


First record of mating behaviour and induced parturition of the Brazilian endemic Lutz's stingray *Hypanus berthallutzae*

Vanessa B. Bettcher^{1,2}  | Nayara Bucair³ | Marta Granville⁴ | Amanda Góes⁴ | Liana de Figueiredo Mendes⁵ | Fabio Di Dario⁶ | Rodrigo Leão de Moura⁷ | Ricardo Garla⁸ | Bianca S. Rangel⁹

¹Programa de Pós-Graduação em Ecologia e Evolução, Rua São Francisco Xavier, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

²Laboratório de Ictiologia Aplicada, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

³Departamento de Oceanografia Biológica, Laboratório de Ecologia da Reprodução e do Recrutamento de Organismos Marinhos, Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brazil

⁴Barracudas – Comércio e Serviços Fotográficos e Cinematográficos Ltda, Fernando de Noronha, Brazil

⁵Laboratório do Oceano, Departamento de Ecologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, Brazil

⁶Instituto de Biodiversidade e Sustentabilidade – NUPEM/UFRJ, Universidade Federal do Rio de Janeiro, Macaé, Brazil

⁷Instituto de Biologia and SAGE/COPPE, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

⁸KAUST Beacon Development, King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia

⁹Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil

Correspondence

Vanessa B. Bettcher, Programa de Pós-Graduação em Ecologia e Evolução, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524, CEP 20550-900, Rio de Janeiro, RJ, Brazil.
Email: vanessa_bettcher@hotmail.com

Funding information

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: 88887.370376/2019-00; Conservando o Futuro do Fundo Brasileiro para a Biodiversidade e Instituto Humanize, Grant/Award Number: 98/2019; Fundação de Amparo à Pesquisa do Estado de São Paulo, Grant/Award Number: 2017/25273-0; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: PROTAX 443302/2020; FAPERJ, Grant/Award Number: E-26/210.290/2021

Abstract

The recently described Lutz's stingray *Hypanus berthallutzae* is endemic to the Brazilian Province, including oceanic islands. Although it is expected to have life-history traits similar to the southern stingray *H. americanus*, little is known about its reproductive biology. Here we present the first observations of courtship behaviour ($n = 4$), copulation ($n = 3$) and an induced parturition of *H. berthallutzae* at the Fernando de Noronha Archipelago, an insular Marine Protected Area from the Equatorial Atlantic Ocean. The mating event records included (1) 'chasing/close following', (2) 'biting/precopulatory biting', (3) 'insertion/copulation' and (4) 'separation'. These results are especially relevant considering that records of reproductive behaviour in the wild are rare for elasmobranchs in general. Mating events occurred in different months, suggesting that the reproductive cycle of *H. berthallutzae* in this insular system is asynchronous, as observed for other stingray species in regions with favourable environmental conditions and abundant food throughout the year. The opportunistic documentation of the induced parturition allowed for direct nonlethal observation of the two pups at or near full term in late May. Although preliminary, these observations should be considered in future management plans as they provide relevant data about the life-history traits and mating behaviour of this endemic and threatened species.

KEYWORDS

courtship, elasmobranch, endangered species, marine protected area, reproduction, South Atlantic Ocean

1 | INTRODUCTION

Sharks and rays (Elasmobranchii) are the second most threatened group among vertebrates, after amphibians (IUCN, 2022). Currently, 36.3% of stingray species (222/611) are under some level of threat (Dulvy et al. 2021). Overfishing threatens 91.8% of all rays, and habitat loss and climate change are additional threats to 20.7% of these species (Dulvy et al. 2021). Despite the alarming conservation status, critical habitats used during their life cycle, such as nursery, maternity, mating and feeding grounds, are still unknown for many ray species (Becerril-García et al. 2022; Jorgensen et al. 2022).

