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Original Article

Reproductive biology and placentotrophic embryonic development of the smalleye smooth-hound shark, *Mustelus higmani*, from the south-eastern Caribbean

Marioxis Macías-Cuyare[1,2,3], Rafael Tavares[3,4] and Maria Cristina Oddone[5]

[1]Departamento de Biología, Universidad de Carabobo, Naguanagua CP 2005, Estado Carabobo, Venezuela; [2]Programa de Pós-graduação em Oceanografia Biológica, Instituto de Oceanografia, Universidade Federal de Rio Grande, Avenida Itália, Km 8, Campus Carreiros, Rio Grande, RS, Brasil, CEP 96203-900; [3]Centro para la Investigación de Tiburones, Av. Don Bosco, Qta. ABC, No. 10, La Florida, CP 1050, Distrito Capital, Caracas, Venezuela; [4]Instituto Venezolano de Investigaciones Científicas (IVIC), Av. Panamericana km 11, San Antonio de los Altos CP 1204, Estado Miranda, Venezuela and [5]Instituto de Ciências Biológicas, Setor Morfologia, Universidade Federal de Rio Grande, Avenida Itália, Km 8, Campus Carreiros, Rio Grande, RS, Brasil, CEP 96203-900

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Author for correspondence:
Rafael Tavares,
E-mail: rafaeltavares2569@gmail.com

Abstract

The reproductive biology and embryonic development of *Mustelus higmani* were examined between January 2015 and December 2016 in the south-eastern Caribbean. Captures comprised 813 females (23.2–72.5 cm TL), and 960 males (22.6–62.5 cm TL). The total length at 50% maturity was estimated as 47.8 and 47.5 cm for females and males, respectively. Uterine fecundity ranged from 1 to 8 embryos and ovarian fecundity between 1 and 9 vitellogenic follicles. The time of parturition and mating season of *M. higmani* may occur throughout the year, peaking between November and February. The presence of pre-ovulatory ovarian follicles along with advanced embryos indicates an annual reproductive cycle for female *M. higmani*. The main embryonic development stages were recorded in the samples, from uterine eggs (1 to 6 per female) to term embryos (23.0–26.0 cm TL). The transition between placental pre-implantation and post-implantation occurs when embryos have attained a TL of 5.0–6.0 cm. The observation of abundant uterine histotrophic secretions in late pregnant and post-partum females demonstrates that histotrophy may intensify close to birth in this species. The local population of *M. higmani* appears to have relatively high productivity; nonetheless, this species is heavily harvested and lacks management measures in the study area.

Introduction

The smalleye smooth-hound (*Mustelus higmani*) is a small, demersal tropical shark, inhabiting coastal waters from the continental shelf to middle slopes, especially on muddy bottoms (Compagno, 1984). The distribution of *M. higmani* is restricted to the central western Atlantic, from the Gulf of Mexico to southern Brazil, including Venezuela, Trinidad and Tobago, Guyana, Suriname and French Guiana (Compagno, 2002). Throughout its distribution range, *M. higmani* is exploited by several coastal fisheries, being captured by shrimp seines, bottom longline and gillnets, and marketed fresh or dried salted for human consumption (Compagno, 2002; Tavares *et al*., 2010). Off Venezuela, the smooth-hound sharks (*Mustelus* spp.) were commonly captured as bycatch by industrial trawl fleets targeting shrimp (FAO, 2001) until the prohibition of this fishery in 2005. *Mustelus higmani* is an abundant shark in the north-eastern region and around Margarita Island where it is the species most frequently caught by the artisanal shark fishery, accounting for 36.2–40.8% of the shark landings numerically (Tavares *et al*., 2010; Márquez *et al*., 2019). Nevertheless, due to the small size of *Mustelus* species, this group represents ~6% of the total elasmobranch production in the eastern Venezuelan Caribbean (Tavares, 2019).

Despite the commercial importance of *M. higmani* as a food resource in the Venezuelan Caribbean, its life history traits are not well known and need further investigation. Moreover, the lack of biological and fishing information has prevented stock assessment and population modelling of this species in the region. However, some information regarding biological descriptions, size structure and reproduction is available for *M. higmani* off the northern coast of South America (Heemstra, 1997; Tavares *et al*., 2010; Tagliafico *et al*., 2015). *Mustelus higmani* is a viviparous placentotrophic species having 3–5 young (Compagno, 1984). Currently, this species is classified as 'Least Concern' (LC) according to the global status of the International Union for Conservation of Nature (IUCN, Faria & Furtado, 2006). In Venezuela, however, the national assessment classified this species as 'Vulnerable' (VU) due to the decreasing abundance trend as a consequence of excessive fishing and the absence of management measures (Tavares, 2015). Although Venezuela has a National Plan of Action for sharks, management and conservation of shark stocks have not been a priority for the governmental institutions with responsibility in fishery administration.

Overfishing and habitat degradation are recognized among the main causes of large population declines in coastal and pelagic shark species (Stevens *et al.*, 2000; Camhi *et al.*, 2009). At present, 25% of shark species are considered threatened with an elevated risk of extinction based on conservation assessments by the IUCN (Dulvy *et al.*, 2017). These negative trends, combined with the commercial and ecological importance of sharks, continue to attract attention from researchers, fishery managers and conservation organizations. The implementation of management and conservation strategies needs to regard all aspects of elasmobranch reproductive systems and processes, particularly because population modelling requires specific information on reproductive parameters (Conrath & Musick, 2012). The objective of the present study was to examine the size structure and several aspects of the reproductive biology of *M. higmani*; in particular, length at maturity, fecundity and reproductive cycle. In addition, observations on the gross morphology of the transition from lecithotrophy to placentotrophy are provided, including both embryos and extra-embryonic membranes.

Materials and methods

Study area

The artisanal fishing fleets based in Margarita Island (Venezuela) operate in a wide extent of the south-eastern Caribbean (10°35′–10°38′N 65°50–61°51′W; Figure 1). The fishing area extends from the marine zone near the continental coastline to ~50 km north of Margarita Island, and from the areas surrounding La Tortuga Island to the boundary with Trinidad and Tobago. The shark fishery is part of the multispecies fisheries exploiting seasonally abundant elasmobranchs and teleosts. The artisanal vessels involved in the fishery are primarily wooden, 8–14 m in length, and are equipped with either outboard or central motors (48–150 hp). The fishing gears used to capture sharks include mainly longlines, bottom-set gillnets and driftnets (Tavares *et al.*, 2010).

Data collection

Between January 2015 and December 2016, 84 surveys (3–4 per month) were carried out at the fishing ports of Juan Griego and Los Cocos, two important localities for the fish trade in Margarita Island. *Mustelus higmani* individuals were sampled immediately after being landed and each specimen was examined and measured for total length (TL cm; with the caudal fin in a natural position) and sexed. Neonates were identified by the presence of an open umbilical scar. When possible, a random subsample of sharks was used for reproductive assessment. In males, the clasper length (CL cm) was measured along the inner margin from the anterior end of the cloaca to the tip of the clasper, and its calcification degree was also registered. Individuals were dissected *in situ* and the reproductive tracts were stored at −5°C and taken to the laboratory for analyses.

Size and sex composition

Size composition by sex was described by grouping the individuals in size classes of 2.0 cm TL. Seasonal variation in sex ratio by month (years combined) was evaluated using a χ^2-test. Mean size at birth of *M. higmani* was estimated based on the TL of all sharks identified as neonates. Statistical analyses were performed using R software version 3.5.2 (R Core Team, 2018).

Reproductive parameters

In order to assess male and female sexual development and the onset of maturity a macroscopic maturity scale of proposed criteria by ICES (2013) for viviparous chondrichthyan species was applied. Slight modifications in the number of categories of this scale were done; the sixth stage for females (regenerating), and the fourth stage for males (regressing) were excluded. The maturity stages considered were: immature (I), developing (II), mature (III), pregnant (IV) and post-partum (V). Stages I–III are common for both sexes, and stages III–V considered as ‘mature’ for deriving estimates of the length at maturity.

For estimating the length at which 50% of individuals were sexually mature ($TL_{50\%}$), a logistic model was fitted to binomial maturity data (0 = unable to reproduce; 1 = able to reproduce), using TL classes of 5 cm in both sexes. The logistic function is as follows: $y = (1 + e^{-(\beta_0+\beta_1 x)})^{-1}$; where, y is the proportion of mature individuals, x is the TL classes, β_0 is an intercept term, β_1 is the effect of size in terms of TL, and the value of $TL_{50\%}$ is given by $-\beta_0/\beta_1$. Model parameters were estimated through maximum likelihood method using the binomial generalized linear model function in R software (R Core Team, 2018). Confidence intervals (95% CI) of the logistic regression curves were estimated by applying bootstrap resampling techniques (N = 1000) from fits of the logistic model to the maturity data. Variation in uterus width (UW mm), oviductal gland weight (OGW g), ovary weight (OW g), clasper length (CL cm) and testis weight (TW g), relative to total length (TL cm), were used to assess sexual development in both sexes. Other reproductive organ measurements (e.g. diameter of vitellogenic follicles, oviductal width, testis length and liver weight) did not provide conclusive information, and were then excluded from this relationship analysis.

