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Evasive mating behaviour by female nurse sharks, *Ginglymostoma* cirratum (Bonnaterre, 1788), in an equatorial insular breeding ground

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Mating events and aggregations of vulnerable nurse sharks *Ginglymostoma cirratum* were recorded in the insular protected area of Fernando de Noronha (FEN), Brazil, between April and August 2015. Female sharks were observed clustering in groups of up to 14 individuals in shallow water adjacent to the shore. Several evasive mating behaviours in the presence of males were noticed, including shoreward movement, positioning ventral side up at the sea surface with emerged pectoral and pelvic fins, body rolling and caudal fin thrashing. Fresh bite marks indicative of male courtship and coupling attempts were visible in female's pectoral and caudal fins. Altogether, the observed behaviours match previous reports of non-cooperative female nurse sharks during mating opportunities. An extended mating season coupled with a persistent use of inshore habitats result in nurse sharks being particularly vulnerable to human pressure during a most sensitive stage of their life cycle. The effective conservation of nurse shark populations from the western South Atlantic may thus depend on the protection of critical habitats where this species aggregates to reproduce. Explicitly addressing environmental requirements by vulnerable species in local management strategies is indispensable to ensure that human pressure, including ecotourism development, does not collide with stipulated conservation aims.

Agregações e eventos reprodutivos do vulnerável tubarão-lixa, *Ginglymosytoma cirratum*, foram registrados na área insular protegida do Arquipélago de Fernando de Noronha (FEN), Brasil, entre Abril e Agosto de 2015. Grupos constituídos por até 14 fêmeas foram observados em águas rasas adjacentes à linha costeira. Perante a presença de machos, diversos comportamentos evasivos por parte das fêmeas foram identificados, incluíndo movimentação para águas ainda mais rasas, posicionamento na superfície do mar com a face ventral orientada para cima e emersão das nadadeiras peitorais e pélvicas, enrolamento do corpo e batimento da nadadeira caudal na superfície do mar. Foram identificadas marcas recentes de mordida nas nadadeiras peitorais e caudais das fêmeas, sugerindo interações de acasalamento com machos. No geral, os comportamentos observados coincidem com os comportamentos exibidos por fêmeas não aquiescentes à cópula reportados no hemisfério norte. Em FEN, a duração relativamente longa do período de acasalamento, juntamente com a utilização persistente dos habitats mais costeiros, promovem a vulnerabilidade do tubarão lixa às pressões antrópicas durante uma das fases mais sensíveis do seu ciclo de vida. A conservação efetiva das populações de tubarão-lixa do Oceano Atlântico Sul ocidental poderá, assim, depender da proteção dos habitats críticos de agregação onde esta espécie se reproduz. Por conseguinte, a abordagem explícita dos requisitos ambientais associados a espécies vulneráveis no âmbito dos planos de manejo locais deve ser considerada como indispensável para assegurar que as pressões antrópicas, incluíndo o desenvolvimento ecoturístico, não comprometerão os objetivos conservacionistas estipulados.

Keywords: Bite marks, Copulation, Fernando de Noronha, Marine protected areas, Reproductive aggregations

Introduction

The nurse shark *Ginglymostoma cirratum* (Bonnaterre, 1788) is a tropical marine chondrichthyan that inhabits insular and continental shelves from the Atlantic and eastern Pacific oceans. Nurse sharks are aplacental viviparous and have

life-history strategies characterized by late maturity and low fecundity, thus being susceptible to overfishing and habitat disturbance. Despite that nurse sharks are globally assessed as Data-deficient, they are considered to be Vulnerable to extinction off South America (Rosa *et al.*, 2006). In Brazil, nurse sharks benefit from no-take protection enforced by

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federal regulations since May 2004 but they are still caught and routinely retained by artisanal fisheries. Furthermore, since nurse sharks make extensive use of coastal habitats and exhibit long-term site-fidelity (Ferreira *et al.*, 2012), they are expectedly exposed to deleterious processes that reduce habitat suitability or availability. Not surprisingly, considerable declines in nurse shark populations from Brazil, including localized extinctions, have been reported (Rosa & Lima, 2008). Research about nurse shark bioecology and essential habitats is thus warranted to design effective conservation strategies in this region.

