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Evidence of mating scars in female tiger sharks (*Galeocerdo cuvier*) at the Fernando de Noronha Archipelago, Brazilian Equatorial Atlantic

Bianca S. Rangel · André S. Afonso · Vanessa Bettcher · Nayara Bucair · Nauther Andres · Leonardo B. Veras · Fábio Hissa Vieira Hazin · Ricardo Garla

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Abstract Although many shark species display predictable and seasonal aggregations related to breeding activities, few studies have reported direct evidence of reproductive interactions of large sharks. In this context, the present study reports the first evidence of mating scars in female tiger sharks *Galeocerdo cuvier* at the Fernando de Noronha Archipelago (FEN), an oceanic remote insular system in the South Atlantic Ocean. Results from the records of seven wounded females suggest that mating activity may occur between February and August. These females measured between 267 and 372 cm in total length, which is aligned with previous studies reporting size at sexual maturity for Atlantic tiger sharks. Although some females exhibited healed wounds, three of them had fresh, open wounds. One of the females also had a swollen and red cloaca. We also report the first presumably gravid female tiger shark for the region. Although sporadic, the observations herein reported indicate the importance of FEN for tiger shark reproduction in western equatorial Atlantic waters. This

In memoriam (Fábio Hissa Vieira Hazin).

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B. S. Rangel (✉)
Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, Cidade Universitária, São Paulo, SP 05508-090, Brazil
e-mail: biarangel.sharks@gmail.com

A. S. Afonso · F. H. V. Hazin
Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco, Recife 52171-900, Brazil

A. S. Afonso
MARE - Marine and Environmental Sciences Centre, ARNET – Aquatic Research Network, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

V. Bettcher
Programa de Pós-Graduação Em Ecologia E Evolução (PPGEE), Universidade Do Estado Do Rio de Janeiro (UERJ), Rio de Janeiro, RJ 20550- 900, Brazil

V. Bettcher
Laboratório de Ictiologia Aplicada (LICTA), Universidade Federal Do Estado Do Rio de Janeiro (UNIRIO), Rio de Janeiro, RJ 22290-240, Brazil

N. Bucair
Departamento de Oceanografia Biológica, Laboratório de Ecologia da Reprodução E Do Recrutamento de Organismos Marinhos (ECORREP), Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brazil

N. Andres
Laboratório de Ecologia E Conservação Marinha LABECMar, Universidade Federal de São Paulo, Santos, SP 11075-285, Brazil

L. B. Veras
Instituto Tubarões de Noronha, Fernando de Noronha, Brazil

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finding is especially relevant as FEN has been identified as an important genetic hotspot for tiger sharks, with the largest global genetic diversity for the species.

Keywords Copulation · Mating ground · Apex predator · Reproduction · Marine-protected area

Introduction

Identifying key habitats associated with reproduction of heavily exploited species, such as sharks, is critical for effective conservation planning in coastal and oceanic regions (Becerril-García et al. 2022). Although many shark species display predictable and seasonal aggregations linked to mating behavior, gestation, and parturition, few studies have found direct evidence of habitats used for reproductive purposes by large pelagic sharks (Chapman et al. 2015). This is the case for the tiger shark, *Galeocerdo cuvier* (Péron and Lesueur 1822), a large-bodied iconic predator found worldwide in warm temperate and tropical seas (Ebert et al. 2021).

Tiger sharks play an important role in the ecosystem, functioning as generalist predators that conduct long-distance and repeated philopatric migrations between insular reefs and open ocean areas (e.g., Hammerschlag et al. 2012; Papastamatiou et al. 2013; Lea et al. 2015). In their reproductive cycle, tiger sharks exhibit long gestation periods (12–16 months), producing relatively large broods (3–70) of large-sized offspring (~ 75 cm in total length; Whitney and Crow 2007; Castro 2011). It has been suggested that females reproduce every 2 (i.e., biennial cycle (Castro 2009)) or 3 years (i.e., triennial cycle (Whitney and Crow 2007)), and they seem to use a mixed capital-income breeding strategy, relying on a mix of energy stores and recently acquired resources to support reproduction (Hammerschlag et al. 2018; Rangel et al. 2021).

