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Reproductive biology of *Acanthurus coeruleus* (Bloch & Schneider, 1801) (Perciformes: Acanthuridae) in the north coast of the State of Pernambuco, Brazil

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Abstract. The objective of this work was to determine aspects of reproductive biology of Blue tang surgeonfish, *Acanthurus coeruleus*. 496 specimens were sampled, between January 2013 and December 2015, out of which, 235 were male and 261 were female. Sexual ratio did not significanlty differ among genders, and a ratio of 1.11 females per 1 male was found. Total length frequency distribution ranged from 14 to 41.7 cm with predominance of females in the 24-32 cm classes and of males in the 26- 32 cm classes. The estimated allometric coefficient (2.7695), suggests that the species presents negative allometric growth. According to the GSI and the gonads histological analysis, the reproductive period of the species occurs from June to January with periods of greater reproductive activity from August. The oocyte diameter-frequency distribution analysis revealed a multimodal distribution, confirming the evidence of multiple spawning and batch fecundity varied from 20000 to 55000 oocytes. Spawning frequency estimates, based on the hydrated oocytes (HO) method indicated that the species spawns once every 3.8 days, while the estimates based on the post-ovulatory follicle (POF) method indicated a spawning every 3.4 days, during a 6-month spawning season lasting from August to January.

Key words: Reef fish, oocyte, spawning, fecundity

Resumo. Biologia reprodutiva de *Acanthurus coeruleus* (Bloch & Schneider, 1801) (Perciformes: Acanthuridae) na costa norte do Estado de Pernambuco, Brasil. Este trabalho buscou determinar aspectos reprodutivos da Caraúna azul. 496 exemplares foram amostrados entre janeiro de 2013 e dezembro de 2015, sendo 235 machos e 261 fêmeas. A proporção sexual encontrada foi de 1,11 fêmeas para 1 macho, não diferindo significativamente entre os sexos. A distribuição de frequência de comprimento total variou entre 14 e 41,7cm, com predominância de fêmeas nas classes 24 a 32 cm e de machos nas classes 26 a 32 cm. O coeficiente de alometria estimado (2,7695), sugere crescimento alométrico negativo para a espécie. Conforme o IGS e a análise histológica das gônadas, o período reprodutivo se estende de junho a janeiro com períodos de maior atividade reprodutiva a partir do mês de agosto. A análise da distribuição de freqüência do diâmetro dos ovócito, revelou uma distribuição multimodal, confirmando a evidência de múltiplas desovas; e a fecundidade em lotes variou de 20,000 a 55,000 ovócitos. As estimativas de freqüência de desova, com base no método dos ovócitos hidratados (HO), indicaram que a espécie desova uma vez a cada 3,8 dias, e segundo o método do folículo pós-ovulatório (POF), ocorre uma desova a cada 3,4 dias, entre os meses de agosto e janeiro.

Palavras-chave: Peixe recifal, ovócito, desova, fecundidade

Introduction

Acanthurus coeruleus (Acanthuridae) is an herbivorous, gonochoristic species with diurnal habitat that inhabits coastal coral reefs and rocky areas where it plays an important ecological role in the dynamics of benthic communities (Mumby *et al*. 2006). In the western Atlantic, its occurrence is from the east coast of the United States, Bermuda to the Gulf of Mexico and Brazil. In the eastern Atlantic, it occurs on Ascension Island (Desoutter 1990).

In the Brazilian coast, this species occurs in greater abundance in the northeast region, where, until recently, it was captured only as a companion fauna of the lobster fishery, being discarded or consumed on a small scale. However, due to the increasing demand for fish consumption and the overexploitation of the main fishing resources traditionally used in this region, several species of reef fish, considered of small importance for fishing as *A. coeruleus* has been target by specific fishing obtaining large volumes of captured fish (Marques & Ferreira 2010, Cunha *et al.* 2012), with a large part of the production assigned for export market (Carvalho 2013).

The studies carried out on this species report its morphometric and meristic characteristics (Szpilman 2000, Figueiredo & Menezes 2000), distribution and habitat (Lawson *et al*. 1999), reproduction and behavior (Morgan & Kramer 2004, Dromard *et al*. 2012) and feeding (Thighman *et al*. 2001). Little is known, however, of the reproductive strategy of this species fish in Brazil, particularly on the population structure and reproduction.

Studies on population structure and reproduction provide important information for the knowledge of the biology of a species and they are essential to establish management measures that can guarantee the maintenance of the stock and the economic potential of this fishery. Hence, the objective of this work was to contribute to the knowledge of some aspects of the biology of this species in Brazil, by analyzing the structure in sex and length and the weight-length ratio in addition to determining from the histological analysis of gonads and monthly evaluation of the gonadosomatic index, the reproductive cycle of the species, estimation of the spawning frequency, estimation of batch fecundity and analysis of the oocyte diameter-frequency distribution, considering that these aspects are the parameters used in the management of the fish resources.