The recently described Lutz's stingray *Hypanus berthallutzae* Petean, Naylor & Lima 2020 is endemic to the Brazilian Province and occurs from the Amazon outflow southwards, including the oceanic islands of the Fernando de Noronha Archipelago and Rocas Atoll (Petean et al. 2020). The species is currently listed as Vulnerable by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species due to habitat loss, and targeted and bycatch fisheries, with a suspected population decrease of 30%–49% over the past 32 years (Charvet et al. 2020).

Before its recognition as a distinct species, *H. berthallutzae* in Brazilian waters was identified as the southern stingray *Hypanus americanus* (Hildebrand & Schroeder 1928). However, recent investigations combining genetics, morphology and ecological niche modelling revealed that the salinity and sedimentation of the Amazon River discharge acted as a barrier separating the two species, with *H. americanus* now restricted to the North Atlantic south to the Caribbean Sea (Petean et al. 2020). *Hypanus berthallutzae* reaches a maximum size of 152 cm disk width (DW) and 142 cm disk length (DL) (Branco-Nunes et al. 2021), with males probably maturing at approximately 60 cm DL

and females at about 85 cm DL (Silva et al. 2007). Due to the relatively recent evolutionary divergence between *H. berthallutzae* and *H. americanus*, the former is expected to have similar life-history traits (Charvet et al. 2020). *Hypanus americanus* reproduction is viviparous, with a gestation period of 5–8 months (in captivity) and annual offspring of two to seven pups with size at birth of 17–19 cm DW (Ramírez-Mosqueda et al. 2012; Last et al. 2016).

Even though *H. berthallutzae* has a wide distribution in Brazilian waters, studies on its reproductive biology have been conducted in just a few regions (Silva et al. 2007; Branco-Nunes et al. 2021). Additionally, specific reproductive aspects (i.e., fecundity, gestation period, mating and parturition period) often differ regionally and among similar species (Ramírez-Mosqueda et al. 2012). Here we present the first observations that *H. berthallutzae* uses the Fernando de Noronha Archipelago, an oceanic Marine Protected Area (MPA), for reproductive purposes (i.e., mating, gestation and parturition).

2 | MATERIALS AND METHODS

The Fernando de Noronha Archipelago (3°52'S, 32°25'W) is an isolated group of volcanic islands located 345 km off the north-east coast of Brazil (Figure 1). A no-take MPA covers 70% of the main island of the Fernando de Noronha Archipelago up to the 50 m isobath (IUCN Category II). The remaining area is an Environmental Protection Area (EPA) for sustainable use (IUCN Category V) that includes two other Brazilian oceanic systems, the Rocas Atoll and the Saint Peter and Saint Paul Archipelago.

The mating activity records were opportunistically obtained through scientific expeditions, citizen scientists' contributions and