Embryos found in pregnant females were examined, measured for TL (cm) and sexed. Uterine and ovarian fecundity were determined by quantifying the number of embryos occupying the uterus (UF) and the number of vitellogenic follicles in the ovary (OF), respectively. Linear regression techniques were applied to the relationships between maternal TL, and UF and OF in order to determine if fecundity increases with maternal size. To examine the reproductive cycle of females, the embryo size (TL cm) and diameter of the largest vitellogenic follicle (FD mm) were plotted against months (years combined). This information, together with the occurrence of neonates was also used to infer parturition time and ovulation. Additionally, embryo size and follicle diameter data were log-transformed and mean values were statistically compared between months by applying one-way analysis of variance (ANOVA-I; R Core Team, 2018).

Embryonic development

Pregnant (IV) (early, mid and late-term pregnant) and post-partum (V) females were randomly chosen for observation and photographic record of the gross morphology of the embryonic development. Descriptive embryology also included observation of the extraembryonic membranes, i.e. the yolk sac (YS) and the YS stalk and later the yolk sac placenta (YSP) and umbilical cord. The descriptions agree with terminology and concepts of Hamlett *et al.* (2005). Early pregnant female uteri were examined for the presence of uterine eggs, the length of which were measured (cm) along the cranial-caudal axis, considering their *in utero* position. Pre- and post-implantation stages were described according with the aspect of embryos and extraembryonic membranes in early, mid, late and term pregnant females. Particular attention was devoted to those embryonic development instances that provided morphological information on the processes of placentotrophy, especially when evidencing the transitional stages from lecithotrophy to placentotrophy/histotrophy.

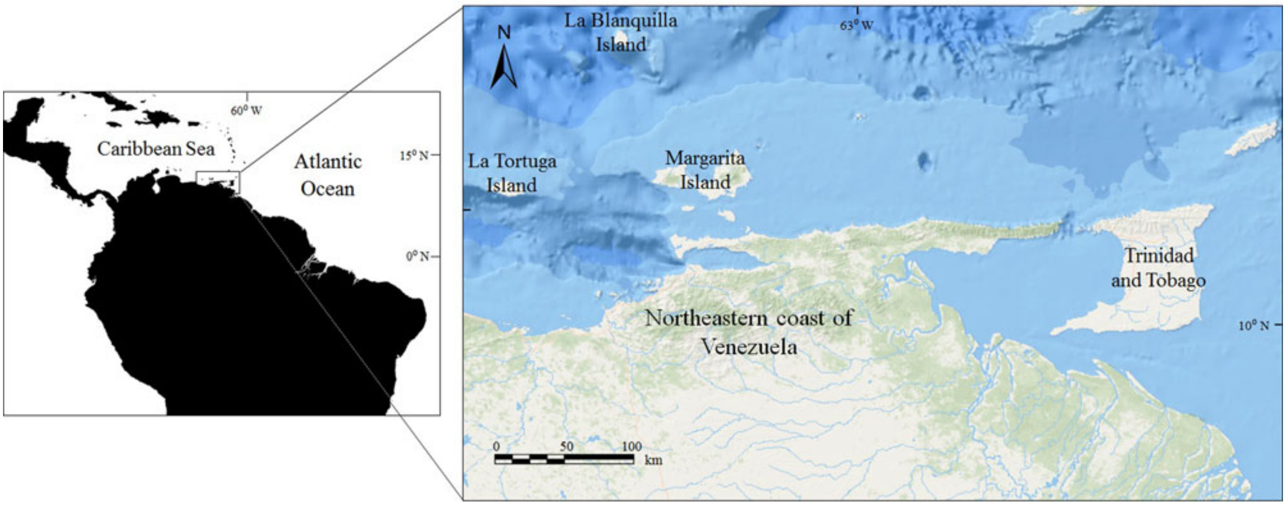

Fig. 1. Map of the study area, south-eastern Caribbean, where *Mustelus higmani* individuals were captured by artisanal shark fishery between 2015 and 2016. (Map sources: Esri, GEBCO, NOAA, National Geographic, Garmin, Here and Geinames.org).

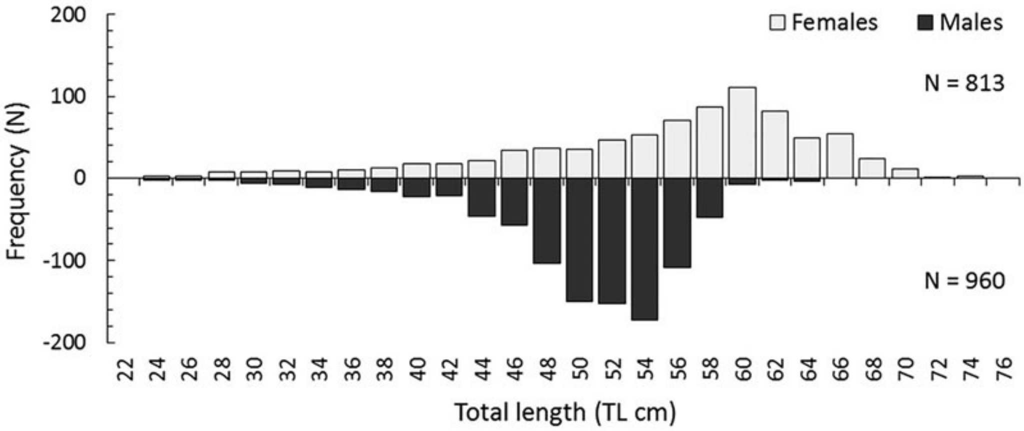

Fig. 2. Size composition by sex of *Mustelus higmani* individuals captured by artisanal shark fishery in the south-eastern Caribbean.

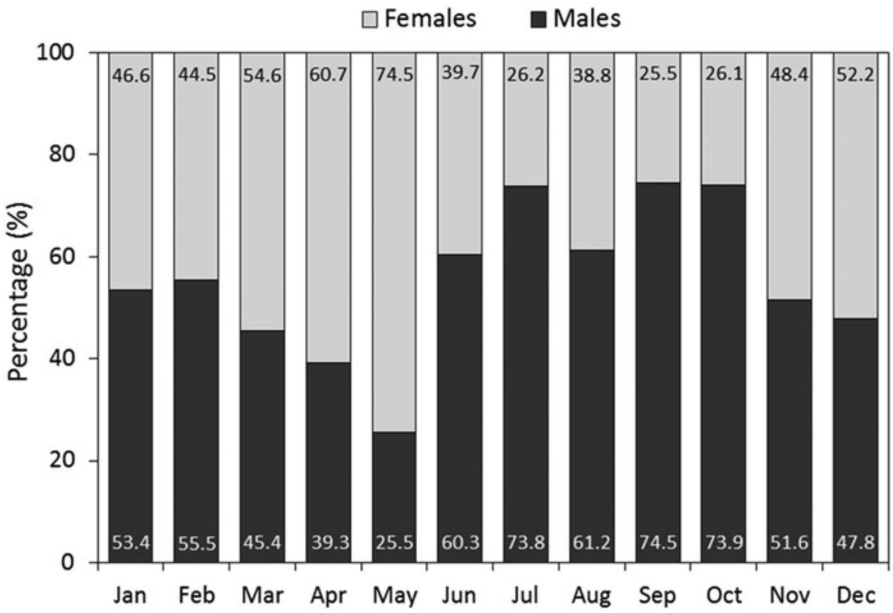

Fig. 3. Monthly sex ratio (years combined) of *Mustelus higmani* individuals captured by artisanal shark fishery in the south-eastern Caribbean.