Reproduction is a key feature in shark bioecology because it encompasses several important aspects of a species' life history that underlie the intrinsic dynamics in shark populations (Walker, 2005). These include age and size at first maturity, fecundity, gestation period, size at birth and longevity. Yet, other species-specific reproductive traits such as mating behaviour and rhythmicity are also expected to influence shark demography (Pratt & Carrier, 2005). For example, mating behaviour could establish reproductive success by either augmenting or diminishing female fertilization rates, which would necessarily produce an effect at the population level. Also, mating periodicity could influence the reproductive output if species, which mate more often turn out to deliver more offspring than species which mate infrequently. Such a relation may be largely obscured by the ability of some species to store spermatozoa in the oviducal glands (Pratt, 1993) and decoupling both mating and fertilization processes, though.

Nurse shark reproduction has been widely addressed in the western North Atlantic (Carrier *et al.*, 1994; Carrier *et al.*, 2003; Castro, 2000; Klimley, 1980; Pratt & Carrier, 2001) but little is yet known about the populations from the southern hemisphere. On that account, this study reports on nurse shark mating events opportunistically witnessed at an insular marine protected area (MPA) off northeastern Brazil in order to contribute for a better acquaintance of nurse shark reproductive behaviours and critical habitats.

Material and Methods

The Archipelago of Fernando de Noronha (FEN) is a small (26 km²) equatorial insular system located ~360 km off northeastern Brazil (Fig. 1). FEN has been designated as an UNESCO World Heritage Site in 2001 and encompasses one of the most prestigious, pristine marine reserves from the western South Atlantic Ocean. The no-take MPA of FEN was established in 1988 and it harbors a flourishing ecotourism industry. Sharks are among FEN's charismatic fauna and are mainly represented by three resident large-bodied species, *i.e.* lemon, *Negaprion brevirostris* (Poey, 1868), Caribbean reef, *Carcharhinus perezi* (Poey, 1876), and nurse sharks. The several contiguous islands composing FEN are aligned in a SW-NE orientation, rendering distinct oceanographic conditions to the northward and southward sections of the MPA. Rocky shores and steep cliffs are

predominant in the south windward side, whereas sandy shores are more common in the north leeward side. The rainy season in FEN spans from February through July but the seawater temperature is relatively steady year-round, averaging 26-27°C.

In several occasions, nurse shark aggregations and mating events were opportunistically witnessed in shallow waters off FEN and recorded with digital video cameras (Panasonic DMC-ZS10 180-dpi and Nikon D5100 300-dpi). In one circumstance, the observer was a tourist who voluntarily provided a video file together with a thorough description of the episode. We also took additional underwater imagery of a nurse shark aggregation in one occasion while free diving. We assembled all video footage and analyzed it in detail using Windows Live Movie Maker in order to inspect for any particular behaviours and body marks that could be indicative of fresh bites or that could be used to identify individual sharks. Also, the sex of the sharks was determined by assessing the presence or absence of claspers or, when visualization of the pelvic region was not possible, by examining individual behaviour during coupling. Typically, male nurse sharks tend to follow females while attempting to grasp one of their pectoral fins (Pratt & Carrier, 2005), hence the distinct behaviours displayed by different sharks expectedly allow for the sex to be determined. We edited the collected footage into WMV video clips and extracted the frames that best depicted behaviours or body marks of particular interest. The reported behaviours were interpreted according to Pratt & Carrier (2001).

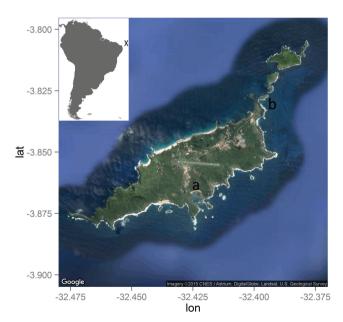


Fig. 1. Map of the Archipelago of Fernando de Noronha (FEN), Brazil, depicting the locations of (a) Baía do Sueste and (b) Enseada dos Tubarões, where nurse shark mating activity was witnessed. The "X" in the inset represents the location of FEN in relation to South America. Map plotted with ggmap package (Kahle & Wickham, 2013) in *R* statistical software.

