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Reproductive biology of the longnose stingray *Hypanus guttatus* (Bloch & Schneider, 1801) from the northeastern coast of Brazil

Victor Emmanuel Lopes DA SILVA[1], Elizabeth Costa TEIXEIRA[2], Nidia Noemi FABRÉ[1] and Vandick da Silva BATISTA[2]
(1) *Laboratório de Ecologia, Peixes e Pesca - Universidade Federal de Alagoas (Instituto de Ciências Biológicas e da Saúde), Maceió, Alagoas, Brasil*
(2) *Laboratório de Conservação e Manejo de Recursos Pesqueiros – Universidade Federal de Alagoas (Instituto de Ciências Biológicas e da Saúde), Maceió, Alagoas, Brasil*
Corresponding author: lopesdasilvavictor@gmail.com

Abstract: Reproductive aspects of the longnose stingray, *Hypanus guttatus* (Bloch & Schneider, 1801), from the northeastern Brazil were estimated from 634 individuals (366 females and 268 males). Sexual dimorphism was found for the species with females reaching larger sizes than males. Females and males reached sexual maturity at 67.1 ± 6.2 and 61.0 ± 4.3 cm, respectively. In females, oocyte diameter and gonadosomatic index indicated that ovulation occurs throughout the whole year. Pregnant females were found in almost all months (except in March and June) with fecundity not exceeding five embryos per litter. The longnose stingray appears to be reproductively active all year round in the studied area with low fecundity.

Résumé : *Reproduction de la pastenague long-nez* Hypanus guttatus *(Bloch & Schneider, 1801) du nord-est du Brésil.* Les aspects de la reproduction de la pastenague long-nez, *Hypanus guttatus* (Bloch & Schneider, 1801), du nord-est du Brésil ont été estimés à partir de 634 individus (366 femelles et 268 mâles). Le dimorphisme sexuel se traduit par une taille des femelles supérieure à celle des mâles. Les femelles et les mâles atteignent la maturité sexuelle à 67,1 ± 6,2 et 61,0 ± 4,3 cm, respectivement. Chez les femelles, le diamètre des ovocytes et le rapport gonadosomatique indiquent que l'ovulation s'effectue tout au long de l'année. Les femelles gestantes ont été retrouvées presque tous les mois (sauf en mars et en juin) avec la fécondité ne dépassant pas 5 embryons par portée. En bref, la pastenague long-nez, *H. guttatus*, semble se reproduire toute l'année sur le site étudié, avec une fécondité généralement faible.

Keywords: Stingray ● Reproduction ● Fish ● Conservation

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Introduction

Elasmobranchs represent a group of species with little information available on their life history traits and reproductive dynamics, characterizing them as one of the most poorly understood groups among the vertebrates (Fowler, 2005; Bradley et al., 2017). In fishery biology, such information is extremely useful for species conservation and the monitoring, management and sustaining of fish stocks (Thorson et al., 2014). For instance, data on reproductive biology may help researchers to assess species vulnerability to collapse and extinction (Le Quesne & Jennings, 2012), as well as in the understanding on how exploitation affects fish population in order to avoid irreparable harms (Frédou et al., 2016). Nevertheless, available knowledge on this subject remains scarce for many stingrays and sharks, especially for those with low economic value.

The longnose stingray, *Hypanus guttatus* (Bloch & Schneider, 1801), for example, is a neotropical species distributed from the Gulf of Mexico to the southern coast of Brazil frequently caught as bycatch in artisanal fisheries with limited data on its biological features (Tagliafico et al., 2013; Teixeira et al., 2017). As a consequence, the species has been categorized as "data deficit" in the IUCN's red list since 2004 (IUCN, 2018). Although there have been some attempts to evaluate reproductive aspects of this species (Silva et al., 2007; Yokota & Lessa, 2006), such studies have mainly focused on its size at first maturity. Hence, the current conservation status of the species cannot be accurately assessed. In this respect, our study aims to provide a better understanding on the reproductive biology of *H. guttatus* from the northeastern Brazil.

Materials and Methods

Study area and data collection

Specimens were sampled monthly from April 2009 to February 2011, during monitoring of artisanal fisheries in the Jaraguá fishing harbor in Alagoas, northeastern Brazil (9°38'S-35°39'W to 9°59'S-35°55'W). Different types of fishing gear (e.g. gill netting, trawl netting, bottom long-line and hand-line) were used to collect fishes from all size-classes (Froese, 2006). Samplings were carried out in different depth zones that ranged from 2 to 60 m, along approximately 100 km off coast.

