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Reproductive Biology of *Cephalopholis fulva* (Linneus, 1758) caught in the north coast of Pernambuco

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Abstract. The objective of this study is to provide information on the reproductive biology of *Cephalopholis fulva* for conservation and management purposes. Fish caught by artisanal fishermen from January 2012 to December 2014 and from February 2017 to October 2017 were analyzed. A total of 199 females, 72 males, and 10 transitional fish were analyzed. The sex proportion was 2.8 ♀: 1 ♂, and the fish reached a maximum total length of 27.5 cm and 28.2 cm for females and males, respectively. The following microscopic stages were identified: in development, capable of spawning, regression, and resting for females and in maturation, releasing, emptied and in regeneration for males. Asynchronous development was observed in the analyzed gonads, characterized by the presence of more than one stage of oocyte development in the gonads as well as individuals with oocyte remnants and early and advanced stages of sexual transition. The highest GSI values occurred from August to October, indicating that this is the reproductive period of the species, with peaks for females and males occurring in October and September, respectively. The average fecundity was estimated at 70,753 oocytes per female.

Key words: conservation, fecundity, gonadal development, management, reproductive season

Resumo: Biologia reprodutiva da *Cephalopholis fulva* (Lineu, 1758), capturada no litoral norte de Pernambuco. O presente estudo objetiva fornecer informações acerca da biologia reprodutiva da espécie para fins de conservação e manejo da pesca. Peixes capturados no período de janeiro/2012 a dezembro/2014 e de fevereiro/2017 a outubro/2017 foram obtidos junto à Colônia de Pescadores de Itamaracá. Foram analisados 199 fêmeas, 72 machos e 10 peixes transicionais. A proporção de 2,8 ♀:1 ♂, atingindo, respectivamente, um comprimento total máximo de 27,5 cm e 28,2 cm para fêmeas e machos. Foram identificados os seguintes estágios microscópicos para as fêmeas: em desenvolvimento, capaz de desovar, regressão e repouso e para os machos: em maturação, liberando, esvaziado e em regeneração. Foi observado o desenvolvimento assincrônico nas gônadas analisadas, caracterizado pela presença de mais de um estágio de desenvolvimento ovocitário nas gônadas, além de indivíduos com a presença remanescente de ovócitos e estágio inicial e avançado de transição sexual. Os valores mais elevados de IGS ocorreram de agosto a outubro, indicando ser este o período de reprodução da espécie, com picos para fêmeas e machos ocorrendo em outubro e setembro, respectivamente. A fecundidade média foi estimada em 70.753 ovócitos por fêmea.

Palavras-chave: conservação, fecundidade, desenvolvimento gonadal, manejo, época de reprodução

Introduction

Artisanal fishing is traditionally responsible for about 50% of estuarine and marine fish production in Brazil, exerting a relevant economic and social function in several regions, mainly in the Northeast, where, in some places, it is the only source of income and food security of the population (Vasconcellos *et al*. 2007). This type of fishery uses several fishing gear, such as nets, handlines and traps, which, for the most part, catch fish from the reef environments which are common in the region. For this reason, in the last 40 years, the reef environment in Brazilian Northeast has suffered from the adverse effects of uncontrolled fishing as well as urban sprawl, aquaculture enterprises, and pollution caused by industrial and domestic effluents (Marques & Padovani 2010).

The Brazilian Northeast has marine environments of high biological diversity and low population density (Jales et al., 2013). Within this scenario, Pernambuco, a northeastern state of Brazil, of great economic potential, holds the largest port industrial complex in the northeast called Suape (Ibge 2017). Thus, Pernambuco reflects the panorama of the biodiversity of the northeast, presenting impacts on the marine environment, by anthropic actions, management and overfishing (Suape 2016, Jales *et al*. 2013).