Results

Size and sex composition

For the study period (January 2015 to December 2016) captures of *M. higmani* were comprised of 813 females between 23.2 and 72.5 cm TL, and 960 males between 22.6 and 62.5 cm TL (Figure 2). Size composition by sex also indicated that females reach a larger TL than males. Regarding sex proportion analysis (Figure 3), a significant difference was not observed from November to March (χ^2 test, $P > 0.05$). However, females

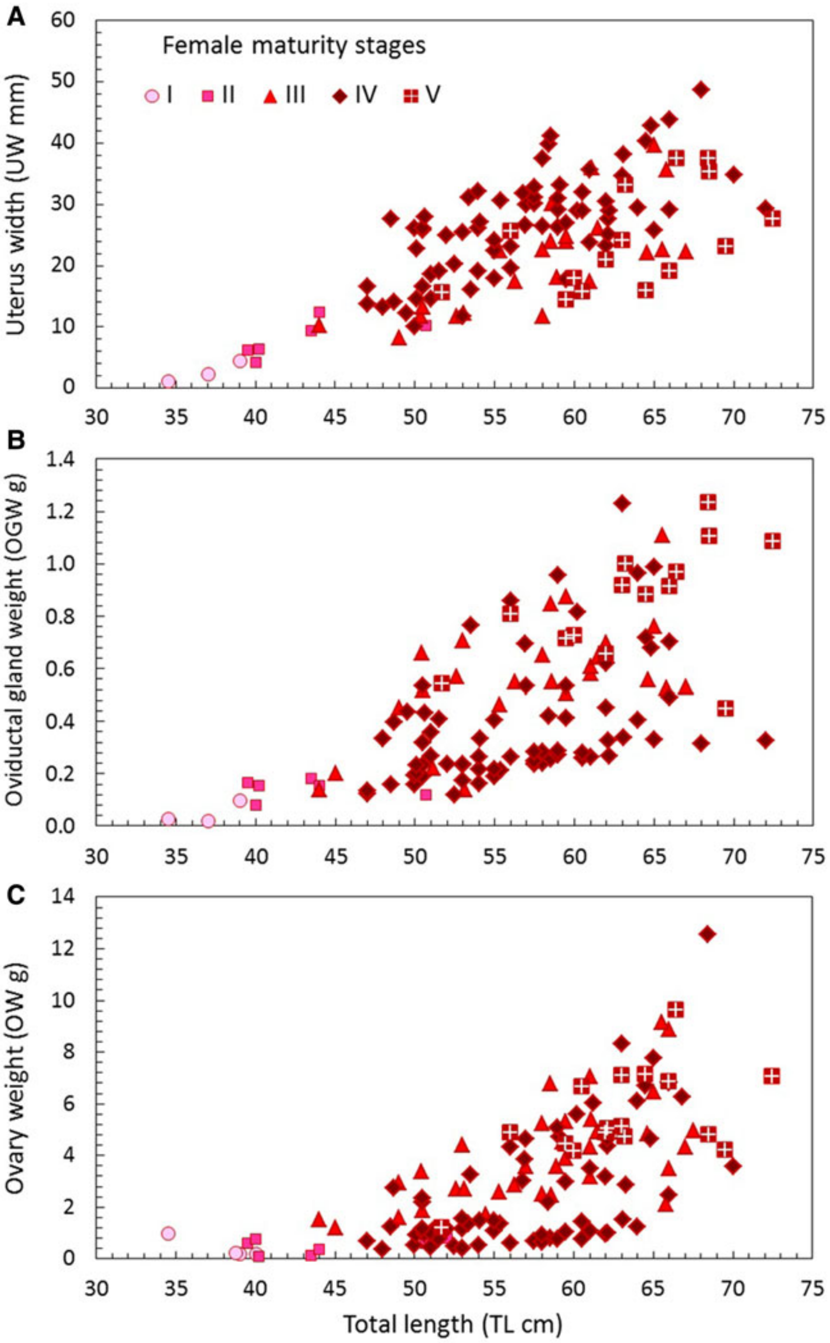

Fig. 4. Relationship between the total length (TL) of female *Mustelus higmani* and (A) uterus width, (B) oviductal gland weight and (C) ovary weight according to maturity stages (immature, I; developing, II; mature, III; pregnant, IV; and post-partum, V).

significantly predominated during April and May (χ^2 test, $P < 0.05$), and males from June to October (χ^2 test, $P < 0.05$). In all, 53 neonates ranging from 22.6–33.8 cm TL (mean: 28.7 ± 2.6 SD) were recorded during the study period, representing 3.0% of the total sample. These neonates were observed throughout the year, in April (N = 2), June (N = 5), July (N = 1), September (N = 2) and October (N = 2), but with a higher occurrence from November to February (N = 38).

Reproductive parameters

A subsample of 143 females (34.5–72.5 cm TL) and 189 males (27.6–59.8 cm TL) was examined for reproductive state. The distributions of maturity stages for females were 2.1% immature (I), 4.9% developing (II), 26.6% mature (III), 55.2% pregnant (IV) and 11.2% post-partum (V), and for males were 15.3% immature (%), 20.1% developing (II) and 64.6% mature (III).

In females, the smallest mature individual was 44.0 cm TL and the largest immature specimen was 50.7 cm TL (Figure 4A–C). Uteri width (Figure 4A) starts active development from TL of 35.0 cm onward. Immature females (N = 3) had UW of up to 4.0 mm, undeveloped OGW and OW of up to 1.0 g. Developing females (N = 6, TL range of 39.5–50.7 cm) had UW of 4.0–12.2 mm, OGW of 0.08–0.18 g and OW of 0.1–0.8 g (Figure 4A–C). Over 47.0 cm of TL, a high overlap in the mature, pregnant and post-partum females was observed, for the three variables under study. Within this range, UW widely varied from 8.1–48.5 mm, with an abrupt growth from TL of 49.0 cm (Figure 4A). Differently, the growth of OGW and the OW with TL was steady but quite gradual (Figure 4B & C). Mature females (N = 38) had UW ranging from 8.1–39.6 mm, OGW from 0.1 to 1.1 g and OW from 1.1 to 9.2 g (Figure 4A–C). In pregnant females (N = 79) UW ranged from 10.0–48.5 mm, the latter corresponding to term pregnant females (Figure 4A). In this category, OGW varied from 0.1–1.2 g and OW from 0.4–12.6 g (Figure 4B & C). In post-partum

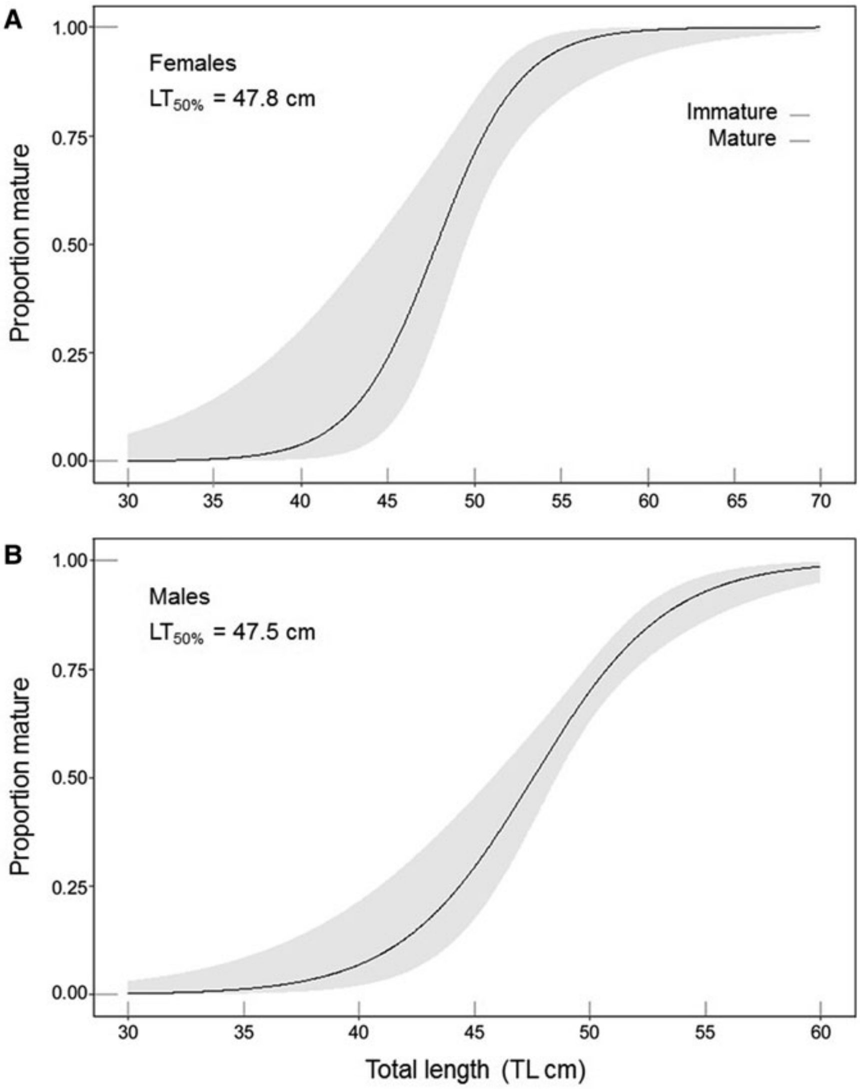

Fig. 5. Length at maturity for (A) females and (B) males of *Mustelus higmani* individuals captured by artisanal shark fishery in the south-eastern Caribbean. Shadow areas correspond to the 95% confidence intervals.

females (N = 16) UW ranged from 14.3–37.3 mm; on the other hand, OGW varied from 0.4–1.2 g and OW from 1.2–9.6 g (Figure 4A–C). The length at maturity ($TL_{50\%}$) for females was estimated in 47.8 cm TL (95% CI: 45.7–50.0 cm), equating to 66.1% of maximum TL recorded (Figure 5A).