Upon capture, fishes were stored on ice to aid preservation until further analysis. In laboratory, each specimen was identified at species level following Figueiredo (1977). Subsequently, individuals were sexed, weighed to the nearest 0.1 g (W) and their disc width (D*w*) was measured in cm. The claspers of males and maximum ova diameter of females were measured with a numeric vernier caliper (0.1 mm) and their gonads were weighed using a digital balance of precision (0.1 g).

Maturity status was assessed following the methodology described by Smith et al. (2007) using measurements mentioned above and macroscopic examination of reproductive tracts. Specimens were assigned to one of the three following categories: immatures, maturing and matures. Specimens in maturing status were identified by presenting little vitellogenic activity (follicles $\leq$ 1 cm diameter) and thin uterus for females, and the clasper partially calcified in males. Females were classified as matures when vitellogenic ova present in ovaries were greater than 1 cm and uterus was well developed, whereas males were defined as matures when their claspers were totally calcified, easily rotated, and with tips able to be expanded.

Statistical analysis

A chi-square test (χ^2) was performed to identify whether sex ratio differed from the expected 1:1 proportion (Zar, 2010). As D*w* data did not meet the assumptions of normality and homoscedasticity even after several transformations, differences in the D*w* between sexes were assessed by non-parametric Kruskal-Wallis test.

Size at first maturity (L_{50}) for females and males were estimated by fitting logistic models using the percentage of mature individuals per size class (King, 2007):

$$Y = 1/[1+e^{-(a+bX)}] \quad (1)$$

models were fitted using log-transformed abundance and the size where 50% of individuals were mature was assessed by $-a / b$. Subsequently, an empirical equation was used to estimate the length at maximum yield per recruit (*Lopt*) from the L_{50} (Froese & Binohlan, 2000):

$$\log Lopt = 1.053 \times \log_{50} - 0.0565 \quad (2)$$

The gonadosomatic index (GSI) was calculated for females and males as an indicator of seasonal variability of reproductive status (Saadaoui et al., 2015; Araújo et al., 2016):

$$GSI = 100 \times (\text{gonad weight / total body weight}) \quad (3)$$

Additionally, Fulton's condition factor (*K*) for separately sexes was estimated to evaluate changes in the energy status throughout the year (Froese, 2006):

$$K = 100 \times (W / Dw^3) \quad (4)$$

Fulton's condition factor was chosen for being an indirect predictor of energy reserve in the body (Chellappa et al., 1995) and for presenting a close relationship with the reproductive cycle of fishes. Differences in GSI and *K* per month and between sexes were tested by non-parametric Kruskal-Wallis test.

We also carried out a regression analysis to test whether the size of the mother was related to the number of embryos. All statistical analyses were performed in the software R statistics at a significance level of $p < 0.05$.

Results

A total of 634 individuals were collected (366 females and 268 males). Samples were mainly comprised of juveniles (79% of total abundance), and sex rate differed from the expected 1:1 proportion ($\chi^2 = 4.18$, $p < 0.05$, ♀:♂ = 1:0.73). Disc width ranged from 14.9 to 108.2 cm for females and from 8.5 to 82.0 cm for males. Sexual size dimorphism was found in the species with females (D*w* = 44.9 ± 24.5 cm) reaching larger sizes than males (D*w* = 36.6 ± 17.1 cm) ($p < 0.05$, Fig. 1). Information on estimated size at first maturity (L_{50}) and length at maximum yield per recruit (*Lopt*) for both sexes is summarized in table 1 and figure. 2.

Macroscopic analysis of reproductive tracts from 137 mature specimens showed that individuals from both sexes capable of reproduction were found in all samples. Higher proportions of adult females were found during the sampling period (94 females and 44 males). No significant differences in gonadosomatic index (GSI) and condition factor (*K*) over the studied period were found ($p > 0.05$). Analysis on 24 pregnant specimens indicated that females present both functional ovaries and uteri. Maximum oocyte diameter (MOD) ranged considerably in most months (Fig. 3), thus, it appears that there is no seasonality in oocyte development. Pregnant females were captured all year round except in March and June (Fig. 4). The smallest pregnant individual measured 60.4 cm and the largest 97.0 cm. In general, *H. guttatus* showed low fecundity with the

Table 1. *Hypanus guttatus*. Estimated size at first maturity (L_{50}) and length at maximum yield per recruit (*Lopt*) for females and males in the coast of Alagoas, northeastern Brazil.

