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OBSERVATIONS ON SMALL-SCALE MOVEMENTS AND HABITAT USE OF LUTZ'S STINGRAY (*Hypanus berthalutzae*) OFF RECIFE, WITH METHOD TAIL TAG ATTACHMENT

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Abstract: Acoustic telemetry is a commonly used method to collect presence and small-scale movement data from various aquatic organisms. Studies that aspire to identify the movement and habitat use of Lutz's stingray, *Hypanus berthalutzae*, are scarce in Brazil and concentrated on oceanic islands. The present research aimed to report a method for acoustic transmitter attachment in a large-bodied coastal stingray species, in addition to describing initial observations of the pattern in movement and habitat use by the Lutz's stingray, off Recife, in Northeast Brazil, tracked in nearshore waters off a 15-km stretch of urban coastline encompassing two estuaries. A total of 25 acoustic receivers were used to monitor the movements of the tagged Lutz's stingray. The detections from the tagged specimen were recorded by 28% of the receivers available in the study area. *H. berthalutzae* was present mostly during the night (96.4% of all detections), whereas only a few detections were registered during the day (3.6%). This study is the first report on the behavior of the Lutz's stingray, off Recife, in addition to improving good tagging practices in studies with rays.

Keywords: acoustic telemetry; behavior; Dasyatidae; ecology.

Batoids (*i.e.*, rays and skates) are the most diverse group of elasmobranchs (~1150 species), with more than 600 species having been identified (Last *et al.* 2016). Stingrays composing the Dasyatidae family are epibenthic mesopredators and play an important role in regulating coastal ecosystems by connecting neritic trophic webs and controlling prey populations directly through predation (Ruiz-García *et al.* 2020). Globally, coastal development and habitat deterioration have increasingly threatened stingray populations, particularly in urban environments, which are often strongly impacted by human action (*e.g.*, fishing, industrial activities, tourism, and leisure) (Gonçalves-Silva & Vianna 2018). Batoids are frequently caught as bycatch in several fisheries (Last *et al.* 2016, Elston *et al.* 2021), and their life history traits, including low fecundity, slow growth rates, and late gonadal maturity, grant them limited ability to recover from population depletion (Stevens *et al.* 2000).

The selection of suitable habitats within the same ecosystem for different purposes is crucial for the survival strategy and reproductive success of marine species (Heithaus *et al.* 2009, Hasler *et al.* 2009, Farrugia *et al.* 2011). Biotelemetry is a useful tool for identifying and characterizing these essential habitats, as well as for understanding how and when individuals move across different micro-habitats in a given ecosystem (Ferreira *et al.* 2012, Afonso 2013). There are two important techniques involving biotelemetry: acoustic telemetry and satellite telemetry. Acoustic telemetry is a commonly used method to collect presence and small-scale movement data from a range of aquatic organisms. In this technique, the movements of tagged individuals with transmitters are detected when they have moved within the detection range of fixed acoustic receivers. This technique differs from satellite telemetry, a technique in which animals are tracked remotely while the tag is attached to an individual, and data are transmitted via satellite in broad geographical and temporal scales. (Mull *et al.* 2022). Although still incipient, acoustic telemetry is one of the most common tagging tools used to investigate the patterns of movement and habitat use of batoids, especially those of the Dasyatidae, Mobulidae, and Pristidae families (Cartamil *et al.* 2003, Setyawan *et al.* 2018, May *et al.* 2019, Lear *et al.* 2024). The present research aimed to report a method for acoustic transmitter attachment to species with large body sizes, in addition to reporting initial observations of the pattern in movement and habitat use by the Lutz's stingray, *Hypanus berthalutzae*, which has recently been identified as an endemic species from Brazil (Petean *et al.* 2020).

