

Reproductive biology of the pelagic stingray, *Pteroplatytrygon violacea* (Bonaparte, 1832), in the equatorial and south-western Atlantic Ocean

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Abstract. From October 2005 to March 2010, a total of 480 pelagic stingray, *Pteroplatytrygon violacea*, specimens, 188 females and 292 males (0.64 female : 1 male), were taken in the equatorial and south-western Atlantic by the commercial tuna longline fishery and their reproductive biology was studied. Disc widths (D_W) ranged from 28.0 to 66.0 cm for females and from 34.0 to 59.6 cm for males. Size at first sexual maturity was estimated at ~ 48.0 cm D_W (first pregnant female) for females and ~ 41.0 cm D_W for males. Ovarian fecundity, considering only follicles larger than 0.5 cm in diameter, ranged from 1 to 17 follicles per female, while the uterine fecundity of embryos in pregnant females in Stages 2 and 3 ranged from 1 to 5 embryos per female. The sex ratio between the embryos was almost equal (1.08 female : 1 male) and the size at birth was 19.0 cm D_W .

Additional keywords: Brazil, by-catch, Dasyatidae, elasmobranchs, maturity, reproduction.

Received 9 January 2014, accepted 10 April 2014, published online 10 October 2014

Introduction

Pelagic sharks and rays form a relatively small group with low diversity, representing $\sim 6\%$ of all living species of elasmobranchs. Only 64 species of sharks and rays inhabit oceanic regions, a number that is very low compared with elasmobranch biodiversity in coastal areas (Camhi *et al.* 2009). A prominent member of the oceanic community is the pelagic stingray, *Pteroplatytrygon violacea* (Bonaparte, 1832), which has been reported as an important by-catch species in many of the tuna and swordfish longline fisheries throughout the world (Wilson and Beckett 1970; Amorim *et al.* 1998; Mollet 2002; Domingo *et al.* 2005; Joung *et al.* 2005; Somvanshi *et al.* 2009). The pelagic stingray is the only species of the batoid family Dasyatidae that is fully pelagic (Wilson and Beckett 1970), and is distributed in oceanic areas within the top 100 m of deep waters (Wilson and Beckett 1970; Last and Stevens 1994). Pelagic stingrays have a worldwide distribution, with highest abundance in tropical and subtropical regions, but are also found

at higher latitudes (Tortonese 1956; Wilson and Beckett 1970; Last and Stevens 1994; Menni *et al.* 1995; Mollet 2002; Hemida *et al.* 2003; Domingo *et al.* 2005; Ellis 2007; Vérias *et al.* 2009; Zacharia *et al.* 2011).

Since February 2007, the pelagic stingray has been listed in the low-risk category of 'Least Concern' by the IUCN (International Union for Conservation of Nature and Natural Resources) (Baum *et al.* 2009), a classification, however, that suffers from a paucity of available life-history and fishery data. There is no comprehensive information regarding the life history of the pelagic stingray throughout its geographic range. Information on the reproduction of the pelagic stingray in the wild is particularly limited, suggesting that reproduction and development is viviparous with uterolactation and a gestation period between two and four months (Lo Bianco 1909; Ranzi 1932, 1933, 1934b; Ranzi and Zezza 1936; Hemida *et al.* 2003; Neer 2008). Data for captive specimens suggest that pelagic stingrays have litters of 4–13 young, with disc widths ranging from 14.0 to

24.0 cm at birth, and that ovulation in captivity may occur twice per year (Mollet *et al.* 2002).

In Brazil, very little has been published on the species, with studies of natural history being especially scarce (Siqueira and Sant'Anna 2007; Ribeiro-Prado and Amorim 2008; Ribeiro-Prado *et al.* 2009; V eras *et al.* 2009). As a result, the actual status of their stock(s) in the south-western and equatorial Atlantic, as in other areas of the world, is still largely unknown. Any fishing activity that results in fishery-related mortality of elasmobranchs, either at-vessel or as catch, whether as a target or especially as poorly recorded by-catch, must always be accompanied by scientific research that addresses life-history and population dynamics in order to allow for proper assessment of the exploited stocks. The present study therefore was undertaken to help fill current gaps in our knowledge of the reproductive biology of pelagic stingrays in the south-western and equatorial Atlantic Ocean.

Material and methods

Data collection

In total, 480 specimens of pelagic stingrays were analysed, of which 292 were male (60.8%) and 188 were female (39.2%). All specimens were collected between October 2005 and March 2010 by onboard observers of the Brazilian National Observer Program monitoring the Brazilian pelagic longline fleet that targets tuna and swordfish. Although the fishing ground was delimited by the area bounded by 09°N, 28°S, 18°W and 53°W, pelagic stingrays were caught only between 06°N, 22°S, 18°W and 37°W (Fig. 1).

Captured specimens were labelled and quickly frozen for days up to months, depending on how long the vessel was at sea. Later, in the laboratory, they were thawed, weighed (g) (total weight: W_T ; eviscerated weight: W_E) and measured (cm) (disc width: D_W ; disc length: D_L). It was not possible to measure the

total length because, for safety reasons, tails were removed after capture.

For females, ovary, oviducal gland and uteri were examined. Data collected included ovary weight (OW), and oviducal gland (OG) width, ovary (OV) width and uterus width. The developmental stage of the ovary was observed macroscopically and the diameter of the largest ovarian follicle (DLOF) was measured (Castro *et al.* 1988; Bridge *et al.* 1998). Ovarian fecundity was estimated by counting the number of ovarian follicles larger than 0.5 cm in diameter (observation of the beginning vitellogenesis process) in each mature female. The uteri were longitudinally sectioned in order to allow the examination of contents. Whenever eggs or embryos were present, they were counted, with embryo sex, total weight, total length, disc width and length being recorded. Uterine fecundity was estimated by counting the number of embryos in each female during pregnancy.