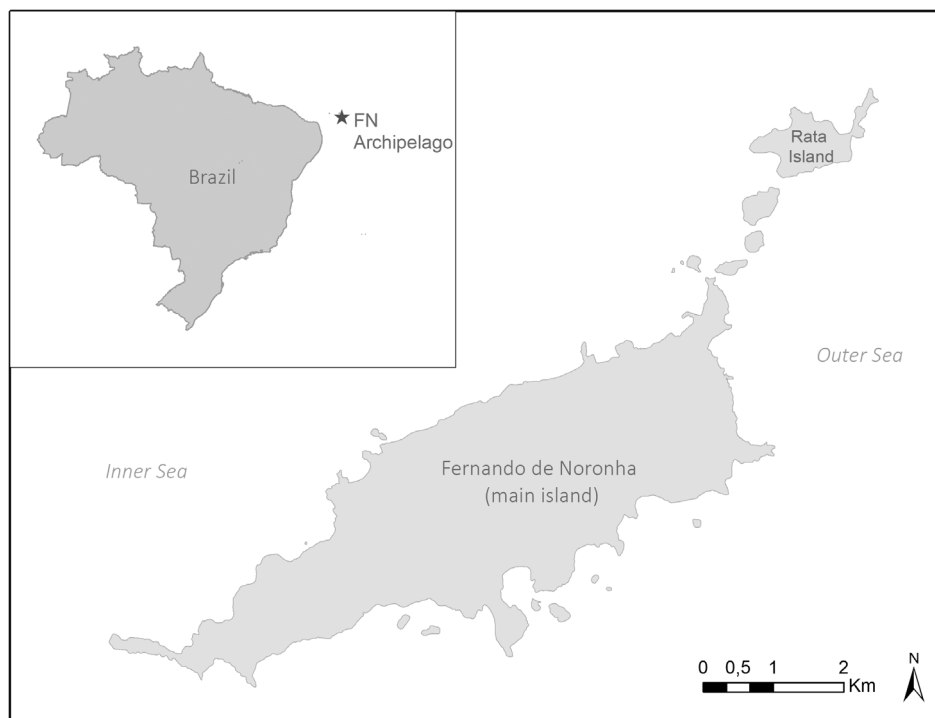


FIGURE 1 Geographical location of the Fernando de Noronha Archipelago in the western South Atlantic Ocean and Rata Island, where all mating events were observed

local underwater media company cooperation. The photos and videos analysed include metadata (*i.e.*, date, time and location) and additional information related to divers' reports.

An induced parturition of one specimen of *H. berthallutzae* was also recorded during a scientific capture for shark monitoring in the archipelago. The specimen was incidentally captured by a drumline, which consisted of a submerged weight with two attachment points: (1) a line running to the surface with buoys floats and (2) a swivel connecting a 23 m monofilament gangion line ending in a baited 16/0.5° offset circle hook (Rangel *et al.* 2022). The immersion time of each drumline was approximately 90 min. After parturition, the adult female and juveniles were released live. This study was conducted under permits approved by the Instituto Chico Mendes para a Conservação da Biodiversidade (ICMBio no. 80761).

3 | RESULTS

All sightings of the mating behaviour of *H. berthallutzae* were made in the leeward side of Rata Island (3°48'49.2''S, 32°23'25.8''W) in the Fernando de Noronha Archipelago. Mating event 1 occurred between 09:00 AM and 11:00 AM on February 1997 and involved a female of approximately 110 cm DW being closely followed by two smaller males (approximately 80 and 60 cm DW), which swam juxtaposed over the female's dorsum. The larger male remained in close contact with the female right pectoral fin for 1.5 min, frequently biting and scaring the female's skin, while the smaller male swam in contact over the larger male (Figure 2a). The female bore wounds on the dorsum of left pectoral fin, indicating previous courtship activity. After this initial courtship behaviour, the larger male rolled laterally under the female, in a ventral to ventral position (Yano *et al.*, 1999), and started copulating. The female immediately arched its posterior dorsum upwards, in a posture which apparently helped the insertion of the male's clasper (Figure 2b). Only the right clasper was inserted, while the left one remained beneath the female's pelvic fins. In the meantime, the smaller male also rolled under the female, remaining under and slightly posteriorly to the larger male, without copulating (Figure 2b). The copulating behaviour lasted for 30 s, and a cloud of semen was observed 6 s after the insertion of the larger male's clasper. No biting or gripping on the female's pectoral fins or venter was observed and both males remained swimming parallel to the female. After copulating, the larger male ceased to follow the female and the smaller male initiated a sequence similar to that performed previously by the larger male, but we could not observe if the smaller male also copulated. All events occurred while the trio slowly swam in an approximately straight line, about 1 m above the bottom. A copy of the video tape is deposited at the Museu de História Natural, Universidade Estadual de Campinas (ZUEC, tape no. 4).

