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**Short Communication**

# Aspects of reproductive biology of the humpback smooth-hound shark (*Mustelus whitneyi*) off northern Peru

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**Abstract.** The humpback smooth-hound shark (*Mustelus whitneyi*) is one of the most captured shark species in the south-east Pacific and is classified as vulnerable, yet its reproductive biology has been poorly studied. The aim of this study was to increase our knowledge of the reproductive biology of the humpback smooth-hound shark. In all, 41 pregnant females, 386 macroscopically visible embryos *in utero* and 16 neonates were sampled off northern Peru. Pregnant females measured between 73- and 118-cm total length (TL) and the number of embryos per litter ranged from 6 to 18, with a mean of 10. Size at birth ranged between 21 and 22 cm TL. Litter size increased with the TL of the mother, yet this relationship was not strong ($r^2 = 0.36$). The gestation period had a minimum duration of 7 months and a synchronous cycle in which birth occurred in September. The humpback smooth-hound shark is a placental viviparous elasmobranch. This study represents the most comprehensive research of the reproductive biology of the humpback smooth-hound shark, and is the first time the embryonic development is described for this species. These findings could contribute to the design and implementation of local management plans for this species.

**Additional keywords:** embryonic development, litter size, pregnancy, south-east Pacific.

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## Introduction

Understanding the reproductive strategy of species is one of the most important factors in stock assessments (Walker 2005). Reproductive parameters (e.g. litter size, sex ratio at birth, size at maturity) are used to establish capture limits and minimum catch sizes that are fundamental components of management plans. The humpback smooth-hound shark is endemic to the Humboldt Upwelling System (off Peru and Chile), one of the most productive marine ecosystems in the world (Pennington *et al.* 2006). Within this system, this species is one of the most frequently captured shark species, yet its reproductive biology has been poorly studied, preventing sound fishery management. In Peru, this triakid species is identified as the fourth most captured shark species and the most captured coastal demersal species by Peruvian small-scale fisheries, and Peru has the highest accumulated historical shark landings in the eastern Pacific Ocean (González-Pestana *et al.* 2014). In addition, this species is incidentally captured and discarded at sea by the Peruvian hake trawl fishery (Céspedes 2013). Although this species is distributed from northern Peru to central Chile, it is rarely caught in artisanal and commercial fisheries in Chile (Romero 2007; Bustamante *et al.* 2014). Therefore, throughout its distribution range, Peruvian fisheries may be its biggest anthropogenic threat, and management efforts should be focused in Peruvian waters. This shark species is classified as vulnerable in the Red List of Threatened Species of the International Union for the Conservation of Nature (IUCN; Romero 2007).

Information about the reproductive biology of the humpback smooth-hound is very limited. Compagno (1984) established that the smallest adult female recorded measured 74-cm total length (TL), and the smallest adult male measured 68 cm TL. In addition, the size at birth was estimated at 25 cm TL and the litter size ranged from 5 to 10 embryos (Compagno 1984). Yet, that report specified neither the sample size nor the sample location. Based on four specimens from northern Peru, Chirichigno (1973) reported that sizes larger that 50 cm TL had gonads in a maturing state, and one pregnant female of 86.7 cm TL had 12 embryos *in utero*. To the best of our knowledge, this represents the only information published of the reproductive biology of the humpback smooth-hound shark. The aim of this study was to increase our knowledge of the reproductive biology of the humpback smooth-hound shark off northern Peru by determining size at birth and maternity, gestation period, litter size and sex ratios of embryos *in utero*. In addition, embryo development is described through observations of embryo morphology. These findings could contribute to the design and implementation of local management plans for this species.

