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Edson da Silva
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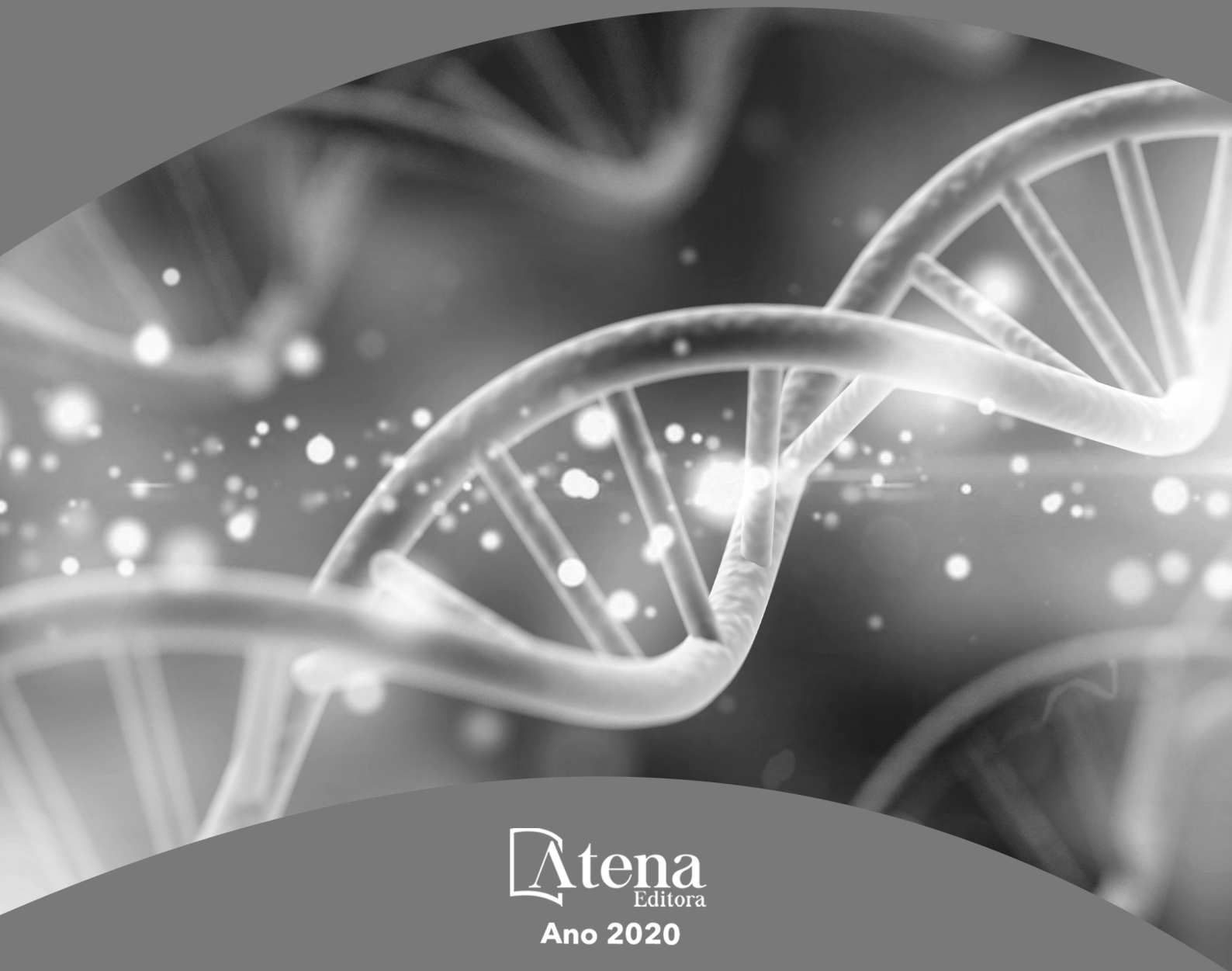

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APRESENTAÇÃO

A coleção “Tópicos Multidisciplinares em Ciências Biológicas” é uma obra composta por estudos de diferentes áreas das ciências biológicas e da saúde. A obra foi ampliada e recebeu mais 47 capítulos distribuídos em três volumes. Os e-books foram organizados por trabalhos resultantes de pesquisas, ensaios teóricos e vivências dos autores.

As ciências biológicas englobam áreas do conhecimento relacionadas às ciências da vida e incluem a biologia, a saúde humana e a saúde animal. Nesta obra, apresento textos completos e atuais sobre estudos desenvolvidos durante a formação acadêmica ou na prática profissional. Os autores são filiados a diversos cursos de graduação e de pós-graduação em ciências biológicas, saúde, tecnologia e áreas afins.

Em seus 15 capítulos o volume 2 aborda, de forma categorizada, os trabalhos de pesquisas, revisões narrativas e ensaios teóricos que transitam nos vários caminhos da atuação em ciências biológicas e áreas correlatas. Neste volume você encontra textos sobre biologia celular e molecular, aquicultura e pesca, anatomia, fisiologia, microbiologia, fitoterapia e muito mais.

