REPRODUCTIVE BIOLOGY OF THE STINGRAY *HYPANUS MARIANAE*, AN ENDEMIC SPECIES FROM SOUTHWESTERN TROPICAL ATLANTIC OCEAN

BIOLOGIA REPRODUTIVA DA RAIA *HYPANUS MARIANAE*, UMA ESPÉCIE ENDÊMICA DO SUDOESTE DO OCEANO ATLÂNTICO TROPICAL

BIOLOGÍA REPRODUCTIVA DE LA RAYA *HYPANUS MARIANAE*, UNA ESPECIE ENDÉMICA DEL SUROESTE DEL OCÉANO ATLÁNTICO TROPICAL

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Abstract

The Brazilian Large-eyed stingray *Hypanus marianae* is the smallest species of the family Dasyatidae in Brazil. This study aims to provide data on the reproductive biology of this species captured in artisanal fisheries from Ceará State. A total of 299 individuals of *H. marianae* were recorded at monitoring landings and adult male to female sex ratio was significantly different (1:2.9), indicating a possible spatial segregation between males and females. The size range was from 13.0 to 36.2 cm in disc width (DW). Females reached greater size and body mass (36.2 cm DW and 1855 g) than males (29.3 cm DW and 915 g). The reproductive system analyses were based on 81 preserved specimens. The DW_{50} parameter was estimated at 26.1 cm DW for females, and 23.8 cm DW for males. Only the left uterus is functional, and birth size was estimated at 13.0–14.0 cm DW. Vitellogenesis occurred concurrently with a short gestation (shorter than 6 months) and uterine fecundity is only one embryo per reproductive cycle, which seems to be asynchronous.

Keywords: maturity; fecundity; birth; embryos; Dasyatidae.

Resumo

A raia Mariquita *Hypanus marianae* é a menor espécie da família Dasyatidae no Brasil e este trabalho tem como objetivo reportar informações acerca da sua biologia reprodutiva a partir de capturas da pesca artesanal no estado do Ceará. Um total de 299 indivíduos de *H. marianae* foram registrados durante o monitoramento dos desembarques e a razão sexual entre machos e fêmeas apresentou-se significativamente diferente (1:2,9), indicando uma possível segregação espacial entre os sexos. O intervalo de tamanho variou de 13,0 a 36,2 cm na largura de disco (LD).

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Fêmeas alcançaram tamanhos e pesos maiores (36,2 cm LD e 1855 g) que machos (29,3 cm LD e 915 g). A análise do sistema reprodutivo foi realizada em 81 espécimes fixados. O parâmetro LD_{50} foi estimado em 26,1 cm LD para fêmeas, enquanto nos machos foi de 23,8 cm LD. Somente o útero esquerdo é funcional e o tamanho de nascimento foi estimado entre 13,0 e 14,0 cm LD. A vitelogênese é concomitante à curta gestação (menor que 6 meses) e a fecundidade uterina é de apenas um embrião por ciclo reprodutivo, que parece ser assincrônico.

Palavras-chave: maturidade; fecundidade; nascimento; embriões; Dasyatidae.

Resumen

Hypanus marianae es la especie de raya más pequeña de la familia Dasyatidae en Brasil. El objetivo de este trabajo es reportar información sobre la biología reproductiva de esta especie capturada en la pesquería artesanal. Se registraron un total de 299 individuos de *H. marianae* durante el monitoreo de los desembarcos. La proporción de sexos entre machos y hembras fue significativamente diferente (1:2.9), indicando una posible segregación espacial entre machos y hembras. El rango de tallas varió de 13.0 a 36.2 cm en el ancho del disco (AD). Las hembras alcanzaron tamaños y pesos mayores (36.2 cm AD y 1855 g) que los machos (29.3 cm AD y 915 g). El análisis del sistema reproductivo se realizó en 81 muestras fijadas en formaldehído. El parámetro L_{50} se estimó en 26.1 cm AD para las hembras, mientras que en los machos fue 23.8 cm AD. Sólo el útero izquierdo es funcional y la talla de nacimiento se ha estimado entre 13.0 y 14.0 cm AD. La vitelogénesis es concomitante con la gestación corta (menos de 6 meses) y la fecundidad uterina es de sólo un embrión por ciclo reproductivo, el cual parece ser asíncrono.

Palabras clave: madurez; fecundidad; nacimiento; embriones; Dasyatidae.

INTRODUCTION

The Brazilian Large-Eyed Stingray *Hypanus marianae* (Gomes, Rosa & Gadig, 2000) is an endemic species that inhabits tropical waters of the north to central Brazilian coast, and it is associated with coraline, sandstone and rocky reefs, as well as seagrass beds (Gomes et al. 2000; Costa et al. 2015). This stingray is the smallest species of the family Dasyatidae in Brazil reaching the maximum size of 38.0 cm DW (Motta et al. 2009). Besides the relatively large eyes, *H. marianae* is characterized by the dorsal and ventral colour patterns (Santos et al. 2006), which is mainly golden yellow on the dorsal surface and white with peculiar dark blotches ventrally.

There is few published information on the biology of *H. marianae*, which is currently classified as Data Deficient (DD) in International Union for Conservation of Nature's Red List (IUCN) (Rosa and Furtado 2004). The previous published studies have presented ecological and populational aspects of the species, as colour patterns (Santos et al., 2006), reproduction (Yokota and Lessa 2007; Motta et al. 2009), population characteristics and habitat use (Costa et al. 2015), diet (Motta et al. 2009; Shibuya and Rosa 2011; Costa et al. 2015) and ecological barriers for the species (Costa et al. 2017), in addition to records in checklists (Rosa and Gadig 2014). Although the reproduction of the species has been previously studied, there is a lack of information about the size of maturity for males and females, embryo development, confirmation of litter size, and reproductive cycle.

The data provided herein will partially fill the knowledge gap for this Brazilian endemic species and highlights the importance of studies on the biology of *H. marianae*, which has the lowest fecundity among dasyatids, and occupies a different ecological niche from its congeners in Northeastern Brazil (Yokota and Lessa 2006; Costa et al. 2015). Several populations of elasmobranchs are facing severe declines due to high fishing pressure (Stevens et al. 2000; Dulvy et al. 2014), and the study of reproduction can contribute with biological information to subsidy conservation and management plans of these animals. Along with the mortality rate and other parameters, reproductive data are necessary for the evaluation of the conservation status of populations of chondrichthyan species, according to criteria of the International Union for Conservation of Nature and Natural Resources (IUCN). Furthermore, the studied species has medical and economic importance, both for ecotourism and as ornamental fish (Rosa and Furtado 2004; Costa et al. 2015). The aim of this study was to provide data on the reproductive biology of *H. marianae*.