## Materials and methods

Samples were collected from the small-scale gill net fishery between March and July 2013, as well as between May and September 2016, at two landing points in northern Peru: Cancas, Tumbes (3°56′43.4″S, 80°56′22.6″W) and San Jose, Lambayeque (6°46′07.0″S, 79°58′19.0″W). Specimens were measured (TL) and sexed. Their reproductive tracts (i.e. oviduct, oviductal gland and uterus) were removed and preserved in 10% formalin solution. Females were classed as pregnant if they met either one of two criteria: (1) pregnant with visible embryos; or (2) pregnant with eggs *in utero* (Walker 2005). All specimens collected were pregnant females, because these were the only specimens available at the collection sites. Therefore, only the range and mean size of pregnant females was determined. Embryos were counted, sexed and measured. The null hypothesis of no difference in the number of embryos between the right and left uteri in females was tested with a paired *t*-test. The total number of embryos per uterus was calculated to determine litter size. The sex ratio of embryos *in utero* was calculated against the null expectation of a 1 : 1 ratio using a paired *t*-test (Walker 2005). Birth size was estimated by comparing the largest embryo with the smallest free-living neonate (Walker 2005). The period of gestation was estimated by plotting the body size (TL) of embryos observed in pregnant females against month, and then evaluating the seasonal pattern (Walker 2005). To determine whether significant differences existed in body sizes of embryos by month, one-way analysis of variance (ANOVA) was used, followed by Tukey's honest significant difference (HSD) *post hoc* test.

A linear regression analysis was performed between the size of the mother and the number of macroscopically visible embryos *in utero* (i.e. litter size). Assumptions of the model (i.e. homogeneity, normality, fixed *X*, independence and no residual patterns) were assessed through model validation graphs (e.g. quantile–quantile plots (Q–Q), Cook's distant plot) and the Shapiro–Wilk test.

Embryos were classified ontogenetically based on morphological characteristics, following criteria proposed by Hamlett *et al.* (2005). Musick and Ellis (2005) list eight modes of reproduction that are categorised as oviparity and viviparity. Triakids exhibit two modes of reproduction: yolk sac viviparity or placental viviparity (Musick and Ellis 2005).

Statistical analyses were performed using R (ver. 3.2.2, R Foundation for Statistical Computing, Vienna, Austria, see https://www.R-project.org/).

## Results and discussion

In all, 41 pregnant females, 386 macroscopically visible embryos *in utero* and 16 neonates were sampled. All adult specimens sampled were pregnant females that measured between 73 and 118 cm TL, with a mean (±s.e.m.) size of 88.1 ± 17.8 cm TL. The size of the smallest pregnant female found in this study is similar to the smallest adult female observed by Compagno (1984), which was 74 cm TL. Therefore, Compagno (1984) reported maturity sizes that are higher than the pregnancy sizes reported in this study. This suggests that the smooth-hound attains sexual maturity at sizes smaller than 74 cm TL, as reported by Chirichigno (1973). For all females sampled, both the left and right ovary and uterus were functional, and equal numbers of embryos were observed in the two uteri (paired *t*-test, d.f. = 40, $P > 0.05$). The number of embryos per litter ranged from 6 to 18 with a mean (±s.e.m.) of 10 ± 4. The maximum litter size found in this study is within the range of values found for other *Mustelus* species, which range from 6 for *M. dorsalis* (Rojas 2014) to 57 for *M. antarcticus* (Walker 2007). However, in this study the litter size may be an underestimate because the samples were obtained from gill net fisheries, in which capture-induced parturition (either premature birth or abortion) may have occurred (Adams *et al.* 2018). For *Mustelus* species, one study determined that of 15 pregnant *M. canis* females, two exhibited capture-induced parturitions, yet the fishing method was longline (Zagaglia *et al.* 2011).

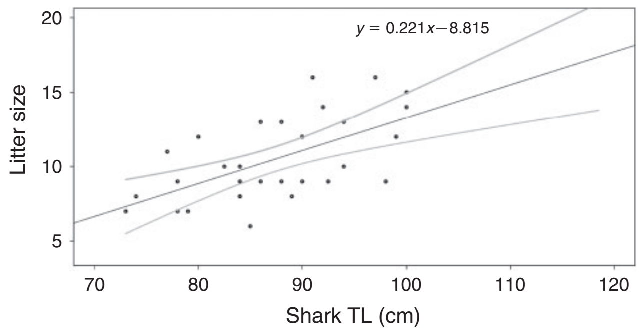


**Fig. 1.** Relationship between maternal body size and litter size (number of embryos per mother; $r^2 = 0.36$) of the humpback smooth-hound shark (*Mustelus whitneyi*). Grey lines represent 95% confidence intervals. TL, total length.