Only few studies have examined the reproduction of tiger sharks, although they do have distinct characteristics from other sharks. Tiger sharks exhibit a unique reproductive mode among elasmobranchs (i.e., sharks, skates, and rays) known as embryotrophy, in which embryos are nourished first by yolk and, later, by an additional energy-rich uterine fluid called embryotrophe in utero (Castro et al. 2016). In contrast to many other carcharhiniforms, tiger sharks seem to be genetically monogamous, i.e., there is no evidence for multiple paternity in the species (Holmes et al. 2018; Pirog et al. 2020). Additionally, tiger sharks apparently lack well-defined parturition and pupping areas (Holland et al. 2019). Results from previous studies have suggested that some oceanic islands are, at least, partially essential to the reproductive cycle of tiger sharks. Examples of these areas include the Hawaiian Islands (Whitney and Crow 2007; Papastamatiou et al. 2013), the Bahamas (Sulikowski et al. 2016), Reunion Island (Jaquemet et al. 2012; Pirog et al. 2020), Galápagos Islands (Acuña-Marrero et al. 2017), and the Cocos Island (Cambra et al. 2021). Given the importance of identifying critical areas associated with tiger shark reproduction for the conservation of this species, the present study reports on the first evidence of mating scars and pregnancy in female tiger sharks at the Fernando de Noronha Archipelago (FEN), a marine-protected area in the western equatorial Atlantic Ocean.

R. Garla
Beacon Development, KAUST – King Abdullah University of Science and Technology, 23955-900 Thuwal, Kingdom of Saudi Arabia

Materials and methods

Fernando de Noronha Archipelago (03°51′S, 32°25′W) (Fig. 1a) is an isolated group of volcanic islands located 345 km off north-eastern Brazil. Part of the archipelago comprises a no-take marine-protected area (MPA), the Fernando de Noronha Marine National Park, which protects nearshore ecosystems up to the 50-m isobath. The other part consists of a sustainable use area (i.e., the Fernando de Noronha–Rocas–São Pedro and São Paulo Environmental Protected Area), where fishing is allowed with some restrictions, and shark fishing is prohibited (ICMBio 2017). The region is under the influence of the South Equatorial Current and experiences a warm tropical oceanic climate, comprising a rainy season (February–July) and a dry season (August–January) (Barcellos et al. 2011). The seawater temperature and salinity average 26 °C and 36‰, respectively, and are relatively steady year-round.

Individuals were captured using bottom longlines and drumlines in waters, ranging from 40- to 60-m depth. Longlines were composed of a 275-m-long braided polypropylene mainline 8 mm in diameter, equipped with ten branch lines, which were attached

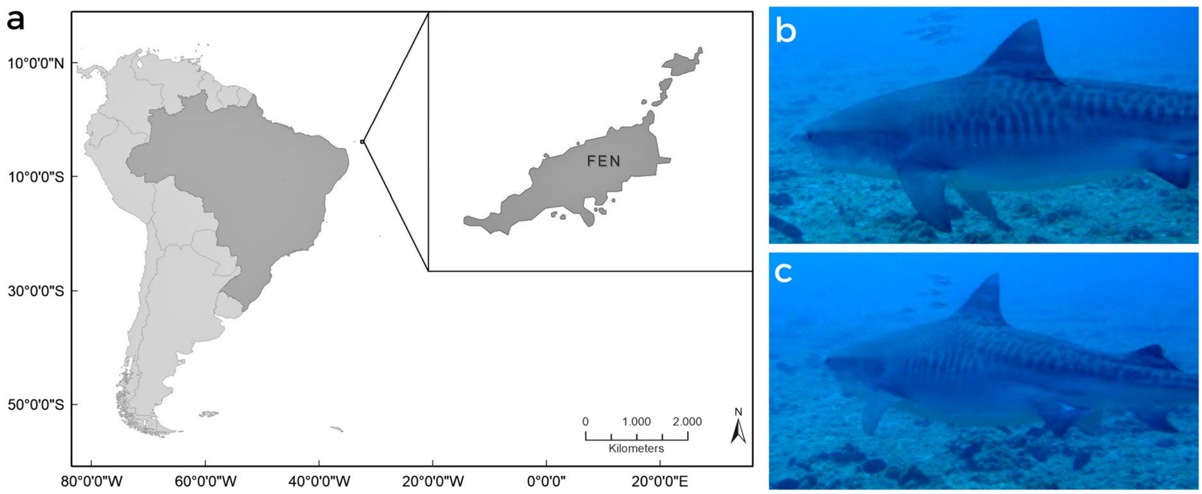

Fig. 1 Geographical location of the Fernando de Noronha Archipelago in the western South. Atlantic Ocean. **b**, **c** female tiger shark (*Galeocerdo cuvier*) #1 presumably pregnant