The Lutz's Stingray occurs in the Southwest Atlantic Ocean, from the mouth of the Amazon River to the state of São Paulo in Brazil. The species also occur in the Brazilian oceanic islands of Atol das Rocas and Fernando de Noronha. On these islands, a consistent predominance of females is noted, indicating that female stingrays probably use these environments for reproduction (Petean *et al.* 2020). *H. berthalutzae* feeds primarily on teleost fishes and cephalopods and, as a result, occupies a higher trophic level than other sympatric stingrays do, *e.g.*, *Hypanus guttatus* and *Hypanus marianae* (Queiroz *et al.* 2023). The abundance levels along the coast of Brazil are relatively different. On the coast of Recife, northeastern Brazil, low levels of abundance have been reported (Branco-Nunes *et al.* 2021), unlike the higher occurrences reported for the species in part of the Ceará coast (Santander-Neto & Faria 2020).

This study was conducted off an ~15-km stretch of coastline in the Metropolitan Region of Recife (8°10´S, 34°53´W). The high incidence of shark attacks on humans off Recife prompted the development of the Shark Monitoring Program of Recife (SMPR), which was based on the capture of potentially aggressive species (*e.g. Galeocerdo cuvier* and *Carcharhinus leucas*), as well as other (bycatch) non-target species (*e.g. Ginglymostoma cirratum* and *Hypanus berthalutzae*) to have their movements monitored by an acoustic array (Afonso 2013). The technique used in the SMPR made it possible to monitor several species simultaneously on the ~15km stretch of beach where the acoustic telemetry system was installed. Among the species captured within the scope of the SMPR, the Lutz's stingray is a coastal, benthopelagic species, with more restricted movements and is considered resident in the areas where it occurs, similar to other species of the Dasyatidae family (Branco-Nunes *et al.* 2016). In this sense, since its captures were reported on the coast of Recife (Branco-Nunes *et al.* 2021), associated with the lack of information available in the literature about the movement pattern executed by the species, the investigation of its movements can contribute to understanding the behavioral ecology of the species on the coast of Recife.

A total of 25 acoustic receivers (VR2W, Innovasea, Canada) were used to monitor the movements of the *H. berthalutzae*, tagged within the study area. The receivers were deployed alongshore the study region and were kept vertical in the middle of the water column at depths varying from 10 to 15 meters with a moored line and a float. The *H. berthalutzae* were caught by the longline gear, composed of a moored, 4-km long mainline to which 100 equally-spaced branch lines were attached.

The branch lines were made of an 8-m long, 3 mm in diameter monofilament line connected to a 2-m long stainless-steel leader and a 17/0 circle hook. Longlines were deployed longshore at the 11-15 m isobaths, approximately 1.5–3 km away from the coastline. The bait was mainly composed of *Gymnothorax* moray eel.

After capture, the rays were carefully brought onboard and placed in a plastic pool (~ 2000 L) on the vessel's deck. In this pool, the animals were stabilized, their visual conditions were checked, they were measured, sexed, and, subsequently, tagged (Fig. 1a). A monofilament thread was connected to a stainless-steel applicator to attach the transmitter through the base of the rays' tails (Fig. 1b). The acoustic transmitters were attached externally. The stainless-steel applicator was inserted into the muscle dorsolaterally on one side of the tail, exiting ventrally. On the other side, the stainless-steel applicator was then inserted ventrolaterally. In both perforations, the applicator was inserted approximately 2 to 3 cm from the specimen's vertebrae. The two loose monofilament strands exiting the tail dorsally were joined together to the acoustic tag (Fig. 1c). Subsequently the tagging procedure the Lutz's stingray were released back to the sea (Fig. 1d). Previously the biological information was collected and the animals were tagged, due to the presence of barbs in the species' tails, nylon clamps were used for mobilization, with the expectation that the next steps would be executed safely for the researchers (Fig. 1b). The capture and

Figure 1. The technique used for tagging the Lutz's stingray, *Hypanus berthalutzae*, with an acoustic transmitter off Recife, Northeast Brazil. a) after capture, the animals were carefully brought on board and placed in a pool on the vessel's deck, where they were measured and sexed; b) the barb on the tail was immobilized using nylon clamps with the expectation that the next steps would be executed safely for the researchers; c) details of the tagging procedure with an acoustic transmitter carried tag attachment on the tail; d) release of the Lutz's stingray back to the sea.

handling of rays was approved and carried out in full compliance with the recommendations of the Commission of Ethics on the Usage of Animals of the Federal Rural University of Pernambuco (license no. 041/2009).

