern Brazil (6°53'50"S, 34°51'01"W), from December 2016 to November 2017, except in May due to a series of meteorological events that hampered the fishing activity (Fig. 1).

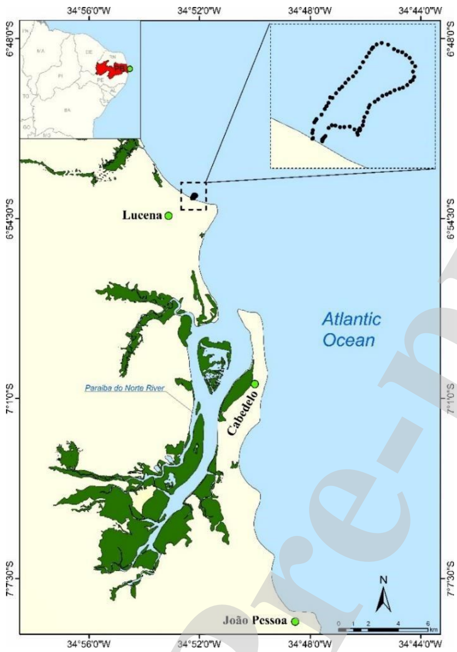


Figure 1. Study area on the north coast of Paraíba State, northeastern Brazil. Black dots represent a real example of a fishing operation (Passarone et al., 2019).

The collection was performed monthly through beach seining, deployed from 500 meters off (depth of 6 meters to the surf zone), and pulled toward the coast, using a non-motorized craft, employed by the local fishers. The net had 2 cm body mesh side length and 1.5 cm cod-end mesh side length (entrance dimension horizontal x vertical: 120 x 6m). Fish collected were kept on ice then transported to the laboratory.

Total length (TL) and the total weight (TW) were recorded and, since data did not follow the necessary assumptions for the parametric test, the nonparametric Kruskal-Wallis followed by Dunn's post-hoc test was used to test for months and sexes differences (see Zar, 2009). The sex-ratios (males individuals/females individuals) determined totally and by size classes (1.0 cm) (Supplementary Material 1), were statistically tested for significant deviations from the expected 1:1 ratio with a χ^2 test ($p<0.05$) (Dagnelie, 1975). The length-weight relationship (LWR) was considered isometric when $b=3$, negative allometric when $b<3$, and positive allometric when $b>3$ (Froese, 2006). The allometry coefficients were further tested for significant deviations from $b=3$ by Student's t-test. A maximum likelihood ratio test was used in comparisons of LWR between the sexes.

The gonads were removed and weighed for sex and maturation stage determination. Microscopic analysis was carried out in 250 ovaries to confirm the macroscopic characterization and describe the maturity stages. Samples were taken from the median portion of the ovary, fixed in10% buffered formaldehyde for 24 hours, cleaved, fixed again for another 24 hours, and transferred to 70% alcohol for conservation. The ovary fragments were dehydrated, cleared in xylol, embedded in paraffin at 60°C, cut in slices of 5 µm, and stained with hematoxylin/eosin-phloxine. Maturational stages were identified through slide analyses and ovary sections photographed using an optical microscope LEICA DM500 (LEICA, Wetzlar, Germany).

The ovaries were classified macroscopically and microscopically in different reproductive phases (Brown-Peterson et al., 2011) according to the most advanced oocyte stage present (West, 1990). It was measured, at most, fifty oocytes per category using the software Image Tool© version2.0 for Windows. The mean and the standard deviation to each specimen's oocyte diameter of the different germ cells were obtained. Oocyte diameters were taken in the cross-section of the ovary.

The mean length at first maturity (L_{50}; mean length at which 50% of the individuals attain gonadal maturity for the first time) was obtained only for females. To achieve it, the percentage of adults (microscopic stages II, III, IV, and V) was estimated for each length class. These values were adjusted by the least-squares method to a logistic curve, which is given according to King (2007): $P_i=1/(1+\exp[-r(L_i-L50)])$, where P_i is the proportion of adult individuals for each class i, L_i is the length at each class i, L_{50} is the length that corresponds to 0.5 proportion (50%) of adults in the population and r is the logistic curve slope.

The spawning season was evaluated through the monthly relative frequency of the gonadal maturation stages and by calculating the Gonadosomatic Index (GSI) for females: GSI=GW/EW, where GW is the gonad weight and EW is the eviscerated weight of the specimen. To test for significant differences in GSI between months, the Kruskal-Wallis test was performed (Sokal and Rohlf, 1987). Immature specimens were excluded from this analysis.