In Pernambuco State, the production of marine and estuarine fish is almost totally generated by artisanal fishing (Revizee 2006). Among the reef fish exploited by this activity is *Cephalopholis fulva*, which belongs to the Epinephelidae family and is a non-migratory, protogynous hermaphrodite fish. It is carnivorous and reaches a maximum total length of about 43 cm and a lifespan of 25 years. *Cephalopholis fulva* is an important fishing resource along the coast of the state, presenting high commercial value due to the appreciation to the taste of its meat (Nobrega *et al*. 2009). This is the case of the island of Itamaracá where *Cephalopholis fulva* is frequently caught using baskets, a practice widely spread among local fishermen (Marques & Padovani 2010).

An obstacle for this activity is that *Cephalopholis fulva* is recorded in association with other species, such as grouper and *Haemulon sciurus*, resulting in uncertain estimates of its specific catches (Marques & Padovani 2010). This fact is very worrying because it may mask the real situation in which its stock is found, leading to a possible situation of over-exploitation of the species in a short period, without providing any management and conservation measures (Viana 2013). Although it is classified as least concern (LC) in the list published by the International Union for Conservation of Nature, the species presents a decreasing population trend (Iucn 2017); thus, it deserves investigation into its biological characteristics and management of its fisheries.

In this specific case, studies on reproductive biology are of fundamental importance for fisheries management and the conservation of the exploited population. In Brazil, studies on *Cephalopholis fulva* were carried out to deepen the knowledge about behavioral biology, population biology, morphometric relationships, morphological changes, and genetic interconnection between coastal and oceanic stocks. In Pernambuco, a study on reproduction, age and growth of *Cephalopholis fulva* was carried out by Marques (2011), with samples collected in 2008 and 2009 in different parts of the coast of the state.

In this context, the objective of this work was to study the reproductive aspects of the species on the northern coast of Pernambuco, aiming to contributing with the supply of new information and knowledge for the purposes of fishery management and conservation of the species in the state.

Material and Methods

This work was conducted in Itamaracá, in the north coast of Pernambuco. It is a very productive region due to the vast estuarine and mangrove ecosystems in its inner region and coastal reefs in the marine region, making it a particularly important fishing region in the state, including *Cephalopholis fulva* fishing utilizing baskets.

Specimens of *Cephalopholis fulva* were collected monthly from January 2013 to December 2014 and from February 2017 to October 2017 directly from the artisanal fishermen who practice basket fishing in the coastal zone of the island of Itamaracá.

Following catch and conditioning on ice, the specimens were transported in thermal boxes to Laboratório de Ecologia Marinha – LEMAR (Marine Ecology Laboratory) the Fisheries and

Aquaculture Department of UFRPE, in Recife, where biometry of the collected individuals was conducted. This included total length (TL), standard (SL) length, total weight (TW), and eviscerated (EW) weight.

After evisceration of the specimens, the gonads were extracted and analyzed macroscopically using the methodology by Brown-Peterson *et al.* (2011) adapted to the *Cephalopholis fulva*, which displays the different stages of changes in the ovaries. The adaptation of the methodology to the species under study is necessary because it is a species of a different location and physiology. After analysis, the gonads were fixed in 10% formalin solution for 48 h and then stored in 70% alcohol. For the microscopic analysis, the gonads were cleaved and dehydrated in alcohol in the concentrations of 80%, 90%, and 100% (I and II) for 30 minutes. Then, for diaphanization, the samples spent 20 minutes in the 50% alcohol + 50% xylol and another 20 minutes in 100% xylol (I and II), impregnated, and embedded in histological paraffin a 60 ºC. Subsequently, the paraffin blocks were cut into a micrometer in 5 µm of thickness. The slides were stained with Eosin-Hematoxylin and visualized under a microscope to identify the stages of maturation, according to a procedure adapted from Mackie & Lewis (2001).

To classify the maturation stages of the gonads, the scale of Brown-Peterson *et al*. (2011) was adapted to the species for both sexes. These stages were defined for the females as immature, in development, capable of spawning, regression, and resting. For the males, these stages were defined as immature, in maturation, releasing, emptied, and in regeneration.