Espero que as experiências compartilhadas neste volume contribuam para o enriquecimento de novas práticas profissionais com olhares multidisciplinares para as ciências biológicas e suas áreas afins. Agradeço aos autores que tornaram essa edição possível e desejo uma ótima leitura a todos.

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ABSTRACT: Despite their important evolutionary history and trophic role as a top predator, little is known about the reproductive biology of deepwater sharks. Due to the lack of studies and available information on the basic biological aspects of sharpnose sevengill shark *Heptranchias perlo*, the aim of the present study was to provide key biological information for the species in western Southern Atlantic, with emphasis on its reproductive aspects, including size-at-maturity, reproductive seasonality, size-at-birth, fecundity and morphological descriptions. The sharpnose sevengill sharks were obtained during 10 scientific expeditions from April 1994 to May 1995, and during the monitoring of 18 surface longline fishing targeting tuna from August 1997 to February 1999. Our results support evidence for a biennial reproductive cycle for females, with gestation period of 9 to 12 months and 1-year resting. The size-at-birth was recorded between 26 to 27 cm total length, with parturition occurring in spring and summer. Annual gonadosomatic index values showed a seasonal variation in both sex, with higher values during autumn and low during summer in females, and higher values during autumn, spring and summer, and low during winter in males. Life-history parameters suggested that sharpnose sevengill shark have a relatively low reproductive output. The basic biological data presented here is essential for fishing management and conservation plans for this deep-water shark, since the growing increase of deep-water fisheries may cause pressure in deep-sea species populations.

KEYWORDS: Elasmobranchs, maturity, viviparity, Hexanchidae, reproductive cycle.

INFORMAÇÕES SOBRE A BIOLOGIA REPRODUTIVA DO TUBARÃO-SETE-GUELRAS (*Heptranchias perlo*) NO ATLÂNTICO SUL OCIDENTAL

RESUMO: Apesar da sua importante história evolutiva e papel trófico como predador de topo, pouco se sabe sobre a biologia reprodutiva de tubarões de profundidade. Devido à falta de estudos e informações disponíveis sobre os aspectos básicos do tubarão-sete-guelras *Heptranchias perlo*, o objetivo do presente estudo foi fornecer informações biológicas chave para a espécie no Atlântico Sul ocidental, com ênfase nos seus aspectos reprodutivos, incluindo tamanho de maturidade, sazonalidade reprodutiva, tamanho ao nascimento, fecundidade e descrições morfológicas. Os tubarões-sete-guelras foram obtidos durante 10 expedições científicas entre Abril de 1994 e Maio de 1995, e durante o monitoramento de 18 espinheis pelágicos destinados à captura de atum, entre Agosto de 1997 e Fevereiro de 1999. Nossos resultados suportam a evidência para um ciclo reprodutivo bienal para fêmeas, com período de gestação de 9 a 12 meses e 1 ano de repouso. O tamanho ao nascimento foi registrado entre 26 a 27 cm comprimento total, com o parto ocorrendo no verão. Valores anuais do índice gonadossomático mostram uma variação sazonal para ambos os sexos, com maiores valores durante o outono e baixos no verão em fêmeas, e picos durante o outono, primavera e verão em machos. Parâmetros da história de vida sugerem que o tubarão-sete-guelras tem uma saída reprodutiva relativamente baixa. Os aspectos biológicos básicos apresentados aqui são essenciais para o manejo da pesca e planos de conservação para tubarões de profundidade, uma vez que o crescente aumento da pesca pode causar uma pressão pesqueira nas populações de mar profundo.

PALAVRAS-CHAVE: Elasmobranchii, maturidade, viviparidade, hexanchidae, ciclo reprodutivo.

1 I INTRODUCTION

Currently, overexploitation is the major challenge for shark conservation (Clarke et al. 2006). Caught both as targeted and bycatch, sharks are now facing high risk of extinction (Dent and Clarke 2015). Despite the growing capture effort and the improvement of fishing technology, the annual global catch (without taking into account unregulated and unreported catches) is about 500,000 tons, indicating a strong decline in populations worldwide (Dent and Clarke 2015). Data on specific deep-water sharks and their fisheries are scarce, making it difficult to assess the real status of populations worldwide (Gordon and Shotton 1999).

As one of the oldest and least studied orders among sharks, the Hexanchiformes has an estimated evolutionary divergence of 82 million years, composing a monophyletic group currently represented by two families (Chlamydoselachidae and Hexanchidae), four genera and six species (De Carvalho 1996, Tanaka et al. 2013). With a global distribution, these sharks can be found from coastal areas to depths of 2.500 m, being considered one

of the most primitive groups among modern sharks, easily identifiable by the presence of six to seven pairs of gill openings, one dorsal fin and one anal fin (Compagno 1984, Ebert, Stehmann 2013). Despite its important evolutionary history and trophic role as a top predator, little is known about the reproductive biology of these elusive shark species (Barnett et al. 2012).