In males, the testes were extracted and weighed. The lengths of claspers were also measured (Compagno 2002) and their calcification stage assessed as flexible, semicalcified or calcified, the latter generally regarded as an indication of male sexual maturity.

The reproductive organs of all females and males were measured to the nearest 0.1 mm using Vernier calipers and weighed using digital scales. All material weighing more than 1000 g was weighed on a scale with 10 g accuracy; material weighing less than 1000 g was weighed on a precision scale with 0.1 g accuracy.

Characterisation of maturity

Macroscopic examination allowed for assignment of each specimen to a specific maturity stage. Females were classified on the basis of the dimensions of the ovary, the oviducal gland and the uterus, and by the uterine contents (eggs or embryos). Males were classified on the basis of the calcification of the claspers and testes development.

The reproductive stages of females and males were assigned using the characteristics of their reproductive organs. Depending on the development of the ovary, the oviducal gland and the uteri, females were divided into six sequential stages: juvenile, maturing, preovulatory, pregnant, postpartum and resting. Juvenile females were characterised by having undeveloped and undifferentiated reproductive organs, while maturing individuals had developing ovary with few vitellogenic follicles and little expanded uteri. The preovulatory stage was characterised by a developed ovary and enlarged uteri, while pregnant females had completed ovulation and their uteri contained either eggs or embryos. For a better understanding, pregnant individuals were classified according to the development of uterine content (eggs and embryos) as: Stage 1 (only eggs present), Stage 2 (presence of embryos in early and middle development), and Stage 3 (embryos approaching parturition). Postpartum females contained a developed ovary and enlarged and empty uteri with well developed uterine villi present, while resting females had only a slightly enlarged ovary, and enlarged and flaccid uteri. On the basis of the development of their claspers and testes, males were classified into juvenile, maturing and adult. Of the 292 males sampled, six were assigned to a maturity stage solely based on the disc width and clasper calcification, since it was not possible to collect the reproductive tract.

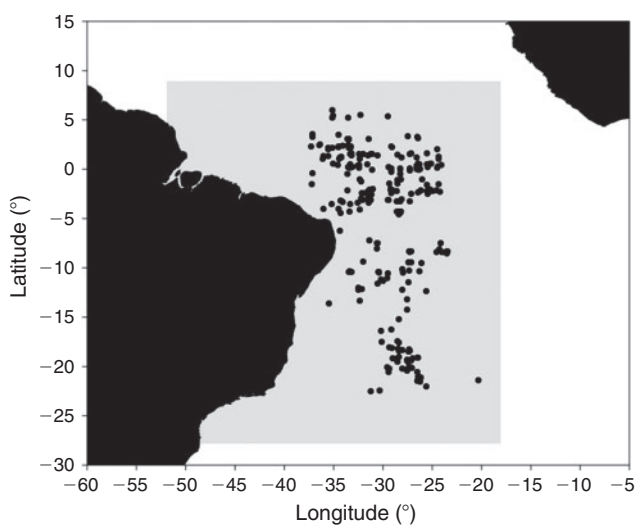


Fig. 1. Sampling area for pelagic stingrays, *Pteroplatytrygon violacea*, in the equatorial and south-western Atlantic Ocean. The light grey rectangle shows the fishing area and the black dots show the latitude and longitude of each set where the pelagic stingrays were caught.

Data analyses

Sexual differences in the size (disc width and length) and weight distribution of males and females were compared using Mann–Whitney *U*-test. The relationships between D_W and D_L , and W_T and W_E between sexes were described using the equations $D_L = a \cdot D_W^b$ and $W_E = a \cdot W_T^b$ (Mollet and Cailliet 1996; Ebert and Cowley 2009). The relationships between lengths (D_W) and weights (W_T and W_E) of both sexes were calculated with power regressions. The regressions were estimated separately for males and females using the equations $\ln(W_E) = \ln(a) + b \cdot \ln(D_W)$ and $\ln(W_T) = \ln(a) + b \cdot \ln(D_W)$, and then compared using Analysis of Covariance (ANCOVA). A paired Student's *t*-test was used to test whether there was a significant difference between the weights of the left and right testes. Since there was a significant difference, power regressions between D_W and W_{TES} left and right were calculated and analysed using ANCOVA. A Chi-square (χ^2) goodness-of-fit test was used to test the hypothesis of a 1 : 1 sex ratio of examined specimens and embryos taken from pregnant females in Stages 2 and 3.

Results

Size composition and sex ratio

The sex ratio of the 480 specimens examined was biased (188 females : 292 males), with a significant difference of the

sex ratio of 0.64 female : 1 male ($P < 0.001$). Statistical differences in sex ratios were observed for January (0.36 : 1, $P = 0.006$), February (0.32 : 1, $P = 0.003$) and July (0.39 : 1, $P = 0.003$). Size distributions differed between males and females, with the highest frequency of males being between 43.0 and 48.0 cm D_W , while the highest frequency of females were between 49.0 and 54.0 cm (Fig. 2). In general, females were larger and heavier than males. Significant differences were observed between sexes in terms of D_W (Mann–Whitney *U*-test, $n_{males} = 291$, $n_{females} = 188$, $P < 0.05$), D_L (Mann–Whitney *U*-test, $n_{males} = 289$, $n_{females} = 187$, $P < 0.05$), W_T (Mann–Whitney *U*-test, $n_{males} = 291$, $n_{females} = 188$, $P < 0.05$), and W_E (Mann–Whitney *U*-test, $n_{males} = 291$, $n_{females} = 188$, $P < 0.05$) (Table 1). Power regressions between D_W and D_L and between W_T and W_E separated for males and females are presented in Table 2.