to the mainline every 25 m with a snap. The branch lines were composed of a 5-m-long monofilament nylon line 2.5 mm in diameter connected to a swivel, a wire leader 1 m in length, and a 17/0 circle hook. Drumlines consisted of a mooring with two attachment points: (1) a line running to the surface with a buoy and (2) a swivel connecting a 23-m monofilament ganglion line to a baited 16/0 circle hook (Gallagher et al. 2014). Both gears were checked every 90 min for shark presence. Upon capture, the sharks were either restrained in the water alongside the boat or placed aboard with their eyes covered and a hose with running seawater fitted into their mouths for ventilation. The sharks were sexed, measured for total length (TL, in cm), tagged with conventional identification tags (Hallprint, www.hallprint.com) for shark identification in case of recapture, and then released.

Additional information acquired through SCUBA diving was also considered based on opportunistically recorded photographs and videos provided by local professional photographers and videomakers. The photos and videos analyzed include metadata (i.e., date, time, and location) and additional information related to divers' reports. Observations and analysis on behavior pattens, maturity, and injuries were manually performed.

This work was conducted under permits approved by the *Instituto Chico Mendes para a Conservação da Biodiversidade* (ICMBio #12,064, #43,305, #80,761) and by the Committee on Ethics for the Use of Animals of the *Instituto de Biociências da Universidade de São Paulo* (CEUA #362/2020) and of the *Universidade Federal Rural de Pernambuco* (CEUA #23,082.025519/2014). Work did not involve anesthesia or any kind of animal euthanasia.

Results

Observations were made on 8 female tiger sharks between 2009 and 2022 (Table 1). One female that lacked bite scars (female #1) appeared pregnant, displaying abdominal distension (Fig. 1b, c; supplementary video S1. All other females displayed mating scars, although the location and nature (e.g., freshness, bite patterns) varied among individuals as reported below in chronological fashion (Table 1). Female #2 showed bite scars in the ventral region near the base of the right pectoral fin and near the gills, exposing integument and dermis (Table 1; Fig. 2a, b). The right pectoral fin also had lacerations on the extremities (Fig. 2a). The shark also had bite marks and superficial scars all over its right flank (Fig. 2c, d). Female #3 exhibited an apparently healed bite on the right side of the posterior dorsal region (Table 1; Fig. 2e). Female #4 showed several bite wounds (Table 1; Fig. 2f, i). It had fresh bite wounds on both the right (Fig. 2f) and left (Fig. 2g) pectoral fins, exposing integument and cartilage. It also exhibited bite injuries over its flank (Fig. 2h) and head (Fig. 2i).

Table 1 Female tiger sharks (*Galeocerdo cuvier*) examined in the present study

Shark	TL	Date	Depth (m)	Method	Animal condition
1	*300	14/Aug/2009	16	SCUBA	Appeared pregnant
2	372	06/Aug/2011	50–55	Longline	Bite wounds
3	*330	02/Mar/2017	5–10	SCUBA	Bite wounds
4	267	20/Feb/2019	50–55	Longline	Bite wounds
5	283	18/Jul/2019	50–55	Longline	Bite wounds
6	308	15/May/2022	42	Drumline	Bite wounds
7	340	18/May/2022	40	Drumline	Bite wounds
8	307	21/May/2022	41	Drumline	Bite wounds

* Estimated size

Female #5 exhibited small, shallow perforations on the ventral side near the cloaca (Table 1; Fig. 2j, k). It also exhibited a swollen and red cloaca (Fig. 2k).

All three females observed in May 2022 had bites in similar body parts, highlighting the perforations observed on the abdomen (Fig. 3b, f, g, j). Female #6 had recent wounds exposing integument and dermis in the region near the left gills and the pectoral fin (Table 1; Fig. 3a; supplementary video S1). Another bite wound was observed on the ventral side close to the cloaca (Fig. 3b). Shallow scratches were also observed on the right side of the first dorsal fin (Fig. 3c) and deeper lacerations on the left side of the first dorsal fin (Fig. 3d). Shallow scratches were observed on the right flank of female #7 (Table 1; Fig. 3e). The shape of the abdominal scar suggests that it derives from a deep bite (Fig. 3f, g; see supplementary video S2). Similar to female #7, female #8 had shallow scratches on the right flank (Table 1; Fig. 3h), although the scratches also extended to the caudal region (Fig. 3i). Compared to females #6 and #7, the abdominal perforations of female #8 were smaller and more superficial (Fig. 3j; see supplementary video S3).