Results

Nurse shark aggregations and mating events were repeatedly observed in shallow waters off the southern side of FEN between April and August 2015. In all circumstances, the sharks were clearly sighted from land as they were in close proximity to the shoreline. Mating activity was first observed on 25 April during the ebb tide, between 11:00 a.m. and 1:00 p.m., in Baía do Sueste (3°51'51.9"S, 32°25'18.8"W; Fig. 1), a shallow, sheltered inshore bay. The weather was clear but poor seawater visibility due to high siltation and abundant Sargassum rafts precluded the observation of submerged sharks. One female and one male were detected while interacting at the sea surface, though. The behaviours observed were clearly indicative of mating because the male relentlessly pursued the female while attempting to grasp onto one of her pectoral fins (Fig. 2-I). The female did not seem to be much cooperative however as she exhibited peculiar behaviours purportedly to deter male initiatives. These included remaining on the surface ventral side up for protracted periods of time with both pelvic fins above the water (Fig. 2-II), raising one pectoral fin in the air (Fig. 2-III), and rolling her body over while repeatedly thrashing her caudal fin on the sea surface (S1 - Available only as online supplementary file accessed with the online version of the article at http://www.scielo.br/ni). Also, and despite that these interactions occurred in very shallow (~0.5 m) water, the female tended to move further shoreward when male initiatives increased in intensity, eventually getting stranded ashore due to wave swash (Fig. 2-IV). Although it was not possible to determine if copulation had occurred, the female's cloaca seemed to be relatively reddish and swollen (Fig. 2-V), suggesting that it did. Moreover, the female had two small, seeminglyfresh, crescent-shaped bite marks on the caudal fin (Fig. 2-VI), which might have been inflicted by the male since he frequently held onto the female to inhibit her from moving shoreward (S1). These coupling interactions lasted for about 40 minutes interspersed by a 15-minute period during which the sharks submerged out of sight, and included one or possibly two additional, presumably male individuals. Thirty minutes after the sharks again submerged out of sight, the observers walked into the area and inadvertently stepped on one of the sharks, which was unreactive and seemingly motionless on the seafloor.



Fig. 2. Nurse shark mating activity witnessed at Baía do Sueste, Fernando de Noronha. The panels depict (I) a coupling male shark (a) grasping the female pectoral fin with his mouth while coiling his body around the female, as evidenced by the alignment of male (b) first dorsal and (c) caudal fins; (II) a female shark ventral side up exhibiting emerged pelvic fins; (III) a female shark ventral side up rising her (d) right pectoral fin and (e) pelvic fins out of the water; (IV) a stranded female shark; (V) a female shark exhibiting f) a cloaca suggestive of earlier copulatory activity while being harassed by (g) a male shark; (VI) a female shark exhibiting (h-i) two small, crescent-shaped bite marks on the caudal fin.

Posteriorly, nurse shark aggregations were observed for a protracted period of time in the northeastern section of the Enseada dos Tubarões (03°50'00.7"S, 32°23'51.3"W; Fig. 1), a narrow, hard-bottom cove that goes virtually dry during spring low tides. These aggregations were first witnessed by a tourist at 3:00 p.m. during the flood tide on 31 July, i.e. 97 days after the mating event described above. Several nurse sharks were seen grouping at this site at least until 16 August, i.e. for a 17-d period, although it was not possible to monitor their presence every each day. Since the seawater was consistently clear, the sharks could be effectively visualized from above (Fig. 3-I; S2 - Available only as online supplementary file accessed with the online version of the article at http://www.scielo.br/ni) and also underwater. The aggregation was comprised of female sharks only and it included at least one pregnant individual (Fig. 3-II). Their size was visually estimated at 200-250 cm in total length (TL), thus likely corresponding to preadult and adult individuals since the first maturation of female nurse sharks occurs at 223-231 cm (Castro, 2000). Females tended to congregate adjacent to the shoreline, clustering against each other while resting on the sea floor (Fig. 3-III; S3 - Available only as online supplementary file accessed with the online version

of the article at http://www.scielo.br/ni), but they would turn agitated and move further shoreward in the presence of males (S4 - Available only as online supplementary file accessed with the online version of the article at http://www.scielo. br/ni). Crescent-shaped bite marks or abrasions found on the pectoral and caudal fins of several females (Fig. 3-[IV-VI]) suggested previous coupling interactions with males. On the other hand, approaching male sharks seemed to focus their attention on one single female while attempting to isolate her from the group and directing her to deeper waters. In one circumstance, a male successfully grasped the left pectoral fin of one female but she did not seem to be acquiescent as she rolled her body and remained at the sea surface ventral side up (S4). For some time, both sharks could be observed motionless in subsurface waters but their bodies were not aligned in such a way that mating could be occurring. The male took 1.17 min to grasp the female after arriving to the group, whereas the coupling interaction lasted for 1.80 min. Overall, the number of sharks participating in the aggregation may have varied, with a maximum of 10 to 14 individual sharks being positively identified from above, although a larger number could have been present in any instance while remaining undetected.