The sharpnose sevengill shark, *Heptranchias perlo* (Bonnaterre 1788), has a wide geographic distribution, being found in tropical and temperate regions (Compagno 1984, Last and Stevens 2009, Ebert and Stehmann 2013). The sharpnose sevengill shark is a benthic/epibenthic species, found mainly in deep-waters, continental regions, islands and high slopes; at depths ranging from 27 to 720 m (Compagno 1984, Ebert and Stehmann 2013). The species exhibit a lecithotrophic viviparous reproductive mode, considered the plesiomorphic reproductive strategy among elasmobranchs (Musick and Ellis 2005), where the development of the embryo depends solely on the nutrition by the yolk, produced by the maternal liver and stored in the yolk sac (Wourms 1981, Hamlett et al. 2005). As a specie with low intrinsic rate of growth, therefore a low resistance to fishing exploitation, sharpnose sevengill shark is assessed as “Near Threatened” by the World Conservation Union’s Red List of Threatened Species Conservation of Nature – IUCN (Paul and Fowler 2003).

Due to the lack of studies and available information on the basic biological aspects of sharpnose sevengill shark (reviewed by Barnett et al. 2012) and considering their conservation status, the aim of the present study was to provide biological information for the species from Southern Atlantic, compiled between 1994 and 1999, with emphasis on its seasonal distribution and reproduction. Since sharpnose sevengill shark is an important apex predator of deep environments, the reduction in its abundance can lead to an ecosystem disruption as already observed in shallow-waters. Therefore, the proper assessment of basic biological and ecological data for the species is imperative for future management plans in front of continuous increase in deep-water fisheries.

2 | MATERIALS AND METHODS

The sharpnose sevengill sharks analyzed were captured along the continental shelf (19º-28ºS e 38º-47ºW) from the Espírito Santo state to Santa Catarina state, Brazil (Figure 1). The sharks were obtained during 10 scientific expeditions (Orion-NPq Cruise), totaling 340 sets of bottom longline fishing, from April 1994 to May 1995. The animals were also obtained during the monitoring of 18 surface longline fishing targeting tuna in Santos and Guarujá, São Paulo state, from August 1997 to February 1999. The specimens were preserved in ice in the boats and donated to the authors. The animals were then dissected for the assessment of the reproductive stages, measurement and quantification of oocytes, gonads and embryos and hepatic function.

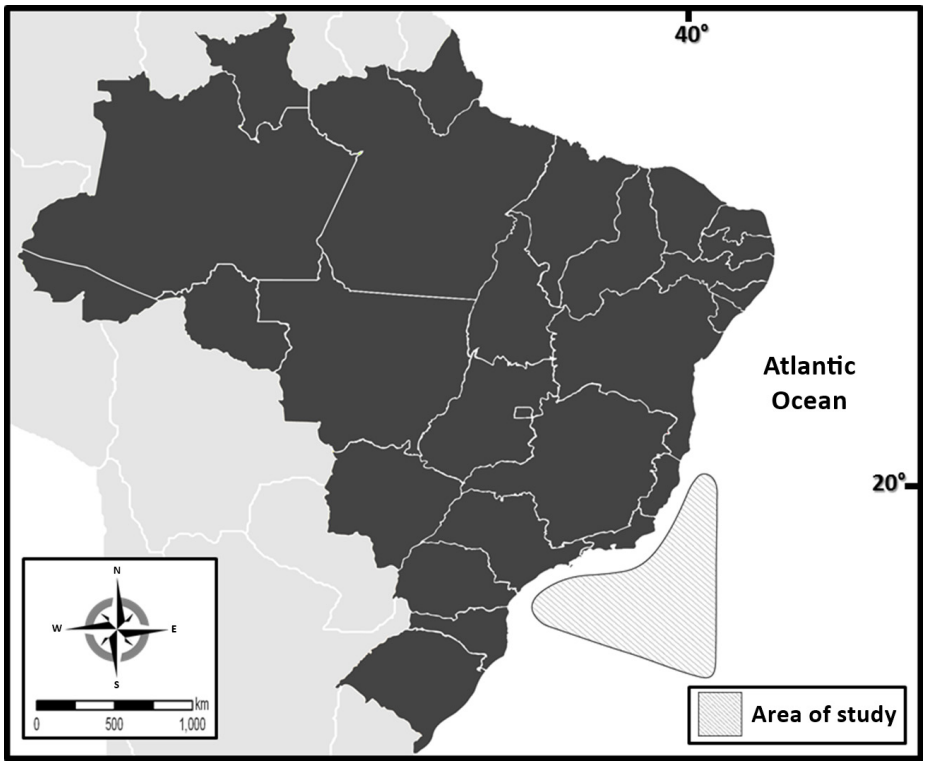

Figure 1 – Area of capture of sharpnose sevengill shark, *Heptranchias perlo* in southern Brazil.

For all specimens dissected, total length (TL) was recorded of males, females and embryos. Total mass and gonad mass was recorded for males and females, juveniles to adults. For analysis of the reproductive aspects of the species, the average TL of first gonadal maturation (L_{50}) was determined according to Vazzoler (1996). Gonadosomatic index (GSI) was calculated as the gonad mass divided by total mass. Were described the morphological characteristics of the reproductive tract of males and females and external characteristics of the embryos. In the present study, it was not possible to perform the statistical analysis of the complete data, since the results were taken from a dissertation (Carvalho 2002), where full data were not more available. However, due to the rarity of the data, we described the results that we considered most important.