Species	**Sex**	**n**	**Disc width range (cm)**	**L_{50} (cm)**	***Lopt* (cm)**
Hypanus guttatus (Bloch & Schneider, 1801)	Female	366	14.9 – 108.2	67.1 ± 6.2	73.7
	Male	268	8.5 – 82.0	61.0 ± 4.3	66.6

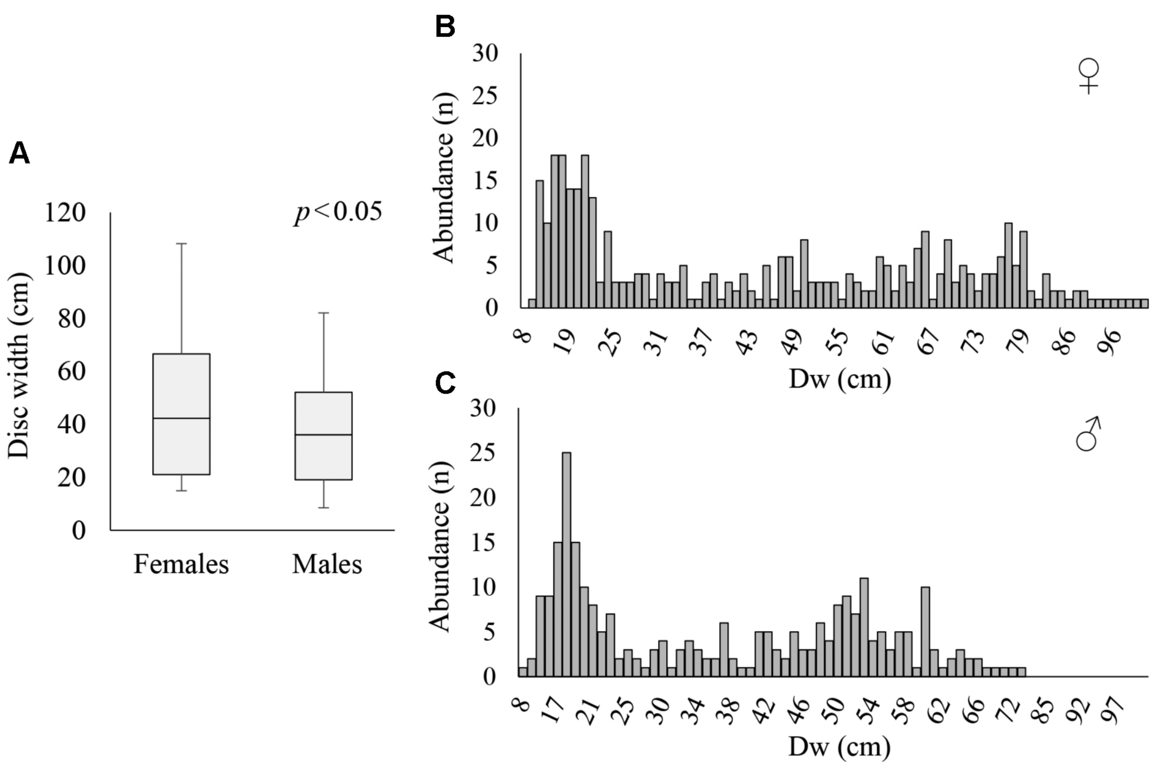

Figure 1. *Hypanus guttatus*. Comparison of disc width between sexes (**A**) and length frequency distribution for females (**B**) and males (**C**) in the coast of Alagoas, northeastern Brazil.