In males, the smallest mature individual was 45.0 cm TL and the largest immature specimen was 49.1 cm TL; with most individuals beginning to mature at ~47.0 TL (Figure 6A & B). Clasper length varied from 1.1–5.3 cm in immature (N = 29), 4.0–6.7 in developing (N = 38) and 5.0–6.5 in mature males (N = 122, Figure 6A). In immature males, TW ranged from 0.2–2.5 g, developing males from 1.0–3.6 g and mature from 1.7–5.3 g (Figure 6B). The length at maturity ($TL_{50\%}$) for males was estimated in 47.5 cm TL (95% CI: 46.1–48.6 cm), which equates to 80.5% of maximum TL recorded (Figure 5B).

The number of embryos (UF) per female varied between 1 and 8 (mean: 3.8 ± 1.9 SD), and the TL of the embryos ranged from 3.0–26.5 cm. A weak relationship ($r^2 = 0.24$) between maternal TL and UF was found, but with a significant positive slope (*t*-test; $t = 2.6$, $P < 0.05$). The analysis to compare sex proportion of embryos did not show significant difference between females and males (χ^2 test, $P > 0.05$). Mean embryo size by month varied between 6.6 (± 1.0 SD) and 22.6 (± 1.1 SD) cm TL, with maximum values observed from September to February (Figure 7A). Comparison between monthly mean values was highly significant (ANOVA-I, $F = 41.7$, $P < 0.000$). The number of vitellogenic follicles (OF) ranged between 1 and 9 (mean: 4.7 ± 1.8 SD). The relationship between TL and OF was also weak ($r^2 = 0.30$) with a highly significant positive slope (*t*-test; $t = 6.3$, $P < 0.000$). Regarding the seasonal variation in follicle growth, the analysis showed that mean diameter by month varied between 9.5 (± 3.2 SD) and 11.7 (± 1.1 SD) mm FD, with maximum values recorded at the end of the year, between September and December (Figure 7B). However, the analysis to test for statistical difference between mean follicle diameters by month was not significant (ANOVA-I, $F = 1.4$, $P > 0.05$). The information on embryo and follicle development, together with the occurrence of neonates showed that time of parturition and mating season of *M. higmani* may occur throughout the year, though both processes may be more frequent between November and February. Six pregnant females (51.5–65.1 cm TL) simultaneously bearing vitellogenic follicles and embryos in an advanced stage of development were recorded in February (N = 2), June (N = 1), August (N = 1), September (N = 1) and December (N = 1), indicating an annual reproductive cycle. However, the length of gestation could not be determined as both folliculogenesis and gestation are not synchronized to the population level.

Descriptive gross morphology of placentotrophic embryonic development

Pre-implantation stages

Uterine eggs were observed in seven early pregnant females and had lengths ranging from 2.1–2.5 cm (N = 3). The encapsulated

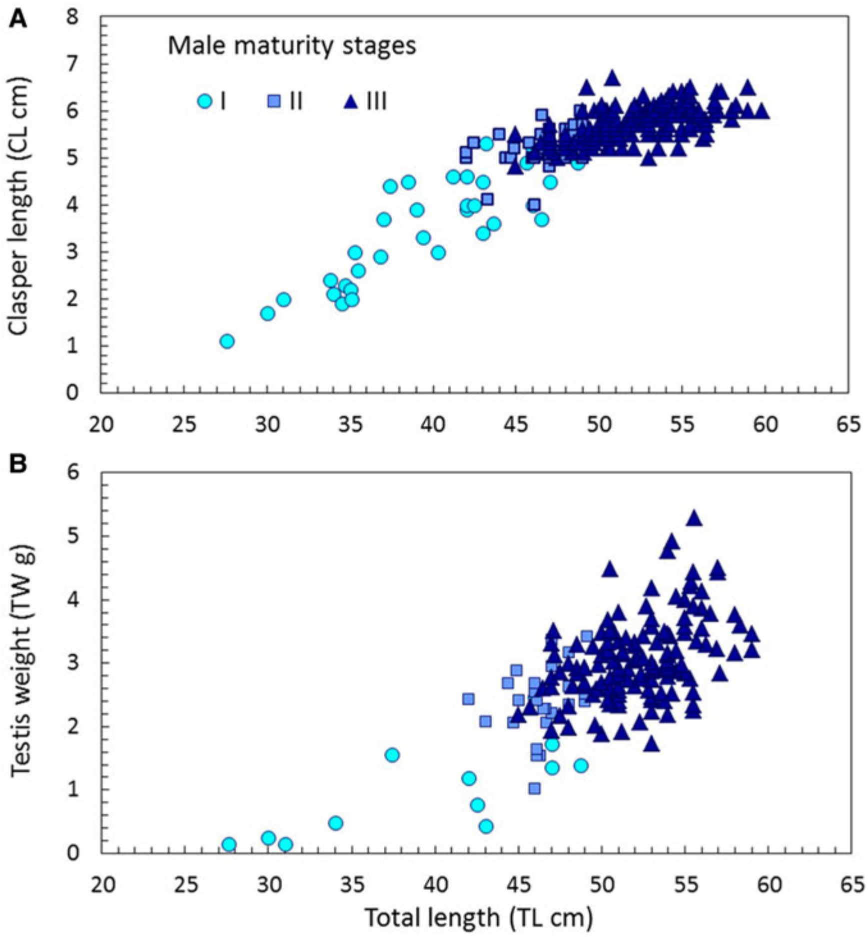

Fig. 6. Relationship between the total length (TL) of male *Mustelus higmani* and (A) clasper length and (B) testis weight according to maturity stages (immature, I; developing, II; and mature, III).

eggs were ellipsoid in shape. The number of uterine eggs per female varied from 1–6 (mean: 2.9 ± 1.7 SD). External eggs colouration varied from yellow to amber (Figure 8A–D). The diagonal position of the uterine eggs inside the uterus indicates that compartments formation may be in early progress (Figure 8A). In uterine eggs bearing females, the uterine epithelium was highly vascularized (Figure 8B). The tertiary egg envelope was light yellow and folded on the egg edges, forming the egg envelope reservoir; and once stretched; the egg occupied the centre of the envelope (Figure 8C & D).

The smallest visible recorded embryos were 3.0 cm TL and bore external highly vascularized gill filaments (Figure 8E). In these embryos, the YS withered and started to lose the typical round shape to assume an irregular one, as visible in embryos of 6.0 cm TL onward (Figure 8F). Embryos of 3.0–6.0 cm TL presented conspicuous, lengthened YS stalks, vitelline artery and vitelline vein visible by transparency through the omphalopleure (Figure 8E & F).

Post-implantation stages

On a 6.0 cm TL embryo the external yolk sac was no longer visible as such and the umbilical cord was connected to the proximal aspect of the YSP, indicating that implantation had occurred (Figure 9A). The umbilical cord was formed by an umbilical artery and umbilical vein. On the other hand, a 5.0 cm TL embryo (the other uterus contained a 5.7 cm embryo) showed a YS anchored to the uterus epithelium, suggesting the recent formation of the placental connection.

The jelly, diaphanous umbilical cord of a 10.6 cm TL embryo, revealed a conspicuous ductus vitellointestinalis filled with yolk and a term YSP. The female bearing this embryo bore another one, on the other uterus, with TL of 10.9 cm. Abundant periembryonic fluids were noted in the two latter cases (Figure 9B & C). Four term embryos (two per uterus) with TL of 23.3, 22.7, 23.2 and 23.3 cm, were attached to full term placentas (Figure 9D). Term placentas were also recorded in three term embryos measuring 25.6, 26.1 and 25.9 cm TL. The umbilical cord of *M. higmani* was smooth along its whole surface, with no ornamentations (Figure 9E). Vitellogenic follicles in the ovaries were present in term pregnant and post-partum females (Figure 9E). The term placenta had a diameter of around 2.0 cm. Post-partum uteri had distended uterine walls and were internally embedded by an abundant yellowish histotrophic secretion (Figure 9F).