3 I RESULTS

The depth of catch for the bottom longline fishing varied from 260 to 500 m, indicating an average depth of 326.7 m. The depth of catch for the surface longline varied between 20 and 40m. Total of 318 sharks was collected between 1994 and 1999, being 194 females (61%) and 124 (39%) males (Figure 2). Total length ranged from 62.9 cm to 114.5 cm, and the total weight ranged from 0.51 kg to 5.4 kg. In 1994, 1995 and 1997, 119 specimens were collected (66 males and 53 females). There was a predominance of males in March, August, September, October and November. The predominance of females occurred in February, March and December. In May, the number of males and females was equal. In

1998 and 1999, 198 specimens were captured (61 males and 137 females). Females were more frequent in all months, except for November where the number of males was equal (Figure 2). The total number of individuals caught was 318, 194 females (61%) and 124 (39%) males. The TL ranged from 62.9 cm to 114.5 cm, and the total mass ranged from 0.51 kg to 5.4 kg. The sexual ratio for the entire study period was 0.64:1 for adults and 1:0.9 for embryos (n=101 from eight pregnant females). The amplitude was 62.9 to 114.5 cm TL and 0.51 to 5.4 kg total mass.

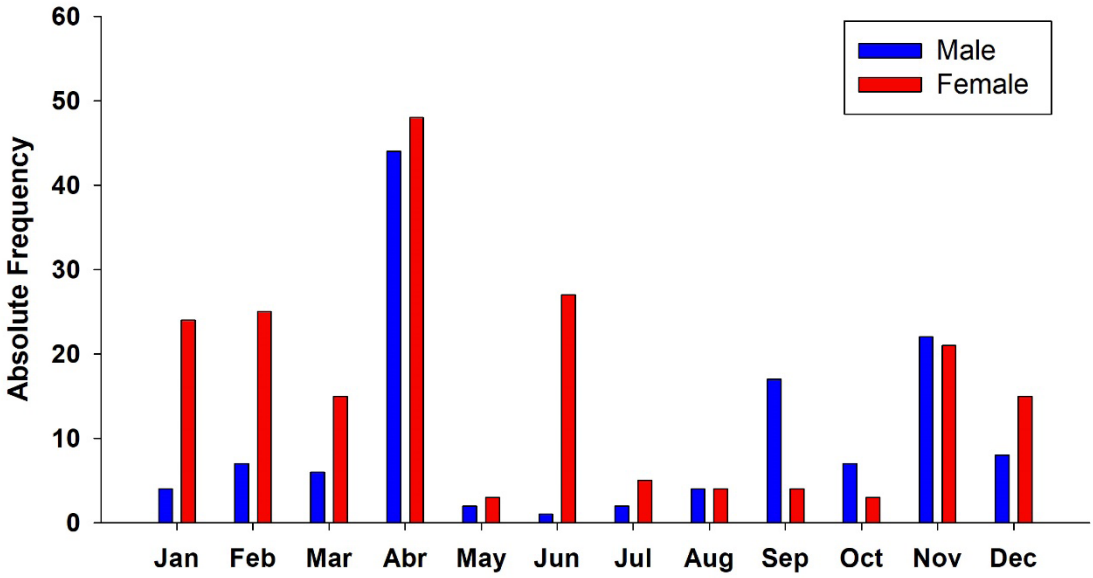

Figure 2 – Absolute frequency of sharpnose sevengill shark, *Heptranchias perlo* captured in the southeast and south of Brazil, from May 1995 to August 1997.

In females, the value of mean GSI per class of TL began to rise in the 85.1-90.0 cm class range. This pattern of GSI elevation was observed up to the 105.1-110.0 cm class range. The class that included the highest (110.1-115.0 cm TL) presented a decrease in mean GSI, possibly related to emptying of the uterus, characterizing the end of the reproductive cycle. The GSI was higher in May (autumn), intermediate between June and September (winter) and low from November to January (spring and summer, Figure 3a). In males, there was an increase in the mean GSI value per class of TL, from the first class (65.0-70.0 cm) to the last one (101.1-105.0 cm). GSI was higher from January to April (summer to autumn). From July to September (winter) there was a decrease followed by an increase to intermediate levels from November to December (spring to summer; Figure 3b).