In mating event 2 (September 2008) the male rotated his body in a ventral to ventral position, slightly laterally of the female's body, in such a way that he was almost perpendicular to her followed by the insertion of the right clasper into the female's cloaca (Figure 3a–c). Mating events 3 (July 2014) (Figure 3d) and 6 (February 2022)

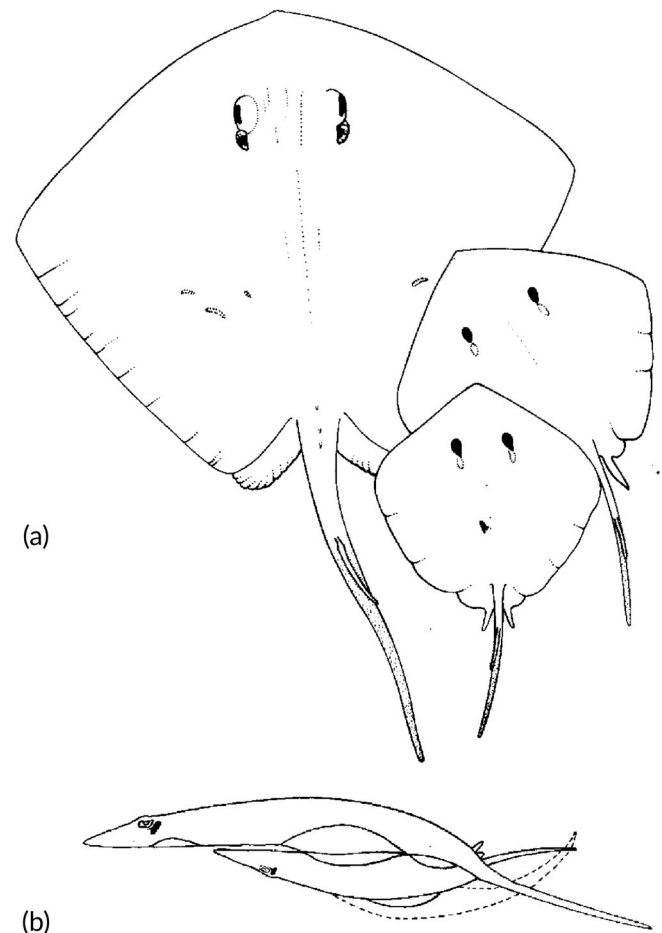


FIGURE 2 Mating event 1 of *H. berthallutzae*, which included both courtship behaviour (a) and copulation (b), and involved a female being closely followed by two smaller males

(Figure 3j, k) show one male juxtaposed over the right inferior dorsum of a resting female. Mating event no. 4 (August 2018) recorded the close-following of the male to the female, followed by the male's biting on the female pectoral fin and an almost perpendicular ventral to ventral position (Figure 3e, f). Mating event 5 (December 2021) occurred in a dive site with a maximum depth of 10 m (Figure 3g–i). First, a female approximately 90 cm DW was sighted resting at 8:57 AM. This female was observed again at 9:00 AM being chased by one mature male stingray approximately 40 cm DW, which was moving over her caudal fin (Figure 3g–i). Two minutes later, the male started grasping the female's right pelvic fin (Figure 3g–i). Both rays swam out of sight and 5 min later, only the male was sighted, swimming in a fast and very agitated way. All females recorded in mating events 1–6 had clear scars on the dorsum indicating previous courtship activity. In the mating event 7 (August 2022) a large female (about 120 cm DW) was recorded swimming slowly about 50 cm over the bottom at approximately 10 m depth. A single smaller male (about 50 cm DW), swimming faster and at first slightly below the female, started approaching her from the rear. On reaching the female, the male started to bite the upper rear portion of the female at the level

#2 (September 8, 2008)