Discussion

The commercial landings of *M. higmani* in the study area comprised a wide range of lengths, 23.2–72.5 cm TL in females and 22.6–62.5 cm TL in males, but with a predominance of captures in the ranges of 52.0–66.0 and 46.0–56.0 cm TL for females and males, respectively. The pattern of size composition observed in this study is similar to those previously reported for the same species in other studies conducted in the north-eastern coast of Venezuela (Tavares *et al.*, 2010; Marquez *et al.*, 2019). The sexually dimorphic maximum sizes (favouring *M. higmani* females) appear to be a common characteristic of mustelid sharks, having also been reported for *M. canis* from the east coast of the USA (Conrath & Musick, 2002) and northern Brazil (Zagaglia *et al.*, 2011), *M. schmitti* in common waters of Uruguay and Argentina (Oddone *et al.*, 2005; Belleggia *et al.*, 2012), *M. henlei* from the Gulf of California, Mexico (Perez-Jimenez & Sosa-Nishizaki, 2008) and *M. asterias* in the north-eastern

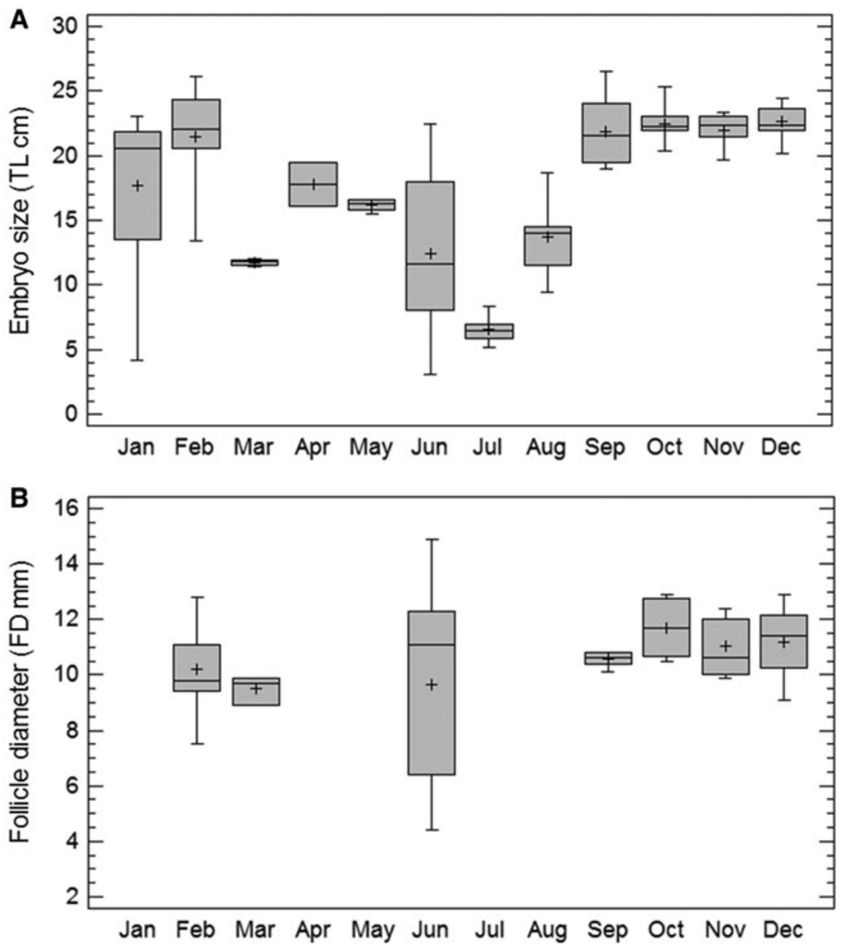

Fig. 7. Seasonal variation in (A) embryo size (TL) and (B) diameter of the largest vitellogenic follicle (FD mm) by months (years combined) of *Mustelus higmani* individuals captured by artisanal shark fishery in the south-eastern Caribbean.

Atlantic (Farrell *et al*., 2010; McCully Phillips & Ellis, 2015). The low number of *M. higmani* caught at a small size (22.0–36.0 cm TL) was likely related to lower gear selectivity (samples were mainly from gillnets; mesh size >7.6 cm) and/or to fishing areas. The capture of small specimens (with lengths around the mean size at birth, 28.7 cm TL) by the recreational reel and rod fishery conducted from land in Margarita Island (Tavares, pers. obs.) suggests that the primary nursery areas of *M. higmani* are distributed near the coast; and then, these shallow areas are out of the depth range for artisanal fishing operations.

Spatial and sex segregation is a widespread behaviour exhibited by several animal groups, including elasmobranchs, and is commonly associated with reproduction, movement patterns and nutritional requirements (Klimley, 1987; Wearmouth & Sims, 2008; Heist, 2012). In several shark populations, seasonal variation in sex proportion appears to be related to differences in the degrees of site fidelity (philopatry) or dispersal by sex (Heist, 2012; Portnoy *et al*., 2015). However, during reproductive periods, the receptivity of females towards males increases and this generally leads to observing similar sex proportions (Magurran & Garcia, 2000). In the present study, the observed sex proportion did not differ from the expected ratio (1:1) during the months of November–March, suggesting that this is the main mating period of *M. higmani*.

Females and males of *M. higmani* matured at similar sizes in the study area with individuals beginning to develop at ~44.0 cm TL. The $TL_{50\%}$ estimates obtained in this study were 47.8 cm TL for females and 47.5 cm TL for males. The first $TL_{50\%}$ estimates reported by Tavares *et al.* (2010) were higher for both females (59.9 cm TL) and males (52.5 cm TL), and this discrepancy might be a consequence of differences in sample sizes. In another recent study, Tagliafico *et al.* (2015) reported $TL_{50\%}$ of 46.7 cm TL for females and 47.6 cm TL for males, similar values to those obtained in our study but in contrast these authors found a $TL_{50\%}$ of males over the females. Although female *M. higmani* reached higher maximum sizes than males, the lengths at maturity observed in the present study were close between sexes. The phenomenon of bimaturism in sharks (Cortes, 2004), where males mature at a smaller size/age than females has been commonly found for congeneric species such as *M. canis* (Conrath & Musick, 2002; Zagaglia *et al*., 2011), *M. schmitti* (Oddone *et al*., 2005), *M. henlei* (Perez-Jimenez & Sosa-Nishizaki, 2008) and *M. asterias* (Farrell *et al*., 2010; McCully Phillips & Ellis, 2015).

The fecundity of *M. higmani* in terms of both uterine fecundity (1–8 embryos) and ovarian fecundity (1–9 vitellogenic follicles) seemed to be relatively low. The similarity between uterine and ovarian fecundity suggests that most fully-yolked oocytes ovulate during the process and settle towards the uterus. Litter size estimates previously documented for *M. higmani* are within the range of 1–10 (Heemstra, 1997; Tavares *et al*., 2010; Tagliafico *et al*., 2015). Most *Mustelus* species exhibit higher fecundity compared with *M. higmani*; with maximum litter sizes >18 embryos (Capapé, 1983; Conrath & Musick, 2002; Perez-Jimenez & Sosa-Nishizaki, 2008; Saidi *et al*., 2008; McCully Phillips & Ellis, 2015; Gonzalez-Pestana *et al*., 2019). Our findings showed an increase in fecundity (UF and OF) with maternal size for *M. higmani*, a reproductive characteristic that is commonly observed among elasmobranch species (Conrath & Musick, 2012).

The information on the capture of neonates, the seasonal development of embryos and follicles, and the sex proportion