Female reproductive cycle

Only the left ovary, oviducal gland and uterus are functional in the pelagic stingray. The species is characterised by aplacental viviparous reproduction and lecithotrophic embryos with a large yolk sac during early development that is fully absorbed before parturition. The uterus has trophonemata, long villous extensions of the uterine epithelium that secrete energy-rich histotrophe or 'uterine milk' that is absorbed first by the external gill filaments and then later ingested by the embryos.

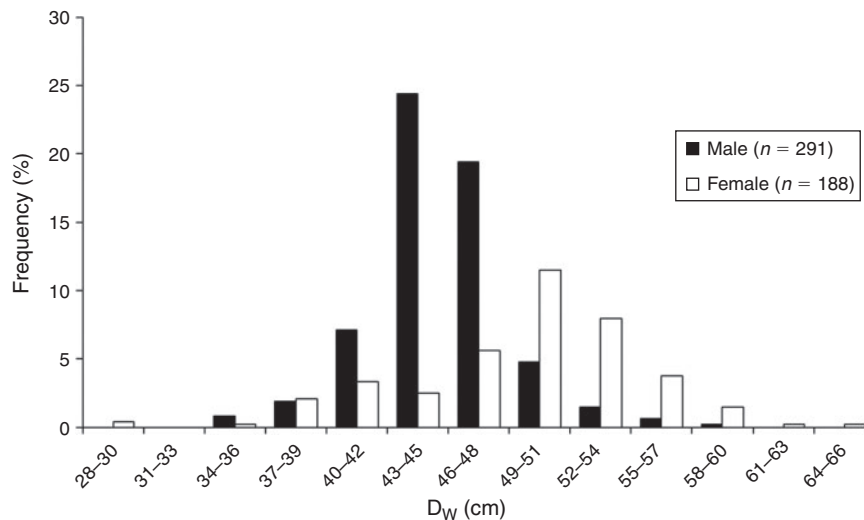


Fig. 2. Disc width (D_W) frequency distribution of the pelagic stingray, *Pteroplatytrygon violacea*, taken in the equatorial and south-western Atlantic Ocean.

Table 1. Length and weight parameters for males and females of the pelagic stingray, *Pteroplatytrygon violacea*, taken in the equatorial and south-western Atlantic Ocean

Parameter	Male		Female	
	Range	Mean ± s.e.	Range	Mean ± s.e.
Disc width (cm)	34.0–59.6	45.5 ± 0.1	28.0–66.0	49.4 ± 0.4
Disc length (cm)	25.0–46.0	33.8 ± 0.2	20.6–50.5	37.1 ± 0.4
Total weight (g)	780.0–3660.0	1924.0 ± 25.6	380.0–6420.0	2732.0 ± 75.2
Eviscerated weight (g)	620.0–3220.0	1656.0 ± 22.7	340.0–5400.0	2223.0 ± 59.1

Table 2. Regressions for females and males of the pelagic stingray, *Pteroplatytrygon violacea*, taken in the equatorial and south-western Atlantic Ocean

For females: $n = 184$, range = 28.0–66.0 cm D_W ; mean = 49.4 ± 0.4 cm D_W . For males: $n = 290$, range = 34.0–59.6 cm D_W ; mean = 45.5 ± 0.1 cm D_W

Females		Males	
Equation	r^2	Equation	r^2
$\ln(D_L) = 1.1126 \cdot \ln(D_W) - 0.7227$	0.9154	$\ln(D_L) = 1.0659 \cdot \ln(D_W) - 0.5429$	0.8183
$\ln(W_T) = 0.941 \cdot \ln(W_E) + 0.2627$	0.9766	$\ln(W_E) = 0.9987 \cdot \ln(W_T) - 0.1414$	0.9522
$\ln(W_T) = 3.1294 \cdot \ln(D_W) - 4.3482$	0.8378	$\ln(W_T) = 2.599 \cdot \ln(D_W) - 2.377$	0.7186
$\ln(W_E) = 2.9591 \cdot \ln(D_W) - 3.8851$	0.8263	$\ln(W_E) = 2.6446 \cdot \ln(D_W) - 2.7026$	0.7104
$\ln(W_T) = 2.6354 \cdot \ln(D_L) - 1.6755$	0.8034	$\ln(W_T) = 2.1401 \cdot \ln(D_L) - 0.0032$	0.6742
$\ln(W_E) = 2.4882 \cdot \ln(D_L) - 1.3443$	0.7904	$\ln(W_E) = 2.1408 \cdot \ln(D_L) - 0.157$	0.6442

Table 3. Characteristics of maturity stages of female pelagic stingrays, *Pteroplatytrygon violacea*, taken in the equatorial and south-western Atlantic Ocean

Information on the different stages can be found in the text. D_W , disc width; OW, ovary weight; DLFO, diameter of the largest ovarian follicle; OGW, oviducal gland width; UW, uterus width