The R software (version 3.4.4) was utilized to perform all statistical analyses (Team R Core, 2018). The package *sizeMat* ("Size at Morphometric and Gonad Maturity in R"; Torrejón-Magallanes, 2016) was used to estimate L_{50} values.

3. Results

A total of 970 specimens of *L. breviceps* were collected: 549 females (56%) and 421 males (44%). Total length ranged from 4.20 to 23 cm (females 4.30–23 cm; males 4.20–22.80 cm, TL), and the total weight from 0.68 to 167.67 g (females 0.77–158.10 g; males 0.68–167.67 g, TW) (Tab. 1). The LWR for females and males did not differ significantly ($p<0.05$) presenting a positive allometric growth in all cases ($b>3$; $p<0.05$) (Tab. 1). In addition, females were statistically predominant over males (1: 0.77; χ^2, $p<0.05$).

Table 1. Descriptive statistics and TL–TW relation parameters of *Larimus breviceps* captured from December 2016 to November 2017 off the coast of Paraíba state, Northeastern Brazil [TL, total length (cm); TW, total weight (g); SD, standard deviation; min, minimum; max, maximum; SL, standard length (cm)].

	Females	**Males**	**Pooled sexes**
Length characteristics			
TL, mean ± SD	11.31±2.94	10.46±2.62	10.97±2.88
TL min–TL max	4.30–23	4.20–22.8	4.20–23
Weight characteristics			
TW, mean ± SD	18.94±17.30	14.58±15.17	17.03±16.54
TWmin–TWmax	0.77–158.10	0.68–167.67	0.68–167.67
Relations			
TL-TW equation	$TW=0.00596TL^{3.22}$	$TW=0.00584TL^{3.23}$	$TW=0.00588TL^{3.23}$
Coefficient of determination (r^2)	0.96	0.96	0.96
t–test (coefficient b=3)	$p<0.05$	$p<0.05$	$p<0.05$
Growth type	Positive allometry (3.22)	Positive allometry (3.23)	Positive allometry (3.23)

The oocyte development was classified in 6 phases (e.g., oogonia, previtellogenic oocyte, cortical alveoli, vitellogenic oocyte, mature, oocyte in atresia) (Fig. 3), as follows:

Oogonia (OO)

The oogonia is the most primitive phase of germinative cells, presenting diameter varying from 25.95±4.14 µm. Its nucleus is wide and located in the center of the cell surrounded by a thin layer of cytoplasm and containing a single and large nucleolus located in the center of the nucleus, which tends to migrate to the periphery as the cell develops.

Previtellogenic oocyte or perinucleolar (PVTO)

In this phase a considerable increase in cellular volume occurs, regarding the previous stage, with a mean diameter of 63.80±3.30 µm. The cytoplasm is larger than the nucleus, presenting peripheric nucleolus in cells nucleus as it develops. These cells reveal strong basophilia and they are found in all ovary development stages (Fig. 3A).

Cortical alveoli oocyte (CA)

The cortical alveoli formation is the main indicator of the beginning of oocyte maturational development. This phase is characterized by the appearance of the oil droplets, which are small spherical vesicles, initially around the nucleus, spreading over the cytoplasm. The cortical alveoli grow in number and size as the oocyte develops. The lipid vacuoles begin to accumulate in the cytoplasm (mean diameter 170.80±19.80 µm) (Fig. 3A).

Vitellogenic oocytes (VTG_1, VTG_2, and VTG_3)