MATERIALS AND METHODS

Study area and sampling

Almofala is a fishermen village located in Ceará state, Northeastern Brazil. The climate is mild tropical semi-arid, the annual rainfall reaches 1,100 mm and the average temperature ranges from 26 to 28 °C. The rainy season begins in January and persists until May, and the period of strong winds occurs from July to November (Governo do Estado do Ceará, 2007). The studied specimens were obtained from November 2007 to October 2008 in a fishing weir (02°56.266'S and 39°48.842'W), with depths ≤ 6 m, and substratum covered by seagrass beds (Fig.1).

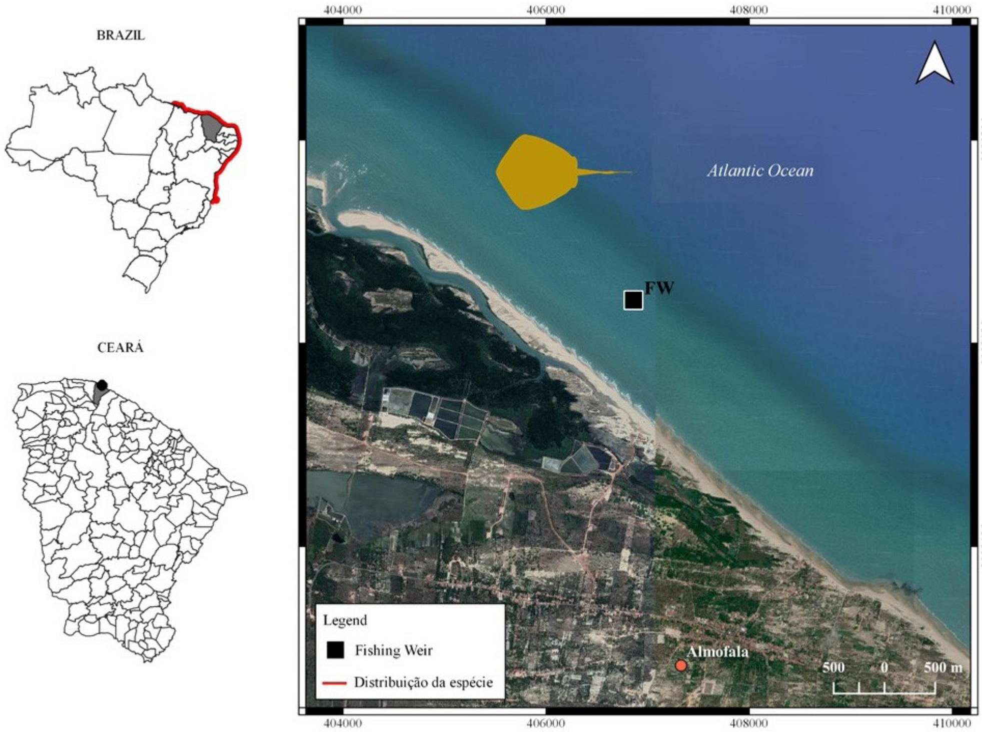

Fig. 1. Location of the monitored fishing weir site at Almofala beach, Southwestern Atlantic Ocean.

All samples (dead rays) were obtained from artisanal fishery landings, with no need of special collecting permit for this type of research in Brazil. All specimens were sexed by the presence or absence of claspers (n = 299), but only part of this total was measured in disk width (DW, n = 152), weighted (n=113), and, from the latter, 81 specimens had the reproductive system analysed. Not all specimens were preserved for this study, as part of the catch was consumed by fishermen. The actual number of samples for the different observations and analyses is indicated in the results.

Reproduction analysis

Forty four males and 69 females of the Brazilian large-eyed stingray were used to analyse the reproductive structures. The morphometric measurements were taken using callipers to the nearest millimetre. Width of the ovaries, width of the oviducal glands, diameter of the largest ovarian follicle, width of the left uterus were measured in females, and clasper length, as the length of the external margin of the left clasper, and width of testicles in males. The weight of these structures was also recorded whenever possible.

The sex ratio was tested using a Chi-square test, while size and mass differences between sexes were tested using a Student *t*-test. Normality condition of each variable was tested by a Shapiro-Wilk test. Development stages of the specimens – immature (neonate and young) and mature (adult) were determined by measurements and macroscopic analyses of their reproductive systems, adapted from Snelson et al. (1988) and Smith et al. (2007): Females – Immature: ovaries not developed, and filamentous uteri. Mature: left ovary with large vitellogenic follicles; left uterus well developed, presence of trophonemata. There was no evidence of the functionality of right ovary and uterus in *H. marianae*. Pregnant females were recorded as mature. Males – Immature: flexible claspers with no calcification, testes as a thin tissue trip. Mature: claspers well calcified, larger than the posterior margin of the pelvic fin; lobular and well-developed testes. Clasper length was measured.

The criterion to determine pregnancy in females was the presence of eggs or embryos in the uteri and uterine fecundity was the number of them (Walker 2005). The gestation period was estimated based on the largest follicle diameter, which is indicative of the month of ovulation, development of embryos and births (Mejía-Falla et al. 2012). Late or near-term embryos were those with an external yolk sac totally absorbed (Mejía-Falla et al. 2012) and neonates are the smallest free living (about near-term DW) with ventral yolk sac scar. Birth size was estimated comparing the largest embryo and the smallest recorded neonate (Yokota and Lessa, 2007).

The size range at first sexual maturity for males and females was calculated as the range between the disc widths of the largest immature individual and the smallest mature individual. The DW_{50} parameters were estimated for females and males based on each reproductive structure by fitting a logistic regression model to binomial maturity data (0, immature; 1, mature) (Mollet et al. 2000). The logistic model was fitted using maximum likelihood (proc logistic, SAS version 9.3, SAS Institute, Cary, North Carolina, USA). The equation is $P = [1 + e^{-(a+bDW)}]^{-1}$, where P is the proportion of mature individuals at DW and a and b are model parameters.

The reproductive cycle was also infered from the gonadosomatic (GSI) and hepatosomatic indices (HSI) in formaldehyde (10%) preserved adult specimens. The equation of the gonadosomatic index, $GSI = (LG_{WE}/ E_W) \times 100$ is the relationship between the weight of the gonad (left ovary or testes) (LG_{WE}) and the total eviscerated weight (E_W). The hepatosomatic index (HSI) is the relationship between the weight of the liver (L_W) and the total eviscerated weight (E_W), given by the equation: $HSI = (L_W/E_W) \times 100$ (Conrath 2004).

RESULTS

Sex ratio and size frequency distribution

A total of 299 individuals of *H. marianae* were recorded in landings monitoring, including 192 females, 60 males and 47 neonates of both sexes. Observed male/female ratio was 1:2.9 (χ^2=21.13; d.f. = 12, p=0.04). The number of captured females was greater than males during the whole study period, except in September and October, when the catches of females and males fell abruptly (Fig. 2A). The ratio between the number of adult females and males per month was significantly different (χ^2=20.40, d.f. =11, *P*=0.04) and higher in February, when the number of females was twenty times greater than males. The size range of *H. marianae* (n = 81) varied between 13.5 and 29.3 cm DW in males, and 13.0 to 36.2 cm DW in females. The modal class of males ranged from 24.1 to 27.0 cm DW (Mean ± S.D. = 25.7±2.2 cm) and the modal class of females ranged from 30.1 to 33.0 cm DW (31.7±1.5 cm) (Fig. 2B).