Stages (n)	D_W (cm)		OW (g)		DLFO (cm)		OGW (cm)		UW (cm)	
	Range	Mean \pm s.e.	Range	Mean \pm s.e.	Range	Mean \pm s.e.	Range	Mean \pm s.e.	Range	Mean \pm s.e.
Juvenile (42)	28.0–47.0	41.1 ± 0.05	0.1–1.9	0.8 ± 0.01	0.3–0.8	0.5 ± 0.0	0.4–1.0	0.8 ± 0.0	0.5–3.0	1.9 ± 0.01
Maturing (67)	45.0–57.7	50.1 ± 0.01	0.4–7.0	2.8 ± 0.04	0.5–1.2	0.8 ± 0.0	1.0–1.7	1.1 ± 0.0	2.3–6.0	3.8 ± 0.01
Preovulatory (28)	49.2–66.0	53.7 ± 0.1	2.1–18.5	9.2 ± 0.2	1.2–2.1	1.6 ± 0.01	1.2–1.9	1.4 ± 0.01	4.3–8.2	5.7 ± 0.04
Pregnant stage 1 (17)	48.0–58.0	52.2 ± 0.1	0.6–1.5	4.4 ± 0.2	0.5–1.8	0.8 ± 0.02	1.0–1.3	1.1 ± 0.01	3.6–6.4	4.5 ± 0.03
Pregnant stage 2 (13)	49.1–60.0	53.4 ± 0.2	0.7–8.5	4.3 ± 0.2	0.6–1.4	0.9 ± 0.03	1.1–1.8	1.4 ± 0.02	5.8–10.5	7.7 ± 0.1
Pregnant stage 3 (2)	50.0–54.0	52.0 ± 1.4	3.4–7.0	5.2 ± 1.2	1.5–2.0	1.7 ± 0.1	1.6–2.0	1.8 ± 0.1	7.4–12.0	9.7 ± 1.6
Postpartum (6)	51.0–63.2	55.4 ± 0.8	3.0–4.0	3.3 ± 0.08	1.1–1.6	1.3 ± 0.03	1.3–1.5	1.4 ± 0.02	5.4–7.1	6.0 ± 0.1
Resting (10)	50.2–56.5	52.9 ± 0.2	0.6–1.8	1.0 ± 0.05	0.3–0.8	0.5 ± 0.02	0.9–1.4	1.1 ± 0.01	2.7–5.5	4.2 ± 0.09

The reproductive organs could be analysed for all but three of the 188 examined females. Females were classified as juvenile ($n = 42$; 22.7%); maturing ($n = 67$; 36.2%); preovulatory ($n = 28$; 15.1%); pregnant, subclassified into three stages: Stage 1 ($n = 17$; 9.2%), Stage 2 ($n = 13$; 7.0%) and Stage 3 ($n = 2$; 1.1%); postpartum ($n = 6$; 3.2%); and resting ($n = 10$; 5.4%) (Table 3).

On the basis of ovary weight, DLOF and width of the oviducal gland and uterus (Fig. 3), females of the pelagic stingray start to mature at ~ 45.0 cm and first maturity is at ~ 48.0 cm D_W , corresponding to 72.7% of the maximum (66.0 cm) D_W observed in this study. The largest juvenile was 47.0 cm D_W , the first maturing and pregnant females were 45.0 and 48.0 cm D_W , respectively, and 67.5% of the 188 females analysed in this study were mature.

Juveniles and preovulatory individuals occurred in all months of the year (Fig. 4). Maturing females occurred throughout the year, except in January. Pregnant females (Stages 1–3) were not encountered in May, August or October, with the highest number ($n = 5$) of Stage 1 females being observed in April. The two pregnant Stage 3 females with highly developed embryos were observed in June and November. Postpartum females were observed in February, March, August, September and November, while resting females were absent in the first four months of the year.

Fecundity and development

Ovarian fecundity, based only on follicles larger than 0.5 cm in diameter, ranged from 1 to 17 (mean \pm s.e. = 5.4 ± 0.3 , $n = 72$). Preovulatory females had the highest number of follicles in the ovary (mean \pm s.e. = 6.8 ± 0.7 , $n = 17$). The uterine fecundity of embryos in pregnant females in Stages 2 and 3, ranging between 1 and 5 (mean \pm s.e. = 3.5 ± 0.3 , $n = 15$), was lower than ovarian fecundity. There was no relationship between the size of the pregnant female and the number of embryos in the uterus ($r^2 = 0.0094$) ($P < 0.001$).

The ova inside the uterus were packaged within an ovoid capsule, while yolk sac-bearing embryos in middle development ($D_W = 4.9$ –12.2 cm, $n = 51$) were found loose. Full-term or near-full-term embryos, with the D_W at or close to the size at birth (14.2 and 18.8 cm, $n = 2$), had consumed all of the yolk sac, and were positioned in the same direction as their mothers. Embryonic heads were pointed towards the maternal head, and the tips of the pectoral fins were rolled ventrally, resembling the shape of a tube. The embryos around 10.0 cm D_W showed start of pigmentation, the full-term embryos exhibited characteristic coloration of adult individuals, i.e. a dark purple to blue-green at dorsum and a deep purple to grey ventrally.

Of the 53 observed embryos, 23 were male (43.4%), ranging in size from 7.5 to 18.8 cm D_W , and 25 were female (47.2%), measuring 5.8 to 12.2 cm D_W . Five (9.4%) 4.9–6.9-cm D_W