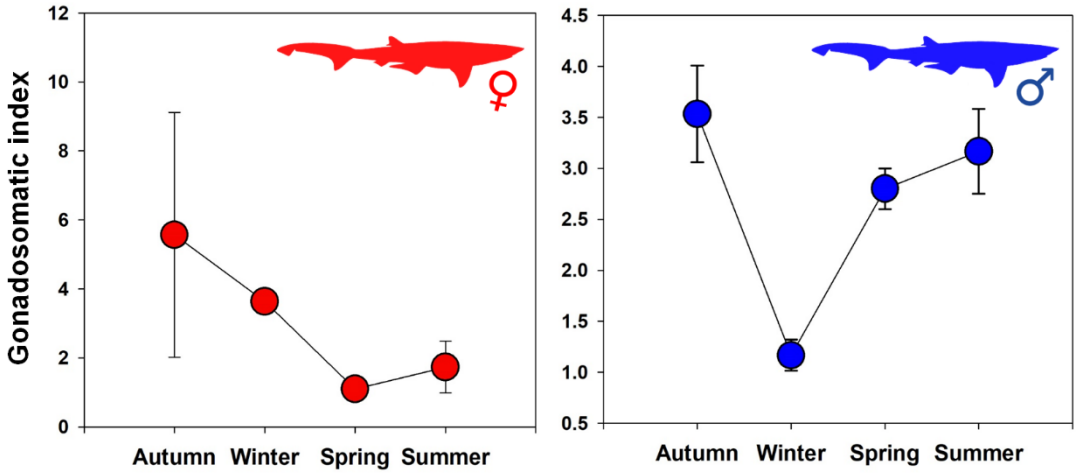

Figure 3- Average of gonadosomatic index (GSI) per season, in **(A)** females and **(B)** males of sharpnose sevengill shark, *Heptranchias perlo.*

The sharpnose sevengill shark exhibited the lecithotrophic viviparity as reproductive mode, where the embryo depends exclusively on the yolk stores. The female reproductive tract was composed of pairs of ovaries, oviducal glands, oviducts, and uterus (Figure 4). Only one ovary develops in each breeding cycle. The reproductive development was divided into five stages of sexual maturity (I – VI; Table I). The pregnancy lasts around 9-12 months, with a probable one-year interval between consecutive pregnancies (biennial reproductive cycle). Of the 141 females sampled, 121 (85.8%) were adult and 20 (14.2%) were immature. The average of first gonadal maturation (L_{50}) was 87.5 cm TL. The TL at which individuals were sexually mature (L_{100}) was 92.5 cm, and 80% of the females (n = 113) had a *TL* greater than that calculated for L_{100}. The frequency of immature females was greater in April, June, September and December.

The vitellogenesis females was more pronounced in April, March, September and December. Females with egg capsules were rare with a peak of occurrence in September. Embryos were observed mainly in September but were also observed less frequently in February, March and June. Finally, females with empty uterus were more frequent in December.

Stage	Description
Juvenile (I)	The ovaries, oviducal glands, and uteri are barely conspicuous. Each ovary with a mean weight of 1 g and a maximum of 2 g; the largest oocytes in the ovaries measuring up to 5 mm in diameter (Figure 4a).
Initial vitellogenesis (II)	Larger oocytes with a mean diameter of 0.86 cm and a maximum of 1 cm; Ovary weighing on average 10g (n = 14) (Figure 4b).
Intermediate Vitellogenesis (III)	When the oocytes reach a maximum diameter of 3.5 cm. The largest had an average diameter of 2.2 cm and the ovary weighs on average 55.8 g (n = 17) (Figure 4c).
Final Vitellogenesis (IV)	The largest oocytes had a diameter of more than 3.5 cm and a mean value of 3.75 cm (n = 7). The ovaries occupy most of the internal volume of the female's abdominal cavity (Figure 4d).
Initial gestation (V)	Characterized by the presence of egg capsules in the uterus. The oocytes show the same development of initial vitellogenesis, stage II (Figure 4e).
Post partum (empty uterus) (VI)	The uterus were distended and emptied. The ovary weighs on average 5.6 g and the mean diameter of the largest oocytes is 0.73 cm. The characteristics were very close to those of stage II, indicating that after parturition the females possibly enter a resting period before new vitellogenesis (Figure 4f).

Table I - Reproductive stages of females sharpnose sevengill shark, *Heptranchias perlo* caught in the Southern Brazil, from April 1994 to February 1999.