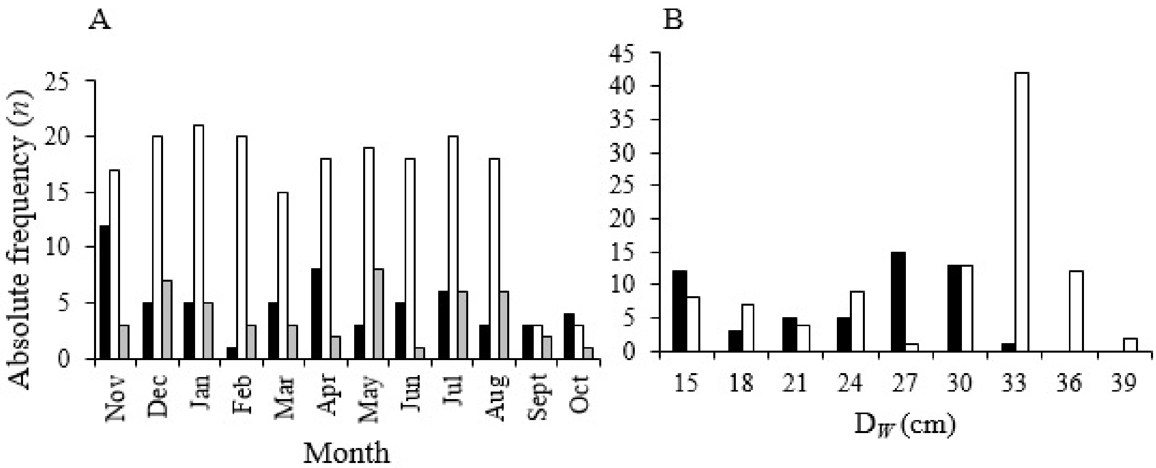

Fig. 2. Samples of *H. marianae* analysed in the study. (A) Monthly sample size of males (black bars, n=60), females (white bars, n=192) and neonates (gray bars, n=47). (B) Disc width frequency in samples. Males (black bars, n=54); females (white bars, n=98).

Size and mass relationships

Sexual dimorphism was observed in the body weight and disc width relationship of the sample, with females reaching larger sizes (DW) and greater body weights ($M_{T=}$ total mass) (13.0-36.2 cm $DW_{;}$ 70-1.810 g $M_{T,}$ n=69) than males (13.5-29.3 cm DW; 85-915 g M_T, n=49). The equations that describe the size and mass relationships are $M_T = 0.0233\ DW^{3.1572}$ (r^2=0.9728) for males and M_T=0.0197$DW^{3.2266}$ (r^2 = 0.9782) for females. The largest female 36.2 cm DW, was 20% larger than the largest male 29.3 cm DW. The maximum weight attained by females (1.855 g) was two-fold the maximum weight (915 g) recorded for males (Fig. 3).

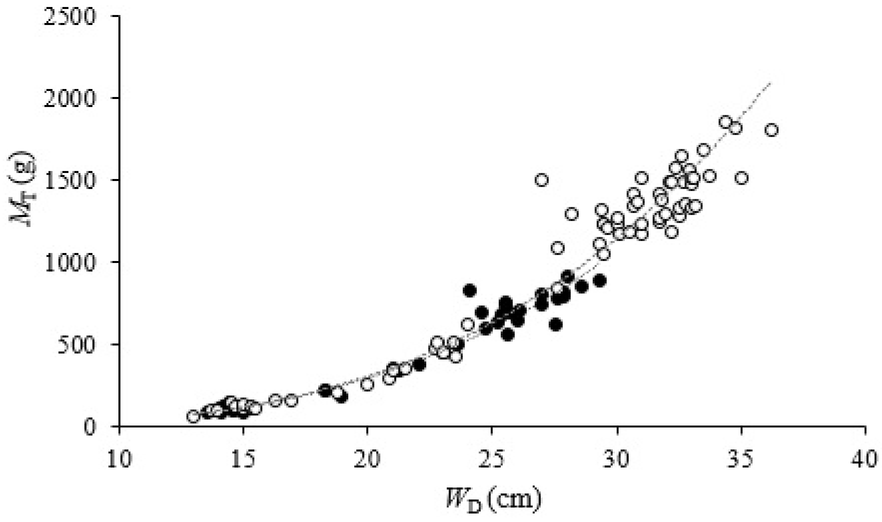

Fig. 3. Total body mass and disc width relationship of *H. marianae* caught in Almofala beach, Southwestern Atlantic Ocean.

Size at maturity in males

Juvenile males had an undeveloped reproductive system macroscopically. The left testes of adult males of *H. marianae* had higher mean weight and width than the right testes. The width of the left and right testis were statistically different (*T-test*= 2.373, $p = 0.02$), with the left testis ranging from 0.05 to 0.20 cm (Mean ± S.D. = 0.13 ± 0.44 cm), and the right ones from 0.05 to 0.17 cm (0.11 ± 0.03 cm) (n=49). The smallest mature male measured 24.1 cm DW and the largest immature male 23.6 cm DW (Fig. 4A).

The monthly distribution of clasper length (C_L) showed two distinct groups and a clear separation interval between them; the first group consisted of neonates and juveniles (13.5-23.6 cm DW), with clasper lengths ranging from 0.4 to 1.2 cm, and the second group, composed by adults (24.1–29.3 cm DW; n=49), with clasper lengths ranging from 2.4 to 3.8 cm. Males with disc width up to 16.5 cm had a mean clasper length of 3.1% DW and individuals between 18.3 and 23.6 cm DW, showed a mean clasper length of 3.8% DW. The largest clasper (3.8 cm of length) was found in a specimen of 27.6 cm DW, whereas the largest examined male had a clasper length of 3.2 cm. Clasper length showed an abrupt increase at 23.6 cm DW onwards, ranging from 4.7 to 13.8% DW. The smallest adult male, based on clasper rigidity, measured 24.1 cm DW and the largest immature male measured 23.6 cm DW (Fig. 4B).