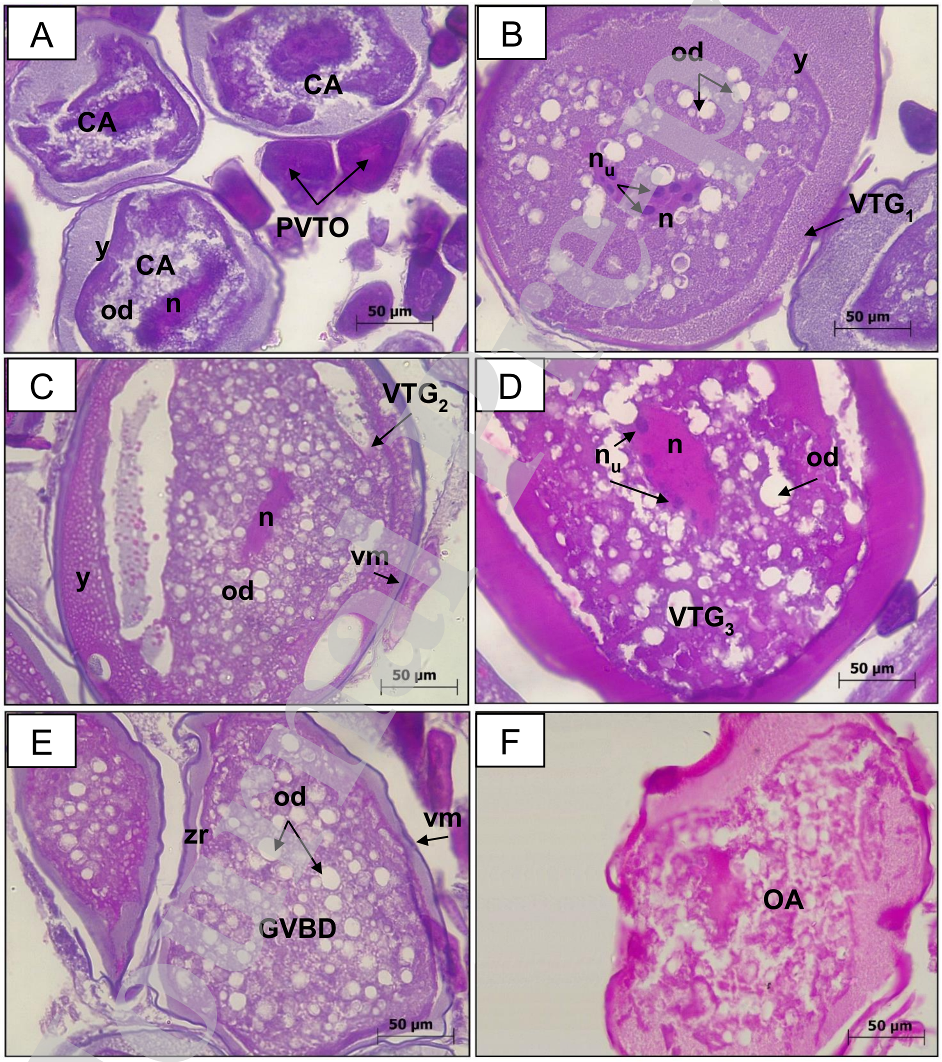
This phase endures from the appearance of egg yolk vesicles in cytoplasm until its fusion through the final maturation. The vitellogenic phase is subdivided into 3 subphases, considering the accumulation of nutrients in the oocyte cytoplasm: *primary vitellogenic oocytes* (VTG_1), the oil droplets occupy the areas around the nucleus (mean diameter 264.60±10.40 µm); *secondary vitellogenic oocytes* (VTG_2), the oil droplets occupy a greater area in the cytoplasm regarding the previous stage and the yolk granules accumulate in the cytoplasm (mean diameter 360.30±10.90 µm); *tertiary vitellogenic oocyte* (VTG_3), the oil droplets are larger than the previous stages and yolk granules spread all over the cytoplasm (mean diameter 412.90±8.90 µm) (Fig. 3B; Fig. 3C; Fig. 3D).

Mature or oocyte undergoing germinal vesicle breakdown (GVBD)

In this phase occur the germinal vesicle migration and breakdown and the yolk granules begin to fuse. It is observed great accumulation of yolk granules in the cytoplasm, provoking a significantincrease in its volume, with a mean diameter 512.30±6.30 µm (Fig. 3E).

Oocyte in atresia (OA)

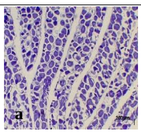
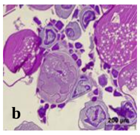
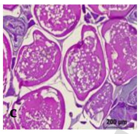
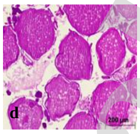
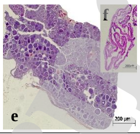
The atretic oocyte is the degeneration of oocyte follicles. Although these cells are observed in higher frequency in the regressing stage, they may occur in all ovary development stages (except in the immature stage). They present an undefined format due to the rupture of the membrane during the process of resorption (Fig. 3F).



"Figure 3. Phases of oocyte development in the *Larimus breviceps* captured from December 2016 to November 2017 off the coast of Paraíba state, northeastern Brazil. A) previtellogenic oocyte phase (PVTO), and oocytes in cortical alveolar (CA) phase; B) oocyte in primary vitellogenic subphase (VTG_1); C) oocyte in secondary vitellogenic subphase (VTG_2); D) oocyte in tertiary vitellogenic subphase (VTG_3); E) oocyte undergoing germinal vesicle breakdown (GVBD); F) oocyte in atresia (OA). n, nucleus; n_u, nucleolus; ca, cortical alveoli; y,yolk granules; od, oil droplets; vm, vitelline membrane; zr, zona radiata."