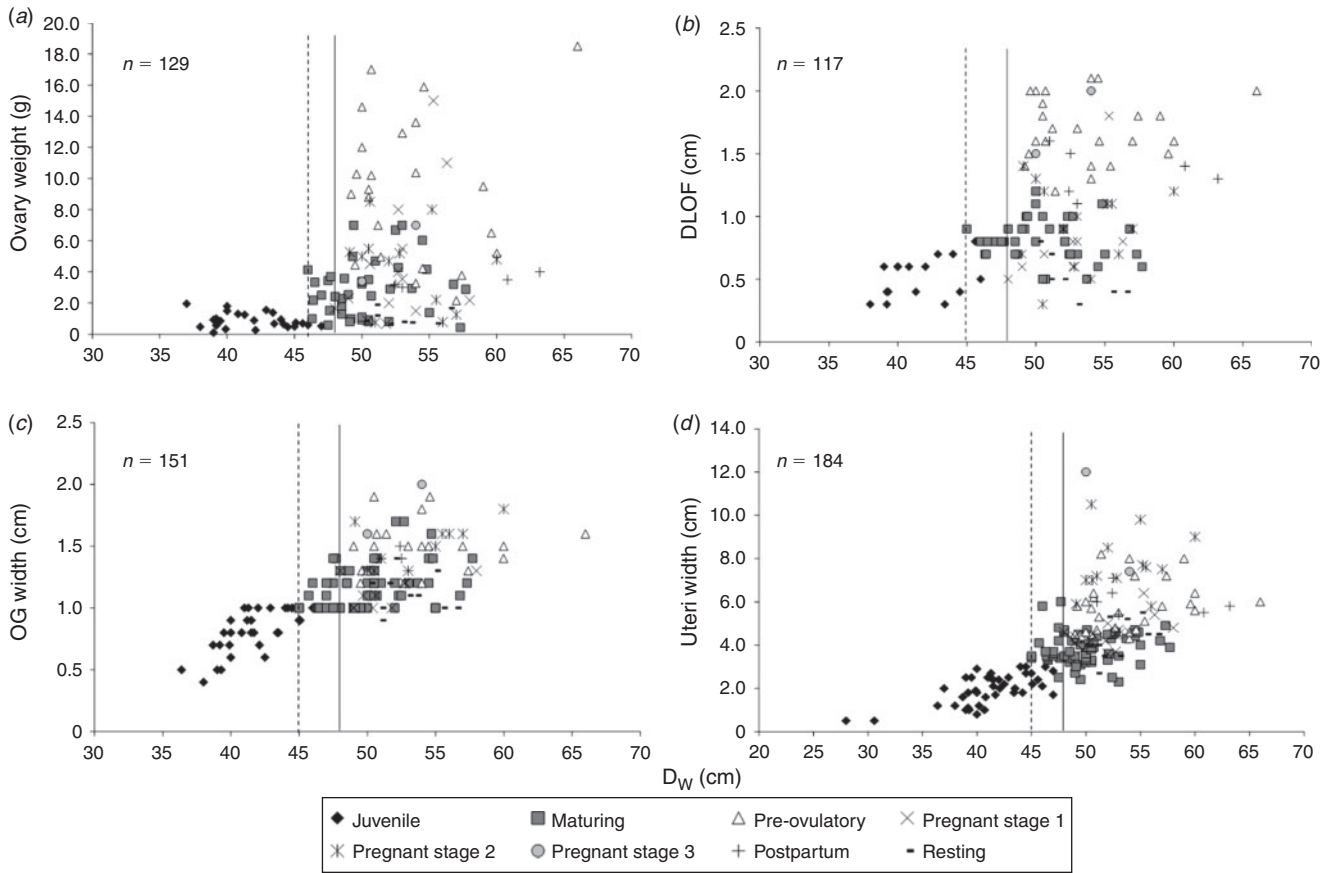


Fig. 3. Relationship between disc width (D_w) and (a) ovary weight, (b) diameter of the largest ovarian follicle (DLOF), (c) oviducal gland (OG) width and (d) uterus width for female pelagic stingrays, *Pteroplatytrygon violacea*, taken in the equatorial and south-western Atlantic Ocean. Dotted line indicates the first maturing individual and solid line the first pregnant one.

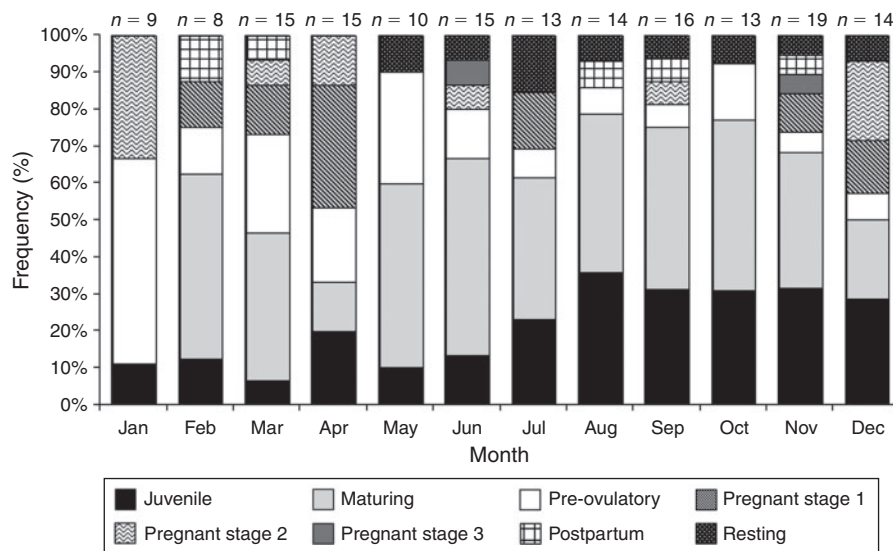


Fig. 4. Monthly frequency of occurrence of the different maturity stages of female pelagic stingrays, *Pteroplatytrygon violacea*, taken in the equatorial and south-western Atlantic Ocean.