Material and Methods

The specimens were collected monthly from January 2013 to December 2015 from the landings of artisanal fishing boats that operate with traps on the Island of Itamaracá, north coast of the State of Pernambuco, Brazil (Fig. 1). For each individual collected, the following were recorded: total length (TL) in centimeters, total weight (TW), eviscerated weight (WE) and weight of gonads (WG) in grams. The gonads were macro and microscopically classified for sex and maturational stage, according to the scale developed by Brown-Peterson *et al*. (2011), adapted for the species under study.

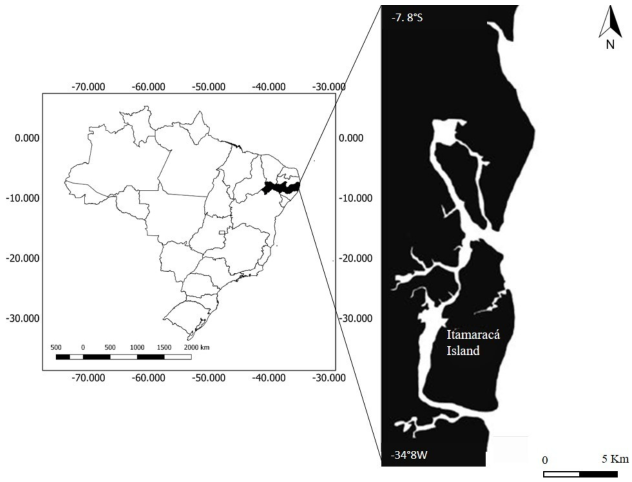

Figure 1. Itamaracá Island, north coast of the State of Pernambuco, Brazil.

The sex ratio was established by the frequency of males and females over the study period. The chi-square test (χ^2) was applied to test the equality hypothesis.

Length structure was based on the distribution of TL frequencies in 3-cm classes, considering the separated sexes. To determine possible differences in lengths between genders, the data were submitted to the Mann-Whitney test.

The weight-length ratio was estimated for males and females from the equation $TW = aTL^b$, where (a) is the linear coefficient, relative to the body shape and (b) is the angular coefficient, which indicates the type of growth. Parameters *a* and *b* were estimated after logarithimic transformation of the data. Possible differences between the weight-length relationship of males and females were verified through analysis of covariance (ANCOVA).

For the histological analysis, medium portions of the gonads were dehydrated, in an increasing alcohol series (80 - 100), diaphanized in xylol, impregnated and included in liquid paraffin at 59°C.

After that, the material was cut at 5µm in a Leica type microtome and stained with hematoxylin and Harris eosin. Histological analysis and images was performed by a light microscope (Leica DM 500). The ovaries classification was based on the most advanced stage of oocyte development. The reproductive cycle of the species was based on the monthly proportion of females at each gonadal maturity phase and the monthly evolution of the gonadosomatic index (GSI), calculated from the equation: GSI = (WG/WE) x 100.

In order to assess the spawning pattern, the oocyte diameters from actively spawning sub-phase females were estimated by stereomicroscope following Brown-Peterson *et al.* (2011). To estimate batch fecundity, the gravimetric methodology of Hunter & Macewicz (1980) and Murua *et al.* (2003) was applied where the number of hydrated oocytes in a small section (0.01 g) of the gonad of 16 ripe females was counted, with batch fecundity being then calculated by the following formula: $BF= [(H_i /W_i)/n) *GoW]$, where: H_i = number of oocytes from each portion, W_i = weight of each portion of the ovary, n = number of repetitions, GoW= weight of each ovary.

Monthly spawning frequency was determined by calculating the percentage of females with postovulatory follicles (POF) hydrated oocytes (HO) from histological examination of ovarian tissues, following the procedures described by Hunter & Macewicz (1980). These provide estimates of fish in the population that will spawn within the next 12 h (HO) or have just spawned in the past 12-24 h (POF). Spawning frequency was determined by dividing 100% (representing all the fish in the sample) by the percentage of fish in the sample with HO or POF in the ovaries. Multiplying the mean spawning frequency by the duration of the spawning season results in a potential total number of spawns during the year (Brown-Peterson *et al.* 2002). An ANOVA test was applied to investigate the diferences between the methods.

Results

A total of 496 specimens of *Acanthurus coeruleus* (235 males and 261 females) were sampled over the study period. By analyzing the frequency distribution of males and females, it was found that there were no statistically significant differences (χ^2 = 1.366, FD = 1, p = 0.2426), with a ratio of 1.11 females to 1 male.

For males, the total length ranged from 19.3 to 41.7 cm, with a mean of 28.8 ($\pm$ 3.9) cm, with most individuals in the 26 to 32 cm classes. In relation to the females, the total length ranged from 14 to 39 cm with an average of 27.7 ($\pm$ 3.6) cm, with an average between the 24-32 cm classes (Fig. 2). No significant differences were observed between sexes (Mann-Whitney test; U = 262.2; *P* = 0.0053).