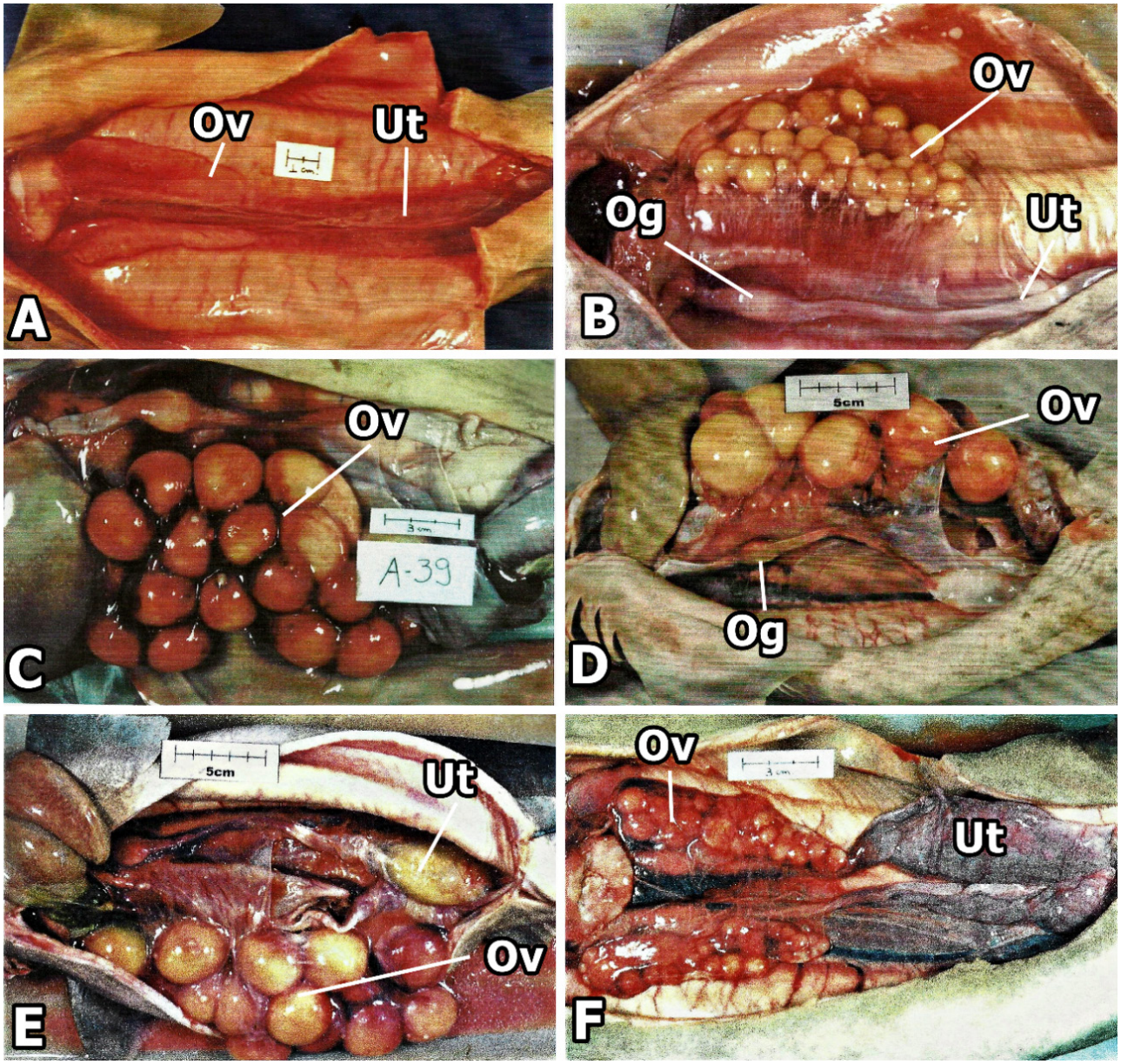

Figure 4 – Stages of development of female reproductive tract of sharpnose sevengill shark, *Heptranchias perlo*. **(A)** Stage I, immature female. **(B)** Stage II, initial vitellogenesis. **(C)** Stage III, intermediate vitellogenesis. **(D)** Stage IV, final vitellogenesis. **(E)** Stage V, presence of egg capsules in the uterus with oocytes in stage II of development. **(F)** Stage VI, emptied uterus. Ov: ovary; Og: Oviducal gland; Ut: uterus.

The male reproductive tract was composed by a par of testicles, epididymis, ductus deferens, spermatic sacs, seminal vesicle, and a pair of clasper (Figure 5). Two categories of reproductive stages were established (Table II).

Stage	Description
Immature (I)	The animals are immature, presenting testicles, vas deferens, epididymis and sperm sacs in development. Of the individuals analyzed, only 4.1% were at this stage (Figure 5a).
Mature (II)	Large amount of sperm in the spermatic bags and clasper. The testicles, ductus deferens, epididymis and spermatic sacs are well defined. Most of the collected specimens (95.3%) were considered mature. The mean weight of the testicles was 27.2 g, ranging from 2.0 to 55.0 g and the mean length was 12.9 cm, ranging from 7.5 to 19.9 cm. The average length of the claspers was 7.0 cm ranging from 5.1 to 8.1 cm. The mean body length was 84.8 cm, ranging from 69.0 to 100.7 (Figure 5b).

Table II – Stages of maturation of males sharpnose sevengill shark, *Heptranchias perlo* captured in Southern Brazil, from April 1994 to February 1999.