Two adult female *H. berthalutzae* were tagged with acoustic transmitters (V16, Innovasea, Canada) with their respective individual identification numbers (IN). The first ray (R1; IN = 33486), measuring 140 cm in disk width, was caught at Boa Viagem beach (8°09'55.6"S, 34°53'15.5"W), whereas the second ray (R2; IN = 33494) was tagged at Paiva beach (8°13'07.7"S, 34°54'17.7"W) and measured 122 cm in disk width. After completion of the acoustic tagging procedure, the specimens also received a conventional 'spaghetti' tag, the nylon clamps on the tail were cut, and the individuals were released for monitoring by an acoustic matrix. Each time a tagged individual moved within the range of a receiver, *i.e.*, ~250 m, the corresponding time, date, and identification number (IN) were registered. Data were periodically downloaded every 4 months using the VUE software (Vemco User Environment, Innovasea, Canada), which allowed combining data from different transmitters, detections, and receivers into a single integrated dataset. From the detections obtained by the acoustic telemetry system, it was possible to calculate the time each individual stayed within the range of the receivers. Since the average transmission period was 60 seconds, the number of detections was considered a proxy of the number of minutes that the animals spent in the area. The acoustic receivers were separated into two main sites, *i.e.*, *i*) Boa Viagem beach (BV): 19 receivers to the north of the Jaboatão Estuary, and *ii*) Paiva beach (PA): 6 receivers in front and to the south of the Jaboatão Estuary (Fig. 2). Since BV corresponded to a more complex habitat than PA due to the presence of a channel bordered by a reef line parallel to the shore (Hazin and Afonso 2013), this site was also subdivided, *i.e. i*) BV-in: inside the channel, and *ii*) BV-out: outside the channel. The significant differences in the number of detections between the diurnal and nocturnal periods among the BV-in and BV-out habitats were assessed with a Mann-Whitney test. The residency and level of site fidelity were then evaluated based on the number of detections from each receiver compared with the total monitoring period.

The monitoring period spanned 483 days, during which only R1 was detected and only during 1.4% of the whole period. The acoustic receivers registered 168 detections from this specimen, which occurred only at BV and were recorded by 28% of the receivers available in the study area (Fig. 2). There was no difference between the total number of detections registered at BV-in (day = 0; night = 76) and BV-out (day = 6; night = 86) habitats ($W = 1$; p-value = 0.666). The Lutz's stingray was detected from September 2012 to December 2012. Among the total detections recorded in this time interval (n=168), 54% occurred in September, distributed over four days, followed by October, when 5% of the total detections were recorded in a single day. After this day (10/21/2012), the specimen returned to the receptor array in November, resulting in 40% of the detections, also in a single day, in addition to a subsequent and last detection in December (12/10/2012) (Fig. 3a). The behavior of the stingray was seemingly related to the diel cycle since it occurred in the study area mostly at night (96.4% of all detections), between 10:00 p.m. and 04:00 a.m. In contrast, only a few (3.6%) detections were registered during the day, mostly from 06:00 a.m. to 10:00 a.m. (Fig. 3b).

Fishing efforts carried out over a decade of SMPR implementation reported low catch rates for the Lutz's Stingray (~86 specimens) over the entire survey period compared with other elasmobranch species also captured in the study region (Afonso *et al.* 2011, Banco-Nunes *et al.* 2021). These results indicate that although the species occurs in the study area, its abundance appears to be low on the coastline of Recife, justifying the difficulties in catching and consequently tagging the specimens in good vital conditions during the present acoustic monitoring. However, this trend is not observed in other Brazilian states. For example, landings of sharks and rays by a small-scale fishing fleet at Fortaleza, state of Ceará, were monitored weekly from 2006 to 2008. The study reported that *H. berthalutzae* was the most abundant species, representing 52% of the total number of specimens landed, probably making Ceará state the region with the largest species aggregation on the Brazilian coast (Santander-Neto & Faria 2020).