The sex ratio between males and females was analyzed monthly. The ratio was calculated using the total number of females upon the total number of males. For the analysis of the gonadosomatic index (GSI), the following equation was used: $GSI = ((GW / (TL^3))10^5)$, where: GW = gonad weight (g), TL = total length (g).

The spawning season of the species was determined by the monthly distribution of maturation stages of the microscopically identified gonads, and the GSI values' peaks for adult females and males.

To estimate the fecundity, the method proposed by Hunter & Macewicz (1985) was adapted for the species and was used by removing a 0.1 g fragment from the central part of the gonad of 10 females at maturation stage characterized mature in three different months, for the counting of the hydrated oocytes, by applying the equation: HO = n(Wg/w), where HO = total hydrated oocytes contained in the ovary, n = total hydrated oocytes contained in each sample, Wg = weight of both ovaries and w = weight of the ovary sample.

Results

During the collection period, 301 individuals, 199 females, 72 males, 10 transitional (individuals in full sex change, characterizing protogynous hermaphroditism), and 20 for which sexual determinations were not possible, were analyzed. The estimated sex ratio was 2,8 ♀:1 ♂. By analyzing the sex ratio monthly using the chi-square method, a significant statistical difference was observed in the months of May, June, August, September, November, and December (Table I).

The total length of the species varied from 15.7 to 27.5 cm for females, with a mode in class 21|-23 cm. However, the length for the males were between 15.8 and 28.2 cm, with a mode in the class 25|-27 cm (Fig. 1). The total weight of females ranged from 61.03 to 410.12 g and from 78.61 to 427.67 g for males.

The sex change was registered between 19 and 27.3 cm in the months of February, March, May, November, and December (Fig. 2).

From August to October, an increase in the value of GSI for females and males was observed (Fig. 3), which is evidence that this is the spawning period of the species, with peaks in September and October for males and females, respectively.

In the analysis of gonadal development, four stages were found for both sexes, described in detail below (Table II): for females, in development, capable of spawning, regression and resting, and for males, in maturation, releasing, emptied and in regeneration.

For females at the in development stage, the start and the end of ovarian development were observed in the cortical alveoli (CA) and primary vitellogenesis phases (VTS1). In the capable of spawning stage, the ovary was fully developed, with the oocytes in secondary vitellogenesis (VTS2) and tertiary vitellogenesis (VTS3) (Fig. 4).

The female in development stage was identified in all months except for October. Resting and regression stages were also recorded in all months of the year except for September and October, respectively. The capable of spawning stage was only identified in August, September, and

Table I: Number of *Cephalopholis fulva* females and males per month and the result of the sex ratio and the chi-square test. *Statistical difference, significance level 5%.

Month	Females	Males	Transitional	Total	1:1 Ratio	X^2 Test	P-value
January	9	4		13	2.25	1.2	0.27
February	12	5	1	17	2.4	2.1	0.15
March	21	14	3	35	1.5	1.0	0.31
April	14	5		19	2.8	2.2	0.14
May	28	13	1	41	2.2	4.4	0.04*
June	12	1		13	12.0	7.7	0.01*
July	9	6		15	1.5	0.3	0.61
August	11	2		13	5.5	4.9	0.03*
September	23	1		24	23.0	18.4	0.00*
October	10	6		16	1.7	0.1	0.81
November	17	1	4	18	17.0	12.5	0.00*
December	18	2	1	20	9.0	12.9	0.00*
Total	**184**	**60**	**10**	**244**	**2.8**	**58.6**	**0.00**

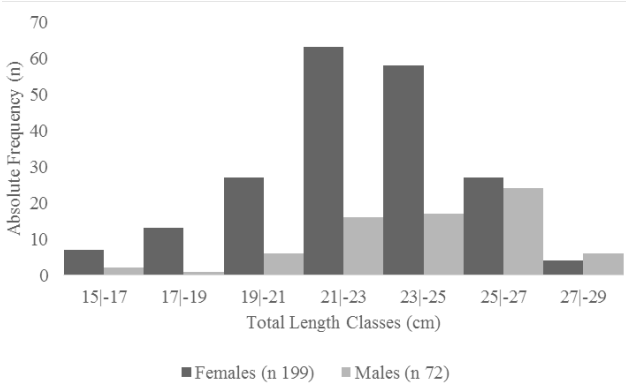

Figure 1. Distribution of the absolute frequency of total length of *Cephalopholis fulva* females (n=199) and males (n= 72) sexually classified.