Table 4. Characteristics of maturity stages of male pelagic stingrays, *Pteroplatytrygon violacea*, taken in the equatorial and south-western Atlantic Ocean
D_W, disc width

Stages (n)	D _W (cm)		Clasper length (cm)		Test weight (g)	
	Range	Mean ± s.e.	Range	Mean ± s.e.	Range	Mean ± s.e.
Juvenile (4)	34.0–36.0	35.0 ± 0.2	8.3–8.5	8.4 ± 0.03	0.4–0.9	0.6 ± 0.05
Maturing (15)	37.3–40.8	39.3 ± 0.07	8.5–10.0	9.0 ± 0.03	1.3–5.7	3.2 ± 0.08
Adult (273)	41.0–59.6	46.0 ± 0.01	8.8–14.6	10.3 ± 0.0	2.0–16.0	7.2 ± 0.01

embryos could not be sexed. There was no significant difference in the ratio of female-to-male embryos (1.08:1, $\chi^2 = 0.8$, $P > 0.05$).

The mean disc width (MD_{WE}) of embryos from pregnant Stage 2 and 3 females did not reveal any seasonal pattern. The smallest embryos (mean ± s.e. = 4.9 ± 0.02 cm D_W) were encountered in April, while larger embryos occurred in March (mean ± s.e. = 12.2 ± 0.06 cm D_W), June (mean ± s.e. = 18.8 ± 0.16 cm D_W, full-term), November (mean ± s.e. = 14.2 ± 0.20 cm D_W, near full-term) and December (mean ± s.e. = 12.2 ± 0.35 cm D_W). Although the relationship between the diameter of the largest ovarian follicle (DLOF) and the MD_{WE} in pregnant Stage 2 and 3 did not show a strong positive relationship (DLOF = 1.0624*MD_{WE}^{0.5098} ($r^2 = 0.1932$, $P < 0.001$), the largest MD_{WE} corresponded to the largest ovarian follicle.

Males

A paired Student’s *t*-test showed a significant difference between the weights of the left and right testes, with the left one being heavier (*t*-test, $P < 0.05$), but power regression between disc width and testes weight showed no difference (ANCOVA, $P > 0.05$).

The collection of the reproductive tract was not possible in six of the 292 males sampled. Males were classified as juvenile ($n = 4$; 1.4%), maturing ($n = 15$; 5.1%) and adults ($n = 273$; 93.5%) (Table 4). Seminal fluid was observed in the vas deferens of both maturing and adult individuals.

A better response on the data was limited by the individuals concentration on specific D_W classes, for males between 40.0 and 50.0 cm D_W. The relationship of D_W to clasper length showed a very weak sigmoid tendency (Fig. 5). The development of claspers is gradual. In juveniles, clasper measurements were ~8.3 cm length on individuals observed in the present study. In maturing individuals there was a rapid increase in relative clasper length through a small range of stingrays D_W, with a slower increase persisting after the attainment of maturity, apparently. The clasper tended to not show a steep slope with increasing D_W. The relationship of D_W and testes weight showed a weak sigmoid tendency, and mature males had heavier testicles (Fig. 5).

On the basis of sexual parameters, such as clasper length and testes weight in relation to disc width (Fig. 5), males of *P. violacea* reach first sexual maturity at ~41.0 cm D_W, which corresponds to 63.6% of the maximum D_W observed in this study. The largest immature male was 36.0 cm D_W and the smallest maturing individual was 37.3 cm D_W. On the basis of

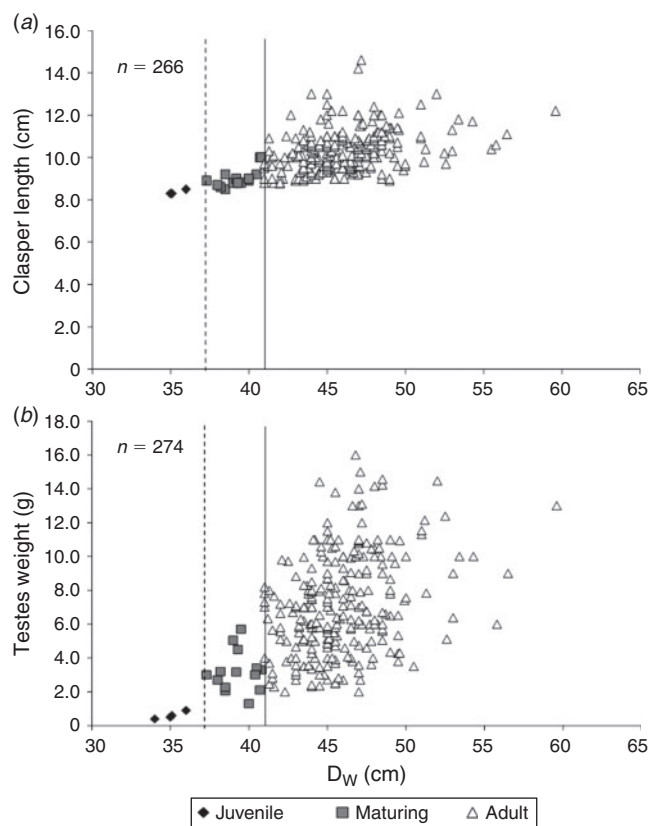


Fig. 5. Relationship between disc width (D_W) and (a) clasper length and (b) testes weight of male pelagic stingrays, *Pteroplatytrygon violacea*, taken in the equatorial and south-western Atlantic Ocean. The dotted line indicates the first maturing individual and the solid line indicates the first adult.

these results, of the 292 males analysed in this study, 93.5% were mature.

Like females, males did not show any seasonal pattern in the frequency of occurrence of maturity stages throughout the year. Through observation of the characteristics of the reproductive organs, adults males occurred in all months, with the highest number observed in September ($n = 16$).