The equations obtained for the weight-length relationship were analyzed separately for males ($TW = 0.0399\ TL^{2.7977}$) and females ($TW = 0.0516\ TL^{2.7361}$). However, no significant differences were found between the sexes (ANCOVA, P < 0.0001). In adition, the weight-length ratio is represented by the equation $TW = 0.0449\ TL^{2.7695}$ obtained for the clustered genders (Fig. 3). The allometry coefficient obtained indicates negative allometric growth for the species.

The results of GSI for females suggest that the reproductive period occurs from July to January. With spawning peaks during the months of September (1.48 $\pm$ 0.76), November (1.65 $\pm$ 1.1) and January (1.32 $\pm$ 1.0), when the highest GSI average values were recorded followed by falls. During this

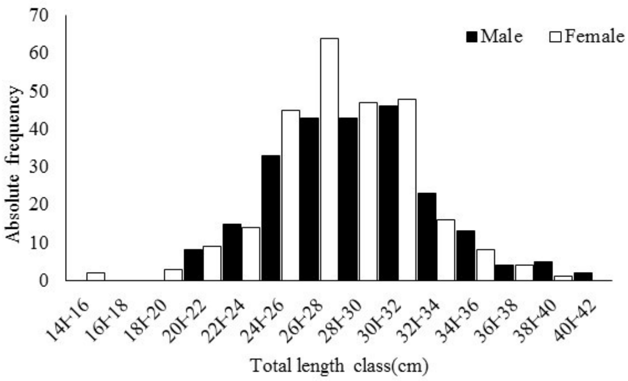

Figure 2. Length frequency distribution of *Acanthurus coeruleus* males and females in the coast of Pernambuco State in Brazil.

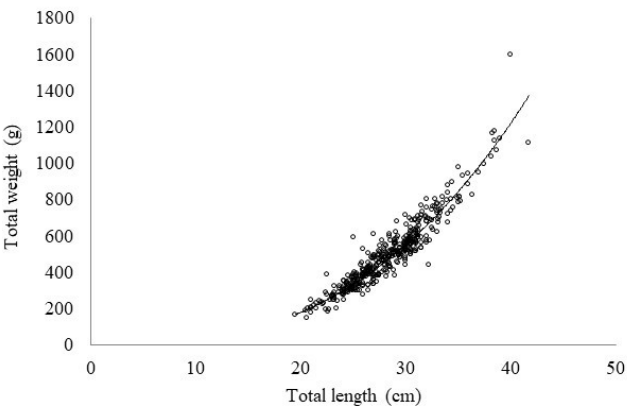

Figure 3. Weight-length ration for clustered genders of *Acanthurus coeruleus* in the coast of the State of Pernambuco, Brazil.

period, the weight of the gonads represented, on average, 1.48%, 1.65% and 1.32% of the gutted weight. From February to June, the GSI presented the lowest mean values, with fluctuations between 0.36 and 0.53. From July, GSI increased (0.84 ± 0.7), marking the beginning of the reproductive period. For males, small variations of average GSI values were recorded throughout the year. However, GSI increases were observed during the reproductive period (between July and January), with the highest average values recorded in July (0.27 ± 0.2), August (0.34 ± 0.2) and December (0.30 ± 0.3), (Fig. 4).

The diameters of oocytes, which corresponds to actively spawning sub-phase, ranged from 16.6 to 600 µm (mean 180.42 ± 133.03) and was multimodal (Fig. 5). The smallest group refers to primary growth and cortical alveoli oocytes, with sizes of 16.6 to 183 µm. The second one was constituted by partially yolked (Vtg1 and Vtg2) and advanced yolked (Vtg3) oocytes, and ranged between 172.0 and 300 µm. The third group was composed by the hydrated (GVM, GVBD and HO) oocytes with diameters from 327.4 to 600 µm.