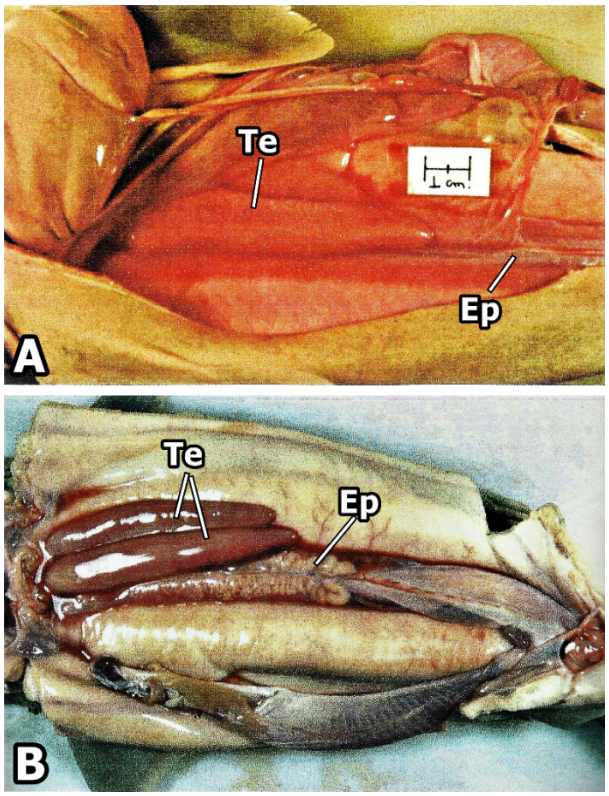

Figure 5 – Development of the male reproductive tract of sharpnose sevengill shark, *Heptranchias perlo*. **(A)** Stage I, immature males. **(B)** Stage II, mature males, with developed organs. Te: testicles; Ep: epididymis.

Embryonic development was divided into seven stages based on TL ranging from 5.75 to 23.9 cm (Figure 6). Anatomical characteristics of each stage was described in Table III.

Embryonic Stage	Description
I – 5.75 cm TL	At this stage of development the embryo exhibit light yellow pigmentation and a small light brown spot on the tip of the caudal fin. The fins are translucent. The eyes are rounded and projected. Sexual dimorphism is already evident; in males the developing claspers are observed (Figure 5a).
II – 6.8 cm TL	It exhibit practically the same anatomical shape of the stage I with a change in coloration, where the tip of the caudal and dorsal fin presents light brown pigmentation. Dorsal canals of the lateral line are visible. Small flexible projections are visible on the surface of the upper jaw epithelium (Figure 5b).
III – 7.9 cm TL	Light brown pigmentation is visible at the dorsal portion. At the tip of the dorsal and caudal fins the spots are more evident with a dark brown tonality. The pectoral fins are still translucent. The head exhibit a light elongation. Head lump was reduced (Figure 5c).
IV – 8.4 cm TL	The brown pigmentation in the dorsal region is more uniform. The oval eyes begin to take shape, being less projected than in the earlier stages. Dental projections on the surface of the jaws are more evident. The head is more elongated and pointed LT (Figure 5d).
V – 10.5 cm TL	It presents dark brown pigmentation in the dorsal region that extends through the body and fins. The nostrils are located in the rostral region, which is more elongated than in the anterior stage. The pores of the lateral line are already visible. The dorsal, pectoral and caudal fins exhibit darker pigmentation, but still translucent (Figure 5e).
VI – 23.3 cm TL	At this stage, much of the volume of the yolk sac was consumed. Regions with brown pigmentation increased compared to stage V. There is no transparency associated with the skin. The eyes no longer stand out. Dermal denticles are present throughout body surface. Upper and lower jaw teeth are already formed (Figure 5f).
VII – 24.0 cm TL	Last stage of embryonic development. At this stage, the embryo resembles the adult in external anatomical features, no longer presenting the yolk sac external to the body, being at this stage prepared for birth (Figure 5g).

Table III – Stages of the embryonic development of sharpnose sevengill shark, *Heptranchias perlo* captured in southern Brazil (April 1994 to February 1999).

Figure 5- Embryos of sharpnose sevengill shark, *Heptranchias perlo* during the stages of development. **(A)** Stage I - 5.75 cm *TL*. **(B)** Stage II - 6.8 cm *TL*. **(C)** Stage III - 7.9 cm *TL*. **(D)** Stage IV - 8.4 cm *TL*. **(E)** Stage V - 10.5 cm *TL*. **(F)** Stage VI - 23.3 cm *TL*. **(G)** Stage VII - 24 cm *TL*. Scale bars: 10 mm.

4 I DISCUSSION

Here we describe preliminary information on key components of life-history traits of the deepwater sharpnose sevengill shark, including size-at-maturity, reproductive seasonality, size-at-birth, fecundity and morphological descriptions. Among species within Hexanchidae family, reproductive data from sixgill shark *Hexanchus nakamurai*, bluntnose sixgill shark *H. griseus* and sharpnose sevengill shark are from sporadic observations,

while for broadnose sevengill shark *Notorynchus cepedianus* more data is available (Barnett et al. 2012; Awruch et al. 2014). Accordingly, we recognize that the lack of raw data affects the presentation of the results; however, we believe that the data presented here about the sharpnose sevengill shark are unique and essential for monitoring programs and conservation of the species, especially considering that information available for the species is very scarce, especially due to the logistical difficulty in obtaining data.

For females, the minimum size-at-maturity was 87.5 cm TL, corroborating previous studies that reported size-at-maturity between 95 and 105 cm TL for the species (Tanaka and Mizue 1977, Ebert and Stehmann 2013). Capapé (1980) observed that the vitellogenesis begins when the females reach 85 cm TL and the largest oocytes are absent in females with less than 100 cm. Our observations suggest that sharpnose sevengill females mature at a smaller size in Southern Brazil. As found by Tanaka et al. (1990) in frilled shark, *Chlamydoselachus anguineus*, and other Hexanchidae sharks (Lucifora et al. 2005), broadnose sevengill shark males also reach sexual maturity at smaller sizes than females. In this study, were found mature males with 69 cm TL, corroborating the maturation stages proposed by Ebert and Stehmann (2013). However, size-at-maturity can vary with the region, for example, Castro (2011) described different sizes, with juveniles less than 80 cm TL and mature male greater than 81 cm TL.