According to the macroscopic and microscopic analysis of 250 ovaries examined, the females were classified in five maturation stages: immature, initial development, advanced development, spawning capable or mature and regressing (Tab. 2). From the total of ovaries analyzed, 46.40% were immatures, 26% in initial development, 6.40% in advanced development, 11% were mature, and 10% regressing.

Table 2. Macroscopic and microscopic photos and descriptions of ovarian development stages of *Larimus breviceps* captured from December 2016 to November 2017 off the coast of Paraíba state, northeastern Brazil.

Stages	Ovaries	Microscopic sections	Macroscopic and microscopic characteristics
I *Immature*	 1 cm		The ovaries are rudimentary, thin, and characterized by a transparent hue, without apparent oocytes and vascularization (0.01 ± 0.004 g). The mean gonadosomatic index (GSI) was 0.15. This stage only presented oogonia and previtellogenic oocytes. The connective tissue found between the follicles are scarce and the ovarian wall is thin. There is no evidence of lipid droplets in oocytes.
II *Initial development*	 1 cm		The ovaries presented a yellow-transparent hue, no apparent oocyte and vascularization (0.03 ± 0.009 g). The mean GSI was 0.20. Microscopically, it was observed the emergence of cortical alveolar (CA), that characterizes the beginning of the reproductive development, and thereafter the primary vitellogenic oocytes (VTG_1), the greater development oocyte of this stage.
III *Advanced development*	 1 cm		The ovaries in this stage are characterized by a yellow hue, oocytes and vascularization may be apparent (0.04 ± 0.02 g). The mean GSI was 0.22. The presence of secundary vitellogenic oocytes (VTG_2) and a few CA and VTG_1 were observed.
IV *Spawning capable* *or Mature*	 1 cm		The ovaries in this stage are turgid and large, with intense orange hue and high vascularization. The oocytes are large, abundant, and visible all over the ovary (0.80 ± 0.90 g). The mean GSI was 0.47. Microscopically it is possible to observe the presence of tertiary vitellogenic oocytes (VTG_3) and oocytes undergoing germinal vesicle breakdown (GVBD). The post ovulatory follicles (POF) are also present, indicating an active spawning. POF is an oocyte residual and confirms that a successful spawn took place, and that no oocyte absorption was held by the fish.
V *Regressing*	 1 cm		The ovaries are flaccid with thick ovarian wall. Although blood vessels are present, the ovaries are less vascularized, regarding the previously stage (0.11 ± 0.14 g). The mean GSI was 0.19. Microscopically it was observed GVBD, atretic oocytes, and POF (Fig. Vf).

The mean length at first maturity (L_{50}) was estimated as 11.10 cm TL (10.90 – 11.30 of confidence interval) for 549 females. The smallest adult individual presented 7.80 cm TL, whereas those with TL above 13.80 cm were adults (Fig. 4). A total of 50.10% of the individuals captured showed TL lower than the L_{50}.

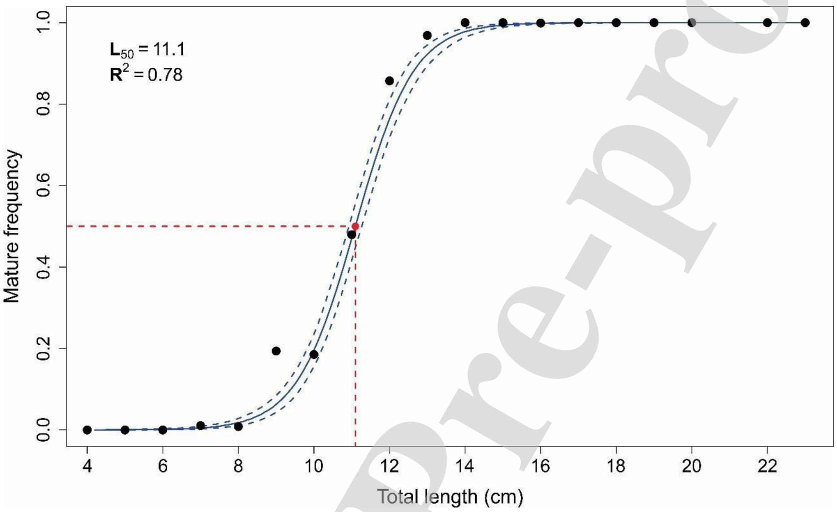


Figure 4. Mean length at first maturity for females of *Larimus breviceps* captured from December 2016 to November 2017 off the coast of Paraíba state, northeastern Brazil (dotted lines represent the confidence interval).