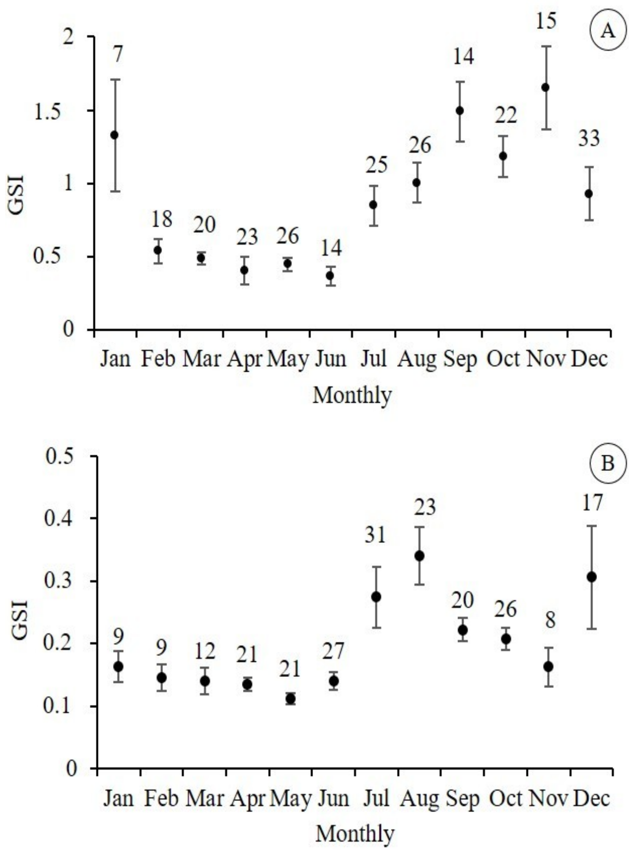

Figure 4. Monthly variation of GSI of *Acanthurus coeruleus* females (a) and males (b) in the coast of Pernambuco State. Bars correspond to the standard error. The number pointed above the bars represent the absolute frequency.

Batch fecundity estimation from 16 in spawning capable maturation phase female (actively spawning sub-phase), with size ranging from 23.2 to 37.2 cm TL, with corresponding weights of 0.521 and 0.997g, were used for the batch fecundity analysis. Batch fecundity ranged from a minimum of 20000 oocytes for a 23.2 cm TL fish weighing 0.412g, to a maximum of 55.000 oocytes for a 30.5 cm TL fish weighing 0.641g, with a mean of 3930988±1198301 oocytes.

According to the histological observations, no immature females were captured in this study. Only four phases of gonadal development and one sub-phase were recorded in this study: development phase, characterized by the presence of cortical alveoli oocytes (CA) and as the phase progressed, the first oocytes in primary (Vtg1) and secondary vitellogenesis (Vtg2); spawn capable phase, characterized by the presence of oocytes in more advanced stages of development (tertiary vitellogenesis, Vtg3), followed by mature oocytes (GVM - germinal vesicle migration and GVBD - germinal vesicle breakdown) and hydrated oocytes (HO) that characterized the active spawning sub-phase.

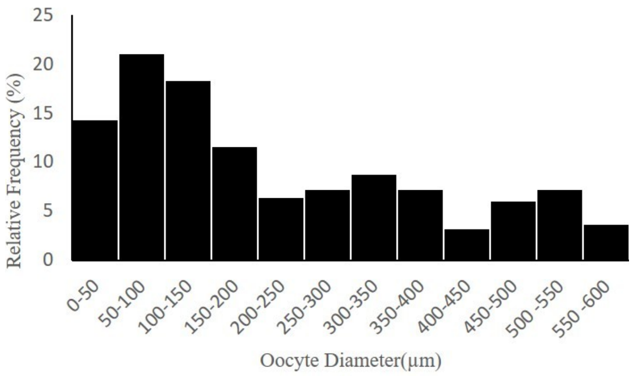

Figure 5. Frequency of oocytes diameter from 16 actively spawning sub-phase females.

The regression phase, was characterized mainly by the abundance of atretic oocytes (A); and the regeneration phase was represented by the presence of primary growing oocytes (PG) and oocytes atresia, in some cases (Fig. 6). Atresic oocytes were observed in all ovarian development phase described in this paper, however, the highest incidence of ovaries containing atresic oocytes were recorded in the spawn capable phase (31.8%) and regression (45.7%). In relation to the others, the number of ovaries with atresic oocytes was 17,5% in the regeneration phase and 5% in the development phase. Post-ovulatory follicles were also visualized