Discussion

Here, we describe the first report of mating scars in female tiger sharks, as well as a possibly pregnant female at FEN. As wounds and scars on females often result from the male's forced attempt to mate, they are commonly used as an indicative of both the mating season (Ritter and Amin 2019) and mating areas in sharks (Sulikowski et al. 2016; Shields 2018; Rangel et al. 2022). Thus, the results herein reported suggest that FEN may be used as a mating ground for tiger sharks in the equatorial Atlantic Ocean. Based on the possibly pregnant females observed, it is possible that, after copulation, they remain in the region. The results of this study are especially relevant, considering that FEN has the largest global genetic diversity of tiger sharks, which exhibit shared haplotypes with all three Atlantic populations (Carmo et al. 2019). In addition to being an important feeding ground, FEN seems to be a key region for the genetic connectivity among tiger shark sub-populations, which should relate to the transoceanic movement behavior this species exhibits in the equatorial Atlantic Ocean (Afonso et al. 2017a).

The size at maturity in female tiger sharks remains poorly understood, as it may vary regionally (Whitney and Crow 2007; Sulikowski et al. 2016; Shields 2018). Previous studies in the North Atlantic Ocean have found that maturity can occur in females as small as 270-cm TL based on the determination of pregnancy and sex hormone concentrations in sharks of that size (Shields 2018). The smallest female with mating wounds in this study had 267-cm TL, which matches those findings from the North Atlantic. Future research combining a long-term shark-monitoring program with ultrasound exams and analysis of physiological markers would be crucial to determine the size at maturity of tiger sharks at FEN.

Mating scars on female tiger sharks have been previously documented in the Bahamas (Sulikowski et al. 2016) SC, USA (Shields 2018), and Hawaii populations (Whitney and Crow 2007; Meyer et al. 2018), suggesting the mating period occurs mainly during northern winter and parturition mainly during the summer. For the Atlantic populations, based on data from the Northwestern Atlantic, the tiger shark reproductive cycle is suggested to be biennial, with a proposed sperm storage period of 6 months