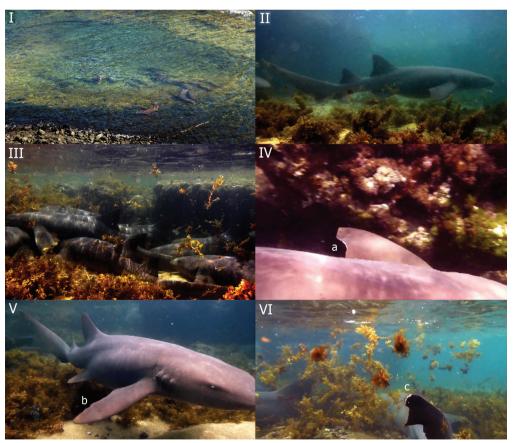


Fig. 3. Nurse shark mating activity witnessed at Enseada dos Tubarões, Fernando de Noronha. The panels depict (I) an aggregation of at least 14 nurse sharks in shallow water adjacent to the shore; (II) a pregnant female shark; (III) a group of 8 female sharks clustering together in shallow waters; (IV) a female shark exhibiting (a) a freshly-inflicted, crescent-shaped bite mark on the left pectoral fin; (V) a female shark exhibiting (b) a freshly-inflicted, crescent-shaped bite mark on the right pectoral fin; (VI) a female shark exhibiting (c) a freshly-inflicted bite mark on the tip of the caudal fin.

Discussion

Opportunistic behavioural observations of free ranging sharks may provide valuable information particularly when biologically-relevant events such as mating interactions remain to be elusive, thus precluding the implementation of robust sampling designs. Albeit being descriptive, this study provides crucial information on the reproduction of a vulnerable species in a region where little is yet known about its bioecology and essential habitats. The collected imagery evidenced that FEN encompasses mating grounds where nurse sharks aggregate for reproducing. Such a function adds on to the nursery role previously suggested by Garla et al. (2009) in depicting the significance of FEN's MPA for the life history of this species. Being classified as a highly valued, unique ecosystem by both national and international institutions and enjoying of strict environmental management, FEN may potentially provide an important framework for the successful conservation of marine wildlife in Brazil and elsewhere. Yet, acquainting the spatial ecology of vulnerable species and MPA habitat functioning is of utmost importance to ensure effective protection of depleted taxa and boosting up population recovery rates. The nurse shark critical habitats herein reported will benefit species conservation and contribute to the optimization of this and other MPAs. Furthermore, the disclosure of rare footage depicting elusive mating behaviours contributes to a better understanding about the intricacies of nurse shark reproduction.

The mating behaviours herein reported match those of conspecifics from the northern hemisphere (Pratt & Carrier, 2001) and should mostly represent evasive behaviours by non-cooperative females. Such an interpretation is sustained by females congregating in extremely shallow habitats, similarly to females from Florida (Carrier & Pratt, 1998), and being seemingly not acquiescent to male coupling attempts. To accomplish copulation, male nurse sharks usually grasp one of the female pectoral fins to facilitate clasper insertion and ensure effective sperm transfer (Carrier et al., 1994), a behaviour also present in several other elasmobranchs (Cornish, 2005; McCauley et al., 2010; Whitney et al., 2004). Non-acquiescent females may seek shallow waters in order to hinder the ability of males to successfully grasp their fins and positioning their bodies adequately (Pratt & Carrier, 2001). Once in shallow water, males frequently hold onto females and attempt to carry them into deeper water so that copulation can be accomplished (Pratt & Carrier, 2001), a behaviour that was also detected in FEN and that could relate to the bite marks found on female caudal fins. In spite of females generally exhibiting an evasive posture against male courtship, evidence of recent copulation was present in at least one individual. Female nurse sharks can copulate several times in a single day (Whitney et al., 2010) but their receptivity to different males might vary (Pratt & Carrier, 2001) and only a small proportion of mating attempts actually result in copulation being accomplished (Carrier et al., 2003). The presence of multiple males in the area could have thus resulted in one, but not the others, having succeeded in courting and mating. Yet, groups comprised of several males attempting to copulate with a single female (Carrier et al., 1994) and cases of multiple paternity within the same brood (Carrier et al., 2003; Saville et al., 2002) have been reported for this species, thus it is conceivable that purportedly non-acquiescent female nurse sharks, such as the one observed in Baía do Sueste, might end up copulating. The unresponsiveness of the shark that was inadvertently stepped on by one observer sustains such a possibility because male nurse sharks may remain motionless on the seafloor following copulation, as if in recovery (Pratt & Carrier, 2005). Further research is necessary to understand the role of evasive coupling behaviours and mate preferences by female nurse sharks, though.