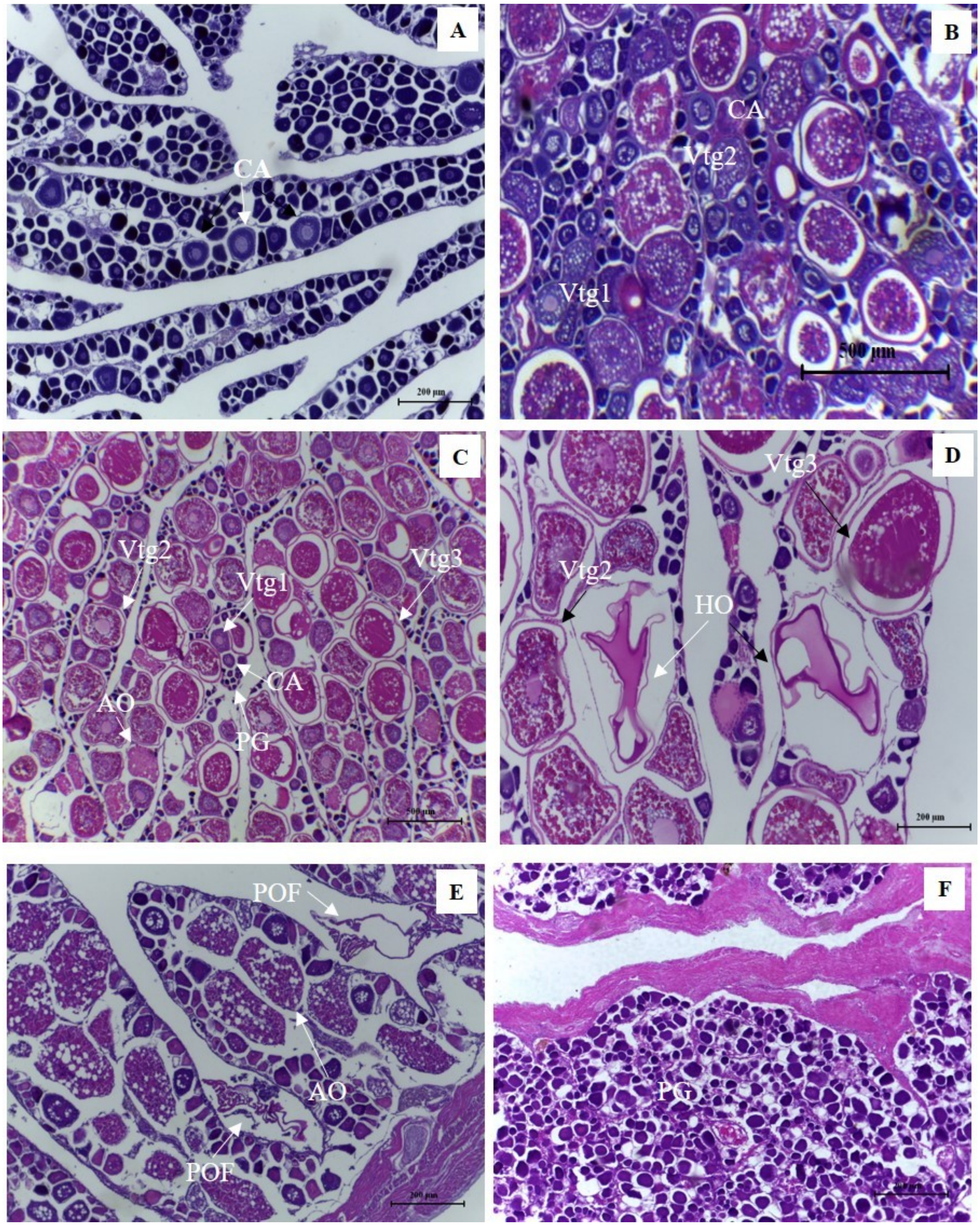

Figure 6. Photomicrograph of *Acanthurus coeruleus* reproductive phases. A-B) development phase; C) spawn capable; D) active spawning sub-phase; E) regression and F) regeneration. PG = primary growth; CA = cortical alveolus; Vtg1 = primary vitellogenesis; Vtg2 = secondary vitellogenesis; Vtg3 = tertiary vitellogenesis; HO = hydrated oocytes; AO = atresic oocytes and POF = post-ovulatory follicle. Staining by HE.

in ovaries in active spawning sub-phase and in the regression phase (Fig. 6E).

In the ovaries of females in reproductive activity, it was possible to identify oocytes in all development stages, indicating that the species present oocytes in asynchronous development (Fig. 5 and Fig. 6C). Out of the 224 analyzed ovaries, 16% were in the development stage, 10.2% at the spawning capable phase, 16% in active spawning, 26.3% in the regression phase and 31.2% in the regeneration phase. Based in the frequency distribution of the different phases of gonadal maturation during the year, ovaries in the development phase had been recorded since April, females in the capable to spawn phase were observed from June to January, however, most females in the active spawning sub-phase were captured in August, September and October. Females in the regression and regeneration phases were observed in all months, except in September when no female in regeneration was recorded (Fig. 7A).

During the spawning months, (August to January), a total of 141 ovaries were sectioned, of which 37 were in the OH stage and 39 in the POF stage (Table I). The mean monthly spawning frequency was 26% using the HO method and 29.1% using the POF method, indicating spawning frequencies of 3.8 days and 3.4 days respectively. There was no statistically significant difference in between the HO method and the POF method (ANOVA, p = 0.7338). Which These figures give a potential number of spawns during the year of 23 and 20, using the HO and POF methods, respectively.

In relation to the males, 178 testicles were histologically analyzed and like the females, no mature individual was recorded, 23.5% were in the development phase, 32.1% were in the capable for spawning phase, 11.9% in the regression phase and 32.5% in the regeneration phase (Fig. 8).