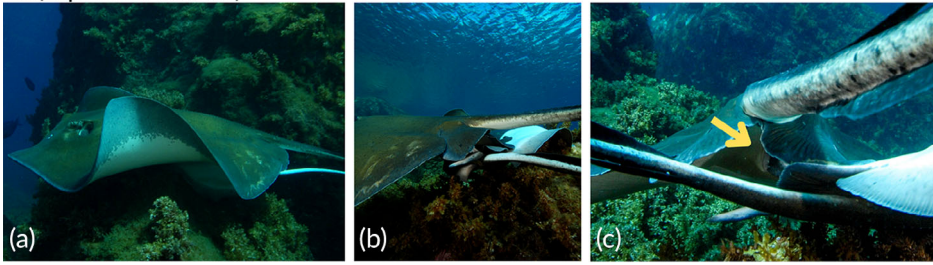
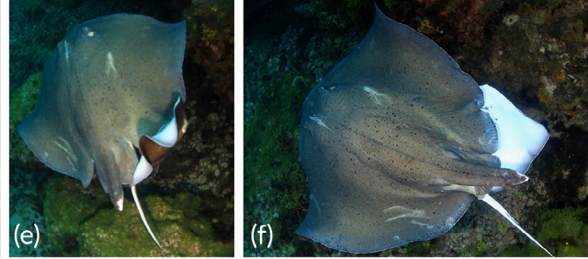


FIGURE 3 Sightings of mating events (i.e., courtship behaviour and copulation) of the Lutz's stingray *Hypanus berthaltutzae* recorded in the Fernando de Noronha Archipelago. The yellow arrow indicates male casper inserted into the female cloaca

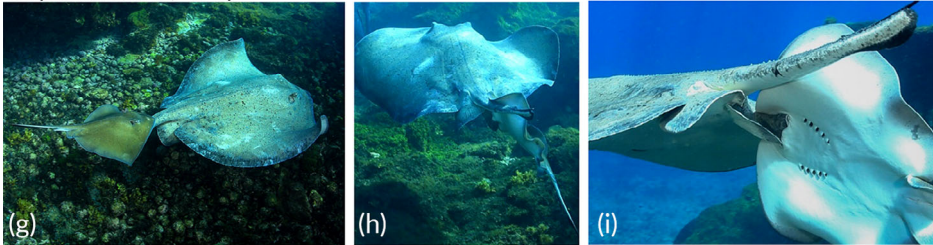
#3 (July 7, 2014)



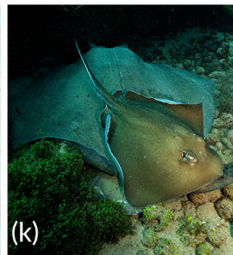
#4 (August 8, 2018)



#5 (December 15, 2021)



#6 (February 12, 2022)



#7 (12 August 2022)

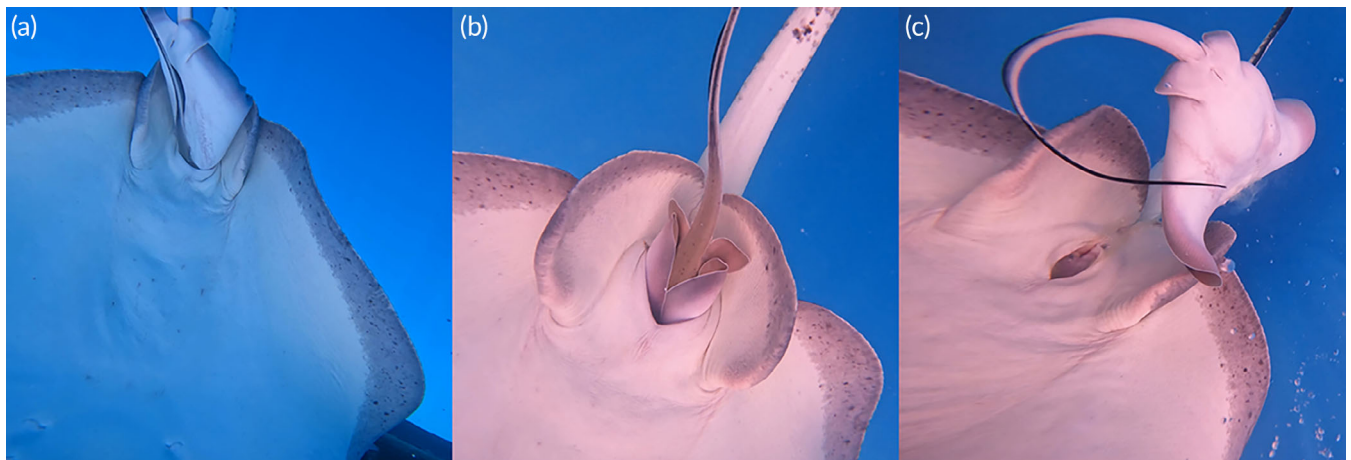


FIGURE 4 Record of the Lutz's stingray (*Hypanus berthaltutzae*)-induced parturition at Fernando de Noronha Archipelago in 2022. (a) Female neonate born. (b, c) Male neonate born