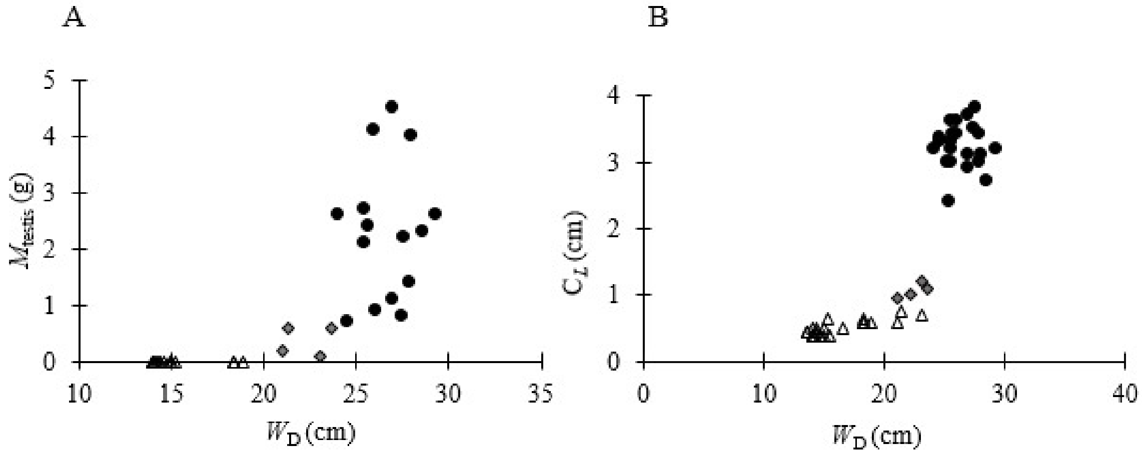

Fig. 4. Relationship between: (A) left testis width (LT_W) and disc width (DW) of males, n=33; (B) outer clasper length (C_L) and disc width (DW), n=49. Neonates (open triangles), juveniles (grey diamonds), adults (solid circles).

Median disk width at maturity (DW_{50}) estimated for males based on clasper calcification stage was the same as that estimated according to gonadal maturation, or 23.8 cm (a=-144.2±122; b=0.605±0.511) (Fig. 5).

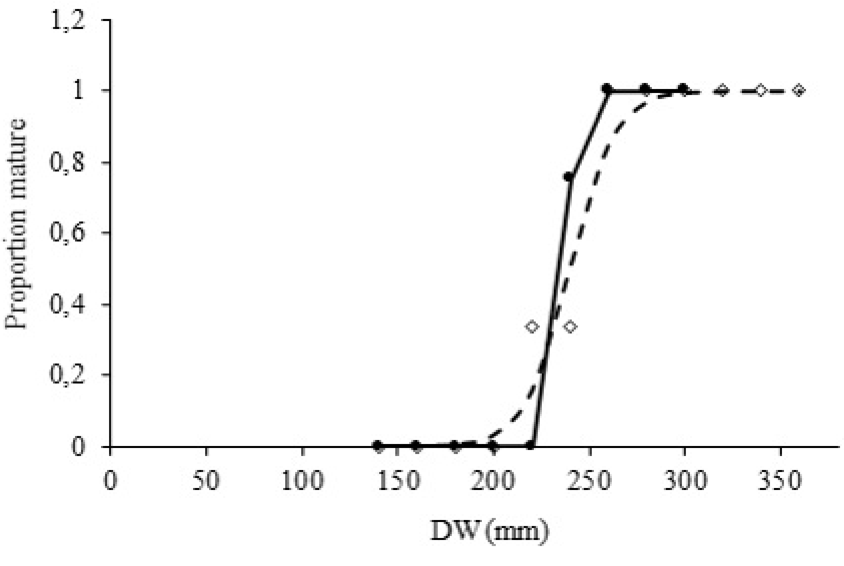

Fig 5. Maturity ogive for males and females of *H. marianae*. Proportion mature males (solid circles), fitted-curve males (solid line). Proportion mature females (open circles), fitted-curve females (dotted line).

Size at maturity in females

The reproductive system of females showed a complete reduction of the right uterus and only the left ovary contained vitellogenic follicles, with the development of a single follicle per reproductive cycle.

The left uterus width of the largest immature female (24.0 cm DW) measured 0.5 cm, while the smallest female with large ovarian follicles (27.0 cm DW) had a left uterus of 2.9cm. A pregnant female of 36.0 cm DW and a fully formed embryo had a left uterus width of 6.6 cm (Fig. 6A). The growth of the oviducal gland matched the disc width increment in females up to a stable maximum gland width of 1 cm.

The left ovary of the largest immature female had a width of 0.3 cm (LO_W), whereas the left ovary of the smallest mature female had a width of 1.9 cm (n=69) (Fig. 6B). The relationship between the weight of the left ovary (LO_{WE}) and disc width showed two distinct groups, as evidenced by the vitellogenic follicles. The smallest female with large ovarian follicles was 27.0 cm DW, and its largest follicle had a diameter of 1.4 cm. The largest follicle measured 3.3 cm in diameter in a 32.2cm DW mature female. An oocyte of 1.6 cm in diameter was found in the abdominal cavity between the ovary and the ostium.

Analysis of the female reproductive structures revealed that the formation of large vitellogenic follicles starts at 27.0 cm DW, which indicates that the size of first maturity is attained at 75% of the maximum recorded disc width (36.2 cm). The smallest mature female was 27.0 cm DW.

Median disk width at maturity (DW_{50}) was estimated for females based on gonadal maturation at 26.9 cm (a=-144.2±35.077; b=0.604±0.151) and DW_{95} parameter was estimated at 26.8 cm DW (Fig. 5).

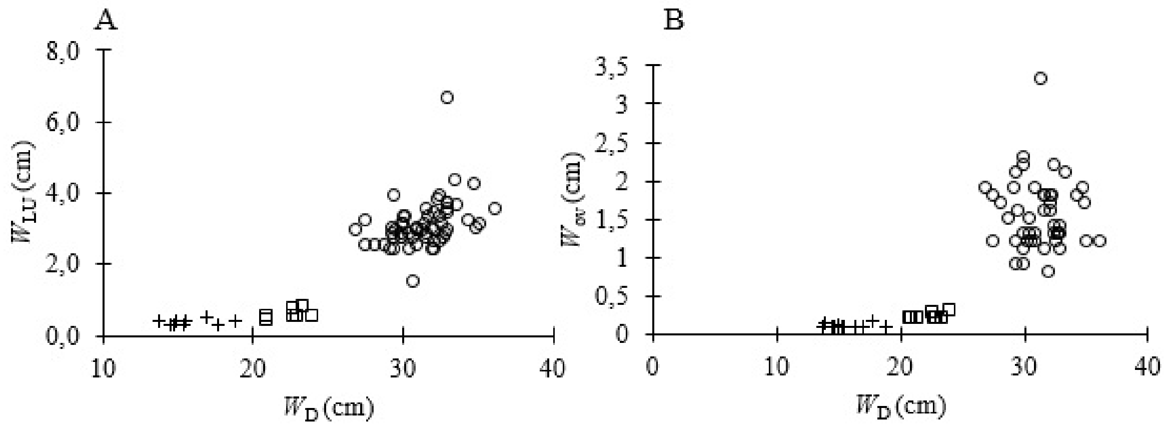

Fig. 6. Relationship between: (A) left uterus width (LU_W) and disc width (DW); (B) left ovary weight (LO_{WE}) and disc width (DW). Neonates (crosses), juveniles (open squares), adults (open circles), n=69.