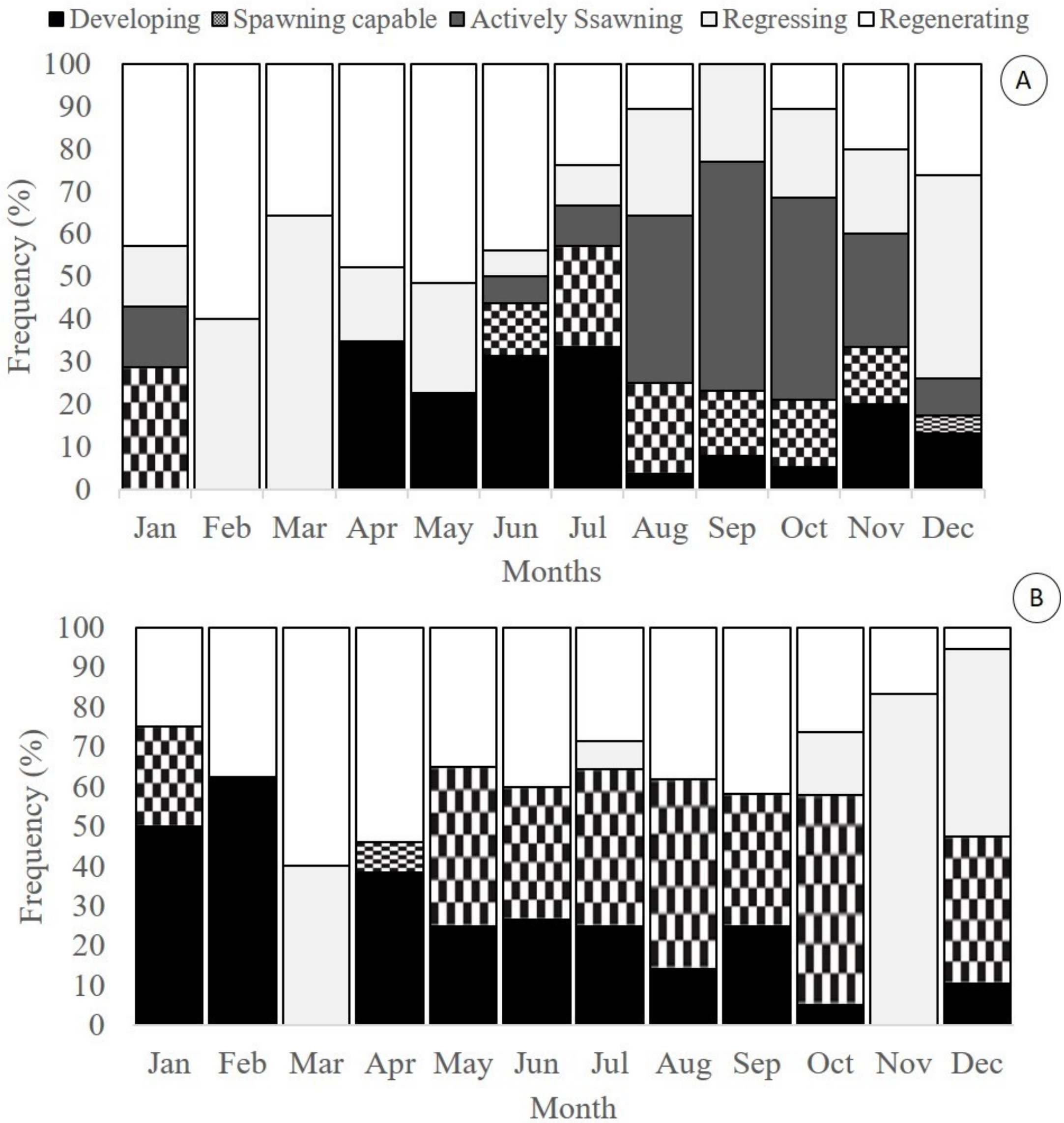

Figure 7. Monthly percentage of the frequency distribution of gonadal maturation phases of *Acanthurus coeruleus* females (A) and males (B) over the months of the year in the coast of the Pernambuco State.

Table I. Spawning frequency of *Acanthurus coeruleus*; N - number of fish sampled; HO - number of fish with hydrated oocyte; POF - number of fish with post-ovulatory follicle.

Month	N	HO	POF	Monthly spawnings (%)	
				HO	POF
June	16	1	2	6.3	12.5
July	19	2	3	10.5	15.8
August	29	11	9	37.9	31.0
September	12	7	2	58.3	16.7
October	20	9	8	45.0	40.0
November	15	4	5	26.7	33.3
December	23	2	6	8.7	26.1
January	7	1	4	14.3	57.1
Mean%				26.0	29.1

Individuals in the development and capable to spawn phases occurred nearly all over the year, expect for the months of March and November, when only individuals in regression or in regeneration were observed. From November to March, the highest frequency of individuals was recorded in the regression phase. Testicles in regeneration were recorded in all months of the year (Fig. 7B).

Discussion

The sexual proportion found for *A. coeruleus* captured by the artisanal fleet that operates with cage traps on the coast of the State of Pernambuco did not show significant variations between months and years sampled. Temporal variations and length classes were not observed either. The same behavior was observed by other authors, when observing that adult individuals of this species form paired aggregations for forage and reproduction (Deloach 1999, Lawson *et al*. 1999, Bell & Kramer 2000, Lukoschek & McCormick 2000, Rocha 2002). Regarding the length frequency distribution, statistical analysis showed no significant differences between sexes, however, it was possible to observe that males reach slightly higher lengths, indicating a possible sexual dimorphism for the species.