For animals to be tracked in aquatic environments using acoustic transmitters, the tag and attachment method must not affect their natural behavior.

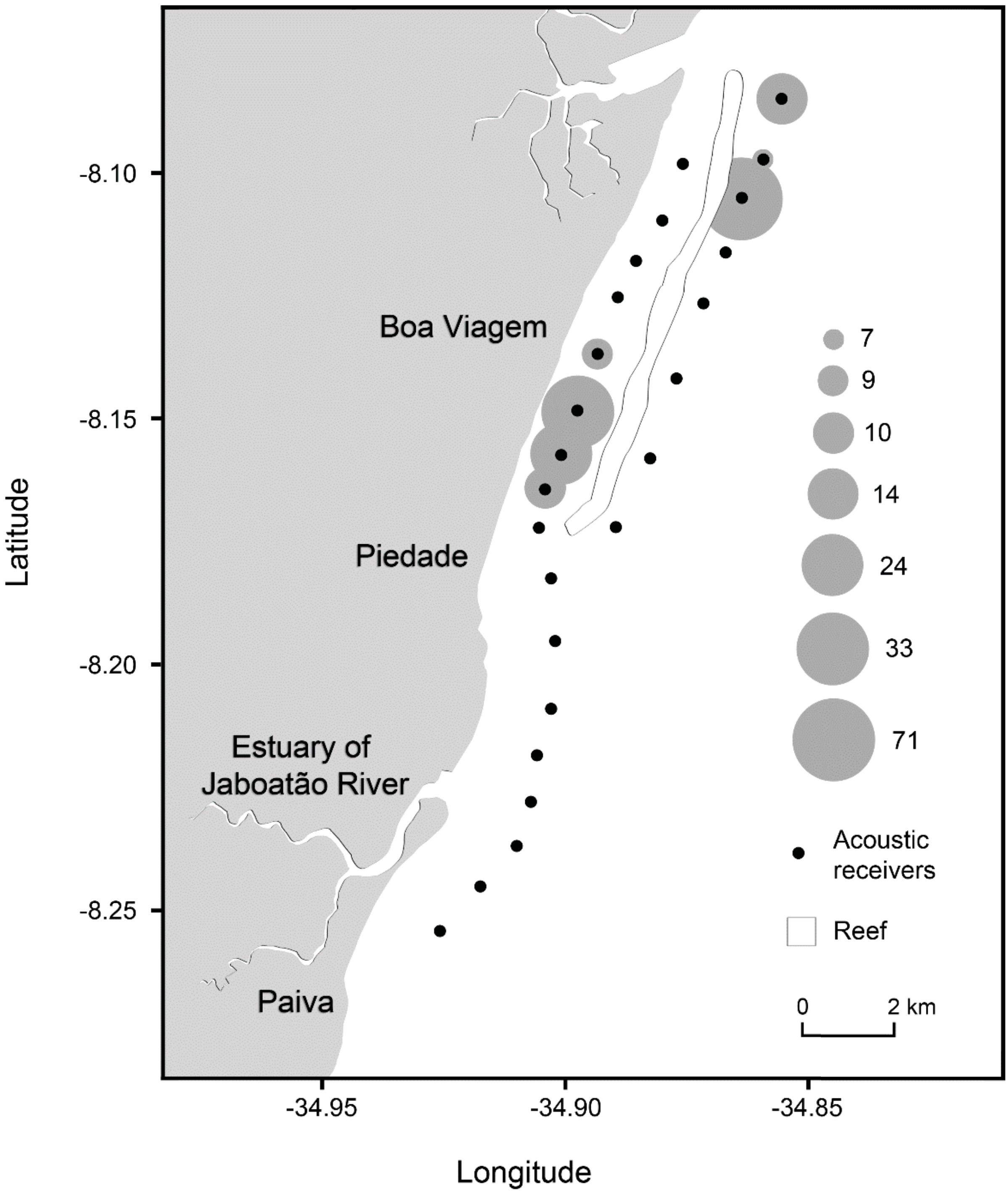

Figure 2. Locations of the acoustic stations (black points) along the study area. The acoustic receivers were positioned off Recife within depths < 14 m (distance from the coast < 3 km) in an alongshore orientation to cover the whole distance between Paiva and Pina beaches. In the northern section of the monitored area, the presence of an alongshore channel next to the beach resulted in a second line of receivers being installed closer to the shore. The shaded circles are the respective total number of detections from R1 (*Hypanus berthalutzae*) monitored in the Metropolitan Region of Recife, Northeast Brazil.

Therefore, a prior assessment of the life history of the animals, available in the scientific literature, associated with the identification of their natural behavior in the marine environment, can facilitate the choice of the best way to attach the transmitters (*e.g.*, internal or external). Lutz's Stingray exhibits a reduced space in its abdominal cavity, making the internal insertion of transmitters more complex,

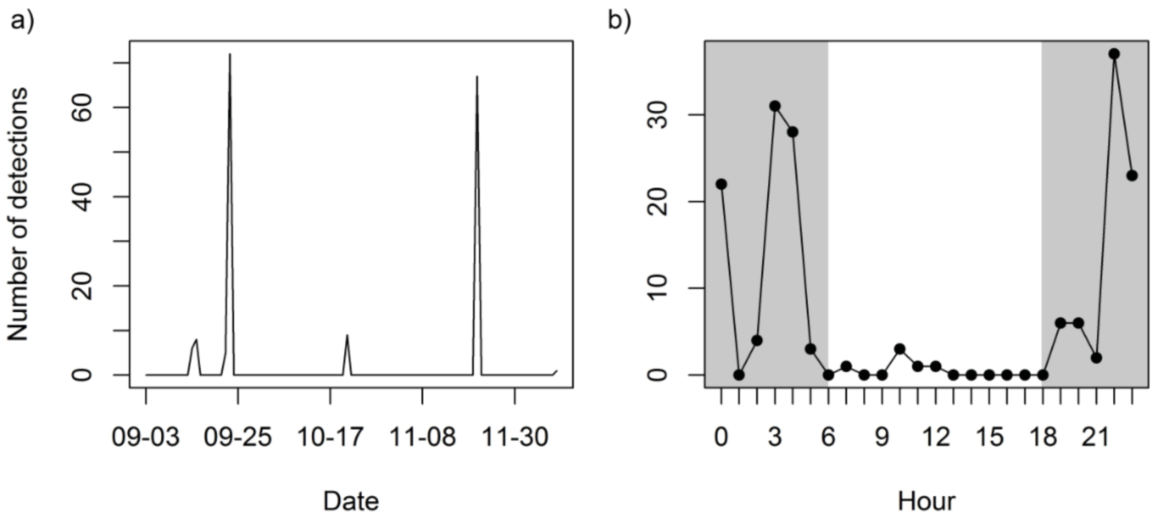

Figure 3. Total number of detections from R1, Hypanus berthalutzae, monitored in the Metropolitan Region of Recife, Northeast Brazil, in year 2012. The acoustic transmitters used in this research did not have depth sensors. Therefore, detailed information on the depth of the detected specimen was not accessed. a) the daily number of detection sand b) the total number of detections throughout the day. The blank and shaded areas in b) depict daytime and nighttime, respectively.

so these transmitters should be attached using an external tail-attachment method. In general, the external attachment of acoustic transmitters in rays can occur on the fins (*e.g.* pectoral and pelvic), muscles and tail (Le Port *et al.* 2008, May *et al.* 2019). External tagging can occur *in situ*, through diving (Branco-Nunes *et al.* 2016), or after catching the specimens (Le Port *et al.* 2008), a method that naturally involves greater animal stress. In the present study, despite the low detection rates, the process of performing external tagging on the tails of the rays occurred in a relatively short period (~5 minutes). In addition, the two individuals were released in excellent vital conditions, possibly reducing the chances of death resulting from the tagging procedure.