In Florida, nurse shark mating aggregations seem to occur during the summer, from June to July (Castro, 2000). In FEN, however, nurse shark mating activity spanned from late April to mid-August, i.e. during the austral winter and for a longer period of time. Little seasonal variability in most environmental features (e.g. seawater temperature and salinity) in this oceanic equatorial region could partially explain such a contrast. Conspicuous nurse shark aggregations in shallow waters off FEN have never been reported despite a continuous use of the area by tourists, researchers and residents. It is thus not possible to ascertain whether the observed seasonality prevailed during previous years neither the periodicity of the mating season. Female nurse sharks seem to breed every other year off Florida (Pratt & Carrier, 2005) hence they might also exhibit a biennial mating cycle off FEN. Notwithstanding, assuming a gestation period of 5-6 months for this species (Carrier et al., 2003; Castro, 2000), parturition would expectedly occur between September and February, i.e. through a longer period of time compared to Florida, where 27-30 cm TL offspring are born mainly during November and early December (Castro, 2000). However, Garla et al. (2009) reported neonates visually-estimated at 30 cm TL to occur off FEN considerably earlier, mostly from July to September, which chronologically matches the observation of a 30-cm individual at the nearby Atol das Rocas in August (Castro & Rosa, 2005). The fact that the presence of yolk sac scars was not assessed, though, together with pigmentation distinctive of newborns persisting until ~55 cm TL (Castro, 2000), both bring uncertainty to the actual free-swimming age of these individuals. Regardless, the currently available information suggests the nurse shark mating season in FEN to be protracted in time compared to tropical latitudes.

Female nurse sharks seem to prefer shallow, sheltered, structurally complex inshore areas off FEN to aggregate. Identifying critical habitats to the life cycle of vulnerable species is essential for designing efficient protection strategies. Although it is unknown whether female nurse sharks aggregate in deeper habitats around FEN as well, they have been reported to mate in deeper waters off Florida (Rouse,

1992), therefore cryptic aggregations in deeper waters off FEN are plausible. Conversely, the unprecedented observations in shallow inshore waters could reflect an intensification of female evasive mating behaviours, resulting in sharks spending more time close to the shore and being more prone to be sighted by humans. An increase in the local abundance of nurse sharks could eventually promote such behaviours, and there is evidence that the abundance of nurse sharks off northeastern Brazil has been rising since 2004 (Afonso et al., 2014), probably as a consequence of the no-take protection status assigned to this species. Albeit female nurse sharks being late maturing, up to 20 years old (Compagno, 2001), their brood size can amount to 50 offspring (Castro, 2000). The removal of fishing mortality in FEN coupled with the availability of high-quality habitats may have synergistically promoted the recruitment of a greater proportion of juveniles into the reproductive population, but no data on local nurse shark abundance and demography are available to examine such a hypothesis.

On the other hand, species-specific behavioural traits during the nurse shark mating season could raise conservation concerns about this particularly sensitive stage. This is because female evasive behaviour against male harassment may result in females spending a considerable amount of time in shallow inshore habitats, which are typically highly susceptible to environmental variability and anthropic interference. In accordance, Carrier & Pratt (1998) report nurse shark mating activity to be greatly disturbed by human presence and boat traffic. Hence, endowing nurse sharks with suitable breeding habitats and enhanced protection from potentially-disruptive anthropic pressure may be indispensable for their effective reproduction. Specific management measures might thus be required to e.g. certify that ongoing ecotourism development does not collide with species conservation. Following Carrier & Pratt (1998), these may include i) acknowledging aggregation grounds off FEN as unique and critical for nurse sharks; ii) conditioning or restricting public access during aggregation periods; and iii) implementing an environmental education program to instruct the public about the ecological requirements of this species. By simultaneously benefiting from no-take legislation and the effective protection of critical habitats identified by dedicated research, depleted nurse shark populations will potentially be able to recover back to sustainable, least-concern numbers.

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