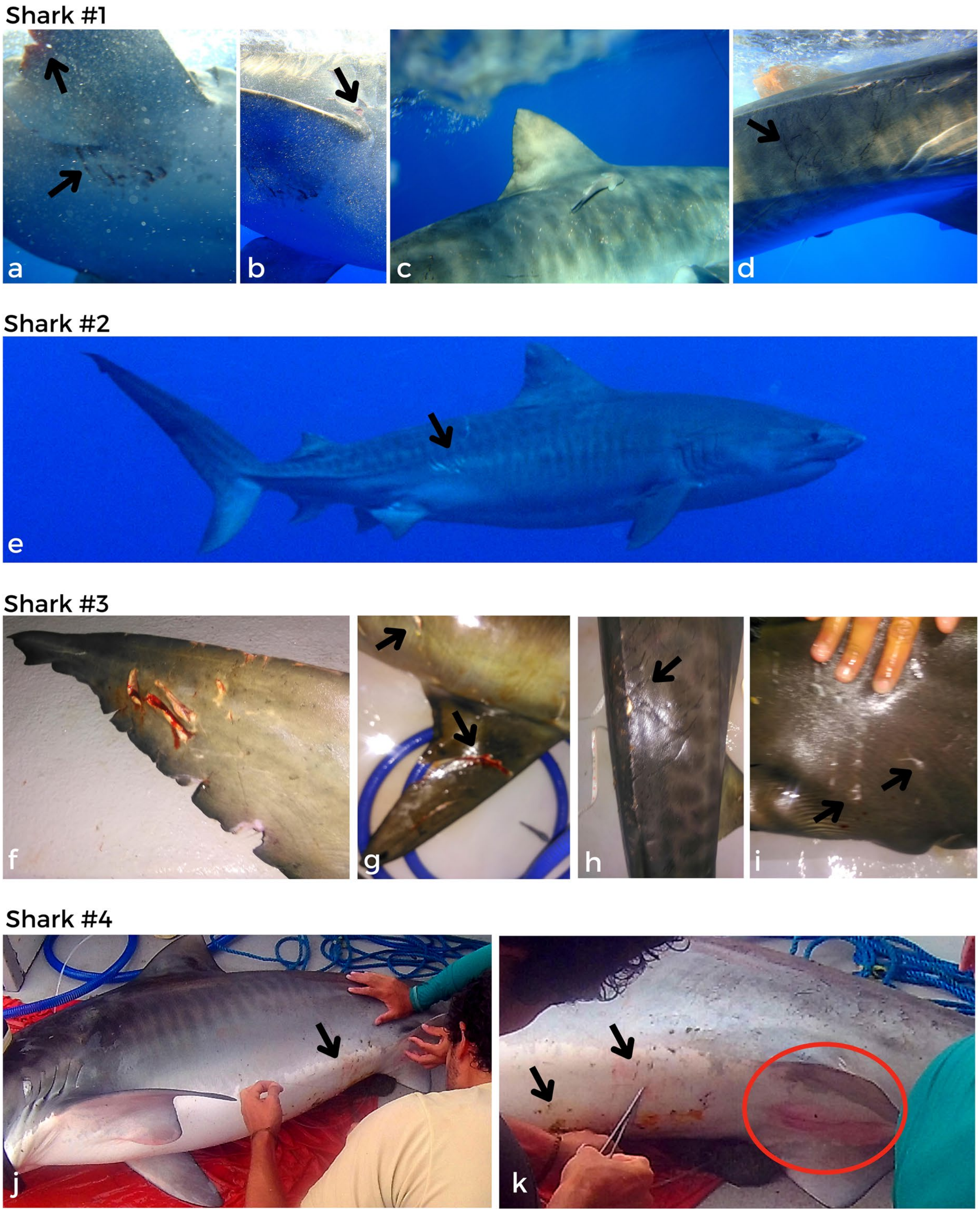

Fig. 2 Mating scars observed on female tiger sharks *Galeocerdo cuvier* at Fernando de Noronha Archipelago between 2011 and 2019. **a**–**d** female #2, **e** female #3, **f**–**i** female #4, **j**, **k** female #5. Arrows indicate the wounds and orange circle indicates the red and swollen cloaca of female #5. Photo (**e**) authored by All Angle