Fecundity, embryos and birth size

Uterine fecundity was determined as a single embryo in the functional left uterus in the seven pregnant females, which was confirmed by a single vitellogenic follicle developed in the left ovary during the ovulation for mature females. The smallest pregnant female measured 30.4 cm DW and the largest one 34.8 cm DW. The size of recorded embryos ranged between 7.5 and 13.9 cm DW. Midterm to fully formed embryos were found with uterine villosities (trophonemata) in their mouths and spiracles. Length of the trophonemata ranged between 0.4 to 1.1 cm. Embryos were found with their pectoral fins folded dorsally, or occasionally with one pectoral fin folded in the opposite way. The fully formed embryo was usually oriented with the head towards the urogenital sinus, but in one birth, the tail was observed to emerge first from the cloaca. The embryo's tail was folded towards the belly and the sting surrounded by protective tegument. At birth, the neonate already displayed the dark blotches on the ventral surface of the disc, which are diagnostic for the species (Fig. 7A-E).

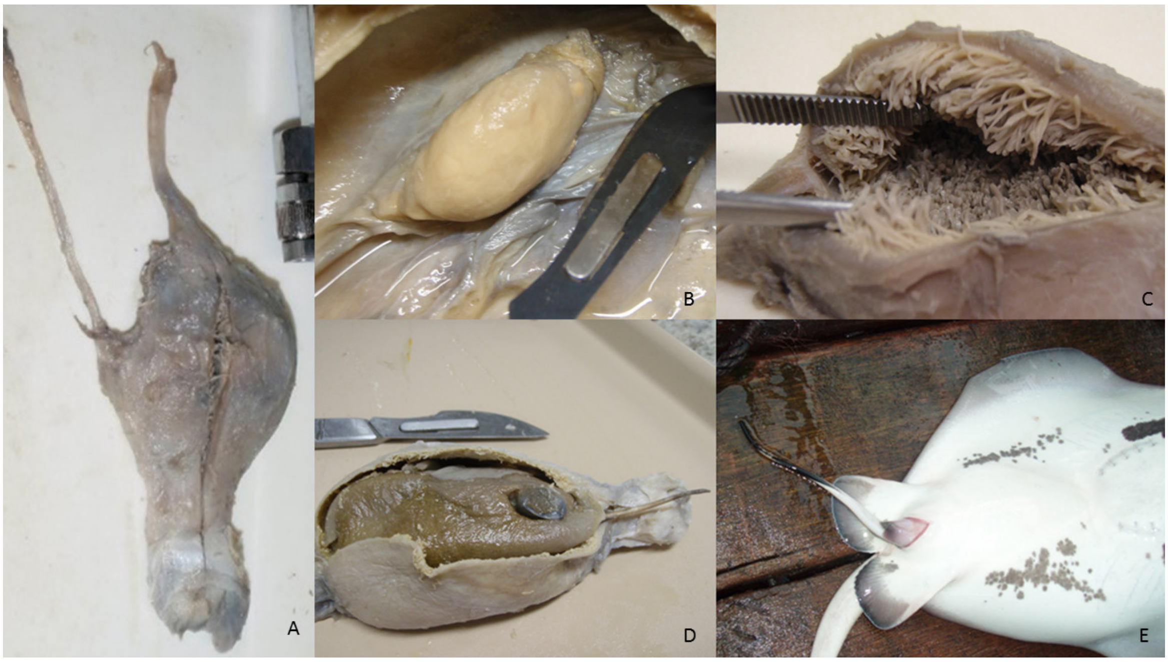

Fig. 7. Female reproductive structures of *H. marianae.* (A) Reduction of the right side and well-developed left uterus. (B) Large vitellogenic follicle. (C) Trophonemata. (D) Midterm embryo. (E) Parturition with the embryo's tail emerging from the cloaca.

The smallest free-living individual recorded was a 13.0 cm DW male and the largest embryo measured 13.9 cm DW, or 41.12% of maternal's DW (33.0 cm). Both were captured in December, which corresponds to the rainy season. From those observations, the size at birth was estimated at 13.5 cm DW.

HSI and GSI

The hepatosomatic index (HSI) of males showed the highest mean value in October. The HSI of females reached peak in December, April and May, indicating periods of high liver reserves for reproduction (Fig. 8A). For males, despite the low sample size and absence of information for some months, it was possible to notice an increment of the gonadosomatic index (GSI) in October, suggesting maturity of gonads. The GSI of females indicated vitellogenesis and displayed peaks in February and July (Fig. 8B). Examination of males revealed that the greatest left testes weights were recorded in October 2008, with an average of 3.6 g.

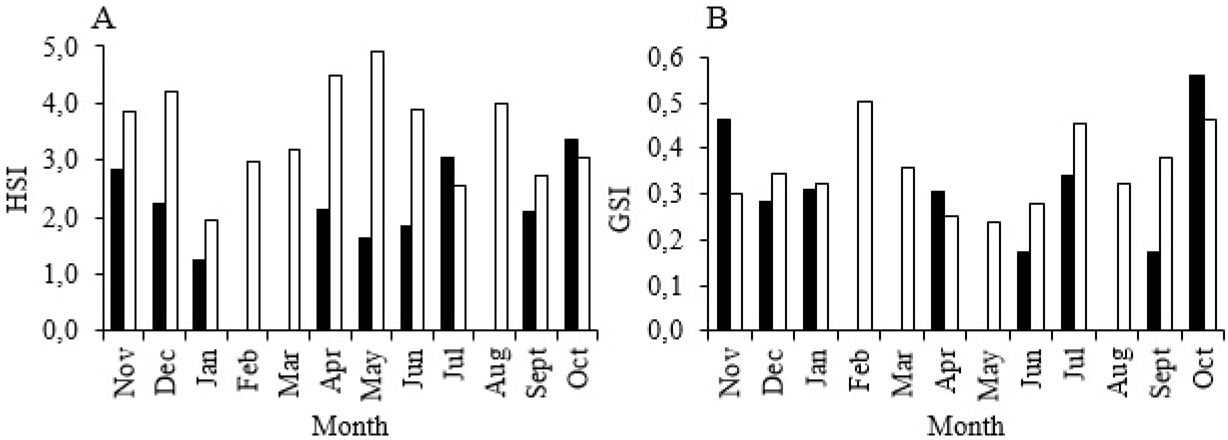

Fig. 8. The hepatossomatic (HSI) and gonadosomatic (GSI) indexes of *H. marianae.* (A) Mean monthly values of the hepatosomatic index (HSI). (B) Mean monthly values of the gonadosomatic index (GSI). Males (black bars, n=32); females (white bars, n=59).