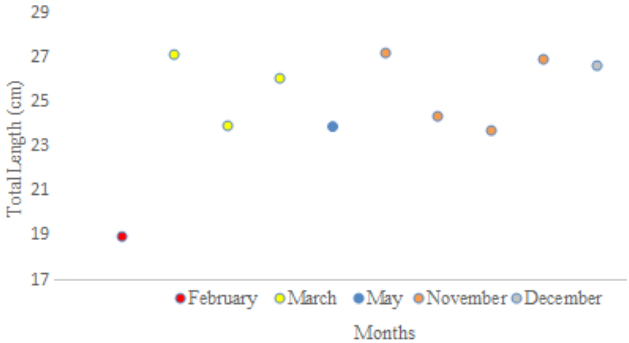

Figure 2: Total length distribution of individuals in the transitional phase of *Cephalopholis fulva*.

October (Fig. 5), indicating that this is the reproductive period for the species.

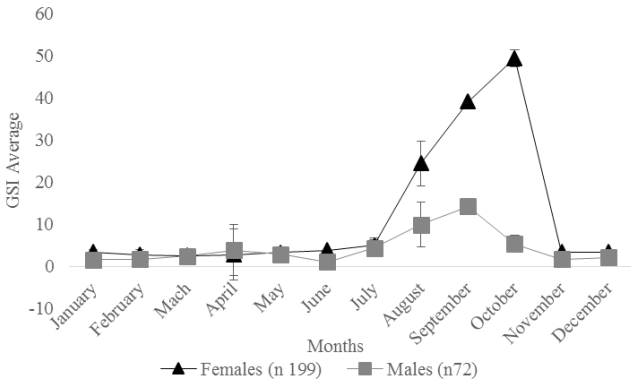

Figure 3: Monthly variation of the average of the gonadosomatic index for *Cephalopholis fulva* males and females.

Fecundity in *Cephalopholis fulva* was established from the analyses of 10 female gonads at the capable of spawning stage (fully mature oocytes), collected in different months, with total lengths ranging from 15.7 to 22.6 cm and total weight ranging from 72.53 to 209.27 g, in which between 61.746 to 83.430 hydrated oocytes were found, with a mean of 70.753. The ratio between the weight increase of the gonad and the number of oocytes is directly proportional (Fig.6).

For males, the in maturation stage was identified in all months except for November. The in regeneration stage was not observed in March, May, June, August, September, October or December. The emptied stage was found in October, December and

Table II: Reproductive stages of *Cephalopholis fulva* females, males and transitional.

Reproductive stages	Females	Males
Immature	No individuals were found in this stage	No individuals were found in this stage
In development (females) and in maturation (males)	Oocytes in developmental process, primary (Vts1) and secondary (Vts2) cortical alveoli; tertiary (Vts3) or POFs oocytes were not found	Presence of seminiferous tubules containing spermatogenesis cells
Capable of spawning (females) and releasing (males)	Large ovaries, visible until macroscopically and the presence of hydrated oocytes incomplete vitellogenesis	Lobules full of spermatozoa
Regression (females) and emptied (males)	Vitellogenic oocytes in atresia.;presence of CA, Vts1 and Vts2 and brown corpuscles and some hydrated oocytes	Emptied seminiferous tubules, indicating that there was a release of spermatozoa, disorganized Sertoli cells
Resting (females) and in regeneration (males)	Oocytes in early perinuclear stage, presence of muscle bundles and atresia, thick ovarian wall, lamellula with some spacing	Initial stage of spermatogenesis and spermatogonia and first stage spermatocytes
Transitional	Ovary showed degeneration in the ovarian tissue and proliferation of sperm tissue with small crypts generally in females in regression and regeneration.	