The equation of the weight-length ratio obtained for males and females showed that the value of the angular coefficient (b) was less than 3 for both sexes, indicating negative allometry for this species, which according to Froese (2006) occurs because the fish grows faster in length than in weight. Other studies conducted in Indo-Pacific waters (Choat & Axe 1996) reported values of the scaling exponent ranging from 2.5 to 3.06 for ten surgeonfish species.

According to the GSI evolution, female gonadal development occurs from July and the period of greatest reproductive activity is from August to January. These results are consistent with histological analysis of the ovaries that show a gradual increase in mature females frequency from June to August, followed by the appearance of post-ovulatory follicles, which reinforces the hypothesis that the major spawning season is from August to January. However, there is histological evidence of less intense spawning events since June. From February, GSI decreased and mature females were no longer observed. The few studies on the Acanthuridae reproductive period show considerable differences between the annual spawning patterns of the species (Bushnell *et al*. 2010). According to Fishelson *et al*. (1987), in the Red Sea region, *Acanthurus nigrofuscus* shows a well-demarcated reproductive cycle and divided into three seasons: the first season is from October to December (winter), when gonads recover from the previous year's reproductive activity; the second season is from January to April (spring) when the reactivation of gametogenesis occurs and the third is from May to September (summer) when the highest values of the gonadal index were recorded. In American Samoa, *Acanthurus lineatus*, *Acanthurus guttatus* and *Acanthurus triostegus* spawn throughout the year, with periods of greater reproductive intensity during the summer months (Craig 1998). In Hawaii, *Acanthurus triostegus sandoicensis* also presented distinct spawning period although it spawns throughout the year (Randall 1961). According to the results obtained in this study, *Acanthurus coeruleus* presents a well-demarcated reproductive period, with optimization of gonadal development during the rainy season, which occurs from March to August in this region, and spawning peaks occurs at the beginning of the dry season, when temperatures increase due to the reduction of the rainfall volume and the proximity with the beginning of summer. The increase in temperatures is probably the main factor to determine the total development of the gonads and consequently spawning peaks.

The spawning period is chosen based on the adequacy of females to accumulate sufficient energy reserves for gonadal development. For *Acanthurus nigrofuscus*, changes in diet between winter and summer exert strong influence on the determination of the reproductive period, so that the winter diet