Regarding the months, it was found a higher frequency of immature females in April (83.50%), initial development in July (60%), advanced development in December (66.60%), spawning capable in February (60%), and regressing in October (27%). Immature individuals were present throughout the year and mature females were only present from November to April. This period of the year also presented the gonadosomatic indexes (GSI) peaks: December and February. After February, the GSI starts to decrease, being relatively constant from April to October ($p<0.05$) (Fig. 5).

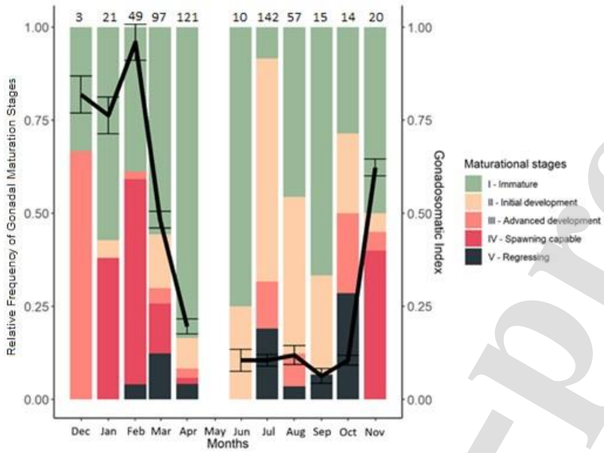


Figure 5. Female monthly gonadosomatic index (±, standard deviation) and maturational stages proportion of *Larimus breviceps* captured from December 2016 to November 2017 off the coast of Paraíba state, northeastern Brazil (black line: GSI; numbers of individuals on the top of the bars).

4. Discussion

This is the first study to analyze the ovary and oocytary development of *Larimus breviceps* caught in shallow waters by artisanal fishermen. Additionally, we brought new information on the sex ratio, length at first maturity, and reproductive season of this species. This data increases biological knowledge of an important bycatch species and may thus support sustainable management and conservation practices.

Larimus breviceps may reach 32 cm TL and occurs up to 60 m depth (Cervigón, 1993; Aparecido et al., 2019). This study performed sampling in shallow waters (< 10 m depth) and specimens ranged from 4.20 to 23 cm TL. Hence, the larger specimens (> 20 cm), which occupy deeper waters, were not fully collected by the beach seine utilized. However, adults and juveniles were similarly represented (50%:50%), indicating a balanced representation of the species ontogeny, raising the concerns of the relevance of *L. breviceps* juveniles within this fishery, which occur throughout the year. This has been also observed in adjacent areas (Silva-Júnior et al., 2015). High catches of juveniles were also observed for motorized bottom trawls, where immature specimens of *L. breviceps* represented up to 80% of the catches (Silva-Júnior et al., 2015; Bomfim et al., 2019), indicating that similar effects over juveniles are observed for both gears.

The catch of juveniles as bycatch is a frequent problem in shrimp fishing in Brazil and elsewhere. In the *L. breviceps* case, this catch may be particularly alarming since this type of fishery encompasses the surfing zone, an important habitat that provides optimal conditions, in terms of food and shelter, for the growth of juveniles (Gibson et al., 1996; Paes, 2002). *Larimus breviceps* is classified as a marine migrant species (Bessa et al., 2013; Passarone et al., 2019), and uses this area as a transition zone between the estuary (Costa et al., 2012) and the adult stock. Therefore, the high catches within this phase/stage of the life history may plummet fish abundance to low levels, jeopardizing ecosystem processes and impacting low-income communities' livelihoods and food security (Baum and Worm, 2009; Cinner, 2014).

Concerning the Length-Weight relationship (LWR) of *L. breviceps*, the positive allometry (b=3.20; p<0.05) found in the present study was within limits indicated by Froese (2006) (2.50 to 3.50), evidencing a greater increment in weight than in length. The positive allometry may be related to the high feeding intensity and/or reproductive events of the species in the area (Silva, 2021). However, in general, this allometry seems to be standard for the species and the genus. This pattern was also observed for *L. breviceps* caught by beach seining in the Brazilian northeast (b=3.06; Ferreira et al., 2017), by bottom trawls in the southern (b=3.20; Freitas et al., 2011) and southeastern Brazil (b=3.10; Vianna et al., 2004), and for species of the same genus in México (*L. acclivis* (b=3.38) and *L. effulgens* (b=3.08); Flores-Ortega et al., 2017).