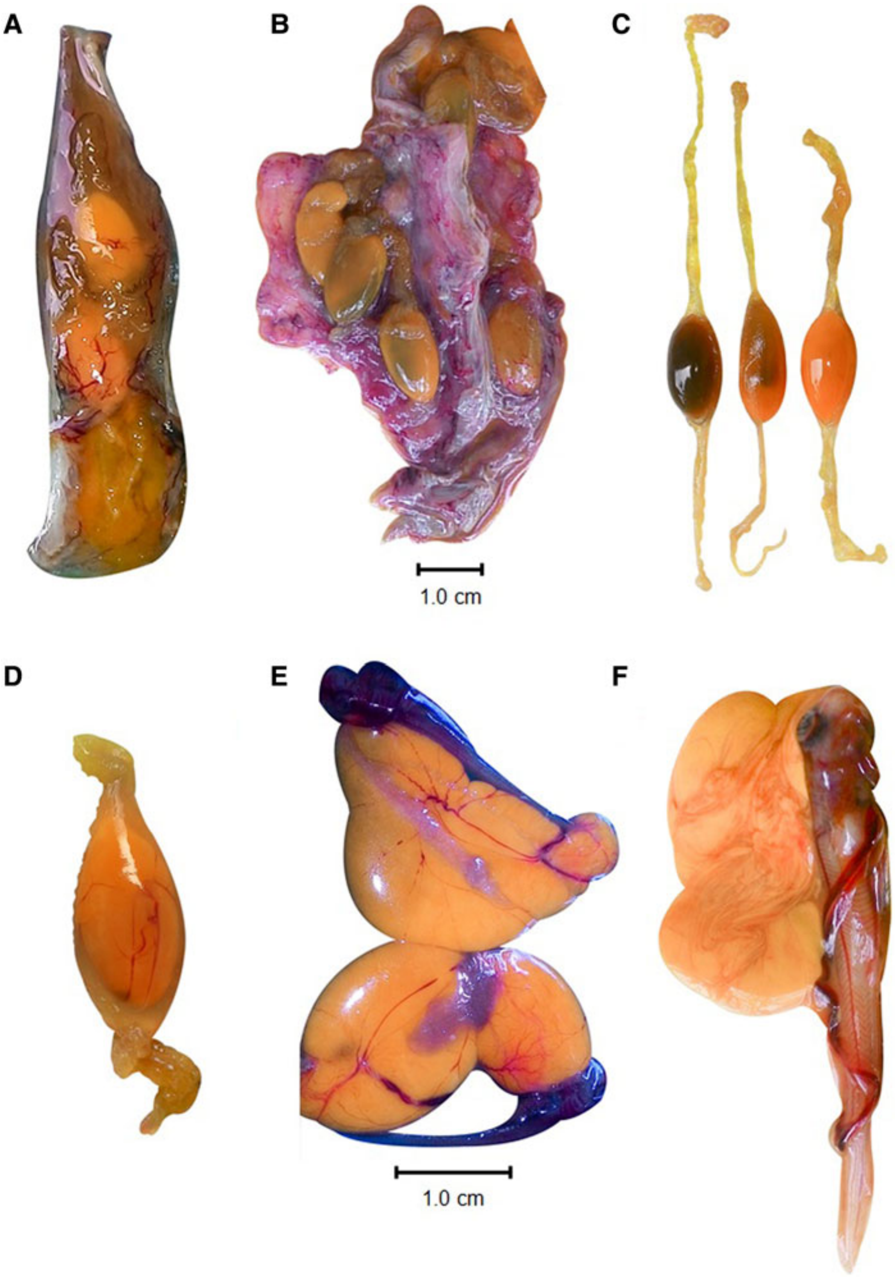

Fig. 8. Instances of early and mid-gestation during the pre-implantation stages of the placentotrophic embryonic development of *Mustelus higmani*, captured by artisanal shark fishery in the south-eastern Caribbean. (A) An early pregnant uterus of a 62.1 cm TL female captured on 20 June 2016, soon after fertilization, with two uterine eggs. (B) A complete set of posterior oviducts extracted from a 62.0 cm TL pregnant female captured on 30 August 2016. The dissection of both early pregnant uteri revealed three uterine eggs per uterus. Compartments are not evident but the uterine epithelium shows rich vascularization. (C) Three uterine eggs with posterior and anterior tertiary egg envelope edges stretched (egg envelope reservoir), extracted from the uterus of a 51.5 cm TL female captured on 20 June 2016. The cause of the variation of colouration on the uterine eggs (yellow to amber) is unknown. (D) A single intrauterine egg extracted *in utero* from a 62.1 cm TL early pregnant female captured on 20 June 2016. The uterine egg had 2.1 cm of length. The egg envelope reservoir was photographed in its natural intrauterine position (folded), visible on both envelope edges. (E) Two embryos extracted from a 59.0 cm TL early pregnant female captured on 24 June 2016. At the embryos' (~3.0 cm TL) cranial end, external gill filaments are visible. Yolk sac at this stage becomes withered. (F) A 6.0 cm TL embryo extracted *in utero* from a 57.5 cm TL early-mid-pregnant female captured on 6 September 2016 presenting an irregularly shaped YS. The external YS stalk has turned into a conspicuous umbilical cord visible around the embryo.

suggest that time of parturition, ovulation and mating of *M. higmani* occurred during a main period of several months, between November and February. An annual and continuous reproductive cycle was supported by the capture of pregnant females carrying concomitantly pre-ovulatory vitellogenic follicles and embryos in an advanced stage of development. This pattern is typical of placentotrophic species that reproduce annually, like *Rhizoprionodon terraenovae* and *M. canis* (Hamlett *et al.*, 2005). Only the left ovary is present in *M. higmani*, in contrast to other species of the genus *Mustelus* (Daniel, 1928; TeWinkel, 1950). Although the gestation period could not be determined due to the wide variability in the ovarian cycle and embryo development, it probably encompasses between 11 and 12 months taking into account the findings related to the main season of parturition and the characteristics of the reproductive cycle. For most studied species of the genus *Mustelus*, an annual reproductive cycle with gestation periods ranging from 10–12 months has been reported (Conrath & Musick, 2002; Oddone *et al.*, 2005; Perez-Jimenez & Sosa-Nishizaki, 2008; Saidi *et al.*, 2008). However, other species with non-placentotrophic reproduction displayed a biennial reproductive cycle, for example *M. manazo* in waters of Japan and Taiwan (Yamaguchi *et al.*, 2000), *M. antarticus* off southern Australia (Walker, 2007) and *M. asterias* from the north-east Atlantic (Farrell *et al.*, 2010).

The lack of a defined seasonal pattern in relation to the development of embryos and follicles in *M. higmani* could be explained in part by the stability of the environmental conditions in the study area, compared with subtropical and temperate regions. The north-eastern shelf of Venezuela is located in a central tropical region of the Caribbean, and is characterized by a wide diversity of fishing resources (FAO, 2020). However, the apparent main season for parturition and mating registered during November–February appears to be related with the seasonal upwelling system that influences the north-eastern coast of Venezuela. This oceanographic event has a major influence during the first months of the year (January–April) when strong winds drive the mixing of water masses and nutrients (Castellanos *et al.*, 2002; Rueda-Roa & Muller-Karger, 2013); and then contributes to the high marine productivity by increasing primary production and abundance of marine fauna in the

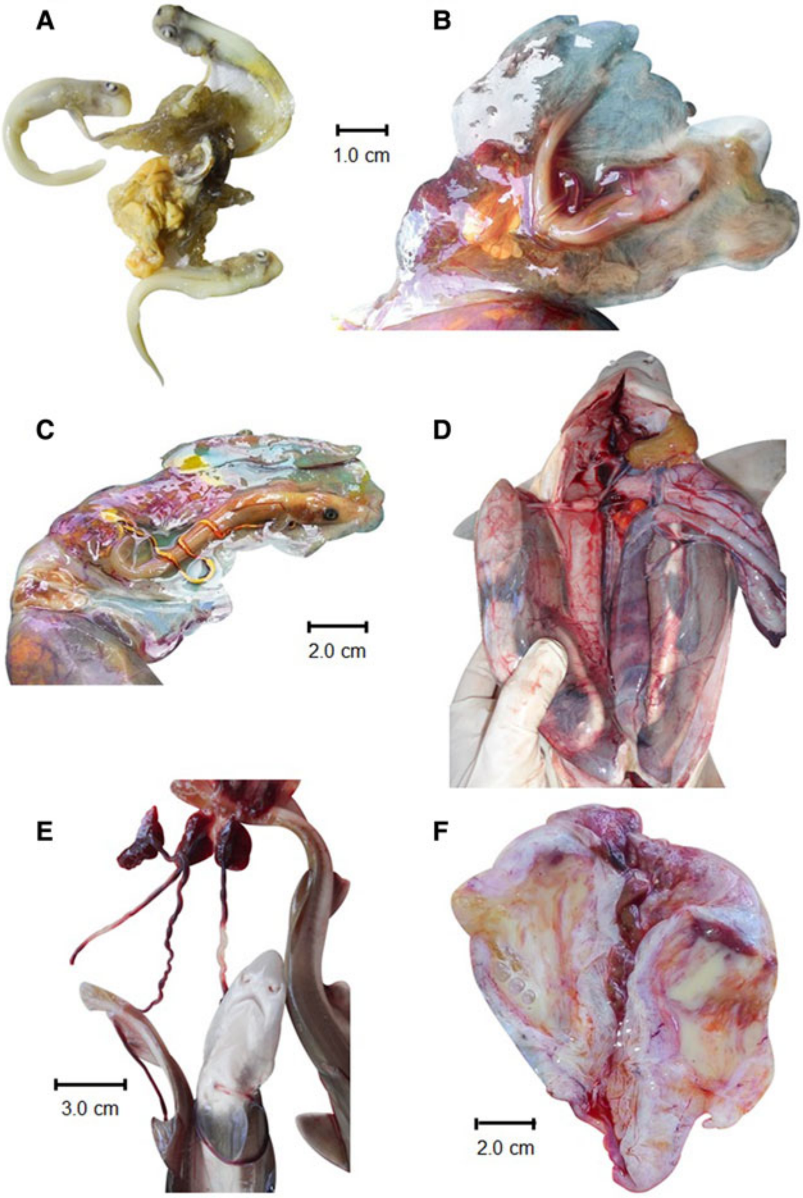

Fig. 9. Instances of mid and late gestation during the pre- and post-implantation stages of the placentotrophic embryonic development of *Mustelus higmani*, captured by artisanal shark fishery in the south-eastern Caribbean. (A) Three 6.0 cm TL embryos extracted from a 55.4 cm TL female captured on 23 February 2016. Yolk sacs are no longer visible and the umbilical cords are connected to the proximal aspect of the YSP. (B) A 5.0 cm TL embryo lying on the dissected uterus, extracted from a 48.5 cm TL female, captured on 16 June 2016. The YS has turn into the embryonic portion of the YSP, anchored to the uterus epithelium. (C) A 10.6 cm TL embryo (the other uterus contained an embryo of 10.9 cm TL) extracted from a 52.5 cm TL mid to term pregnant female captured on 20 June 2016. The embryo is lying on the dissected uterus. The jelly umbilical cord shows a conspicuous ductus vitellointestinalis filled with yolk. Note the abundant periembryonic fluids in both (B) and (C). (D) A dissected 56.8 cm TL term pregnant female, bearing two embryos per uterus (TL of 23.3, 22.7, 23.2 and 23.3 cm) captured on 12 November 2015. The embryos can be seen by transparency across the thin uterus wall. Uteri width was 3.2 cm. Left ovary is visible, presenting ripe vitellogenic follicles. (E) Three term embryos extracted from a term pregnant female (62.0 cm TL) captured on 19 February 2016. The embryos, with full term placenta (embryonic aspect) and smooth umbilical cord, had TL of 25.6, 26.1 and 25.9 cm. (F) Empty uteri of a post-partum 63.5 cm TL female captured on 12 September 2016. The stretched, distended uterine wall is internally embedded by a yellowish histotrophic secretion which avoids visualizing the umbilical scars.