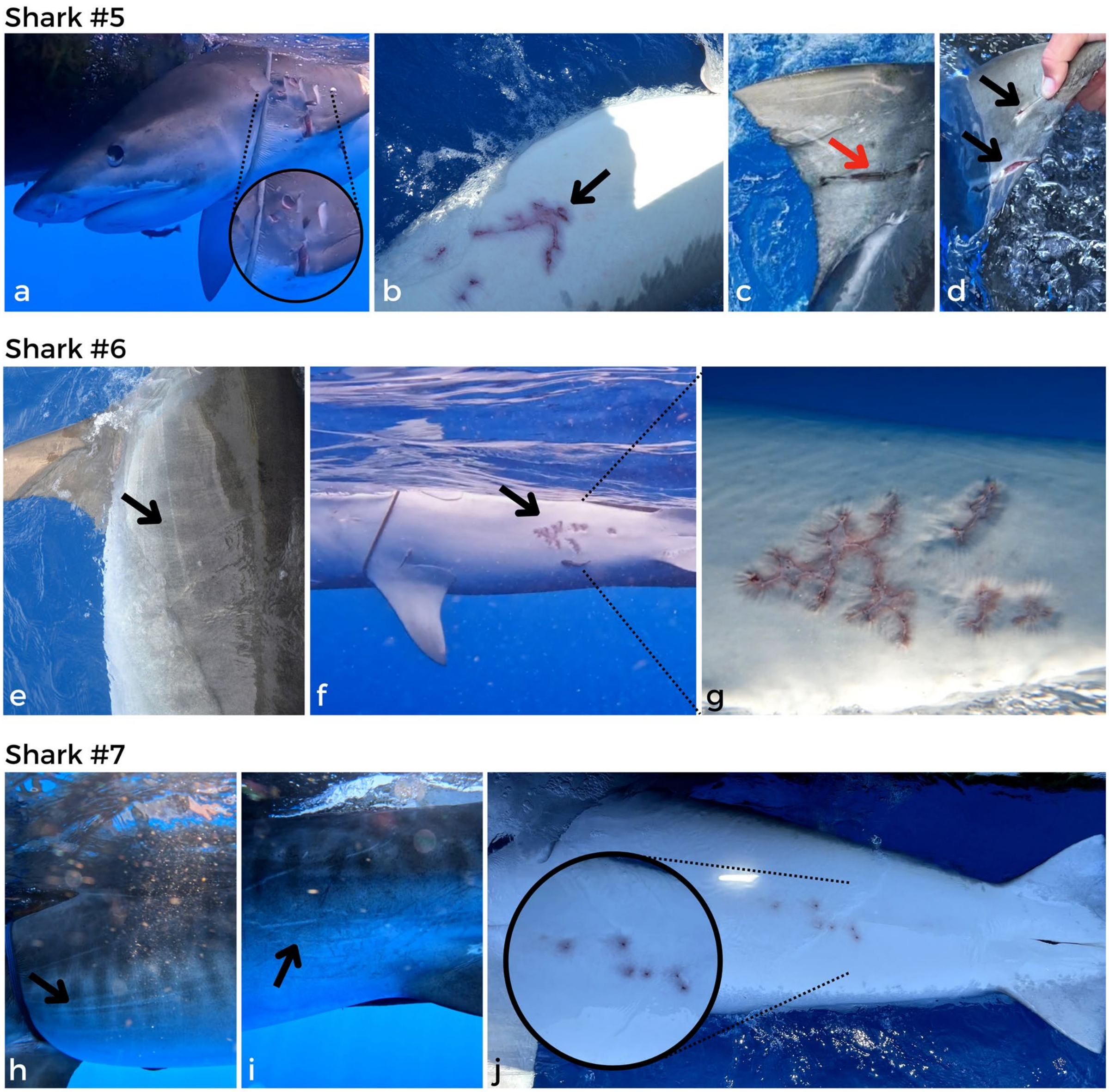

Fig. 3 Mating scars observed on female tiger sharks *Galeocerdo cuvier* at Fernando de Noronha Archipelago in 2022. **a**–**d** female #6, **e**–**g** female #7, **h**–**j** female #8. Black arrows indicate the wounds, and the red arrow in (**c**) indicates an old conventional identification tag

and a gestation period of 12–5 months (Castro 2009; Sulikowski et al. 2016; Shields 2018). In fact, neonates (< 100-cm TL) have been observed only during the first trimester of the year off northeastern Brazil (Afonso and Hazin 2015) and off southeastern Brazil (A. Rodrigues, pers. comm.), although some neonates have also been observed between March and May off Cananéia City (P.R.S. Santos, pers. comm.) and Rio de Janeiro (Araujo et al. 2020), southeastern Brazil. Our results were inconclusive regarding the mating period because both fresh and healed wounds were observed between February and August, a period which comprises the end of the austral summer, autumn, and winter seasons. It is possible that the mating period of tiger sharks at FEN occurs during a protracted period of time in comparison with conspecifics from higher latitudes, since this equatorial region exhibits low-amplitude seasonality in environmental conditions. Therefore, a greater sampling effort is needed to inquire about the synchronization of the mating period for tiger sharks at FEN.

Unlike many other shark species in which polyandrous behavior and multiple paternity are widely documented, it has been suggested that tiger sharks are genetically monogamous, i.e., females produce offspring with a single partner each reproductive cycle (Holmes et al. 2018). If applied to the females at FEN, this hypothesis could contribute to the low frequency of mating activity, as well as the low probability of registering mating marks in females. This could be related to the fact that sharks show a high capacity for bite-wound healing (e.g., Chin et al. 2015), which makes fresh wounds relatively rare to record. However, it is important to note that the offspring monogamy could be more related to post-copulatory events, such as sperm competition or female sperm choice, rather than copulation frequency (Holmes et al. 2018).

To our knowledge, this is the first study reporting a possible critical area associated with mating and gestation of tiger sharks in Brazilian waters. In addition, considering that (1) FEN is an important genetic hotspot for tiger sharks (Carmo et al. 2019), (2) the species is highly exploited in non-protected areas throughout Brazilian waters (e.g., Afonso and Hazin 2014; Leduc et al. 2021), and (3) many tracked tiger sharks seem to exhibit strong fidelity to the Archipelago (Afonso et al. 2017a; Afonso unpublished data), future studies are urgently needed for the development of appropriate management strategies in this region aimed at promoting tiger shark conservation while mitigating potential shark hazard issues associated with a potential increase in tiger shark local abundance (Afonso et al. 2017b; Meyer et al. 2018). Also, a greater sampling effort is crucial to inquire about the synchronization of the mating period for tiger sharks at FEN.

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Author contribution BSR, ASA, VB, and RG envisioned the study. BSR wrote the first draft. ASA, VB, LBV, NA, RG, and NB recorded the video and photos. All the authors contributed critically to the writing of the manuscript and gave final approval for publication.

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Data availability No further data than that presented in this manuscript are available.

Declarations

Ethical approval No animal testing was performed during this study.

Conflict of interest The authors declare no competing interests.

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