As for the LWR, the sex ratio may also supply data on important aspects of the reproductive ecology, providing basic information to access population structure, reproductive potential, and stock size (Stratoudakis et al., 2006). In this study, the sex ratio significantly differed from 1:1 (1 F: 0.78 M; p<0.05). This may be linked to the population's reproductive success since a higher proportion of females may increase the population's reproductive potential (Coelho et al., 1987). In the British Guiana, an equal proportion of sexes captured by bottom trawling was observed (McConnell, 1962). Yet, in the Brazilian northeastern, also using bottom trawling, a higher proportion of males was reported (Silva-Júnior et al., 2015). These divergences may be caused by fishing pressures, trawling area, and intrinsic biogeographic features (Rijnsdorp et al., 2010).

The validation of macroscopic stages with microscopic features gives an accurate description of the reproductive biology of marine organisms and confidence in the analysis and outputs necessary for fishery management and resource conservation. However, this validation is rare in fishes and absent to the species of the genus *Larimus*. Therefore, the comparisons in this study were made to the family level. There are several differences between stages nomenclatures and their respective descriptions among Sciaenidae species (Farmer et al., 2008; Almeida et al., 2016). In addition, we have noticed differences in color and body cavity occupation for most of the stages. Despite that, all Scianidae species studied are group-synchronous or asynchronous batch spawners (Hutchings *et al.*, 2006; Yamaguchi et al., 2006; Dadzie et al., 2007). Additionally, oocyte stages between undergoing germinal vesicle break down (GVBD) and atresia were not encompassed in this study (e.g., germinal vesicle migration and hydrate oocytes) and a low frequency of GVBD was observed, therefore, the species oocyte maturation and spawn may occur rapidly. This strategy results from a large investment by the parents to produce a high number of offspring at each reproductive cycle (Pianka, 1970). Moreover, in our study area, *L. breviceps* spawns throughout the year with a peak reported between November and March. This pattern was also observed throughout the Brazilian coast to motorized bottom trawling (Pernambuco and Santa Catarina; Souza and Chaves, 2007; Silva-Júnior et al., 2015). The energy allocation for reproduction over a wide period defines behavioral strategies to maximize reproductive success and guarantee offspring survival in different environmental conditions, allowing juveniles' development and survival (Yamahira, 2004; Winemiller, 2005).

Overall, the maturation in early ages is typical in Sciaenidae and in short-lived fishes, which tend toward r-strategist life histories (Shlossman and Chitteden, 1981). This was the case of *L. breviceps*, with an estimated L_{50} of 11.10 cm TL, lower than those found in adjacent areas using motorized bottom trawling (13.50 cm TL; Silva-Júnior et al. 2015). These differences may be associated with sampling strategy (depth, gear, and effort), gonadal classification (e.g., this is the first study performing microscopical analysis to validate the macroscopic stages), and/or fishing pressure differences among locations (Ashworth and Ormond, 2005).

In the study area, fishing is a relevant socioeconomic activity, representing a source of food and income for a large part of the population (Nascimento, 2019). The shorthead drum, as a marine migrant species, plays an important role by connecting different areas, using the estuary for breeding, the surf zone for protection and growth, and deeper marine areas for the adult stock, revealing high ecosystemic connectivity that supports the importance of ecosystem conservation (Costa et al., 2012; Bessa et al., 2013). Despite the ecologic importance of *Larimus breviceps*, the lack of studies (e.g., reproduction; diet; age; growth; mortality) prevents a complete assessment of the ecology and hamper the development of management practices that ensure the sustainability of the fishery. However, given the multi-specific nature of this fishery, the shorthead drum must be considered in an ecosystem approach for management, considering other main bycatch and target species.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Credit authorship contribution statement

Lucas Vinícius Santos: Writing - original draft, Investigation, Microscopic analysis, Formal analysis and Review. **Cecília Fernanda Farias Craveiro**: Microscopic analysis and Editing. **Andrey Paulo Cavalcanti Soares:** Formal analysis and Editing. **Leandro Nolé Eduardo**: Editing. **Rafaela Passarone**: Editing. **Emanuell Felipe Beserra da Silva**: Project administration and Editing. **Flávia Lucena-Frédou**: Project administration, Conceptualization, Supervision and Editing.

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Declaration of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.