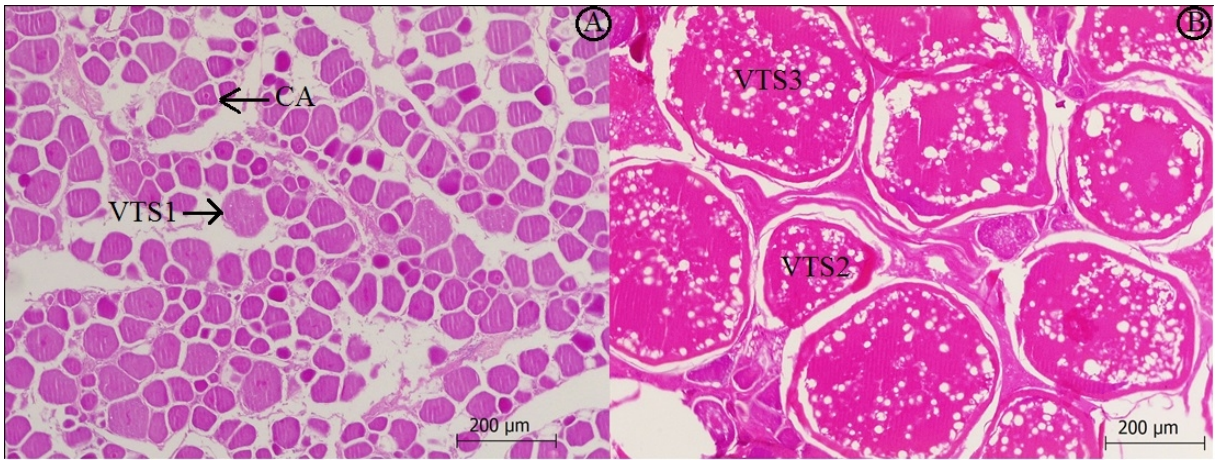

Figure 4: Photomicrography of *Cephalopholis fulva* ovaries on the north coast of Pernambuco in the stages of: (A) - in development (CA - Cortical Alveoli, VTS1 - Primary Vitellogenesis); (B) – capable of spawning (VTS2 – secondary vitellogenesis, VTS3 - Tertiary Vitellogenesis with hydrated oocytes).

February. However, the releasing stage was only observed in July, August and October (Fig. 7).

For males in the maturation stage, the development of the testis was observed, presenting spermatogonia, spermatid (ED), and some spermatozoa. In the releasing stage, a large amount of sperm filled (EZ) the testis (Fig.8).

Transitional individuals were identified in November, December, February, March and May, when both female structures, such as oocytes in primary vitellogenesis (VTS1), and male structures such as spermatozoa (EZ), were observed (Fig.9).

Discussion

The analysis of the obtained data allowed us to observe that artisanal fishery using baskets in the region of Itamaracá, in the northern part of the state of Pernambuco, shows selectivity in catches. Since immature individuals below the minimum registered size of 15.7 cm in total length for females and 15.8

cm for males did not occur in the sample, we could not analyze the average size of first maturation

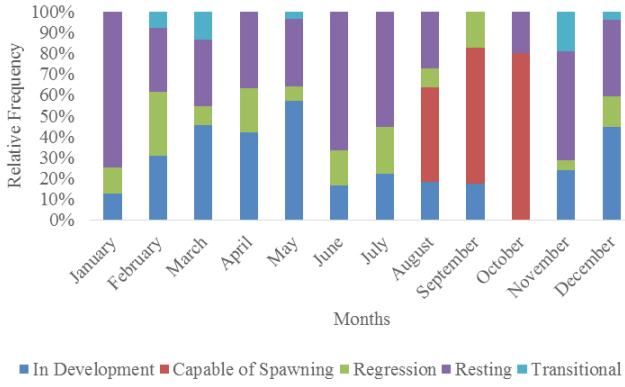

Figure 5: Maturation stage of female gonads over 2013, 2014, and 2017. (n=199).