Taken together, our results suggest that sevengill female sharks exhibit at have at least a biennial reproductive cycle with a non-continuous cycle, in which ovulatory cycle is separated from gestation period (Figure 7). Biennial reproductive cycle is typical found in deep-sea sharks (Kyne and Simpfendorfer 2010; Awruch et al. 2014; Finucci et al. 2016). Our results also suggest that mating probably occurs in late summer and austral autumn (Figure 7).

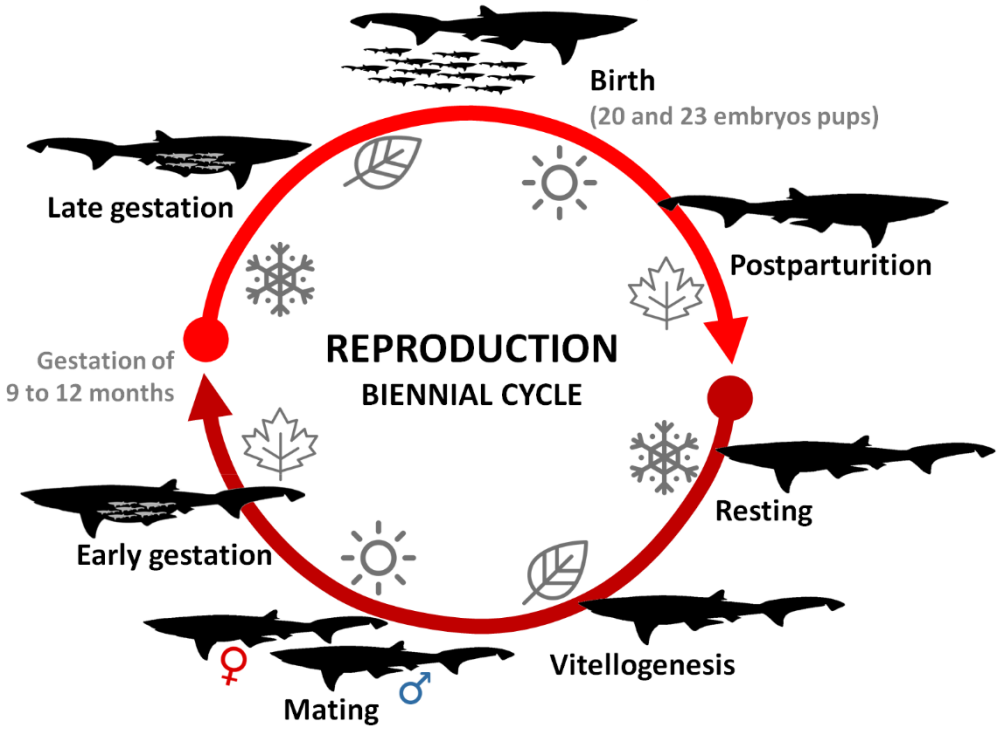

Figure 7 –Scheme of the proposed biannual reproductive cycle for sharpnose sevengill shark, *Heptranchias perlo*. Females being pregnant for 9 to 12 months, spending at least 1 year non-pregnant, with the ovulatory cycle separated from gestation.

In the present study, the two largest litters recorded were of 20 and 23 embryos. The same was observed in northeastern Brazil (22 and 23 embryos; Santander-Neto et al. 2007). According to Compagno et al. (2005) and Ebert and Stehmann (2013), the number of embryos per litter varies between 6 and 20 for the species. Birth probably occurs at the end of austral spring and summer, indicating pregnancy period of 9-12 months followed by a period of at least one year without gestation. As observed by Bigelow and Schroeder (1948) and Tanaka and Mizue (1977), the embryos showed no connection with the uterus wall or uterine compartments. Corroborating previous studies, the maximum embryo length at birth was 26.7 cm TL, similar was reported by other authors (26-27 cm TL, Bigelow and Schroeder 1948, Bass et al. 1975). Little variation was also described by other authors such as 30 cm (Capapé 1980), 25 cm (Castro 2011) and 26-17 cm in total length (Ebert and Stehmann 2013). Neonates were not captured, indicating their absence in tuna fishing areas or selection trough fishing gear.

The present study brings new information about the basic of reproductive aspects of sharpnose sevengill shark in Southern Brazil, a deep-water shark species currently listed as “Near Threatened” by the IUCN (Paul and Fowler 2003). Despite its status, there are no legislation or management plans for the species. Together, these reproductive characteristics suggested sharpnose sevengill shark is likely to have relatively low reproductive output (i.e. slow growth, high longevity and low fecundity). Given that deep-sea fisheries are increasing, basic biological data of key deep-sea species are imperative for fishing management and conservation plans, allowing this way, the implementation of damage reduction plans or catch quotas for the species where its capture is high.

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