Discussion

Dasyatids exhibit sexual dimorphism, with females maturing at, and growing to, a larger size, and older age, than males (Ebert and Cowley 2009). In the present study the largest female

and male were 66.0 cm and 59.6 cm D_W , respectively, corresponding to 73.3% and 66.2% of the largest D_W (90.0 cm). Other authors also reported large pelagic stingray specimens (Bigelow and Schroeder 1962; Wilson and Beckett 1970; Forselleo *et al.* 2008). Mollet *et al.* (2002) cited the largest ever D_W for a female kept in captivity (96.0 cm).

The smallest free-living individual caught in the present study was a 28.0-cm D_W female, equal in size to the smallest female observed by Forselleo *et al.* (2008). The small specimens were not caught, probably, because they were absent in the study area or the fishing gear used did not capture them. The smallest specimen observed in this study was almost 10.0 cm larger in D_W than the full-term embryo observed in this study and by Hemida *et al.* (2003) from the Mediterranean Sea.

The size classes with the highest frequency of females (49.0–54.0 cm D_W) and males (43.0–48.0 cm D_W) found in the present work were similar to those observed in other studies (Mazzoleni and Schwingel 2002; Forselleo *et al.* 2008; Neer 2008). The high frequency of individuals in a particular length class is likely related to the method of capture and gear selectivity. Oliveira *et al.* (2010), working in the tropical western Atlantic Ocean, offered two hypotheses for the high number of individuals within a small size range: (1) that smaller specimens were not caught due to size selectivity of the fishing gear; or (2) that small specimens were not caught because they were absent in the study area, suggesting that the juveniles of this species might be occurring either at a different geographical location or at a different depth range than those where the fishery operates. The same alternatives may apply to the pelagic stingray. Considering, however, the small size of the mouth of the pelagic stingray, like all dasyatids, and the large size of the hooks (circle hooks, size 18/0, 0° offset; and J-style hooks, size 9/0, 10° offset) used in the longline tuna fisheries, the first hypothesis seems more plausible.

Female pelagic stingrays were, on average, ~5 cm D_W larger and ~800 g heavier than males. The typically larger size of elasmobranch females in relation to males is related to the need to accommodate the developing embryos during pregnancy, providing more space for embryos and ova, as well as for more muscle mass and a larger liver for energy storage, the latter needed to support the female reproductive processes (Klimley 1987).

Hemida *et al.* (2003) also noted that the size at sexual maturity and maximum size were slightly different between females and males in the Mediterranean (although different from the sizes observed in this study), but they did not find significant differences between the weight of females and males. Several other authors have also observed a larger size for female pelagic stingrays than for males (Wilson and Beckett 1970; Tortonese 1976; Mollet 2002; Mazzoleni and Schwingel 2002; Forselleo *et al.* 2008; Neer 2008; Ribeiro-Prado and Amorim 2008; Vaske and Rotundo 2012). The sex ratio in our study was 0.64 : 1 (female : male), close to the ratio 0.53 : 1 encountered by Forselleo *et al.* (2008) and observed by Mazzoleni and Schwingel (2002) off southern Brazil (0.45 : 1), but much higher than what was found by Ribeiro-Prado and Amorim (2008) (0.30 : 1) and Ferrari and Kotas (2013) (0.04 : 1) off southern Brazil. Somvanshi *et al.* (2009), in the Indian Exclusive Economic Zone (EEZ), also reported high ratios of males (0.30 : 1). On the other hand, Mollet (2002), off Baja

California and southern California, Hemida *et al.* (2003), in the Mediterranean, and Neer (2008), in the eastern Pacific Ocean, found low ratios of males (7.1 : 1, 1.5 : 1 and 2.3 : 1, respectively). It is likely that these varying rates are related to the season of the year, site of capture, or the type of fishing gear employed. Sexual segregation, a general characteristic of many elasmobranch populations (Springer 1967; Klimley 1987; Sims 2003; Mucientes *et al.* 2009), may be the underlying reason for the higher number of males. Mollet (2002), off Baja California and southern California, noted that the sex ratios observed could be due to sexual segregation and/or higher male mortality, or, less likely, to selectivity of the fishing gear. However, D. P. Véras, M. T. Tolotti, F. H. V. Hazin, I. S. L. Branco and P. E. Travassos (unpubl. data) observed no geographical segregation, despite the higher number of males, between sexes or between different sexual stages of maturity for *P. violacea* in the south-western and equatorial Atlantic. This difference between studies could be related mainly to the area where the study was conducted and the characteristics of the region.

Female pelagic stingrays mature at larger sizes than males. The sizes at first maturity found in the present work, 48.0 cm D_W for females and 41.0 cm D_W for males, are close to most of the previous estimates published in the literature, which ranged from 37.5 to 42.0 cm D_W for males and 42.5 to 50.0 cm D_W for females (Wilson and Beckett 1970; Tortonese 1976; Hemida *et al.* 2003; Siqueira and Sant'Anna 2007; White and Dharmadi 2007; Ribeiro-Prado and Amorim 2008).