region (Freon & Mendoza, 2003). In the study area, water temperature varies between 20.5 and 29.7°C during the principal and relaxation upwelling periods, respectively (Castellanos *et al.*, 2002; McConnell *et al.*, 2009). The synchronization of parturition with the upwelling period is advantageous because it provides abundant food for neonates and juvenile sharks. In the Venezuelan Caribbean, the distribution and abundance of important groups of clupeid fish and oceanic sharks also seem to be influenced by the seasonal upwelling system (Tavares & Arocha, 2008; Rueda-Roa *et al.*, 2017).

Within the genus *Mustelus*, placentotrophic and non-placentotrophic (previously referred to as 'aplacental') embryonic development strategies can be found (Teshima, 1981). Non-placentotrophic strategies in *Mustelus* include strict lecithotrophy (Yamaguchi *et al.*, 2000) and minimal histotrophy (Storrie *et al.*, 2009; Orlando *et al.*, 2015). The morphology of the yolk sac placenta of *M. canis* has been largely studied among *Mustelus* species (TeWinkel, 1963*a*, 1963*b*). However, for the remaining *Mustelus* species declared as placentrotrophic, including *M. higmani*, the placenta have received little or no attention regarding the general gross morphology, changes during the embryonic developmental stages or other basic aspects. TeWinkel (1950) observed that the spherical shape of the vitellogenic follicles of *M. canis* is lost during its passage through the anterior oviduct and oviducal gland, where it is compressed into an ellipsoid shape, just as observed in *M. higmani*. This author also reported brownish colouration in *M. canis* uterine eggs, after fertilization. Our observations demonstrated that the YSP implantation in *M. higmani* occurs when embryos attain a TL of about 5.0–6.0 cm. From this size onward, the YSP develops until term, when attaining a diameter of around 2.0 cm and embryos have grown up to about 20.0 cm TL.

Despite the same embryological origin, there is a wide morphological diversity in the shark YSP. Basically, there are two major types (Hamlett *et al.*, 2005); the first group including those placentae with two morphological distinct portions (proximal and distal), as seen in *Carcharhinus plumbeus* and *R. terranovae*. The second group includes placentae with no distinct proximal portion, as described for *M. canis* (Hamlett *et al.*, 2002). The YSP of *M. higmani* belongs to the second group. Moreover, as in most placentotrophic sharks, the umbilical cord of *M. higmani* is smooth-surfaced, unadorned (Lombardi, 1998), with no appendiculae (Compagno, 1988).

Periembryonic fluids were observed in all embryonic stages of *M. higmani* on record. However, uterine secretion seemed to intensify and change in composition by the end of gestation, according with the observation of late pregnant and post-partum females' uterine internal lining, with abundant yellowish, thicker secretion. In spite of the fact that further studies are needed (coupled with histology), our observations lead us to hypothesize that *M. higmani* histotrophy intensifies late in gestation. According to Hamlett (1989), during the initial lecithotrophic phase the uteri of placentotrophic shark species are able to produce a histotroph (mucoid secretion), which nourishes the embryos during the period between yolk depletion and placenta establishment.

In *M. higmani* 3.0 cm TL embryos had external gills, which represent nutrient uptake sites, as in other matrotrophic development modes (Hamlett *et al.*, 2005). Also, once the YSP has formed, paraplacental sites are able to produce histotroph with nutritive or uterine lubricant functions (Hamlett, 1989). In *M. canis*, the lecithotrophic phase takes 12 weeks. Immediately after (experimentally demonstrated), paraplacental histotrophy takes place, and the yolk sac placenta is formed two weeks later, 14 weeks after fertilization (Hamlett *et al.*, 2005). Even though these time estimates cannot be assessed for *M. higmani*, according to our observations, the period between the yolk sac depletion and the settlement of the YSP in this species may happen when embryos have TL of around 6.0 cm.

The present study showed that the local population of *M. higmani* had a relatively high productivity compared with other shark species; however, *M. higmani* is among the *Mustelus* species with a lower fecundity. Though commonly neglected, the study of embryology, altogether with reproductive parameters, can throw light on key periods in the life history of the species, especially for those in which biological aspects are still unknown. Although *M. higmani* has been subject to fishing for decades, its gross embryonic development had not been studied before. Future studies may focus on the reproductive cycle of *M. higmani*, including the assessment of gestation and the duration of the three major nourishment embryonic phases (lecithotrophy, histotrophy and placentotrophy), as well as the presence of ovarian resting periods. Furthermore, in Venezuela this species is not under any fishing control or regulation by the governmental institutions with responsibility in fishing administration. Taking into account the sizes at maturity obtained in this study, captures comprised 22.8 and 36.6% of immature females and males, respectively. Although more detailed research and fishing monitoring are needed in order to generate accurate data for development of population dynamic models, basic management measures must be implemented such as minimum sizes of capture and fishing restrictions in essential habitats.

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References

Belleggia M, Figueroa DE, Sánchez F and Bremec C (2012) The feeding ecology of *Mustelus schmitti* in the southwestern Atlantic: geographic variations and dietary shifts. *Environmental Biology of Fishes* **95**, 99–114.

Camhi MD, Valenti SV, Fordham SV, Fowler SL and Gibson G (2009) The Conservation Status of Pelagic Sharks and Rays: Report of the IUCN Shark Specialist Group Pelagic Shark Red List Workshop. IUCN Species Survival Commission, Shark Specialist Group. Newbury: IUCN.

Capapé C (1983) Nouvelles données sur la biologie de la reproduction de *Mustelus asterias* Cloquet, 1821 (Pisces, Pleurotremata, Triakidae) des côtes tunisiennes. *Vie et Milieu* **33**, 143–152.

Castellanos P, Varela R and Muller-Karger F (2002) Descripción de las áreas de surgencia al sur del Mar Caribe examinadas con el sensor infrarrojo AVHRR. *Memoria de la Fundación La Salle de Ciencias Naturales* **154**, 55–76.

Compagno LJV (1984) *FAO Species Catalogue. Sharks of the World: An Annotated and Illustrated Catalogue of Shark species Known to Date. Vol. 4. Part 2. FAO Fisheries Synopsis*. Rome: FAO.

Compagno LJV (1988) *Sharks of the Order Carcharhiniformes*. Princeton, NJ: Princeton University Press.

Compagno LJV (2002) Sharks. In Carpenter KE (ed.), *The Living Marine Resources of the Western Central Atlantic: Species Identification Guide for Fishery Purposes*. Rome: FAO, pp. 357–505.

Conrath CL and Musick JA (2002) Reproductive biology of the smooth dogfish, *Mustelus canis*, in the northwest Atlantic Ocean. *Environmental Biology of Fishes* **64**, 367–377.

Conrath CL and Musick JA (2012) Reproductive biology of elasmobranchs. In Carrier JC, Musick JA and Heithaus MR (eds), *Biology of Sharks and Their Relatives*. New York, NY: CRC Press, pp. 291–312.

Cortes E (2004) Life history patterns, demography, and population dynamics. In Carrier JC, Musick JA and Heithaus MR (eds), *Biology of Sharks and Their Relatives*. New York, NY: CRC Press, pp. 449–470.

Daniel JF (1928) *The Elasmobranch Fishes*, 2nd Edn. Berkeley, CA: University of California Press.

Dulvy NK, Simpfendorfer CA, Davidson LN, Fordham SV, Bräutigam A, Sant G and Welch DJ (2017) Challenges and priorities in shark and ray conservation. *Current Biology* **27**, R565–R572.

FAO (2001) *Tropical Shrimp Fisheries and Their Impact on Living Resources*. FAO Fisheries Circular No. 974 FIIT/C974, Rome: FAO.

FAO (2020) Fisheries Global Information System. Rome: FAO Fisheries and Aquaculture Department. Available at http://www.fao.org/fishery/figis/en/ (Accessed 12 March 2020).