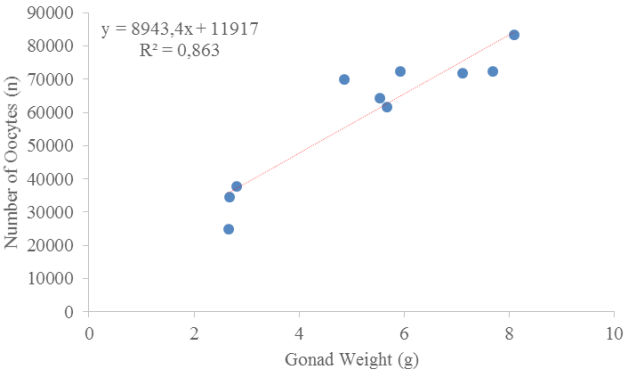

Figure 6: Relationship between absolute fecundity and weights of gonads in *Cephalopholis fulva* females caught on the northern coast of the state of Pernambuco.

(L50), which, according to Fao (2017), was estimated at 16 cm total length. A study with the same species on the southern coast of Pernambuco estimated that the average size of first maturation for females is 18 cm and for males is 15 cm (Marques, 2011), indicating that the geographical distribution of the population may affect growth and maturation parameters. In addition, large reproductive individuals (big, old, fat, fertile, female, fish-BOFFFFs) (Hixon *et al*. 2014) with 30 to 40 cm in total length were not caught, and a maximum catch size of 28.2 cm in total length for males and 27.5 for females was recorded in this study. The lack of immature and large reproductive individuals in the sample is explained to some extent by the 30 cm mouth and 71 cm stream, allowing for the escape of small fish and preventing the entry of large fish.

This may be a positive aspect for fishing *Cephalopholis fulva* at the study site since only intermediate-sized individuals are caught, allowing the young and older adults to remain in the stock (Hixon *et al*. 2014). According to Hixon *et al*. (2014), it is necessary to keep older individuals, the

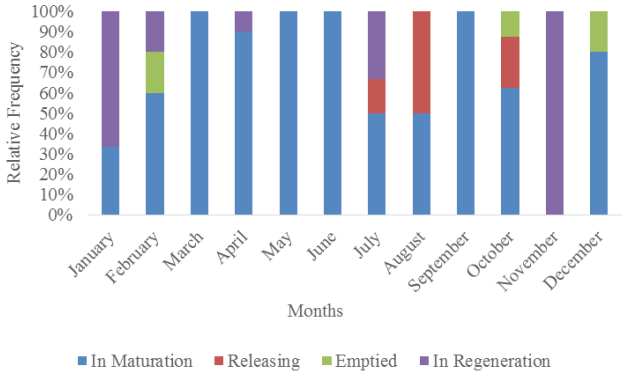

Figure 7: Male maturation stage over the years of 2013, 2014, and 2017. (n=72).

so-called large breeders (BOFFFFs), in the population since they have high fertility rates and thus contribute to the conservation of the species and maintenance of the population's biomass. However, a more detailed evaluation of this issue is necessary via fishery monitoring.

In addition to the aspect previously mentioned, it is also possible that the depth range (>20 m and <40 m) where fishing in baskets occurs contributes to the absence of individuals below and above the sizes mentioned. Burton *et al*. (2015) and Trott (2009), in studies conducted in the southeastern United States and Bermuda Islands, recorded catches of larger fish of the species at depths over 20 m using a different fishing method (line and hook), demonstrating that a vertical distribution of age and size per depth had likely occurred. However, the range of class with the largest abundance of individuals found in this study corroborates other studies carried out by Bezerra & Silva (2011) and Araújo & Martins (2009) in Ceará, Bahia, Espírito Santo, and Rio de Janeiro, where modes of around 23 cm of total length were registered for the same species.

In addition to selectivity of fishing method in relation to fish length, smaller individuals are often found in shallow reef areas. This fact was observed in a study carried out by Marques (2011), for the same species, also on the coast of Pernambuco, where individuals of smaller lengths, between 8 and 10 cm of total length, caught at shallower depths (2-3 m) were registered. The author also presented a total length variation for this work, from 9.5 to 36.8 cm. Of these, 20% of the individuals were collected with hand line, a method which likely caught these smaller individuals.