For females, the months with the greatest left ovary widths were January and July, with sample means of 1.90 and 1.97 cm, respectively. The hightest mean values of the largest oocyte diameter in the functional ovary, which indicates ovulation period, occurred in January and September (Fig. 9). The mean width of the left uterus (LU_W) showed peaks in July and December, and the proportion of the width of the oviducal gland (OG_W) to DW reached its maximum values in January. This indicates that the fertilization periods occur in the subsequent months (January/ February), while the peaks of births occur in May and December. The number of births in September could not be confirmed due to lack of data, as the fishing weir was no longer fully operating at that time.

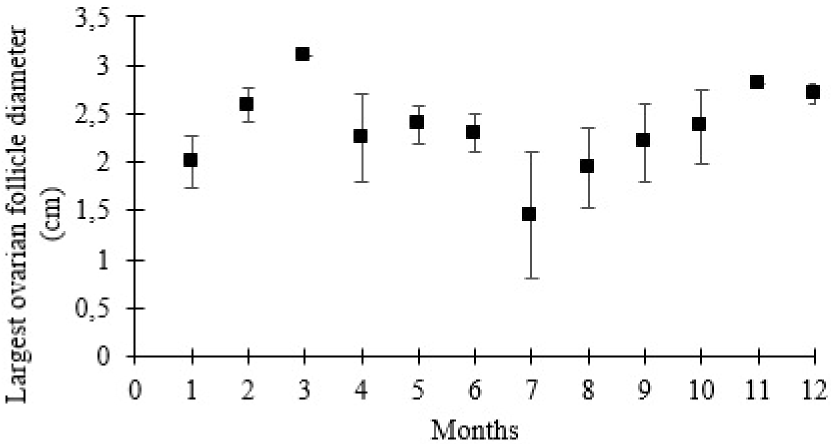

Fig. 9. Monthly variation of the mean of the largest follicle diameter in the functional ovary of female *H. marianae.* (Month 1=November 2007; Month 12=October 2008).

Reproduction notes

An adult female, captured in December 2007, had a corpus luteum near the ostium, weighting 3.6 g. In March 2008, a female of 31.8 cm DW had a corpus luteum in the left oviduct, but its vitellogenic content bursted out into the anterior oviduct and the oviducal gland. In March, April and June, three females had a corpus luteum in the left oviduct with their membranes reaching the oviducal gland and the uterus. Seven pregnant females were recorded, three of them with embryos and four with fertilized eggs in the uterus. Two females had fully formed embryos (of 11.5 and 13.9 cm DW), captured in December 2007 and January 2008, respectively, during the early rainy season. One female captured in September 2008 had an embryo of 7.5 cm DW. Females with fertilized eggs in the uterus were captured in March and April 2008 (Table 1). Bite marks on female pectoral fins were found throughout the year, suggesting continuous copulation (Fig. 10A-E).

Table 1. Records of pregnant females of *H. marianae*. DW_M, maternal disc width (cm); DW_E, disc width of embryo (mm); HSI; GSI; *pregnant females with eggs in the uterus.

Month	DW_M	DW_E	HSI	GSI
November	32.2	*	4.96	0.15
December	33.0	13.9	4.10	0.59
January	30.4	11.5	1.86	0.18
March	31.8	*	2.77	0.16
April	33.0	*	3.77	0.09
April	32.0	*	3.61	0.12
September	34.8	7.5	3.04	0.06

Fig. 10. Structures analysed in females during the reproductive cycle. (A) Oocyte at ostium. (B) Corpus luteum in left oviduct. (C) Bites on disc (pectoral fin). (D) Left uterus with fertilized egg. (E) Near-term embryo.

Vitellogenesis took place concurrently with gestation. Analysis of females with left ovarian follicles and near-term embryos showed the largest mean diameter of ovarian follicles at the beginning and end of embryonic development (Fig. 11).

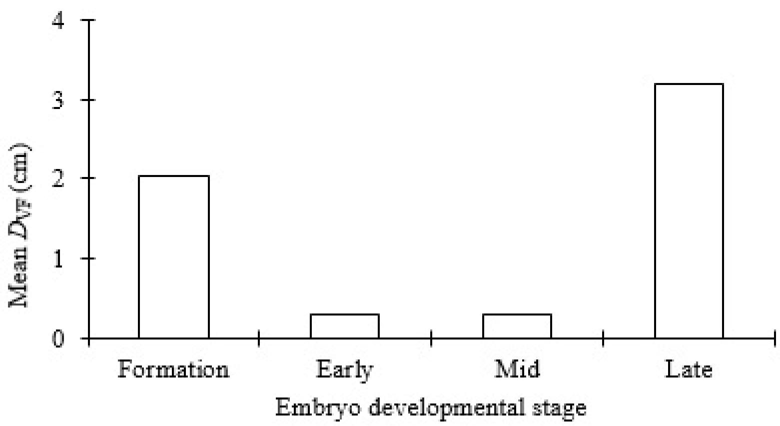

Fig. 11. Mean width of the largest vitellogenic follicle and embryo development stage of *H. marianae* in pregnant females.

Based on this study, it is possible to draw at least one cycle per year, considering that males come inshore in early rainy season (November) with a high IGS incremented by liver resources gained along the previous months (October). Meanwhile, females have high value of IHS in December, which show an increase of reserves to the following months: in January females have large vitellogenic follicles and oviducal glands, an ovulation evidence; and in February they have high IGS values. This seems to reflect the peak of births in May. This interpretation, added to the others analysed structures, could indicate a gestation period of four months for *H. marianae*, with at least two cycles per year (Fig. 12).

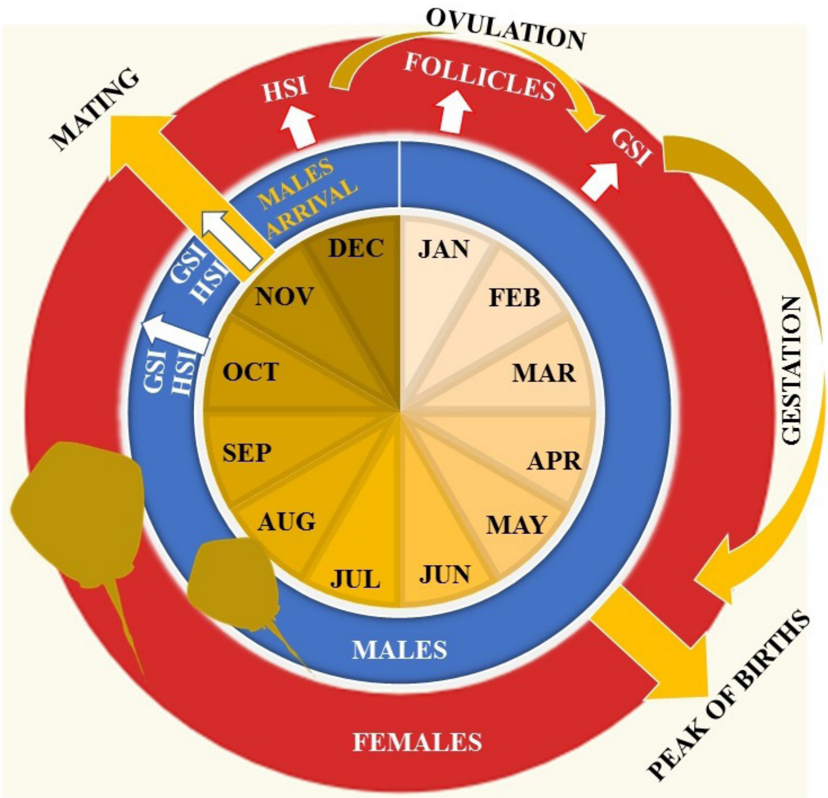

Fig. 12. Diagram of the proposed reproductive cycle for males and females of *H. marianae* based on the present study.