Despite the determination of the first maturity size, a pattern in reproductive seasonality was not observed, perhaps because of the small number of pregnant Stage 3 females ($n = 2$). Reproductive seasonality is one of the most difficult variables to estimate (Oliveira *et al.* 2010). The present data show no clear seasonal or annual pattern in the monthly frequency of different maturity stages, suggesting that parturition occurs throughout much of the year, probably with a rather short reproductive cycle. Forselleo *et al.* (2008) suggested that the pelagic stingray might have a single reproductive cycle per year in the south-western Atlantic Ocean. Lo Bianco (1909), Ranzi (1932, 1933), and Ranzi and Zezza (1936), in the Bay of Naples, reported that gestation was short (2–4 months) and that full-term embryos were observed only in August–September (possible parturition), indicating that pelagic stingrays may give birth even three times per year. The reproductive cycle of the pelagic stingray apparently is variable in its areas of occurrence, certainly a function of local oceanographic variability and food availability. Although it is not possible to infer the gestation period from the present data, it might be sufficiently short to allow for birthing of two litters in a year, assuming a shortened resting period. Indeed, the data do suggest a shortened resting period since the largest ovarian follicles were found in the two pregnant females with the largest embryos, despite the lack of a strong positive relationship between the diameter of the largest ovarian follicle and mean embryo size of pregnant females in Stages 2 and 3. These results also suggest that ovarian development probably speeds up in the later stages of pregnancy, and that females are probably able to ovulate soon after parturition. This condition has been observed in other studies of the pelagic stingray and in other dasyatids (Capapé 1993; Hemida *et al.* 2003; Chapman *et al.* 2003; Janse and Schrama 2010).

An ovarian fecundity higher than uterine fecundity should be expected since during ovulation some oocytes are not released and are reabsorbed by the ovary. The observed uterine fecundity is also lowered by spontaneous abortion during capture, a phenomenon that has been observed for other dasyatids (Struhsaker 1969; Snelson *et al.* 1988; Smith *et al.* 2007). Accordingly, the ovarian fecundity observed for pelagic stingrays in the present study, ranging from 1 to 17 (mean \pm s.e. = 5.4 ± 0.24), was higher than the uterine fecundity, ranging from 1 to 5 (mean \pm s.e. = 3.5 ± 0.32). Hemida *et al.* (2003) reported an ovarian fecundity for the species of 5–10. In their study on captive pelagic stingrays, Mollet *et al.* (2002) documented uterine fecundity of 4–13 embryos. A mean uterine fecundity of 5.4 embryos was reported by Mazzoleni and Schwingel (2002) from 11 pelagic stingrays taken in south-eastern Brazil. Somvanshi *et al.* (2009) examined a single pelagic stingray bearing three embryos. In many species of elasmobranchs there is a positive relationship between fecundity and female size (Conrath 2005). In theory, as a female grows, the increase in length, disc width and girth results in a larger space in the body cavity to accommodate pups. However, pelagic stingrays in this study did not show a positive relationship between the size of the pregnant females and the number of embryos in the uterus. This is consistent with other studies (Hemida *et al.* 2003) that have addressed the pregnancy of pelagic stingrays and some other species of dasyatids (Snelson *et al.* 1988, 1989; Capapé 1993; Johnson and Snelson 1996; Pierce *et al.* 2009).

At the uterus, villi were absent or small in juveniles, present in maturing females, developing or slightly developed in the preovulatory phase, slightly developed in pregnant Stage 1, moderately developed in pregnant Stage 2, highly developed in pregnant Stage 3, slightly developed during postpartum females, and very small in resting females. Wilson and Beckett (1970) also found longer trophonemata in pregnant pelagic stingrays. Lewis (1982) found that trophonemata increased in size as gestation proceeded. Ranzi (1934a) observed that the 'uterine milk' is weak (low protein value) and just enough to wet the embryos and to fill the spaces between the uterine villi. The 'uterine milk' consists of 85.5% water and the remainder is formed by an oily substance. During this study the 'uterine milk' was also very watery.

In this study, based on the maturity criteria used, 67.5% of the 188 females and 93.5% of the 292 males examined were sexually mature. The high proportion of immature females is, therefore, a concern for the sustainability of the stock.

Recently, Dulvy *et al.* (2014) worked with more than 300 scientists around the world to assess the conservation status of all 1041 species of Chondrichthyes. Based on this, they estimate that one in four of these species are threatened with extinction, mainly as a result of overfishing. Moreover, just 389 species (37.4% of the total) are considered to be safe, which is the lowest fraction of safe species among all vertebrate groups studied to date. According to Dulvy *et al.* (2014), the main threats to chondrichthyans are overexploitation through targeted fisheries and incidental catches (by-catch), followed by habitat loss, persecution, and climate change. While one-third of threatened sharks and rays are subject to targeted fishing, some of the most threatened species have declined due to incidental capture in fisheries targeting other species. Besides that, the rise in fishing

effort in pelagic fisheries worldwide has resulted in an increase in by-catch and associated by-catch mortality of pelagic stingrays in some areas (Neer 2008; Baum and Blanchard 2010), a trend that might be expected to continue. Nowadays, even without any directed fishery for the species, *P. violacea* is commonly caught as by-catch in the tuna and swordfish pelagic longline fisheries worldwide, including the south-west Atlantic (Forselledo *et al.* 2008; Zacharia *et al.* 2011). In spite of that, even with considerable participation as by-catch in pelagic longline fisheries, the status of the pelagic stingray populations of the world is still largely unknown, particularly because of the sparse information available about the effects of fishing, distribution and biology. We hope, therefore, that the information generated by this study will contribute to a better assessment of the stocks of pelagic stingrays in the Atlantic Ocean, in order to ensure their conservation through effective management.

Acknowledgements

The first author thanks the CNPq for the Ph.D. fellowship and the Department of Oceanography of the Universidade Federal de Pernambuco, the Department of Fisheries and Aquaculture of the Universidade Federal Rural de Pernambuco, and the Program of Fisheries and Aquatic Science of the University of Florida, where the work was conducted. The authors also thank the Ministry of Fisheries and Aquaculture, who funded this study.

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