The 2.8 ♀: 1♂ ratio found in the northern coast of Pernambuco was slightly higher than that observed by Bezerra & Silva (2011) in a study on

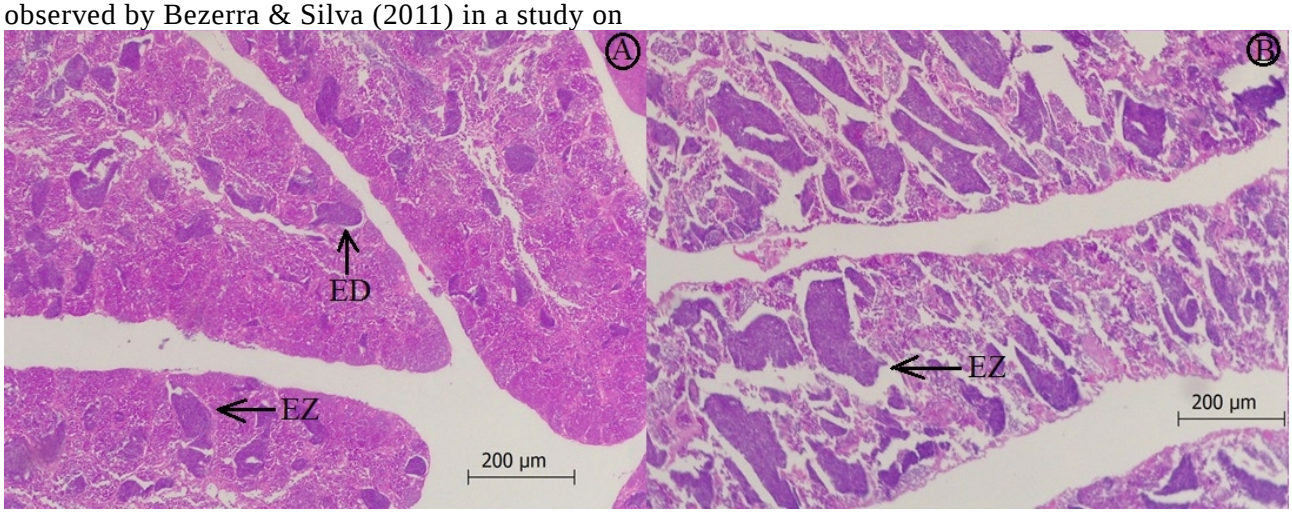

Figure 8: Photomicrography of *Cephalopholis fulva* testicles on the north coast of Pernambuco in the stages of: (A) – In Maturation (ED – Spermatid), (B) - Releasing (EZ – Spermatozoa).

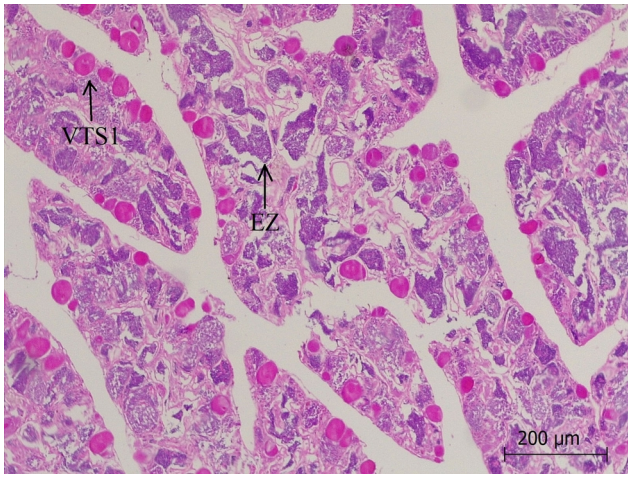

Figure 9: Photomicrography of a *Cephalopholis fulva* transitional individual on the northern coast of Pernambuco; at the extremities, female structures with oocytes in primary vitellogenesis (VTS1), and in the inner parts, male structures with spermatozoa (EZ).