The mean (±s.d.) number of male embryos did not differ significantly different from that of female embryos (5.5 ± 2.2 *v.* 5.2 ± 2.0 respectively). Thus, the sex ratio was not different from 1 : 1 ($t = -0.567$, d.f. = 25, $P = 0.57$). Size at birth ranged from 22 to 23 cm TL, because the largest embryo measured 23 cm TL and the smallest free-living neonate, with open umbilical scars, measured 22.4 cm TL. Free-living neonates were collected in September. The size at birth found in this study was smaller than that reported by Compagno (1984) of 25 cm TL. As discussed above, the small free-living neonates may be aborted embryos from premature births, because the mothers may have experienced capture-induced parturitions (Adams *et al.* 2018).

Model validation graphs and the Shapiro–Wilk test ($P = 0.203$) determined that the data met the assumptions for creating a linear regression model. Yet, Cook's distant plot identified four outliers that were removed from further analysis. The linear regression model showed that litter size increases as the TL of the mother increases, yet this relationship was not strong. The TL of the mother explained 36% of the variation in litter size ($F_{1,29} = 16.257$, $P < 0.001$, $r^2 = 0.36$; Fig. 1). Many studies have shown that for species of the genus *Mustelus*, litter size increases with the TL of the mother (Francis and Mace 1980; Menni *et al.* 1986; Yamaguchi *et al.* 2000; Conrath and Musick

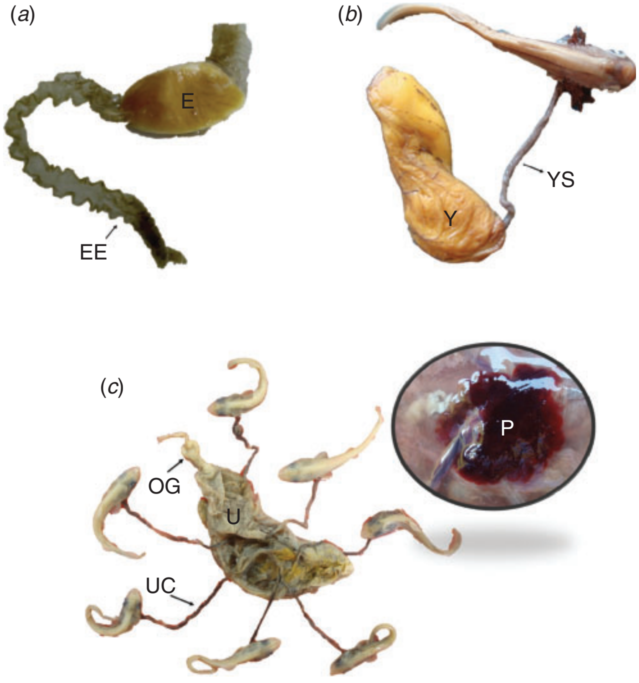


**Fig. 2.** Selected stages of embryo development of the humpback smooth-hound shark (*Mustelus whitneyi*). (*a*) Stage of the eggs *in utero*; (*b*) early lecithotrophic stage of placental yolk sac viviparity; (*c*) late matrotrophic stage of placental yolk sac viviparity. E, eggs; EE, egg envelope; Y, yolk; YS, yolk stalk; U, uterus; UC, umbilical cord; OG, oviductal gland; P, placenta.