of the posterior border of both pectoral fins and apparently the pelvic fins. The female then stopped swimming and landed on the bottom, and at that point the male focused the biting attempts over her left pectoral fin. The female then started to swim slowly again, close to the substrate, when the male moved away. Mating between the pair was not observed at this event (Figure 3l).

The induced parturition was recorded in a pregnant female incidentally caught on 21 May 2022 at a depth of 40 m (3°50'02.2"S, 32°26'33.9"W). The cloaca was darker and dilated, and parturition began before the ray was released. The capture-induced parturition lasted approximately 1 min (Figure 3 and Supporting Information Video S1). The litter consisted of two pups, one female and one male with an estimated 25 cm DW. Both pups were well developed and swam right away. The female was born first and was with the ventral part facing down (Figure 4a). The male came out soon after and was positioned with the ventral part facing up (Figure 4b,c).

4 | DISCUSSION

Records of reproductive behaviour in the wild are rare for elasmobranchs in general. For batoids, copulation and pre-mating behaviour have been observed in only 14 species according to a recently published survey (McCallister et al., 2020). Four stages in the mating behaviour sequence described by Chapman et al. (2003) were observed in the present study, including (1) 'chasing/close following' (Figure 3g), (2) 'biting/precopulatory biting' (Figure 3f,i), (3) 'insertion/copulation' (Figures 2 and 3b,c) and (4) 'separation'. In addition to pectoral fin biting as generally recorded in other studies (McCallister et al., 2020), biting and scars in the pelvic fin were also observed (Figure 3i,j), as well as no biting during the precopulatory behaviour (mating event 1). Further observations are necessary to conclude if there is a preference for such biting behaviour, including the pelvic fins in the species.

Mating events were observed in different months, with two events occurring in December and August, and one in February, July and September, and copulation in August and September. Based on these observations, the most plausible explanation is that the reproductive cycle of *H. berthallutzae* in this insular system is asynchronous, as in the case of other stingrays which live in regions with favourable environmental conditions and abundant food throughout the year (e.g., Ramírez-Mosqueda et al., 2012). The Fernando de Noronha Archipelago has a relatively constant seawater temperature of 26°C and high species richness, especially of decapods crustaceans (Maida & Ferreira 1997; Alves et al. 2008), conditions that likely favour an asynchronous reproductive strategy.

The opportunistic documentation of the induced parturition allowed for direct nonlethal observation of the two pups at or near full term in late May. Although it is not known if additional pups were born before capture or after release, fecundity was within the expected for stingrays, which can vary from two to 10 with a linear

relationship with maternal DW (Ramírez-Mosqueda et al. 2012). Since the *H. berthallutzae* cycle seems to be asynchronous in this oceanic archipelago, it is not possible to estimate the period between mating activity and natural birth. However, considering the gestation period suggested of 7–8 months for *H. americanus* (Ramírez-Mosqueda et al. 2012), it is possible that *H. berthallutzae* reproduces annually. Nevertheless, a biannual cycle for females in the archipelago should not be discarded, as higher temperatures can decrease the gestation period, enabling more than one gestation per year (Henningsen et al., 2004). That situation was observed in nature for *H. americanus*, with a decrease to 4.4–7.5 months gestational time in captivity (Henningsen & Leaf 2010).