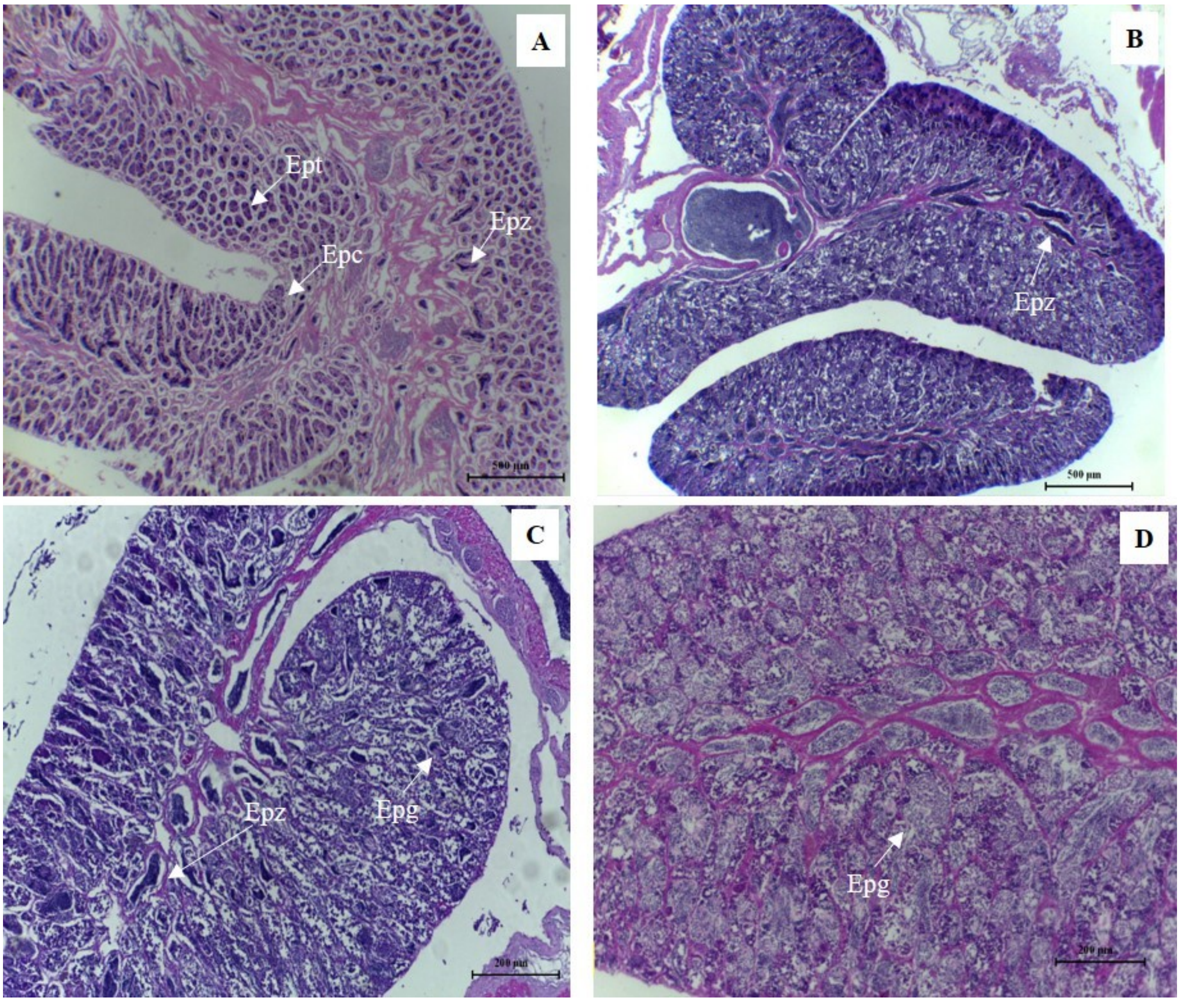

Figure 8. Photomicrography of *Acanthurus coeruleus* development. A) Development phase; B) capable to spawn; C) Regression and D) Regeneration (Epg = spermatogonia; Epc = spermatocytes; Ept = spermatids; Epz = spermatozoa).

provides energy for gonadal development, and the summer diet provides energy for subsistence and maintenance of activity (Fishelson et al. 1987). For *Acanthurus coeruleus*, it was observed that gonadal development occurs mainly during the rainy period of the region, when primary productivity increases due to the greater nutrient supply, and although it cannot be said that there is any relationship between food availability and gonadal development of this species during the dry and rainy periods, it is likely that the increase in food availability during the rainy season is a factor that influences the reproductive cycle of the species.

Oocytes at different development stages were observed in the ovaries of *Acanthurus coeruleus* mature females, which appears to be an indicator of asynchronous development of the oocytes and split spawning, with multiple batches of oocytes being spawned over a prolonged spawning season. Species with a long reproductive period and multiple spawns present advantages that can maximize reproductive success, since a greater number of reproductive opportunities will be able to ensure that their offspring are born in adequate environmental conditions, allowing the development and survival of the offspring (Yamahira 2004). In addition, species with prolonged spawning seasons have an offspring that widely varies with age, resulting in different life histories associated with the traits of the individuals born at the beginning or in the end of the reproductive period, as well as different vulnerability to the minimum size of capture (Lowerre-Barbieri *et al*. 1998). Oocyte diameter-frequency distribution revealed a multimodal pattern, in further confirmation of multiple spawning. Moreover, the asynchronous oocytes development and no hiatus observed between clutches, as presence of hydrated oocytes both post-ovulatory follicles evidences batch spawning. This is an indicative for continuous oocyte recruitment during the spawning season.

The batch fecundity in the *Acanthurus coeruleus* (between 20.000 and 55.000 oocytes) is relatively similar to that of other small surgeonfish worldwide, such as *Acanthurus triostegus sandvicensis* (mean 40.000 batch oocytes) in Hawaiian Islands (Randall 1961) and *Zebrasoma Flavescens* (20.000 and 44.000 oocytes per batch) (Bushnell 2010). Fecundity is regulated in response to environmental conditions, food availability nutrition, predation, and energetic resources to reproductive success (Kjesbu *et al*. 2003). However, low batch fecundity the surgeonfishes is associated with body shape strongly laterally compressed and a sharply asymptotic growth curve both of which contribute to a relatively small range of adult body size and ovary size (Choat & Axe 1996, Bushnell 2010).

The HO and POF methods for estimating spawning frequencies have been used and the results are quite striking and have important implications for estimating annual reproductive output. From the present study females are reproductively active over extended periods and are likely to spawn many times, with spawning occurring in females on average every 3.8 days according to the HO method of estimation, and every 3.4 days as revealed by the POF method, it may be concluded that the potential annual number of spawns in *Acanthurus coeruleus* is 23 and 20, according to the HO and POF methods, respectively. Most of the fish that perform multiple spawning have high spawn frequencies and can spawn successively during the breeding season (Ganias *et al.* 2011). The high spawning frequency of *Acanthurus coeruleus* species compensates for their low batch fecundity.

During this study, immature specimens were not captured because of the high selectivity of the fishing gear for the capture of specimens smaller than 14 centimeters of total length, which does not mean that this population cannot suffer the impacts caused by the fishing, once that gear operates within the spawning area, capturing individuals that would still reproduce. In this study, the smallest mature female and the smallest mature male captured measured 20.6 and 20.4 cm of total length, respectively. In addition, it is known that this type of equipment has led to the reduction of fish stocks by interfering with the dynamics of populations (Wolff *et al*. 1999, Maron & Hunte 2001).

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