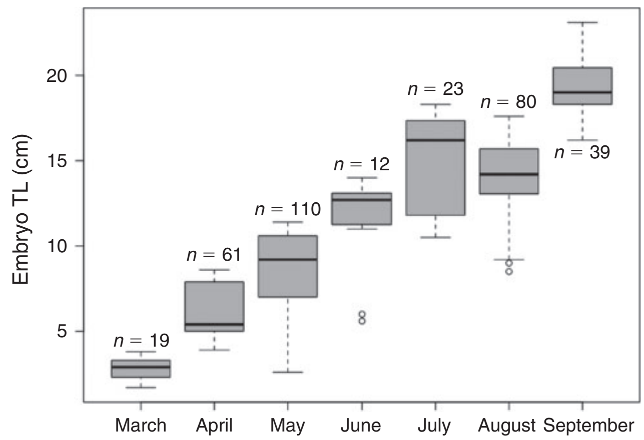


**Fig. 3.** Embryo length of the humpback smooth-hound shark (*Mustelus whitneyi*) plotted against month, with sample size per month (*n*) indicated. The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range. Outliers are indicated by circles.

2002; Pérez-Jiménez and Sosa-Nishizaki 2008; Saïdi *et al.* 2008). Differences in the maximum litter size between the present study ($n = 18$) and that of Compagno (1984; $n = 10$) and Chirichigno (1973; $n = 12$) may be explained by the increase in litter size as the mother's TL increases, because, in the present study, the largest female measured 118 cm TL, whereas in the other studies the largest females measured 87 cm TL (Compagno 1984) and 86.7 cm TL (Chirichigno 1973).

Embryos *in utero* were observed during the 7-month period March–September, and eggs *in utero* (Fig. 2*a*) were observed during March in three pregnant females. The smallest embryos were recorded in March and the largest were recorded in September (near-term embryos; Fig. 3). Comparisons of the data among months revealed significant differences in the TL of embryos (ANOVA, $F = 174.32$, $P < 0.001$). Of the 21 pair-wise comparisons between months, 20 were significantly different (Tukey's HSD test, $P > 0.001$); only in the months of July and August was there no significant difference in the TL of embryos. Thus, the minimum gestation period may have a duration of 7 months and a synchronous cycle. Birth likely occurred in September, because, in this month, we found the largest size range of embryos that were close to the size at birth (Fig. 3). Most species of the genus *Mustelus* have a gestation period between 10 and 11 months (Francis and Mace 1980; Yamaguchi *et al.* 1997; Cousseau *et al.* 1998; Conrath and Musick 2002; Pérez-Jiménez and Sosa-Nishizaki 2008; Saïdi *et al.* 2008). In the present study, a minimum of 7 months was estimated as the gestation period. Further research should sample months before March in order to estimate the total length of the gestation period. The period in which the eggs are developing may occur before March because, of the 19 females recorded in March, only three had eggs.

*M. whitneyi* exhibits a placental viviparity, which is one of two modes of reproduction found among triakids (Musick and Ellis 2005). Embryos were found to be at an early embryonic developmental stage, with a yolk sac viviparity nutrition mode (Fig. 2*b*), in March, at an intermediate stage in April and May and at a final embryonic developmental stage with a matrotrophy placental nutrition mode between May and September (Fig. 2*c*). This suggests that embryo growth is rapid during the first months (March–May).

We recommend that future studies include a wider range of body sizes to determine the size at maturity. In addition, the ovarian and reproductive cycles and a more precise gestation period need to be determined; therefore, samples should be collected throughout the year. Finally, these reproductive parameters should be determined for other areas, such as central and southern Peru, because reproductive characteristics can vary between populations of the same *Mustelus* species (Yamaguchi *et al.* 2000; Walker 2007). The only management measure for this species in Peru is a legal minimum size of 60 cm TL. Therefore, all the specimens were above this size. However, all specimens sampled were pregnant females, suggesting that small-scale gill net fisheries primarily capture this life history stage, which could be detrimental to the population. Therefore, we recommend that future studies assess the degree of life stage-specific vulnerability to overfishing of this commercial shark species and, if needed, implement additional measures (e.g. spatial bans, maximum size of capture). This study represents the most comprehensive research of the reproductive biology of the humpback smooth-hound shark, a highly commercialised species with limited biological studies, and is also the first time that embryo development has been described for this species.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

## Declaration of funding

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