Although acoustic telemetry is a non-lethal technique and is considered efficient in monitoring the movement of aquatic animals, the transmitters and receivers also have limitations and thus should be considered. In the present study, the detection range of the acoustic receivers was 250 m, which is substantially lower than the detection ranges informed by the fabricator (500–600 m). In general, the detection range greatly depends on the physical properties of the water and local environmental features (Heupel *et al.* 2010). The high turbidity and freshwater runoff from the Estuary of Jaboatão River could have reduced the detection range by the receivers and, consequently, lowered the acoustically-monitored area off Recife (Afonso 2013). In addition to the intrinsic characteristics of the receiver array installed on the coast of Recife, the low number of detections may also be related to habitat deterioration in the study area because of urban development, which can significantly influence animal behavior in coastal environments (Oleksyn *et al.* 2021).

Stingray R1 was considerably more active within the study area during nocturnal periods, similar to the trends observed for this species at the Rocas Atoll Biological Reserve (Branco-Nunes *et al.* 2016). This diel rhythmicity is likely related to a nocturnal foraging strategy reported in *Hypanus stingrays* (Gilliam & Sullivan 1993, Ebert & Cowley 2003). For example, in the Cayman Islands, the Southern Stingray exhibits limited movements during the day and much more extensive movements at night, with nocturnal foraging taking place across large activity spaces but remaining mostly stationary during the day (Corcoran *et al.* 2013). This behavior has also been reported for several other coastal elasmobranch species in coastal shallow waters (Vaudo & Lowe 2006, Collins *et al.* 2007). Areas with strong anthropogenic influence (*e.g.* ecotourism) can significantly affect the natural movement patterns of animals (Corcoran *et al.* 2013). Human-sourced supplemental feeding has altered activity patterns and habitat use of Southern stingrays at Stingray City Sandbar (Grand Cayman) compared with those of wild animals at control sites. The specimens that received supplementary feeding during ecotourism became more active throughout the day, whereas the wild specimens of Southern stingrays showed naturally active behavior at night (Corcoran *et al.* 2013). Notwithstanding that the coast of Recife is a region impacted by a series of activities of anthropogenic origin, the circadian rhythm of Lutz's stingray, in the present study, did not appear to be altered. In addition, the two individuals were released in excellent vital conditions, possibly reducing the chances of death resulting from the tagging procedure.

Although unusual, research involving a small number of tagged stingrays (n = 2) of the same sex and monitored by telemetry has already been conducted and provides a starting point for further tagging and elucidating possible life history differences between this and other stingray species (Le port *et al.* 2008, Branco-Nunes *et al.* 2016). In this sense, the results reported here provide the first information related to the movement patterns and habitat use of the Lutz's stingray, a recently identified species (Petean *et al.* 2020), in a coastal region of Brazil, historically impacted by anthropogenic activities. These findings can help to better understand the natural behavior of this species, which is endemic to the Brazilian coast.

This research also highlights procedures for capturing, handling, immobilizing, and tagging relatively large ray species, which naturally involves great difficulties. The use of nylon clamps, an efficient and low-cost alternative for immobilizing and handling the spines of rays, was reported in this research for the first time. To elucidate the movement pattern and habitat use of the *H. berthalutzae*, further research should include increasing the sample size in both sexes and at different stages of sexual maturation. The results presented here can support the development of research with similar technology in areas of the Pernambuco coast, which show more favorable oceanographic conditions for the use of acoustic telemetry, combined with the organization of an overlapping array of receivers to monitor the movement of Lutz's stingray in detail.

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