the population of *Cephalopholis fulva* in the state of Ceará, where the sexual ratio was 2♀: 1♂. However, this result diverges from the works of Burton *et al*. (2015), who found a 1♀: 1♂ ratio in studies in the southeastern United States; Trott (2006), who found a proportion of 1.15♀: 1♂ in Bermuda; and Araújo & Martins (2009), who found a proportion of 1♀: 1♂ in Espírito Santo. However, Marques (2011), in a study carried out on the southern coast of Pernambuco, found a ratio of 1♀: 1♂. The sex ratio found in this study has significant importance for the population in the presence of adaptive factors of imbalance and population decline, mainly caused by anthropic activities such as fishing due to protogynies (sex change). This phenomenon of sexual transition was registered in 10 females, from 19 cm in total length, close to the value found in Marques and Ferreira (2016) in some females of the species, with this transition from 20 cm of total length. According to Erisman *et al*. (2010), this attempt of population balance occurs because genus *Cephalopholis* presents the reproductive behavior of sexual intercourse in pairs and low average GI of males, indicating a low sperm competition.

The monthly variation of the gonadosomatic index (GSI) of males and females indicates an evolution of gonadal maturation starting in August, with the peak of reproduction occurring from this month to October. This peak of reproduction corroborates a study carried out by Marques (2011) for *Cephalopholis fulva* on the coast of Pernambuco, in which the spawning period occurred from August to October. This result may be associated with the end of the rainy season, which is a reproductive strategy that some species adopt to spawn at a time when there is a greater food availability and decreased predatory threats to eggs and larvae (Araújo 2009). This fact is corroborated by the work on the continental shelf off Recife, where river discharges on the coastal environment were observed. This generated higher concentrations of food due to the enrichment of nutrients in the coastal zone, soon after the rainy period between the months of September and October, characterizing a spring

bloom in the region (Ressureição *et al*. 1996). This was also observed by Trott (2006), who recorded an increase in gonadal weight in the period of lower rainfall intensity in the Bermuda.

In addition to confirming the reproductive period already indicated by the GSI, the microscopic analysis of the gonads showed that, over the year, the species presents individuals in development, resting, and regression stages for the gonads of both sexes, with multiple spawning stages, a result that corroborates with that found by Marques (2011) on the southern coast of Pernambuco. This characteristic is considered a reproductive strategy for fish from tropical and reef zones, which are directly associated with favorable environmental conditions (Araújo & Martins 2009). In the stage capable of spawning, hydrated oocytes and tertiary, secondary and primary vitellogenesis were found. This was also found in the work of Marques (2011), where these same cells were visualized for this stage of development.

Some species of Epinephelidae family, such as *Alphestes afer* (Marques & Padovani 2016) and *Epinephelus marginatus* (Ximenes-Carvalho *et al*. 2012), present protogynies, promoting the change of sex from immature female to males. In *Cephalopholis fulva*, it was possible to observe this transition, which was related to the strategy of population sexual balance. In this case, the female population was larger than that of males. Ten individuals were recorded in November, December, February, March, and May, undergoing different degrees of transition and making it possible to observe the start of this sexual transition in females that were undergoing gonadal regression.

The average fecundity of 71,000 oocytes per spawning female estimated in this study diverges from Freitas *et al*. (2003), who found an estimated average fecundity of 200,000 oocytes per mature female in the Atol das Rocas and the coast of Ceará, environments that are similar to the present study. Such important differences were evaluated by Araújo & Martins (2009) by comparing the absolute fecundity found by Randall (1961) and Thompson & Munro (1978) for *Epinephelus guttatus* mature females, with a record of 40 and 160 thousand oocytes per female, respectively, in studies carried out in the same area at different periods. Therefore, the occurrence of variations in the average fecundities of the same species in distinct environments and periods is possible, and it is not indicative of reproductive failure for *Cephalopholis fulva* in the northern coast of Pernambuco.

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