DISCUSSION

The monthly distribution of captures showed the predominance of females in all months, except in September and October, when fish production of the fishing weir decreased due to intensive wind (trade winds). The wind season begins on August and goes to November in this region, so at this period the fishing weirs are very worn and the catches inoperative. The significant difference in sex ratio could indicate sexual segregation, with females concentrated in shallower depths such as those of the fishing weir (4 to 6m), whereas males seem to stay in deeper waters and channels, returning to shallow waters close to the beach during mating periods. Spatial segregation between males and females, tends to be a seasonal phenomenon, since part of the population of adult males and females must gather for reproductive purposes (Wourms 1977; Wearmouth and Sims 2010). The same pattern has been observed in several elasmobranch species, including stingrays (Babel 1967; Springer 1967; Pratt 1979; Thorson 1983; Klimley 1987). During mating periods, when males approached females, their numbers increase in shallow waters. Adult females of *H. marianae* have been reported mainly in seagrass beds at Rio Grande do Norte state, while males in coral reef areas and the deeper areas of the reef front (Costa et al. 2015).

The size distribution of sampled specimens also suggests an ontogenetic segregation, since only neonates and adults (mostly females) were captured in the study area. Feeding habits can influence ontogenetic segregation, since animals of different sizes and maturity stages can occupy different habitats (Ebert and Cowley 2003; Aguiar et al. 2009). In the state of Rio Grande do Norte, young individuals of *H. marianae* were found closer to the beach feeding on prawns, while adult females have been sampled mostly on seagrass beds with crabs and lobsters in their stomachs (Costa et al. 2015). Congeners also show the same segregation pattern, such as *Hypanus americanus* in Fernando de Noronha Archipelago (Aguiar et al. 2009) and *Hypanus say* in Florida coastal lagoons (Snelson et al. 1989). Segregation by size is often found in some species of sharks, as a putative strategy to protect neonates and juveniles from cannibalism (Springer 1967; Ebert et al. 2002; Grubbs 2010).

Elasmobranchs often use shallow areas, usually close to the shore, as nurseries where predation pressure upon neonates and juveniles is lower and food availability is high (Branstetter 1990). Considering this, newborn and young-of-the-year *H. marianae* stay in shallow and turbid waters, where less preferred prey with lower energy value occur instead of areas with more adequate prey, but deeper and unprotected, as verified in other places (Costa et al. 2015). Even nursery areas are exposed to predators and strategies are needed for young to escape them (Heupel et al., 2007), considering, for example, the predation record of a young *H. marianae* measuring 13.6 cm, which was found in the stomach of a similar species, *H. americanus* in shallow water near Recife, Brazil (Branco et al., 2016).

The largest observed individual of *H. marianae* had a similar DW to the maximum sizes estimated in the literature for this species (31 to 38 DW) (Gomes et al. 2000; Yokota and Lessa 2007; Motta et al. 2009; Costa et al. 2015), being smaller than the others Brazilian congeners *H. americanus,* that attains 150 cm DW; *H. guttatus*, with maximum size of 180 cm DW; and *H. say*, that reaches 78 cm DW (Last et al. 2016). The Atlantic stingray *Hypanus sabinus* sampled in Florida coastal lagoons reached a maximum size of 37 cm DW (Snelson et al. 1988). In other studies, *H. sabinus* attained larger sizes, up to 45 cm DW (Bigelow and Schroeder 1953; McEachran and De Carvalho 2002; Last et al. 2016a). Other species have been reported to reach similar maximum sizes, such as *Neotrygon kuhlii* (Java morphotype) recorded off eastern Indonesia, which reached a maximum disk width of 37.9 and 32.4 cm, for females and males, respectively (White and Dharmadi 2007). Thus, *H. marianae* is the smallest species of all dasyatids found in Southwestern Atlantic Ocean (Last et al. 2016b).

Females of *H. marianae* mature at a larger size than males, as found in congeners, such as *H. say* (Snelson et al. 1989), *H. americanus* (Ramírez-Mosqueda et al. 2012) and *H. guttatus* (Da Silva et al., 2018). Usually, females reached greater weights and lengths than males because they require additional body space to bear eggs and embryos, as well as space for the large liver that stores energetic reserves for reproduction (Walker 2005).

Previous studies have not determined maturation sizes for *H. marianae* since all individuals in their samples were adults (Yokota and Lessa 2007). Costa et al. (2015), although not specifically on reproduction, have estimated the size at maturity for males and females as 24 and 27 cm DW, respectively, based on macroscopic analysis. These sizes were corroborated in the present study. For *H. sabinus* studied in Florida and *N. kuhlii* (Java morphotype) studied in Indonesia, sizes of first maturation were close to those of *H. marianae*: *H. sabinus* was 20.0 and 24.0 cm DW for males and females (Snelson et al. 1988), respectively, and *N. kuhlii*, 23.7 cm DW for both sexes (White and Dharmadi 2007). The other congener recorded in Southwestern Atlantic Ocean, *H. guttatus*, presented 61 cm WD of sexual maturation size for males and 67 cm WD, for females (Da Silva et al. 2018).

The abrupt increment change in clasper size indicates the transition from juveniles to adults, at the onset of sexual maturation of batoids (Babel 1967; Struhsaker 1969; Martin and Cailliet 1988; Snelson et al. 1988; Snelson et al. 1989).

The uterine fecundity of a single embryo per female in *H. marianae* was also reported in a mother of 38 cm DW female that bore one 17 cm DW full-term embryo (Motta et al. 2009) and the size at birth was estimated at 13-14 cm DW (Yokota and Lessa 2007). All other Brazilian dasyatids with available information showed higher uterine fecundity (Bigelow and Schroeder 1953; McEachran and De Carvalho 2002; Yokota and Lessa 2007; Véras et al. 2009; Ramírez-Mosqueda et al. 2012). Uterine fecundity of *H. sabinus* was one to four embryos, possibly because of its small size (Snelson et al. 1988), while, on a global scale, *Neotrygon cf. kuhlii*, found in eastern Indonesia, had one embryo per female in all 56 analysed pregnant females (White and Dharmadi 2007).