Although preliminary, the records presented here add important information to the biology of this recently recognized species, which is already threatened with extinction. All mating events recorded were observed at the same islet of the Fernando de Noronha Archipelago (i.e., Rata Island), suggesting that this site might be an important reproductive ground for *H. berthallutzae* in the region. However, we recognize that there might be a bias in this conclusion, since more recreational diving takes place in this island compared with other regions of the archipelago. Furthermore, although the size of all males' claspers and the females' body sizes suggest that courtship and copulation were effective, it was not possible to confirm whether fertilization actually happened. This would be especially relevant to confirm whether copulation by multiple males is evidence of polyandrous mating for Lutz's stingray (e.g., Chapman et al. 2003).

In summary, this is the first study presenting records of mating behaviour and parturition of *H. berthallutzae*. Since Fernando de Noronha is a remote archipelago in the South Atlantic Ocean, it is possible that individuals of the species spend their entire life cycle in the region. The data presented here demonstrate that Fernando de Noronha Archipelago provides favourable conditions for the species' reproduction, but future studies are needed, both locally and along the Brazilian coast, to determine the *H. berthallutzae* reproductive cycle in a broader context and enable future recovery plans for this threatened species.

AUTHOR CONTRIBUTIONS

V.B.B. and B.S.R. conceived the study. V.B.B. wrote the first draft. V.B.B., F.D.D., L.F.M. and A.G. recorded the videos and photos. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

The authors thank Ricardo Araujo from Núcleo de Gestão Integrada de Noronha - NGI Noronha/ICMBio (Instituto Chico Mendes para a Conservação da Biodiversidade) and the ICMBio local team for all their support. We are grateful for the support of the fishermen Carlinhos Gomes and Weliton da Silva. We also thank the photographers for allowing the use of the images: Jefferson Silva (mating event 2), Juliana Viana (mating event 3), Luisa Schneider (mating event 4), and Andressa Aviz (mating event 6).

FUNDING INFORMATION

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES no. 88887.370376/2019-00), Code 001 (doctorate scholarship to VBB). Bolsas FUNBIO – Conservando o Futuro do Fundo Brasileiro para a Biodiversidade e Instituto Humanize (no. 98/2019) and Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (no. 2017/25273-0, PhD scholarship to B.S.R.). Conselho Nacional de Desenvolvimento Científico e Tecnológico – PRO-TAX 443302/2020 and FAPERJ (grant E-26/210.290/2021) to F.D.D.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

No animal testing was performed during this study.