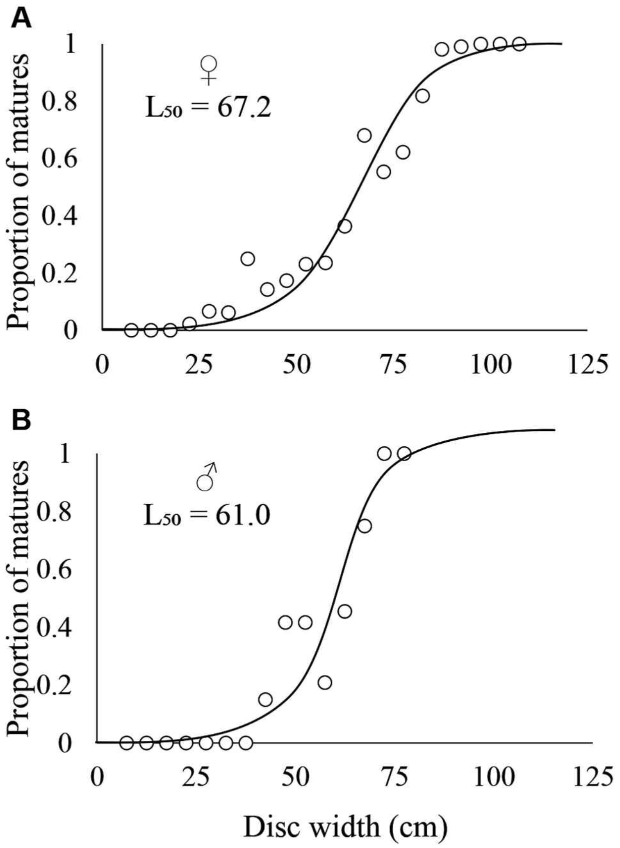

Figure 2. *Hypanus guttatus*. Size at first maturity for females (**A**) and males (**B**) in the northeastern coast off Brazil.

number of embryos in each uterus varying from 1 to 5. A relationship between the mother's size and the number of embryos was not found ($p > 0.05$). Embryos ranged from 2.4 to 51 cm in length (D*w*) and 1.6 to 95.8 g in weight.

Discussion

In tropical regions, coastal areas play a key role as nurseries for elasmobranchs, providing shelter and greater food abundance for many species (Andrade et al., 2008). Previously studies in the northeastern Brazil have shown that *Hypamus guttatus* uses coastal waters, mainly, as primary nursery ground (Yokota & Lessa, 2006). Our study supports this information as samples were mainly comprised of juveniles which depend on these areas to feed and grow until recruitment. Nevertheless, it is important to be noticed that even though we tried to remove bias towards sampling methodology by using different fishing gears (Froese, 2006), the low selectivity of trawl netting may have influenced the percentage of juveniles observed. Furthermore, the longnose stingray followed the nursery criteria defined by Heupel et al. (2007), such as greater

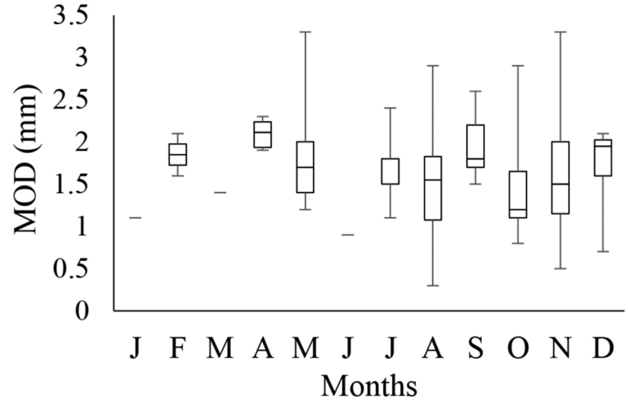

Figure 3. *Hypanus guttatus*. Monthly variation in maximum oocyte diameter from the northeastern Brazil.

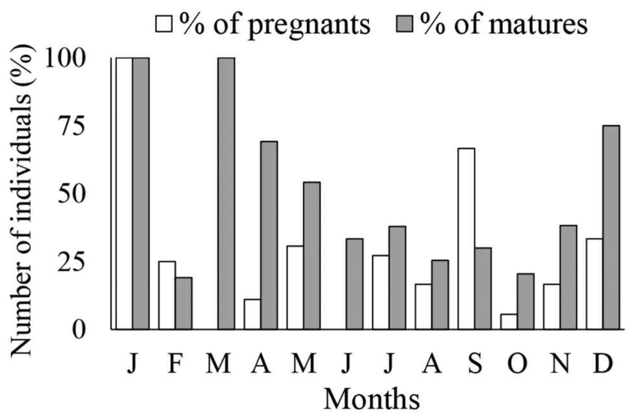

Figure 4. *Hypanus guttatus*. Proportion of mature females and pregnant individuals caught in the northeastern Brazil.

density in the studied area, tendency to remain or return for extended periods (presence of adults and pregnant females in many samples), and habitat use across years.