The low fecundity of *H. marianae* may be related to the relatively small size of this species when compared to most congeners, and also to the relatively large size at birth, which is nearly half of the maternal size in terms of disc width. This strategy of giving birth to a single individual possibly reduces the mortality of neonates, given their larger size. As only the left reproductive organs are developed, the embryo is able to occupy a larger space in the abdominal cavity, whereas the reduction of the reproductive organs on the right side may represent an economy of energy to be conveyed to the developing embryo. Viviparous batoids may show the right ovaries and oviducts in different degrees of reduction or loss (Wourms 1977), as reported for *Pateobatis bleekeri* (Alcock 1967), *H. sabinus* (Snelson et al. 1988) and *H. guttatus* (Yokota and Lessa 2007).

The HSI of males was higher in all months that preceded the increment of the HSI of females thus confirming the synchrony of the pre-copulatory periods of males and females in the population, given that liver reserves are used for developing gonads in the beginning of the reproductive cycle (Maruska et al. 1996; Carrier et al. 2004; Pratt and Carrier 2005). Females had heavier livers than males, indicating a different energetic role of this organ for each sex. Male sharks have the liver thinner and less oily than females (Springer 1967), possibly due to the competitive pre-copulatory behavior and production of large quantities of sperm (Pratt and Carrier 2005). In females, the liver produces nutrients that will contribute to the growth of the oocyte and the yolk that will nourish the embryo (Hamlett et al. 2005).

Climatic and oceanographic factors in Almofala may influence the occurrence of ovulation and mating in the reproductive cycle of *H. marianae*, such as the dry season that starts in September and runs through March. The adult male's arrival, mainly on November, coincides with the ovulation peak in the female population (December and January, which presented the higher values of diameters follicles). Considering the possibility of a sexual segregation, the onset of these events could be environmental signals, as it has been suggested in the ovulation cycle of viviparous species of elasmobranchs (Lutton et al. 2005).

The bite marks on the pectoral fins of females of *H. marianae*, a pre-copulatory behavior, may indicate courtship and mating throughout the year (Pratt and Carrier 2005). This was supported by births all over the year, suggesting an asynchronous cycle. Byte marks were also reported in other dasyatids, such as *Bathytoshia centroura* (Reed 1981), *H. americanus* (Chapman et al. 2003) and *H. sabinus* (Kajiura et al. 2000).

Even if an asynchronous cycle is considered, reproductive data from the present study indicates the occurrence of reproductive peaks along the year with a gestation period of approximately four months (or shorter than six months). This short period of gestation is also reported for the congeners *H. sabinus* in Florida (Snelson et al. 1988), *B. centroura* (Capapé 1993) and *Dasyatis marmorata* in Tunisian coasts (Capapé and Zaouali 1995). The gestation period of viviparous batoids is shorter than most of the sharks (Wourms 1977; Hamlett and Koob 1999), as in *Pteroplatytrygon violacea*, with the shortest known period of only two months (Ranzi 1977). Some large-sized species of stingrays may have longer gestation periods, such as *H. guttatus* with five to six months (Yokota and Lessa 2007) or *H. americanus* with five to eight months (Henningsen 2000; Ramírez-Mosqueda et al. 2012). A gestation period of three months has been reported for *Urobatis halleri* in California, where two female populations showed non-synchronized ovulatory cycles, with intervals of six months between them (Babel 1967). This may be the case of *H. marianae*, in which only part of the female population would mate in each reproductive peak. However, in order to assure the female population proportion for each reproductive peak, a larger sample period would be required.

Ovarian cycle and gestation were concurrent, as found for other dasyatids, such as *H. guttatus* (Yokota and Lessa 2007), *P. violacea* (Véras et al. 2009) and captive *H. americanus* (Henningsen 2000). In wild populations of *H. americanus* the ovarian cycle and gestation were consecutive (Ramírez-Mosqueda et al. 2012).

Neonates of *H. marianae* were found throughout the year in Almofala, as for *H. americanus* in the southern Gulf of Mexico (Ramírez-Mosqueda et al. 2012) and *Gymnura micrura* in Caiçara do Norte (Yokota and Lessa 2007). In tropical regions, the water temperature varies slightly between seasons (~3 °C) and newborns can be expected throughout the year (Wourms 1981), although a 1 °C gradient may strongly influence stingrays parturition (Wallman and Bennett, 2006).

The stress of capture is known to cause abortion in female elasmobranchs (Snelson et al. 1988; Mejía-Falla et al. 2012; Véras et al. 2009; White et al. 2001), followed by death caused by homeostatic disruption (Wosnick et al. 2018). This fact may have reduced the accuracy in describing the reproductive cycle of *H. marianae* herein, given that the complete embryonic development could not be analysed and part of the neonates may have been near-term embryos instead. The uterus width and flabbiness may indicate females in *post-partum* condition, but this stage was not observed since the uterus remained with a high elasticity in all samples. The expulsion of recently fertilized eggs and the rupture of their contents in the uterus, as observed in this study, were setbacks for the precise determination of the ovarian and uterine cycles.

The Almofala beach can be considered a conservation priority area for *H. marianae*, given the abundance of pregnant females and neonates throughout the year, a similar condition to Caiçara do Norte, in the neighboring state of Rio Grande do Norte (Yokota and Lessa, 2006). Furthermore, additional criteria for the recognition of elasmobranch nursery areas (Heupel et al. 2007; Martins et al. 2018) could be met for *H. marianae* at Almofala beach, such as: 1) the species is more frequent and abundant in this area than in adjacent ones, as batoids' research developed in another areas of Ceará state do not show the same abundance or frequency observed so far (Basílio et al. 2008); 2) tendency to remain or return during long periods of time, which is observed in the area; 3) and use of the area by the species for many years; both facts 2) and 3) attested by fishermen and researchers of the marine turtle conservation project TAMAR that have monitored the same fishing weir for many years at Almofala's beach. There is no evidence to consider the area a refuge for the juveniles, since they appeared in low numbers in the catches. However, more studies on the young size-classes of *H. marianae* are necessary to define their relevance on the species population maintenance, considered the most important for the intrinsic population growth rate of some elasmobranchs (Cortés 2000; Kinney and Simpfendorfer 2009).

CONCLUSION

Finally, the reproductive biology of *Hypanus marianae* highlights one of the strategies of small-size batoids, in which a low fecundity is associated with the reduction of the right side of its reproductive system, allowing a larger embryo per gestation. A short gestation period of four-six months is proposed considering studies on the left uterus and parturition events. This endemic species has the lowest fecundity of all Brazilian dasyatids, which renders the species vulnerable to the increasing fishing pressure in coastal waters of Southwestern Atlantic Ocean. This also emphasizes Almofala's beach as one of the priority conservation areas for *H. marianae*. A continuous fishing monitoring program is required in order to evaluate the population trends, since courtship, mating and parturition occurs in the area.

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