ORCID

Vanessa B. Bettcher  <https://orcid.org/0000-0003-0762-5663>

REFERENCES

- Alves, M. D. L., Ramos-Porto, M., & Viana, G. F. S. (2008). Checklist of the decapods (Crustacea) from the Fernando de Noronha archipelago, Brazil. *Zootaxa*, 1881(1), 43. <https://doi.org/10.11646/zootaxa.1881.1.2>.
- Becerril-García, E. E., Arauz, R., Arellano-Martínez, M., Bonfil, R., Ayala-Bocos, A., Castillo-Géniz, J. L., ... Godard-Codding, C. A. (2022). Research priorities for the conservation of chondrichthyans in Latin America. *Biological Conservation*, 269, 109535.
- Branco-Nunes, I., Yuri, N., Hazin, F. H. V., Creio, E., De Oliveira, P. G. V., & Afonso, A. S. (2021). Abundance dynamics of a new, endemic batoid from Brazil: The Lutz's stingray, *Hypanus berthallutzae*. *Regional Studies in Marine Science*, 48, 102059–102066.
- Chapman, D. D., Corcoran, M. J., Harvey, G. M., Malan, S., & Shivji, M. S. (2003). Mating behavior of southern stingrays, *Dasyatis americana* (Dasyatidae). *Environmental Biology of Fishes*, 68, 241–245. <https://doi.org/10.1023/A:1027332113894>.
- Charvet, P., Derrick, D., Faria, V., Motta, F., Dulvy, N.K. (2020). *Hypanus berthallutzae*. In: *IUCN Red List of Threatened Species* Version . Available at <https://www.iucnredlist.org/species/181244306/181246271> (last accessed June 11, 2022).
- Dulvy, N. K., Pacoureau, N., Rigby, C. L., Pollom, R. A., Jabado, R. W., Ebert, D. A., ... Simpfendorfer, C. A. (2021). Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Current Biology*, 31, 1–15. <https://doi.org/10.1016/j.cub.2021.08.062>.
- Henningsen, A. D., Smale, M., Garner, R., & Kinnunen, N. (2004). Reproduction, embryonic development, and reproductive physiology of elasmobranchs. In M. Smith, D. Warmolts, D. Thoney, & R. Hueter (Eds.), *The elasmobranch husbandry manual: Captive care of sharks, rays and their relatives* (pp. 227–236). Ohio: Biological Survey.
- Henningsen, A. D., & Leaf, R. T. (2010). Observations on the captive biology of the southern stingray. *Transactions of the American Fisheries Society*, 139, 783–791.
- IUCN (2022). The IUCN red list of threatened species. Version 2022-1. <https://www.iucnredlist.org>. Downloaded on (June 15, 2022).
- Jorgensen, S. J., Micheli, F., White, T. D., Van Houtan, K. S., Alfaro-Shigueto, J., Andrzejczek, S., ... Ferretti, F. (2022). Emergent research and priorities for shark and ray conservation. *Endangered Species Research*, 47, 171–203.
- Last, P. R., Naylor, G. J. P., Séret, B., White, W. T., de Carvalho, M. R., & Stehmann, M. F. W. (2016). *Rays of the world*. Clayton South, Australia: CSIRO Publishing.
- Maida, M. & Ferreira, B.P. (1997). Coral reefs of Brazil: An overview. In *Proceedings of the Eighth International Coral Reef Symposium*. 263–274.
- McCallister, M., Mandelman, J., Bonfil, R., Danylchuk, A., Sales, M., & Ajemian, M. (2020). First observation of mating behavior in three species of pelagic myliobatiform rays in the wild. *Environmental Biology of Fishes*, 103, 163–173.
- Petean, F. F., Naylor, G. J. P., & Lima, S. M. Q. (2020). Integrative taxonomy identifies a new stingray species of the genus *Hypanus* Rafinesque, 1818 (Dasyatidae, Myliobatiformes) from the tropical southwestern Atlantic. *Journal of Fish. Biology*, 97, 1120–1142. <https://doi.org/10.1111/jfb.14483>.
- Ramírez-Mosqueda, E., Pérez-Jiménez, J. C., & Mendoza-Carranza, M. (2012). Reproductive parameters of the southern stingray *Dasyatis americana* in southern Gulf of Mexico. *Latin American Journal of Aquatic Research*, 40(2), 335–344.
- Rangel, B. S., Afonso, A. S., & Garla, R. (2022). Female wound records suggest mating periods for the Caribbean reef shark at an insular marine protected area from the equatorial Atlantic Ocean. *Journal of Fish Biology*, 1–4. <https://doi.org/10.1111/jfb.15212>
- Silva, G. B., Basílio, T. H., Nascimento, F. C. P., & Fonteles-Filho, A. A. (2007). Tamanho na primeira maturidade sexual das raia *Dasyatis guttata* e *Dasyatis americana*, no litoral do estado do Ceará. *Arquivos de Ciências do Mar*, 40, 14–18.
- Yano, K., Sato, F., & Takahashi, T. (1999). Observations of the mating behavior of the manta ray, *Manta birostris*, at the Ogasawara Islands, Japan. *Ichthyological Research*, 46, 289–296. <https://doi.org/10.1007/BF02678515>.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bettcher, V. B., Bucair, N., Granville, M., Góes, A., Mendes, L. d. F., Di Dario, F., de Moura, R. L., Garla, R., & Rangel, B. S. (2022). First record of mating behaviour and induced parturition of the Brazilian endemic Lutz's stingray *Hypanus berthallutzae*. *Journal of Fish Biology*, 1–6. <https://doi.org/10.1111/jfb.15249>