In our study, females of *H. guttatus* presented a larger disc width than males and they also reached maturity at a larger size. Sexual size dimorphism in dasyatids has been widely reported in literature (Capapé, 1993; Smith et al., 2007; Tagliafico et al., 2013), with females growing and maturing to a larger size than males in many species (*e.g. Bathytoshia centroura* (Mitchill, 1815) and *Dasyatis chrysonota* (Smith, 1828). Differences in the size of specimens among sexes for stingrays may be related to a variety of factors, including their reproductive modes (Ebert & Cowley, 2009). For instance, in viviparous species such as *H. guttatus*, females tend to reach larger sizes to maximize uterine capacity and support a greater number of embryos (Charvet-Almeida et al., 2005). This strategy is used to increase reproductive success and offspring survival by increasing the size at birth of neonates.

The size at first maturity (L_{50}) for *H. guttatus* estimated in our study was different from the ones previously reported in the literature for the species (Silva et al., 2007; Yokota & Lessa, 2007; Tagliafico et al., 2013). Differences in the L_{50} of stingrays may result from latitudinal variation, changes in environmental conditions, different sample sizes and biological discrepancy between geographically isolated subpopulations (Menni & Lessa, 1998; Charvet-Almeida et al., 2005; Barrios-Garrido et al., 2017). However, identifying which factor is better correlated to these changes is complex and hence require a more direct experiment that encompasses different seasons, years and latitudes.

The length at maximum yield per recruit (*Lopt*) for *H. guttatus* was greater than its L_{50}, which is a typical reproductive strategy of large and long-lived fishes (Beverton, 1992). Compared to bony fishes, elasmobranchs have a smaller predation risk, and since maximum possible yield is determined by the ratio between growth and mortality, the species in this group tend to reach the *Lopt* during older ages in a trade-off of reproduction and survival (Jensen, 1996; Froese & Binohlan, 2000). Although comparisons between these two traits are important to a better comprehension of fish reproductive strategies, no previously comparisons on literature for stingrays were found.

The absence of significant difference in the IGS and Fulton's condition factor (*K*) over time for both sexes along with the occurrence of mature and pregnant individuals in all samples suggest that *H. guttatus* is reproductively active all year round in the region. Although not common in the Dasyatidae family (Yokota & Lessa, 2007; Ebert & Cowley, 2009), asynchronous cycle were previously reported for other species, such as *Hypanus americanus* (Hildebrand & Schroeder, 1928) in the Gulf of Mexico (Ramírez-Mosqueda et al., 2012), *B. centroura* in the Tunisian coast (Capapé, 1993), and *Neotrygon kuhlii* (Müller & Henle, 1841) and *Telatrygon zugei* (Müller & Henle, 1841) in the eastern Indonesia (White & Dharmadi, 2007). Asynchronous reproductive cycle in elasmobranchs is frequently associated with the occurrence of species in low latitude regions which presents stable environmental conditions and greater food availability throughout the year (Yokota & Lessa, 2007; Castro, 2009). In the study region, seasonality plays an important role in food availability of coastal areas which, consequently, results in changes in the structuring of fish assemblages (Passos et al., 2016) as well as in the life history traits of fish species, such as their growth characteristics (Sousa et al., 2015).

Hypanus guttatus showed a low fecundity rate, with the number of embryos ranging from 1 to 5, with an average of 1-2 embryos per litter. Low fecundity is a common pattern found among dasyatids which usually have an average of 2-3 embryos per litter (Smith et al., 2007; Ebert & Cowley, 2009; Ramírez-Mosqueda et al., 2012). Nevertheless, it has been discussed that observed fecundity in these species are usually underestimated due to the huge number of abortions caused by stress from capture and the elevation of individuals from the water (Struhsaker, 1969; Snelson et al., 1988; Capapé, 1993). Therefore, such information should be treated with caution.

In summary, our study provides some insights about the reproductive cycle of *H. guttatus* in the northeastern coast off Brazil. In general, the longnose stingray is a species with sexual dimorphism, low fecundity and late maturity, which are typical features of elasmobranchs. Such characteristics are of concern as these parameters suggest that this dasyatid has low biological productivity and limited resilience to fishing pressure. Furthermore, our results indicate that the coast of Alagoas in the northeastern Brazil act as an important nursery ground for *H. guttatus*, which is also concerning since the species is the most common ray caught as bycatch in the region. We expect that the information herein may be useful for future research, especially for those that aim the management and conservation of this species.

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