Faria V and Furtado M (2006) *Mustelus higmani*. The IUCN Red List of Threatened Species. e.T60204A12318622.

Farrell ED, Mariani S and Clarke MW (2010) Reproductive biology of the starry smooth-hound shark *Mustelus asterias*: geographic variation and implications for sustainable exploitation. *Journal of Fish Biology* **77**, 1505–1525.

Freon P and Mendoza J (2003) *The Sardine* Sardinella aurita*: Its Exploitation and Environment in Eastern Venezuela*. Montpellier, Paris: Editions IRD.

Gonzalez-Pestana A, Alfaro-Shigueto J and Mangel JC (2019) Aspects of reproductive biology of the humpback smooth-hound shark (*Mustelus whitneyi*) off northern Peru. *Marine and Freshwater Research* **70**, 1185–1188.

Hamlett WC (1989) Evolution and morphogenesis of the placenta in sharks. *Journal of Experimental Zoology* **252**, 35–52.

Hamlett WC, Musick JA, Hysell CK and Sever DM (2002) Uterine epithelial-sperm interaction, endometrial cycle and sperm storage in the terminal zone of the oviducal gland in the placental smooth-hound, *Mustelus canis*. *Journal of Experimental Zoology* **292**, 129–144.

Hamlett WC, Kormarik CG, Storrie M, Serevy B and Walker TI (2005) Chondrichthyan parity, lecithotrophy and matrotrophy. In Hamlett WC (ed.), *Reproductive Biology and Phylogeny of Chondrichthyes*. Enfield, NH: Science Publishers Inc., pp. 395–434.

Heemstra PC (1997) A review of the smooth-hound sharks (genus *Mustelus*, family Triakidae) of the western Atlantic Ocean, with descriptions of two new species and a new subspecies. *Bulletin of Marine Science* **60**, 894–928.

Heist EJ (2012) Genetics of sharks, skates, and rays. In Carrier JC, Musick JA and Heithaus MR (eds), *Biology of Sharks and Their Relatives*. New York, NY: CRC Press, pp. 487–504.

ICES (International Council for the Exploration of the Sea) (2013) Report of the workshop on sexual maturity staging of elasmobranchs (WKMSEL). Lisbon: ICES, CM 2012/ACOM: 59.

Klimley AP (1987) The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. *Environmental Biology of Fishes* **18**, 27–40.

Lombardi J (1998) *Comparative Vertebrate Reproduction*. New York, NY: Springer.

Magurran AE and Garcia CM (2000) Sex differences in behaviour as an indirect consequence of mating system. *Journal of Fish Biology* **57**, 839–857.

Márquez R, Tavares R and Ariza LA (2019) Elasmobranch species in the artisanal fishery of Sucre state, Venezuela. *Ciencias Marinas* **45**, 181–188.

McConnell MC, Thunell RC, Lorenzoni L, Astor Y, Wright JD and Fairbanks R (2009) Seasonal variability in the salinity and oxygen isotopic composition of seawater from the Cariaco Basin, Venezuela: implications for paleosalinity reconstructions. *Geochemistry, Geophysics, Geosystems: An Electronic Journal of the Earth Sciences* **10**, 1–15.

McCully Phillips SR and Ellis JR (2015) Reproductive characteristics and life-history relationships of starry smooth-hound *Mustelus asterias* in British waters. *Journal of Fish Biology* **87**, 1411–1433.

Oddone MA, Paesch OL and Norbis W (2005) Reproductive biology and seasonal distribution of *Mustelus schmitti* (Elasmobranchii: Triakidae) in the Rio de la Plata oceanic front, south-western Atlantic. *Journal of the Marine Biological Association of the United Kingdom* **85**, 1193–1198.

Orlando L, Pereyra I, Silveira S, Paesch L, Oddone MC and Norbis W (2015) Determination of limited histotrophy as the reproductive mode in *Mustelus schmitti* Springer, 1939 (Chondrichthyes: Triakidae): analysis of intrauterine growth of embryos. *Neotropical Ichthyology* **13**, 699–706.

Perez-Jimenez JC and Sosa-Nishizaki O (2008) Reproductive biology of the brown smoothhound shark *Mustelus henlei*, in the northern Gulf of California, Mexico. *Journal of Fish Biology* **73**, 782–792.

Portnoy DS, Puritz JB, Hollenbeck CM, Gelsleichter J, Chapman D and Gold JR (2015) Selection and sex-biased dispersal in a coastal shark: the influence of philopatry on adaptive variation. *Molecular Ecology* **24**, 5877–5885.

R Core Team (2018) *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available at https://www.R-project.org/

Rueda-Roa D and Muller-Karger FE (2013) The southern Caribbean upwelling system: seasurface temperature, wind forcing and chlorophyll concentration patterns. *Deep Sea Research Part I: Oceanographic Research Papers* **78**, 102–114.

Rueda-Roa D, Mendoza J, Muller-Karger F, Cárdenas JJ, Achury A and Astor Y (2017) Spatial variability of Spanish sardine (*Sardinella aurita*) abundance as related to the upwelling cycle off the southeastern Caribbean Sea. *PLoS ONE* **12**, e0179984.

Saidi B, Bradai MN and Bouain A (2008) Reproductive biology of the smooth-hound shark *Mustelus mustelus* (L.) in the Gulf of Gabes (south-central Mediterranean Sea). *Journal of Fish Biology* **72**, 1343–1354.

Stevens JD, Bonfil R, Dulvy NK and Walker PA (2000) The effects of fishing on sharks, rays, and chimaeras (Chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science* **57**, 476–494.

Storrie MT, Walker TI, Laurenson LJ and Hamlett WC (2009) Gestational morphogenesis of the uterine epithelium of the gummy shark (*Mustelus antarcticus*). *Journal of Morphology* **270**, 319–336.

Tagliafico A, Hernández-Ávila I, Rangel S and Rago N (2015) Size of catch, reproduction and feeding of the small-eye smooth-hound, *Mustelus higmani* (Carcharhiniformes: Triakidae), in Margarita Island, Venezuela. *Scientia Marina* **79**, 443–452.

Tavares R (2015) Condrictios. In Rodriguez JP, García-Rawlins A and Rojas-Suarez F (eds), *Libro Rojo de la Fauna Venezolana*. Caracas: Provita & Fundacion Empresas Polar, pp. 270–282.

Tavares R (2019) Current status of sharks and rays (Pisces: Elasmobranchii) in the Venezuelan Caribbean Sea: biodiversity, fishery and conservation. *Ciencia Pesquera* **27**, 33–52.

Tavares R and Arocha F (2008) Species diversity, relative abundance and length structure of oceanic sharks caught by the Venezuelan longline fishery in the Caribbean Sea and western-Central Atlantic. *Zootecnia Tropical* **26**, 489–503.

Tavares R, Sanchez L and Medina E (2010) Artisanal fishery and catch structure of the smalleye smooth-hound shark, *Mustelus higmani* (Springer and Low 1963), from the northeastern region of Venezuela. *Gulf Caribbean Fisheries Institute* **62**, 446–449.

Teshima K (1981) Studies on the reproduction of Japanese smooth dogfishes, *Mustelus manazo* and *M. griseus*. *Journal of the Shimonoseki University of Fisheries* **29**, 113–199.

TeWinkel LE (1950) Notes on ovulation, ova and early development in the smooth dogfish, *Mustelus canis*. *Biological Bulletin* **99**, 474–486.

TeWinkel LE (1963*a*) Notes on the smooth dogfish, *Mustelus canis*, during the first three months of gestation. I. Components of the egg, early embryos, and yolk-sacs. *Journal of Experimental Zoology* **152**, 115–122.

TeWinkel LE (1963*b*) Notes on the smooth dogfish, *Mustelus canis*, during the first three months of gestation. II. Structural modifications of yolk-sacs and yolk-stalks correlated with increasing absorptive function. *Journal of Experimental Zoology* **152**, 123–137.

Walker TI (2007) Spatial and temporal variation in the reproductive biology of gummy shark *Mustelus antarcticus* (Chondrichthyes: Triakidae) harvested off southern Australia. *Marine and Freshwater Research* **58**, 67–97.

Wearmouth VJ and Sims DW (2008) Sexual segregation of marine fish, reptiles, birds and mammals: behaviour patterns, mechanisms and conservation implications. *Advances in Marine Biology* **54**, 107–170.

Yamaguchi A, Taniuchi T and Shimizu M (2000) Geographic variations in reproductive parameters of the starspotted dogfish, *Mustelus manazo*, from five localities in Japan and in Taiwan. *Environmental Biology of Fishes* **57**, 221–233.

Zagaglia CR, Damiano C, Hazin FHV and Broadhurst MK (2011) Reproduction in *Mustelus canis* (Chondrichthyes: Triakidae) from an unexploited population off northern Brazil. *Journal of Applied Ichthyology* **27**, 25–29.